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Research paper

# Preparation and in vitro study of lipid nanoparticles encapsulating drug loaded montmorillonite for ocular delivery

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ABSTRACT

Solid lipid nanoparticles (SLNs) are effective for drug delivery as they can improve the drug release and its bioavailability while reducing its side effects. An acid-treated montmorillonite (acid-Mt) was first intercalated with Betaxolol Hydrochloride (BH) in the interlayers and this nanocomposite was encapsulated by SLNs (Mt-BH-SLNs) using an emulsion evaporation-low temperature solidification method. The successful intercalation of BH in montmorillonite was verified by showing an increase in $d_{001}$ value from 1.54 to 1.93 nm using X-ray diffraction (XRD). The successful encapsulation of BH intercalated Mt (Mt-BH) and formation of a new nanocomposite of Mt-BH-SLNs was indicated by the disappearance of the characteristic band at 1512 $cm^{-1}$ representing C-H aromatic ring of BH in infrared spectrum (IR). The mean particle size of Mt-BH-SLNs was ca. 150 nm, which is favorable for cornea permeation. The experimental results of in vitro release, ocular irritation and in vitro corneal penetration indicated that the Mt-BH-SLNs possessed a controlled release of BH for a period of 12 h and led to a lower stimulation than that of BH solution.



## 1. Introduction

Glaucoma is the second leading cause of blindness, which is a group of ophthalmic diseases with the major risk of intraocular optical pressure (IOP) rise (Caprioli and Coleman, 2008). Betaxolol hydrochloride, with a formula as $C_{18}H_{29}NO_3 \cdot HCl$ (BH, Fig. S1), can treat this condition because it has a selective $\beta_1$-receptor blockers and calcium channel blocking ability. These features can effectively inhibit the generation of aqueous humor and increase its effusion, leading to a reduction in IOP and thus slowing down the disease progression. Therefore, BH is the most widely used drug for ocular hypertension and open-angle glaucoma in clinical therapeutics.

Low bioavailability and pre-ocular retention, and some side effects are the main obstacles for BH use as a glaucoma drug. The low bioavailability of BH drug is mainly due to the permeation barriers of eyeball. Cornea is the largest static barrier inhibiting the transportation of drug molecule into the eye. Its three layers, i.e., epithelium, stroma and endothelium, hinder absorption of hydrophilic, lipophilic and hydrophilic drug molecules (Barar et al., 2008). Therefore, the corneal epithelium is the biggest factor affecting the transportation of hydrophilic drug molecule (BH) into intraocular tissues. In recent years, many ocular drug delivery systems were investigated to improve their pre-ocular retention and drug absorption. For example, poly (lactic-co-glycolic acid)-polyethylene glycol nanoparticles (PLGA-PEG-NPs) (Vasconcelos et al., 2015), ion sensitive in situ gels (Yu et al., 2015), solid lipid nanoparticles (SLN) (Leonardi et al., 2014; Liu et al., 2011; Hippalgaonkar et al., 2013), liposome (Li et al., 2012), microspheres (Giannola et al., 2008) etc. were used. SLNs is one of those most promising ophthalmic drug delivery systems, because it is a highly biocompatible, biodegradable and controlled release drug carrier, which can encapsulate both hydrophilic and lipophilic drugs. However, there are still some unsolved issues restricting its further exploitation in ocular delivery applications, such as low corneal permeability and poor physical stability. The improved methods are to use macromolecules to change the SLNs surface charge toward positive. These macromolecules include penetration enhancers such as Pharmasolve® (Li et al., 2009), positively charged lipids (Leonardi et al., 2014), bioadhesives and positively charged chitosan (Nair et al., 2012; Ridolfi et al., 2012), and their derivatives. The electrostatic interaction can improve the physical stability of SLNs. The positively charged SLNs can electrostatically interact with the negatively

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charged mucin on corneal epithelium, which can promote the corneal permeation of drug molecule. However, high mucoadhesiveness enables the products to improve the pre-corneal retention and the penetration of the water-soluble drugs.

Montmorillonite (Mt) is a TOT type clay mineral. It has an alumina octahedral sheet sandwiched by two silica tetrahedral sheets on both sides in a single layer. Due to the isomorphous substitution in its tetrahedral sheet, Mt possesses a high cation exchange capacity (CEC). Therefore, the physical and chemical properties of montmorillonites can be adjusted via cation exchange reactions and to carry drug molecules with different surface affinities. Acid activated Mt can encapsulate more drug molecules and retain them for sustained release. In the last several decades, the unique properties of Mt have attracted great interest in drug design research. Drug molecules with different structures and properties have been intercalated into the Mt interlayer space, and the products exhibited improved drug release properties. These molecules include 5-fluorouracil (Lin et al., 2002), promethazine hydrochloride (Gereli et al., 2006), ibuprofen (Zheng et al., 2007) and timolol maleate (Joshi et al., 2009) etc. Our previous study showed that the morphology of BH loaded Mt can be readily controlled to form microspheres. This kind of microspheres possessed a sustained-release of BH, and its release duration was prolonged from 2.5 h (raw BH) to 10 h (Hou et al., 2014).

In this study, after loading of BH in Mt, it was encapsulated by SLNs (Mt-BH-SLNs) using an emulsification evaporation-low temperature solidification method. The structural, spectroscopic, thermal decomposition and morphological properties of Mt-BH-SLNs were measured by a combination of X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), thermogravimetry (TG), and transmission electron microscopy (TEM). The quality of formulations was evaluated by in vitro corneal penetration and irritation tests. This kind of solid lipid nanoparticles encapsulating montmorillonite-drug nanocomposites can serve as promising ocular drug delivery systems.

