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# Development of chitosan nanoparticles as drug delivery system for a prototype capsid inhibitor

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ABSTRACT

Oral delivery of biopharmaceutics drug disposition classification system (BDDCS) Class II or IV drugs with poor aqueous solubility and poor enzymatic and/or metabolic stability is very challenging. Bay41-4109, a member of the heteroaryldihydropyrimidine (HAP) family, inhibits HBV replication by destabilizing capsid assembly. It pertains to class II of the BDDCS which has a practically insoluble solubility which is 38 μg/mL (LYSA) and the oral delivery resulted in low bioavailability. The purpose of the current research work was to develop and evaluate Bay41-4109 loaded chitosan nanoparticles to increase the solubility and bioavailability for treatment of HBV. The Bay41-4109 nanoparticles were prepared by gelation of chitosan with tripolyphosphate (TPP) through ionic cross-linking. A three-factor three-level central composite design (CCD) was introduced to perform the experiments. A quadratic polynomial model was generated to predict and evaluate the independent variables with respect to the dependent variables. Bay41-4109 was encapsulated in the chitosan nanoparticles were demonstrated by PLM, FTIR, DSC, XRD and TEM etc. The in vivo results suggest that Bay41-4109 nanoparticles have better bioavailability and would be a promising approach for oral delivery of Bay41-4109 for the treatment of HBV.



## 1. Introduction

Poor aqueous solubility and intrinsic dissolution rate (mass of the drug dissolved per time unit and area) are the major factors that affect oral delivery of many existing drugs (Preshita et al., 2012). Moreover, around 40% of the new chemical entities generated via drug discovery screens exhibit poor aqueous solubility (Gursoy and Benita, 2004). Typically, such drugs belong to Class II or IV as per BDDCS (Amidon et al., 1995) and their oral delivery often results in low bioavailability, erratic absorption, large variations in intra- and inter-subject pharmacokinetics and lack of dose proportionality (Date et al., 2010). These molecules are either abandoned early on their development process or the products are launched with suboptimal properties including poor bioavailability, lack of fed/fasted equivalence, lack of optimal dosing, presence of extra excipients that pose limitations with respect to dose escalation, and ultimately, poor patient compliance. To generally describe “solubility” the Pharmacopoeia (USP) uses seven different solubility expressions as shown in Table 1.

Bay 41-4109 (methyl-4-(2-chloro-4-fluorophenyl)-2-(3,5-difluoro-2-pyridinyl)-6-methyl-1,4-dihdro-pyrimidine-5-corboxylate) has been identified as an effective inhibitor of HBV replication in cell cultures and in an HBV transgenic mouse model. It has been demonstrated, in vitro, that Bay 41-4109 was equally effective at inhibiting HBV DNA release and the cytoplasmic HBcAg level (Deres et al., 2003; Hacker et al., 2003; Stray et al., 2005; Stray et al., 2006). According to LYSA result and USP guideline, Bay41-4109 (MW 395.77) belongs to practically insoluble (PI) range. Because of the poor aqueous solubility which is only 38 μg/mL and poor bioavailability, oral delivery system development of Bay41-4109 is very challenging.

Various nanotechnologies have been employed for improving oral drug delivery (Preshita et al., 2012). Natural polymer chitosan and their derivatives have been widely used for the development of

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**Table 1**
Solubility definition in USP.

| Description forms (solubility definition) | Pars of solvent required for one part of solute | Solubility range (mg/mL) | Solubility assigned (mg/mL) |
|---|---|---|---|
| Very soluble (VS) | <1 | >1000 | 1000 |
| Freely soluble (FS) | From 1 to 10 | 100–1000 | 100 |
| Soluble | From 10 to 30 | 33–100 | 33 |
| Sparingly soluble (SPS) | From 30 to 100 | 10–33 | 10 |
| Slightly soluble (SS) | From 100 to 1000 | 1–10 | 1 |
| Very slightly soluble (VSS) | From 1000 to 10,000 | 0.1–1 | 0.1 |
| Practically insoluble (PI) | >10,000 | <0.1 | 0.01 |

self-assembled nanoparticles which could increase the drug oral bioavailability (Cho et al., 2011; Chung et al., 2013; Lee et al., 2011; Nogueira et al., 2013). Due to their good muco adhesive character, they are used as pharmaceutical carriers for sustained drug release (Hirano, 1996). Chitosan derivatives are used in tissue engineering, wound heal (Jayakumar et al., 2005; Madhumathi et al., 2010) and to enhance the bioavailability and dissolution rates of hydrophobic drugs (Miyazaki et al., 1981). This nanoparticle drug delivery system could enhance stability of labile drugs, drug bioavailability and controlled drug release owing to the fact that particles in the nanosize ranges are efficient in crossing permeability barriers (Sharma et al., 2004).

