European Journal of Pharmaceutical Sciences 91 (2016) 144–153

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Preparation and evaluation of chitosan-based nanogels/gels for oral delivery of myricetin

Yashu Yao [a,c], Mengxin Xia [a], Huizhen Wang [a], Guowen Li [b], Hongyi Shen [a], Guang Ji [d], Qianchao Meng [e], Yan Xie [a,d,*]

[a] Research Center for Health and Nutrition, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
[b] Pharmacy Department, Shanghai TCM-integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200082, China
[c] Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
[d] Institute of Digestive Diseases, Long Hua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China
[e] Center for Drug Safety Evaluation, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form 6 June 2016
Accepted 17 June 2016
Available online 18 June 2016

Keywords:
Chitosan
β-glycerol phosphate
Myricetin
Nanogels
In vitro release
Pharmacokinetics

ABSTRACT

A novel nanogel/gel based on chitosan (CS) for the oral delivery of myricetin (Myr) was developed and evaluated comprehensively. The particle size of the obtained Myr-loaded CS/β-glycerol phosphate (β-GP) nanogels was in the range of 100–300 nm. The rheological tests showed that the sol-gel transition happened when the nanogels were exposed to physiological temperatures, and 3D network structures of the gelatinized nanogels (gels) were confirmed by Scanning Electron Microscopy. Myr was released from CS/β-GP nanogel/gel in acidic buffers *via* a Fickian mechanism, and this release was simultaneously accompanied by swelling and erosion. Moreover, the nanogel/gel exhibited no cytotoxicity by MTT assay, and the oral bioavailability of Myr in rats was improved with an accelerated absorption rate after Myr was loaded into CS/β-GP nanogel/gel. In summary, all of the above showed that CS/β-GP nanogel/gel was an excellent system for orally delivering Myr.

1. Introduction

Nanogels are nanosized networks of chemically or physically crosslinked polymers that can load drugs through self-assembly mechanisms involving electrostatic, Van der Waals, and/or hydrophobic interactions between the drug molecules and the polymer (Kabanov and Vinogradov, 2008; Vinogradov et al., 2006). Nanogels exhibit several advantages that were embodied in both hydrogels and nanoscale carriers. For example, nanogels can be easily administered and can swell 1–30 times with a three-dimensional structure, from which drugs are expected to a sustained release (Moya-Ortega et al., 2012; Oh et al., 2008; Samah et al., 2010). Furthermore, nanogels can lead to a high specific surface area because of the nanoscale size and consequently increase the solubility and pharmacokinetics of the conjugated drug molecules (Li et al., 2011; Pérez et al., 2014). Thus, nanogels can regulate the drug release and pharmacokinetic properties and have a great potential in drug delivery systems.

Chitosan (CS), *i.e.*, poly [β-(1–4)-linked-2-amino-2-deoxy-D-glucose], is a natural, linear, and cationic polyaminosaccharide obtained from the alkaline deacetylation of chitin. Being biocompatible, biodegradable, and muco-adhesive (Liu et al., 2008; Park et al., 2010), CS and its derivatives have been used as drug vehicles in the forms of films (Zhao et al., 2009), beads (Chen et al., 2006; Guo et al., 2005), and nanogels, *etc.* In recent years, nanogels based on CS have received considerable attention because they can encapsulate various types of drugs, such as small molecules, peptides, proteins, *etc.*, to minimize burst release, regulate the release rate, enhance the efficiency, and improve the selectivity of the therapy. For example, an ethosuximide (ESM)-loaded nanogel based on CS displayed an extended release without burst release both *in vitro* and *in vivo*, and therapeutic drug levels were maintained until day 4, while for free ESM, the drug level was still low after 24 h (Hsiao et al., 2012). Furthermore, covalently crosslinked hybrid nanogels based on CS chains provided a pH-regulated release of the anticancer drug temozolomide in the typical abnormal pH range of 5–7.4 found in a pathological zone and consequently enhanced the therapeutic efficiency (Wu et al., 2010). Lastly, CS-based nanogels decorated with hyaluronate improved the selective uptake of three different anionic photosensitizers and increased their retention

* Corresponding author at: Research Center for Health and Nutrition, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China.
E-mail address: rosexie_1996@hotmail.com (Y. Xie).

http://dx.doi.org/10.1016/j.ejps.2016.06.014
0928-0987/

in inflamed tissues, thus exhibiting the potential to treat inflamed articular joints (Schmitt et al., 2010). From these studies, it is clear that CS can be used as nanogels carrier for effective drug delivery.