## 2. Experimental

### 2.1. Materials

Na-montmorillonite (Na-Mt) with a cation exchange capacity (CEC) of 0.90 meq $g^{-1}$ was obtained from Zhejiang, China. The chemicals used in this research include betaxolol hydrochloride (BH), phosphatidylcholine (PC), glycerol monostearate (GMS), Tween 80 and PEG 400. All the chemical reagents were of HPLC or analytical grade and were used as received.

### 2.2. Sample preparation

#### 2.2.1. Acid treatment of Na-Mt

Na-Mt was acid activated by sulfuric acid. Briefly, this procedure involves treatment of Na-Mt with 5% sulfuric acid in a 50 mL centrifuge tube for 0.5 h at 70 °C with a solid-to-liquid ratio of 1:10. The mixture was then washed using deionized water to neutral pH. After separating the solid and liquid phases by centrifugation, the solid product (acid-Mt) was dried, ground, and stored for the further tests.

#### 2.2.2. Preparation of Mt-BH

One hundred milliliters of 3 mg $mL^{-1}$ BH solution and 100 mg acid-Mt were mixed in a beaker. After the above dispersion pH had been adjusted to 4, the mixture was left static for adsorption to occur in a 50 °C water bath for 6 h. The solid and liquid phases were separated by centrifugation, the solid (Mt-BH) was dried, and then stored for further treatment. The BH loading rate (Q) was calculated from ultraviolet spectrophotometry at 275 nm. After loading, the BH concentration (C) was calculated from the obtained ultraviolet absorption ($A_{H2O}$ = 0.004C + 0.0361, the extinction coefficient $R^2$ = 0.9994), and the results using the following formula:

$$Q\,(\mathrm{mg}\cdot\mathrm{g}^{-1}) = \frac{(C_0 - C)\times V_{BH}}{M_{\text{acid-MMT}}}\times 1000 \tag{1}$$

where $C_0$ is the BH concentration (mg $mL^{-1}$) before loading onto acid-Mt in solution, respectively, $V_{BH}$ (mL) is the volume of BH solution and $M_{\text{acid-Mt}}$ is the mass of acid-Mt (mg).

#### 2.2.3. Preparation of Mt-BH-SLNs

Mt-BH-SLNs were prepared by using emulsion evaporation-low temperature solidification method (Hao et al., 2011; Mohanty et al., 2015). Briefly, PC, GMS, BH and Mt-BH were added into 5 mL ethanol as organic phase. The mixture was dissolved using a water bath at 75 °C. In addition, Tween 80, PEG 400 and sodium deoxycholate (SDC) were added into 15 mL deionized water as internal water phase. Mt-BH was ultrasonified for 10 min in an organic medium, and then heated to 75 °C. After that, the mixture was slowly injected into the internal water phase while stirring at the rate of 1000 r $min^{-1}$ and evaporated at 75 °C, until a-5 mL first-emulsion was formed. The newly formed first-emulsion was quickly injected into the external water phase with 3.6% mannitol (osmotic medium) and 0.01% benzalkonium bromide (BZK, preservative) with an ice-bath for 2 h. The obtained dispersions (Mt-BH-SLNs) were stored at 4 °C for further tests.

#### 2.2.4. Entrapment efficiency (EE) and drug-loading rate (Q′)

The freed BH was separated by dialysis method using a dialysis bag which can retain a molecular weight of 12–14 kDa. The procedure is as follows: 1 mL of Mt-BH-SLNs dispersions were transferred into dialysis bags along with10 mL of deionized water as solvent medium and stirred at 120 r $min^{-1}$ at 34 °C. The BH concentration released into the solvent medium from samples was measured by HPLC (Agilent Instrument 1200, USA) with a Shimadzu column (250 × 4.60 mm). A 30:70 (v/v) mixture of acetonitrile and 0.05% trimethylamine (phosphoric acid adjust the pH value to 3.0) were chosen as mobile phase. The detector flow rate, wavelength, and column temperature was 1 mL $min^{-1}$, 275 nm and 30 °C, respectively. The EE and Q′ of Mt-BH-SLNs were calculated using the following Eqs. 2 and 3, respectively:

$$EE = \frac{C_0' - C'}{C_0'}\times 100\% \tag{2}$$

$$Q' = \frac{EE\times M_{BH}}{M_{\text{MMT-BH-SLNs}}}\times 100\% \tag{3}$$

where $C_0'$ is the BH concentration (mg $mL^{-1}$) of Mt-BH-SLNs without dialysis treatment; $C'$ is the BH concentration (mg $mL^{-1}$) in solvent medium after dialysis treatment; $M_{BH}$ is the initial mass of BH (mg) and $M_{\text{Mt-BH-SLNs}}$ is the mass of Mt-BH-SLNs (mg).

### 2.3. Characterization

#### 2.3.1. Sample characterization

A VERTEX 70 Fourier transform infrared (FTIR) spectrometer was used. All FTIR spectra were collected at room temperature (RT) in the range of 400 to 4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ and using 64 scans for each spectrum. Thermogravimetric analysis (TG) was performed on a Netzsch STA 409 PC/PG instrument, with a heating rate of 10 °C $min^{-1}$ from RT to 1000 °C under a nitrogen flow of 60 $cm^3$ $min^{-1}$. X-ray diffraction (XRD) patterns were obtained using a Bruker D8 advance diffractometer from 3° to 70° with a scan rate of 3.0° (2θ) $min^{-1}$, by using CuKα radiation with a generator voltage of 40 kV and a generator current of 40 mA. The particle size and zeta potential of Mt-BH-SLNs were determined using Zeta potential and laser particle size analyzer (Beckman Coulter, USA) at room temperature.