In the present study, an attempt was made to prepare the chitosan nanoparticles as carriers for the hydrophobic Bay41-4109. Bay41-4109 was encapsulated in chitosan nanoparticles by the ionic gelation process and characterized using PLM, FTIR, DSC, XRPD and TEM etc. Cytotoxicity and in vivo pharmacokinetics studies were also performed to see the effectives of this chitosan nanoparticle delivery system. After evaluating the main and interaction variables which affect entrapment efficiency (EE), particle size and drug loading (DL), a three-factor three-level central composite design was employed to schedule and perform the experiments. Optimized Bay41-4109 nanoparticles were prepared on the basis of the predicted optimum levels of the independent variables of the factorial design.

## 2. Materials and methods

### 2.1. Materials

Chitosan, low molecular weight (deacetylation ≥75.0%, viscosity in 1% acetic acid: 20–300 cps), was purchased from Sigma–Aldrich (China). Bay41-4109 was synthesized by Roche Pharma Research and Early Development China. Sodium tripolyphosphate granular (TPP) was also purchased from Sigma–Aldrich (China). Sodium hydroxide, acetic acid and methanol were obtained from Merck (China). All organic solvents were analytical grade reagents.

### 2.2. Methods

#### 2.2.1. Central composite factorial design (CCD)

After opting for the most important factors (drug, ratio of chitosan and TPP) influencing the physicochemical properties of the produced Bay41-4109 nanoparticles, a three-factor, three-level CCD was developed to explore the optimum levels of these variables. This methodology consisted of three groups of design points, including three-level factorial design points, axial or star points, and center points. Therefore, three selected independent variables (drug concentration ($A$), chitosan concentration ($B$) and TPP concentration ($C$)) were studied at three different levels coded as −1, 0 and 1. Physicochemical properties of the produced nanoparticle, i.e. particle size ($R_1$), EE ($R_2$) and DL ($R_3$) were selected as dependent variables. The coded and actual values of the variables are given in Table 2. According to the CCD matrix generated by Design-Expert software (Trial Version 9.0.3, Stat-Ease Inc., MN), a total of 17 experiments, including three factorial points, three axial points and three replicated center points for statistical assessment the pure error sum of squares, were constructed (Gonzalez-Mira, 2011).

#### 2.2.2. Preparation of Bay41-4109 loaded chitosan nanoparticles

The chitosan nanoparticles were prepared by the ionic gelation process (Calvo et al., 1997; Vila et al., 2002; Aktas et al., 2005) of chitosan with sodium tripolyphosphate. Sodium tripolyphosphate is a non-toxic inorganic polyanion. The molar ratio between chitosan and sodium tripolyphosphate has been shown to be fundamental for the formation of nanoparticles and especially to the achievement of good drug release characteristics (Calvo et al., 1997). The formation of nanoparticles was a result of the ionic interaction between the positively charged amino groups of chitosan and negatively charged sodium tripolyphosphate. The ratio between chitosan and sodium tripolyphosphate is critical and does control the size and PDI of the nanoparticles. The nanoparticle suspensions (Fig. 1) were gently stirred for 1 h at room temperature before using subjected to further analysis.

Chitosan was firstly dissolved in the aqueous acetic acid solution, and sodium tripolyphosphate was dissolved in distilled water. Bay41-4109 dissolved in ethanol was dropped into the sodium tripolyphosphate solution at different concentrations before the synthesis of chitosan nanoparticles. All of these solution concentrations were prepared according to the CCD design. Then, sodium tripolyphosphate solution containing Bay41-4109 was dropped into chitosan solution under magnetic stirring (1000 rpm) at room temperature. Chitosan nanoparticles were formed instantaneously. Chitosan nanoparticle suspension was kept stirring for 30 min for further crosslinking of nanoparticles. After

**Table 2**
Independent variables and their levels of experiment design.

| Independent variables | Levels | | |
|---|---|---|---|
| | −1 | 0 | 1 |
| $A$:drug concentration (mg/mL) | 0.1 | 0.3 | 0.5 |
| $B$: chitosan concentration (mg/mL) | 1 | 2 | 3 |
| $C$: TPP concentration (mg/mL) | 0.25 | 0.5 | 0.75 |
| Dependent variables | Constraints | | |
| $R_1$ = particle size (nm) | Minimize | | |
| $R_2$ = entrapment efficiency (EE%) | Maximize | | |
| $R_3$ = drug loading efficiency (%) | Maximize | | |