Oral delivery is the most convenient approach for administering drugs due to the many advantages when compared to other prescribed methods, including ease of use, ease of dose adjustment, and low cost, *etc.* However, the poor solubility or instability of many drug candidates in the gastrointestinal (GI) fluid decreases the gastric residence time and consequently lead to the low bioavailability after oral administration (Karnoosh-Yamchi et al., 2014; Qi et al., 2015; Senanayake et al., 2013). Nanogels are a promising oral drug delivery system because they can increase the residence time of drugs in the GI tract, protect drugs from degradation in the GI fluid, obtain the appropriate drug release in the GI tract, and consequently increase the absorption of drugs across GI membranes. For instance, nanogel conjugates of gemcitabine were relatively stable in gastric conditions and were able to actively penetrate through the gastrointestinal barrier, which possibly resulted from an increased GI adsorption (Senanayake et al., 2013). Furthermore, the anti-norovirus activity of interferons (IFN) showed a steep drop (by 80%) in PBS in 3 days of incubation at 4 °C, while the activity of IFN-loaded nanogels decreased only by up to 17% during the same period. Moreover, IFN was released as an intact form the nanogels, and thus the bioavailability of IFN was not reduced (Kim et al., 2011). Lastly, nanogels composed of p(NIPAAm) and p(MAA-gEG) increased the permeation of peptide across the small intestine, owing to its release in a pH-dependent manner, a good cytocompatibility, and the cellular tight junction opening effect (Ichikawa et al., 2006).

Myricetin (3, 5, 7, 3′, 4′, 5′-hexahydroxyflavone, Myr) is a flavonol that is present in many fruits, vegetables, and herbs, such as grapes, berries, onions, tea, and *Abelmoschus moschatus*, *etc.* (Li and Ding, 2012). As a natural antioxidant, Myr has a variety of therapeutic applications including as a cardiovascular protective agent, a hepatoprotective agent, and a potential agent for the treatment of colorectal carcinoma (Dajas et al., 2003; Liu et al., 2007; Maheshwari et al., 2011). However, Myr is essentially insoluble in water (16.60 μg mL^{-1}) and has a slow intrinsic dissolution rate of 11.66 ± 0.82 g min^{-1} cm^{-2} and a rapid degradation rate of 0.1860 ± 0.0094 h^{-1} and 0.3343 ± 0.0547 h^{-1} at pH 4.5 and 6.8, respectively (Yao et al., 2014a), which probably resulted in its poor oral bioavailability in rats (Dang et al., 2014). Fortunately, the solubility and the dissolution rate of Myr were greatly enhanced by forming a Myr/hydroxypropyl-beta-cyclodextrin (HP-β-CD) inclusion complex, and accordingly the *in vitro* antioxidant activity and *in vivo* oral bioavailability in rats were effectively improved. However, the mean residence time (MRT) in plasma was significantly shortened ($p < 0.05$), and a burst release of Myr from *in vitro* dissolution (over 98% within 10 min) was observed (Yao et al., 2014b), which would probably limit the therapeutic effects of Myr and cause the potential side effects. Therefore, it is necessary to explore an effective formulation for the oral delivery of Myr to overcome the above mentioned problems.

In the present study, a novel oral delivery nanogel/gel based on CS containing a myricetin/HP-β-CD inclusion complex is developed, and the related *in vitro* and *in vivo* evaluations are investigated. Briefly, the particle size and zeta potential of the nanogels, and the rheology, morphology, swelling, and erosion properties of the nanogels/gels are presented. The *in vitro* drug release in different buffers, the biocompatibility of the nanogels/gels, and the *in vivo* pharmacokinetics of Myr in the nanogels/gels are also evaluated. The present research will provide some beneficial results for effectively delivering Myr and enriching the administration route of nanogels/gels.

