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Nanostructured lipid carrier (NLC) coated with Chitosan Oligosaccharides and its potential use in ocular drug delivery system

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ABSTRACT

The objective of the present investigation was to explore the potential of the Chitosan Oligosaccharides (COS)-coated NLC (nanostructured lipid carrier) for ocular drug delivery. NLC loaded with flurbiprofen was prepared by melt-ultrasonic method and then coated with COS with a molecular weight of 3000–6000 kDa. After coating, the particles reflected spherical morphology with smooth surface under transmission electron microscope (TEM) analysis and a changed zeta potential from −0.446 mV to +20.7 mV. The ocular bioadhesion property was evaluated by Gamma scintigraphic technique, revealing that the clearance of the formulations labeled with radioactive ^{99m}Tc-DTPA was significantly delayed in the presence of COS, and the AUC of the COS-coated formulation had a 7.7-fold increase comparing with non-coated ones. Additionally, enhanced transcorneal penetration was achieved by using the COS coating with a corresponding apparent permeability coefficients (P_{app}) which had a 2.4-fold increase comparing with the reference. Consequently, COS coating modified the properties of NLCs and presented a series of notable advantages in ophthalmic application.

1. Introduction

Pharmaceutical preparations are applied topically to eyes to treat surface or intraocular conditions. Since the capacity of the eye can only retain a limited volume, excessive liquids, both normally produced and externally delivered, rapidly drain from the eye. Therefore, topical drug administration is restricted in small amount. On the other hand, due to the dynamics of the lacrimal system, the retention time of an ophthalmic solution on the eye surface is short, and the amount of drug absorbed is usually only a small fraction of the quantity administered. In addition, corneal and conjunctival epithelia of human eye, along with the tear film, construct a compact barrier preventing the drug absorption into the intraocular area (Li et al., 2009). This mechanism removes the exogenous substances involving entraps debris, microorganisms, and even drugs from the ocular surface, assisted by frequent blinking. These are the dominating reasons that many drugs have the difficulty in penetrating the ocular barrier and reaching the target tissues. Therefore, in clinical use of eye drops, frequent instillations are often required to get the expected therapeutic efficacy, which may lead to inconvenience and other system side effects through nasolacrimal absorption as well.

In recent years, studies on novel ocular drug delivery systems have been reported, such as in situ gel, microemulsion, microspheres, liposomes and solid lipid nanoparticles (SLN) (Qi et al., 2007; Chan et al., 2007; Gavini et al., 2004; Cavalli et al., 2002) all of which aim to prolong the pre-ocular retention and promote the absorption of the drug. As the second generation deriving from SLN (Müller et al., 2002), nanostructured lipid carriers (NLC) based on mixture of solid lipids with spatially incompatible liquid lipids (Müller et al., 2002) combines many features for application of pharmaceutics, i.e. controlled release of actives, drug targeting, and increasing the amount of drug penetrating into mucosa. On account of the physiological and/or biodegradable lipids, this carrier system also exhibits an excellent tolerability. Nevertheless, efforts are still needed to improve the drug delivery efficiency, especially, aiming to prolong the retention time of drug on the corneal surface to some extent.

Chitosan Oligosaccharides (COS), obtained through the decomposition of Chitosan, is a cationic polymer of low molecular weight. Unlike Polysaccharides Chitin and Chitosan, which are not water-soluble and therefore have some limitation in the use of pharmaceutics, high water solubility of COS makes it suitable for pharmaceutical application which is correlated to its structure and unique biological activities including favorable biocompatibility and mucoadhesiveness, in addition, its special property of

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Table 1
Composition of 0.03% flurbiprofen NLC dispersion.

Ingredient	Quantity (mg)
Oil phase	
Flurbiprofen	6
Compritol ATO 888	250
Miglyol 812	100
Gelucire 44/14	100
Water phase	175
Solutol HS 15	
Tween 80	75
Glycerol	400
Water ad	20

antimicrobial (Xia et al., 2010) is also taken into consideration in ophthalmic application.

It is reported that cationic polymers were probably admirable mucoadhesive materials due to an ability to develop molecular attraction forces by electrostatic interactions with the negative charge of the mucus (Ludwig, 2005). Taking this into account, the polycationic COS has been investigated as an ophthalmic vehicle and its superiority was extensively studied in drug delivery. The linking of COS which has a molecular weight of 3000–6000 kDa with the surface of the nanoparticles would presumably modify the action of NLC and ameliorate its efficiency in ocular drug delivery.

