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Optimization and evaluation of bioactive drug-loaded polymeric nanoparticles for drug delivery

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ABSTRACT

The premise of the present study was to suitably select or modify the constitution of the polymer matrix to achieve significantly high entrapment of hydrophilic drugs within polymeric nanoparticles (NPs). Glycyrrhizin (GL), the bioactive drug was selected as a representative hydrophilic drug. Ionotropic gelation technique was used for the preparation of glycyrrhizin-loaded NPs. Concentration of polymers were optimized by 3-level factorial design which affected the particle size and encapsulation efficiency. The formulation was subjected to morphological, physiochemical and *in vitro* drug release studies. Mean particle size of nanoparticles was around 181 nm as estimated with particle size analyzer. TEM observations revealed spherical shape and size in the range of 140–200 nm. Fourier transform-infrared analysis did not reveal any chemical interaction among the drug and polymers used for the nano-formulation. A release study conducted *in vitro* over a period of 24 h indicated primarily burst release after that controlled release of glycyrrhizin from the formulation. Antibacterial activities of glycyrrhizin, blank chitosan–gum arabic NPs and glycyrrhizin-loaded chitosan–gum arabic NPs were tested against two Gram negative and two Gram positive bacteria. The study demonstrates the benefit of excipient screening techniques in improving entrapment efficiency of a hydrophilic drug.

1. Introduction

Transport and delivery of the drug molecules specifically, gradually or securely to the site of action with the help of effective drug delivery systems (DDS) is becoming a highly important research area for the pharmaceutical researchers. Nanotechnology provides an effective DDS for delivering at the targeted sites and release of therapeutic compound in a controlled manner. Nanoencapsulation is the formation of drug-loaded NPs that increase the efficiency, efficacy, protect drug from degradation and maintains functional activity of encapsulated drugs [1]. Unique features of nanosized particles expand the scope of nanotechnology to many fields, especially in nanomedicine which namely include diagnosis, prevention and treatment of disease. Several nanoscale DDS have been used like liposomes, solid-lipid nanoparticles, micelles, hydrogels and dendrimers. Therefore NPs are fabricated from natural and synthetic macro-molecules for the fulfillments of drug delivery purpose [2,3]. Polysaccharides have gained attention due to mucoadhesive properties, biodegradability, sustainability, lack of toxicity and non-antigenicity, therefore they are the most abundant industrial raw material for pharmaceutical applications [4]. For polymeric nanoparticulate system, several natural polymers like chitosan, starch, dextran, albumin, gelatin, alginate, gums and synthetic polymers like polylactic acid (PLA), poly-(lactide-*co*-glycolide) (PLGA), polyanhydrides, poly-ε-caprolactone (PCL) have been used [5]. The polymeric materials that are to be engulfed, injected or implanted in the body should have the properties of biodegradability and biocompatibility. Several reports exist in the literature wherein polymeric NPs have been used for delivery of formulation to the oral [6], parental [7], and ocular purpose [8]. The polymeric material can protect the labile molecule from enzymatic and hydrolytic degradation in the gastrointestinal tract and enhances the transport across the systemic circulation [5,9]. Some researchers have used polymer in conjugation with other polymers [10–12] so as to enhance the solubility, encapsulation efficiency, reducing the degradation of drug and prolonging the residence time by increasing the contact time between therapeutic molecule and biomembrane at the target or absorption site.

Ionotropic-gelation is a method for the encapsulation of macromolecules such as insulin [11], using natural polysaccharides; chitosan (CS) and gum arabic (GA). Chitosan and gum arabic are naturally occurring polysaccharides, basically polycationic and

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polyanionic, respectively, and are biodegradable, biocompatible and less toxic [13–15]. Cationic nature of chitosan shows good mucoadhesive and membrane permeability properties [16]. The mechanisms of mucoadhesive and membrane permeation is based on interaction of positive charges with negatively charged cell membrane and prevent structural recognition by membrane associated gate proteins, respectively [16]. Negative charge of GA helps in the interaction with positively charged chitosan polymer leading to the formation of polymer matrix for the purpose of encapsulation. Along with these polymers, surfactant is also used for enhancing the interaction and for reducing the particle size.

Glycyrrhizin is bioactive compound extracted from the roots of liquorice (*Glycyrrhiza glabra*). After hydrolysis glycyrrhizin changed into two molecules of D-glucuronic acid and one molecule of aglycone named 18 β-glycyrrhetinic acid. Pharmacological actions, including antiviral activity [17,18], anti-hepatotoxic activity [19], anti-allergic activity [20], anti-inflammatory activity [21], protection against autoimmune disorders [22] and anti-hyperglycaemic effects [23,24] have been reported for glycyrrhizin and 18 β-glycyrrhetinic acid. Commercial products of glycyrrhizin are available in oral (25 mg/tablet) and intravenous (2 mg/ml glycyrrhizin solution containing glycine and L-cysteine) formulations. The intravenous formulation is administered 2 or 3 times per week over a longer time. Frequent intravenous injections increase patient non-compliances. Therefore, the formulations which are compatible for the patient will be more suitable.

