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Preparation of ibuprofen-loaded chitosan films for oral mucosal drug delivery using supercritical solution impregnation

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ABSTRACT

Drug-loaded chitosan films suitable for oral mucosal drug delivery were prepared using supercritical solution impregnation (SSI) technology. Firstly, chitosan films were obtained via casting method, and the film properties including water-uptake, erosion and mucoadhesive were characterized. SSI process was then employed to load the drug of ibuprofen onto the prepared chitosan films, and the effects of impregnation pressure and temperature on morphologies of the ibuprofen-loaded chitosan films and drug loading capacity (DLC) were studied. The SEM and X-ray diffraction patterns suggested that distinct ibuprofen shapes such as microparticles, flake, rod-like and needle-like occurred after impregnation at different pressures, and DLC varied from 7.9% to 130.4% during the SSI process. The *ex vivo* release profiles showed that ibuprofen-loaded chitosan films could deliver the drug across the rabbit buccal mucosa, and up to 70% of the ibuprofen was released from the matrix in 460 min. SSI process is a promising method to prepare drug-loaded film formulations for oral mucosal drug delivery, which provides the advantages of low solvent residual and sustained- and controlled- release behavior.

1. Introduction

Oral mucosal drug delivery nowadays has attracted great attentions, which could avoid hepatic first-pass metabolism and gastrointestinal drug degradation existed in oral administration (Bruschi and de Freitas, 2005). Moreover, oral mucosal drug delivery can achieve not only local drug delivery but also systemic drug delivery (Morales and McConville, 2011). The oral cavity has many advantages for oral mucosal drug delivery. For example, the oral mucosa is permeable with a rich blood supply, which can easily recover from stress of damage and has good patient compliance. The relatively low enzyme activity and the lack of Langerhans cells make the oral mucosa a suitable site for drug delivery (Shakya et al., 2011). For these reasons, various mucoadhesive formulations like disks (Jay et al., 2002), tablets (Llabot et al., 2002), gels (Ayensu et al., 2012; Şenel et al., 2000a), ointments (Petelin et al., 2004), patches (Nafee et al., 2003; Onishi et al., 2005), and films (Cui et al., 2007; Watanabe et al., 2009) have been developed. Among these formulations, films are especially flexible and elastic, making the patients more comfortable and compliable. Besides, films also ensure more accurate dosing of the drug compared to gels and ointments.

For successful oral mucosal drug delivery, the rapid elimination of the formulation due to the flushing action of saliva is a major difficulty, which limits its application in clinical therapy. This problem could be considered to be solved by using formulations based on mucoadhesive polymers. Mucoadhesive polymers are synthetic or natural macromolecules which could attach to mucosal surfaces to prolong residence time of the formulation (Grabovac et al., 2005), and it could also favor the drug absorption and localization to improve the bioavailability of drugs (Pedro et al., 2009). Chitosan is a typical mucoadhesive polymer due to the interaction between its positively charged amino groups and negatively charged sialic acid residues in mucus (Lee et al., 2000), and has been proposed as an ideal carrier in oral mucosal drug delivery (Abruzzo et al., 2012; Aksungur et al., 2004; Portero et al., 2007) due to its good biocompatibility, biodegradability and favorable toxicological properties (Bernkop-Schnürch and Dünnhaupt, 2012; Saranya et al., 2011). In addition, chitosan could enhance the absorption of drugs during trans-mucosa delivery by opening the tight junction (Şenel et al., 2000b), and demonstrate antimicrobial effect (Knapczyk and Macura, 1992).

In most cases, soaking the polymer matrices in a drug solution is a conventional method to make the drug-loaded formulation. However, this soaking method presents several drawbacks, such as possible use of toxic organic chemicals, undesired drug reactions, drug degradation, heterogeneous drug incorporation and low processing efficiency (Costa et al., 2010). In recent years,

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supercritical fluid has been proved to be an alternative green process for pharmacy and pharmaceutics (Pasquali and Bettini, 2008). Drugs could also be impregnated into polymer matrices by dissolving them in supercritical fluid (usually $SCCO_2$), in which the binary mixture of drug and $SCCO_2$ could facilitate the mass transfer in the drug loading process. After depressurization, the drug is entrapped in matrices, and finally homogeneous drug-loaded polymer matrices would be obtained. This process, known as supercritical solution impregnation (SSI), has been reported to be successfully implemented in fabricating several polymer based drug-loaded formulations. For example, flurbiprofen was impregnated in P(MMA-EHA-EGDMA) for ophthalmic drug delivery (Duarte et al., 2007); and PLLA film loaded with roxithromycin was used as an antibacterial implant (Yu et al., 2011). But the application of SSI in oral mucosal drug delivery was rarely reported. Compared with soaking method, SSI allows the drug impregnation of polymer matrices without altering or damaging their physical, chemical and mechanical properties and would not cause degradation of the impregnated drug (Costa et al., 2010). Moreover, drug loading can be controlled by the manipulation of several operational parameters. This method also permits $SCCO_2$ to remove the residual of organic solvent used in the polymer matrices producing process (Reverchon et al., 2008). Therefore, more successful cases would be necessary to enrich this novel process in medical and pharmaceutical applications.