2. Materials and methods

2.1. Materials

Compritol 888 ATO (glyceryl behenate) and Gelucire 44/14 (polyoxylglycerides) were obtained as a gift from Gattefosse (France), Solutol HS-15 (polyoxyethylene esters of 12-hydroxystearic acid) was kindly supplied by BASF (Germany), Miglyol 812N (C8–C12 triglyceride) was given by Sasol (Germany). Flurbiprofen (FP) was supplied by Hangzhou Keben Chemical Co., Ltd. (Zhejiang, China). Chitosan Oligosaccharides (COS) was purchased by Tianjin Hiromi Biotechnology Development Co., Ltd. (Tianjin, China). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of FP loaded NLC

The formulations of FP-NLCs, listed in Table 1, were prepared by melt-emulsification and ultrasonication technique. Briefly, appropriate amounts of FP, Compritol 888 ATO, Miglyol 812N and Gelucire 44/14 were blended and melted at 85 °C to form a uniform and clear oil phase. Meanwhile, the aqueous phase consisting of dispersing surfactant Solutol HS-15 and Tween 80 in double distilled water was added dropwise to the oil phase at the same temperature by the aid of agitation at 600 rpm for 10 min. The coarse emulsion was then treated by probe-ultrasonic (JY-92-II, Xinzhi, China) cell disruptor for 5 min (active every 3 s for a 3 s duration, 400 W). Subsequently the dispersion was cooled to room temperature to solidify nanoparticles and stored at 4 °C.

2.3. COS coating of NLC

For COS-coated NLCs, appropriate amounts of the polymer was dissolved in water in order to form a series of various concentrations (0.1%, 0.3%, 0.5%, 1%, w/v), then mixed with FP loaded NLC dispersions. In each case, an aliquot of NLC was mingled with an equal volume of COS liquor by adding it dropwise to the polymer solution under continuous agitation at room temperature (20 °C) for a 30-min incubation.

2.4. Drug encapsulation efficiency (EE)

Free FP (non-incorporated in the FP-NLC) was separated by ultrafiltration centrifugation technique (Zhuang et al., 2010). Briefly, 1 mL of FP-NLC colloidal solution was placed in the upper chamber of a centrifuge tube matched with an ultrafilter (Amicon ultra, Millipore Co., USA, MWCO 10 kDa) and centrifuged for 15 min at 4000 rpm. The total drug content in FP-NLC was determined as follows: aliquots of 1 mL FP–NLC dispersion were diluted appropriately by ethanol to dissolve the lipid ingredient and then the obtained suspension was filtrated through 0.45 μm membrane filters. The resulting solution was analyzed by HPLC. HPLC conditions were as follows: a Diamasil® C18 column (200 mm × 4.6 mm, 5 μm, Dikma, China) was used. The mobile phase was a mixture of methanol, water and glacial acetic acid (73:22:5). The flow rate was 1.0 mL min^{-1} and the column temperature was 35 °C. The drug loading content was the ratio of incorporated drug to lipid (w/w). The encapsulation efficiency (EE) and drug loading could be calculated by the following equations, respectively:

$$\text{EE} \quad (\%) = \frac{W_{\text{Total}} - W_{\text{Free}}}{W_{\text{Total}}} \times 100$$

$$\text{Drug} \quad \text{loading} \quad (\%) = \frac{W_{\text{Total}} - W_{\text{Free}}}{W_{\text{Lipid}}} \times 100$$

where W_{Total}, W_{Free}, W_{Lipid} were the weight of total drug in NLC, the weight of unentrapped drug in ultrafiltrate and the weight of lipid added in system, respectively.

2.5. Particle size and zeta potential measurement

The average particle size and polydispersity index (PI) of FP–NLCs were determined by Laser Particle Size Analyzer (Coulter LS-230, Beckman Coulter Co. Ltd., USA). The zeta potential was analyzed by a Nano-ZS zeta sizer (Malvern Instruments, Malvern, UK) at 25 °C after appropriate dilution with ultra-purified water. Each measurement was made in triplicate.

2.6. TEM analysis

The morphological observation of COS-coated NLC was performed by transmission electron microscopy (JEM-1200EX, JEOL). The sample demanded was prepared by placing a drop of formulation which was diluted 50-fold with double-distilled water onto a 400-mesh copper grid coated with carbon film and followed by negative staining with 1% phosphotungstic acid.

2.7. Corneal penetration study

The penetration-enhancing effect of COS-coated NLCs was evaluated on isolated rabbit corneas (available areas 0.70 cm^2) using a perfusion apparatus (Camber, 1985). Albino New Zealand albino rabbits (male, weighing 2.5–3.0 kg) were used. Resumptively, 1 mL sample and 7.8 mL glutathione bicarbonate Ringer (GBR) buffer (O'Brien and Edelhauser, 1977) were applied into the epithelial (donor) side and endothelial (receptor) side of the cornea, respectively. The apparatus were maintained at 35 °C. At 40-min intervals, 1.0 mL sample was withdrawn from the receiving compartment, and was immediately replaced with an equal volume of preheated GBR buffer. Each experiment was continued for 4 h in triplicate. The osmolality of all perfusion solutions was adjusted with glycerol to 292–298 mOsm/kg, evaluated by freeze 256 drying point measurements on a Fiske osmometer.