2. Materials and methods

2.1. Materials

CS with a deacetylation degree of 90% and a molecular weight (Mw) of 20 kDa was supplied by Aladdin, Inc., St (Shanghai, China). β-glycerol phosphate (β-GP), β-glucuronidase, sulfatase, formic acid, and MTT [3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide] were purchased from Sigma Aldrich (St. Louis, MO, USA). Myr with purity >98% (standard substance in HPLC analysis) was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China), and the raw Myr material (yellow powder, purity ≥90%) was purchased from Shanghai DND Pharm-Technology Co., Inc. (Shanghai, China). Myr/HP-β-CD inclusion complex was prepared by a method described previously (Yao et al., 2014b). HPLC grade acetonitrile and methanol were purchased from Honeywell Burdick & Jackson (Ulsan, Korea). Ultra-pure deionized water was generated from a Millipore Milli-Q Gradient System (Billerica, MA, USA). All other chemicals and solvents used were of analytical grade.

Caco-2 cells (ATCC, Manassas, VA, USA) were grown in DMEM media supplemented with 10% FBS, 1% non-essential amino acids, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C under a humidified atmosphere of 5% CO_2. Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Thermo-Fisher Biochemical Product (Waltham, MA, USA). Fetal bovine serum (FBS) and Hanks balanced salts solution (HBSS) were purchased from Gibco Laboratory (Grand Island, NY, USA).

Sprague-Dawley rats (280–300 g) were supplied by the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine (Shanghai, China).

2.2. Preparation of CS/β-GP nanogels

In a typical procedure, CS powder (200 mg) was slowly added to a 0.1 mol L^{-1} hydrochloric acid solution (9 mL) under stirring, and then the mixture was continuously stirred overnight to make a clear solution (2.22%, w/v). After that, the CS solution was chilled to 4 °C in an ice bath. Meanwhile, β-GP was dissolved in purified water (56%, w/v) and was chilled alongside the CS solution in an ice bath. Then, the β-GP solution (1 mL) was added dropwise into the CS solution (7 mL) under vigorously agitation, and the mixture was stirred for 10 min to obtain a CS/β-GP solution, which was used as the blank nanogel matrix. For the Myr-loaded nanogels, an appropriate amount of the Myr/HP-β-CD inclusion complex (the loading efficiency was about 5%) was added slowly to the blank nanogel matrix under constant stirring for 30 min, and the final concentration of Myr was 1.33 mg mL^{-1}. Then, the solution with or without Myr was sonicated for 4 min (the pulse was turned off for 2 s every 2 s) using a probe-type sonicator (Qsonica, Newtown, CT, USA) at 100 W in an ice bath to obtain blank or Myr-loaded nanogels.

2.3. Characterization of the nanogels/gels

2.3.1. Particle size and zeta potential measurements

Particle diameter, polydispersity index (PDI), and zeta potential of the blank and Myr-loaded nanogels were determined by a laser light diffraction system using a Mastersizer (ZEN3600 Nano ZS, Malvern Instruments Ltd., Malvern, UK). Samples were diluted with cold deionized water and measured at 4 °C. The obtained data were analyzed automatically using Mastersizer software supplied by the manufacturer (Malvern Instruments Ltd., Malvern, UK). All measurements were made in triplicate, and each measurement represented 20 runs ($n = 3$).

2.3.2. Rheological properties

The rheology study of the nanogels/gels was carried out using an Anton Paar MCR 101 rheometer (Anton PaarTrading Co., Ltd., Shanghai, China) equipped with a stainless steel parallel plate measuring system (50-mm plate diameter). The sample was placed in the center of the bottom parallel plate, and the top plate was moved to the measuring position (a 1-mm gap size was used). Afterwards, the sample was trimmed using a spatula such that the sample edge was approximately flush with the top parallel plate. The viscosity as a function of temperature (10–45 °C) was measured, and changes in the elastic modulus (G′) and the