The present work is undertaken with an aim to form glycyrrhizin-loaded nanoformulation for drug delivery with increased efficacy and sustained release, using biocompatible polymers. Ionotropic gelation method was used for the preparation of nanoformulations and optimization of concentration of polymers (CS and GA) was done with the help of factorial design with aim of minimum particle size (PS) and maximum encapsulation efficiency (EE). Synthesized glycyrrhizin-loaded CSGA-NPs were further characterized by FTIR ((Fourier Transform Infrared Spectroscopy) for interactive studies and TEM (Transmission Electron Microscope) for microscopic evaluation. Furthermore, *in vitro* evaluation of antibacterial activity has been conducted against various bacterial strains and *in vitro* release of the nanoformulation was evaluated in phosphate buffer saline (PBS) at physiological condition (pH 7.4) using dialysis sac method.

2. Experimental

2.1. Materials

Chitosan (CS), gum arabic (GA) and polysorbate-60 were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbia, India). Bioactive compound, glycyrrhizin was procured from Sigma-Aldrich. The other materials used in experiment were of pharmaceutical and analytical grade. Bacterial cultures *i.e. Bacillus ceresus* NCDC no. 240, *Bacillus polymyxa* NCDC no. 068, *Pseudomonas aeruginosa* NCDC no. 105, *Enterobacter aerogenes* NCDC no. 106 were procured from National Collection of Dairy Cultures (NCDC), NDRI, Karnal.

2.2. Protocol for nanoparticles synthesis

Glycyrrhizin-loaded chitosan–gum arabic nanoparticles (GL-loaded CSGA-NPs) were formulated by ionotropic gelation method using chitosan as primary polymer, gum arabic as secondary polymer *i.e.* co-polymer and polysorbate-60 as surfactant. CS solution (1–1.5%) was prepared in 2% (v/v) acetic acid solution and GA solution was prepared in distilled water by stirring. Thereafter, GA solution was added to CS solution during stirring. Finally surfactant (1% of the total solution) was added to the mixture and stirring was continued to achieve reduced size of nanoparticles. Glycyrrhizin-loaded CSGA-NPs were prepared by gradual addition of aqueous solution of glycyrrhizin in CS solution initially and then same procedure was followed. Polymer–drug ratio was taken 7:1. The obtained nanosuspension was analyzed for PS and % EE. PS was determined by particle size analyzer and % EE was determined by estimating amount of free drug by UV–vis spectrophotometer. The sediment having NPs freeze dried at −80 °C for 4 h followed by lyophilization using a lyopholizer (Alpha 2–4 LD plus, Martin Christ, Germany) for 24 h at −90 °C and 0.0010 mbar, using mannitol (1% w/v) as cryoprotectant.

Table 1
Actual values of parameters for glycyrrhizin loaded CSGANPs.

Coded values	Actual values	
	Concentration of CS (%)	Concentration of GA (%)
−1	1	0.1
0	1.3	0.15
1	1.5	0.2

2.3. Optimization by factorial statistical design

The factorial design was applied for the optimization of formulation ingredient to develop a nanoformulation. Based on the preliminary experimental observations, concentrations of polymers were found to be more affective on PS and EE. A 3-factorial design was chosen for optimization of nanoformulation to achieve minimum PS and maximum EE. The concentration parameters for factorial design of glycyrrhizin-loaded CSGA-NPs are depicted in Table 1. Design Expert Software Version 8.0.7.1. was used for the data analysis and for plotting of contour plots and 3-D response surface plots.

3. Characterization

3.1. Particle size (PS) and encapsulation efficiency (EE)

The particle size of glycyrrhizin-loaded CSGA-NPs samples were determined by the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The % EE was determined by UV–vis spectrophotometer (Shimardzu) by estimating the free amount of drug in the clear supernatant by using supernatant of blank-CSGA-NPs as basic correction. The absorbance was recorded at 258 nm for GL in supernatant.

$$\%\ \mathrm{EE} = \frac{(\mathrm{Total\ \ glycyrrhizin} - \mathrm{Unbound\ \ glycyrrhizin})}{\mathrm{Total\ \ glycyrrhizin}} \times 100$$

3.2. Zeta potential

Zeta potential of the optimized final batch was measured by Zetasizer Nano ZS (Malvern Instruments, Malvern, UK).

3.3. Transmission electron microscopy of nanoformulation

TEM (TEM Morgagni 268D, Fei Electron Optics) analysis was used to study the morphology of prepared nanoparticles. The lypholized sample was diluted with distilled water and homogenized using an ultrasonicator. A drop was casted on a copper grid, air dried for 5 min and loaded in the goniometer. The processed sample was observed under microscope and TEM images were recorded.