Ibuprofen is a non-steroidal drug used for anti-inflammatory and analgesic therapies, and it exhibits poor solubility in water and would stimulate the gastrointestinal tract in oral administration. In this study, ibuprofen is chosen as a model drug to prepare drug-loaded chitosan film formulations for oral mucosal drug delivery. The effect of SSI parameters on the morphology and drug loading capacity (DLC) of the chitosan film will be investigated. Finally, *in vitro* and *ex vivo* ibuprofen release profiles as well as bacterial inhibition of drug-loaded chitosan films are evaluated to indicate the potential application of the SSI process in the oral mucosal drug delivery.

2. Materials and methods

2.1. Materials

Pseudomonas aeruginosa (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were provided by Hangzhou Tianhe Microorganism Reagent Co., Ltd. (Zhejiang, China). Chitosan (deacetylation degree of 95%, viscosity of 186 mPa·s) was obtained from Golden-Shell Biochemical Co., Ltd. (Zhejiang, China), and ibuprofen (98% purity) was from Sangon Biotech Co., Ltd. (Shanghai, China). Carbon dioxide (99.99% purity) was supplied by Hangzhou Jingong Gas Co., Ltd. (Zhejiang, China). All other reagents were of analytical grade and used as received.

In this work, female New Zealand white rabbits from Zhejiang University Laboratory Animal Center were used, and buccal mucosae were taken according to the method described in the reference (Abruzzo et al., 2012). All the animal procedures were reviewed and approved by the Committee of Animal Research of Zhejiang Province, and under the supervision of Animal Ethical Committee of Zhejiang University.

2.2. Fabrication of chitosan film and its properties

Chitosan was dissolved in 1% v/v acetic acid to obtain 1% w/v chitosan solution. About 50 ml of chitosan solution was poured into 90 mm petri dish, and pre-heated at 60 °C for 6 h. Then 20 ml of 0.5 M NaOH was added into the dish to form chitosan gelatin. The chitosan gel was washed overnight with deionized water. After lyophilization of the gel, chitosan film with the thickness of 1.5 mm was obtained. Surface pH value of the chitosan film was determined using a pH test paper.

Water-uptake (WU) and erosion studies of the prepared chitosan film (10 mm in diameter) were performed in phosphate buffer (pH 6.8) that mimicked human saliva, and the weight change of chitosan film was measured at predetermined periods of time. Finally the samples were desiccated completely in an oven at 60 °C for 24 h, and the weights of the dried samples were recorded. WU and erosion were calculated according to following equations:

$$\mathrm{WU}(\%) = \frac{(W_2 - W_1)}{W_1} \times 100\% \quad (1)$$

$$\mathrm{Erosion}(\%) = \frac{(W_1 - W_3)}{W_1} \times 100\% \quad (2)$$

where W_1 was the initial weight of chitosan film, W_2 was the weight of chitosan film after immersion, and W_3 was the weight of chitosan film after erosion, respectively.

The *in vitro* mucoadhesion properties were determined in terms of the residence time of the chitosan film adhered to a rabbit buccal mucosa. Chitosan film was wetted with 200 μl of phosphate buffer (pH 6.8) and adhered to the rabbit buccal mucosa fixed to the inside wall of a 250 ml glass beaker. Then 200 ml of same buffer was poured into the beaker to submerge the chitosan film and rabbit buccal mucosa; it was rotated at 120 rpm and kept at 37 °C. The adhesion time for chitosan film was defined as the time when the chitosan film detached from the rabbit buccal mucosa.

2.3. Preparation of ibuprofen-loaded chitosan film using SSI

Ibuprofen-loaded chitosan film was prepared using SSI process. The SSI apparatus was described in previous work (Yu et al., 2011), which mainly consists of a high pressure stainless vessel, a mechanical stirrer with a rotating speed of 60 rpm, an electrical heater and a high pressure pump. The pressure and temperature of the high pressure system was well controlled within ±0.1 MPa and ±1 °C respectively. In a typical SSI process, CO_2 was liquefied and compressed to get the operating pressure via the high pressure pump, and then the fluid was pre-heated to the desired temperature. The powder of drug was placed at the bottom of the cell, while the chitosan film wrapped in a piece of filter paper was fixed on the top of the cell with no direct contact with the drug. The amount of the drug in the cell should ensure to be saturated in the CO_2. After the desired temperature and pressure were attained, the system was kept at constant conditions over 30 min. At the end of impregnation, the system was slowly depressurized at approximately 3 MPa/min, and the collected samples were stored in the desiccator.

2.4. Drug loading capacity of ibuprofen-loaded chitosan film

Drug loading capacity (DLC) of ibuprofen-loaded chitosan film was determined using UV–vis spectrophotometer (Ultrospec 3300 pro, GE Healthcare, USA). Briefly, 50 mg of ibuprofen-loaded chitosan film was immersed in 20 ml of phosphate buffer (pH 6.8), and ultrasonically processed for 30 min to completely extract ibuprofen. Samples were centrifuged and the supernatant was determined by its absorbance at 264 nm. DLC was defined as the drug mass loaded onto chitosan film divided by the mass of polymer matrix.

2.5. Characterization of ibuprofen-loaded chitosan film

The morphology of drug-loaded chitosan film was examined by scanning electron microscopy (JSM-6390A, JEOL, Japan). The