International Journal of Biological Macromolecules 118 (2018) 149–159

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Facile synthesis and characterization of tailor-made pectin-gellan gum-bionanofiller composites as intragastric drug delivery shuttles

Hriday Bera [a,b,*], Sanoj Kumar [b], Sabyasachi Maiti [c]

[a] Faculty of Pharmacy, AIMST University, Semeling, Kedah 08100, Malaysia
[b] Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad 500090, India
[c] Department of Pharmacy, Indira Gandhi National Tribal University, Amarkantak 484887, Madhya Pradesh, India

ARTICLE INFO

Article history:
Received 26 February 2018
Received in revised form 6 June 2018
Accepted 17 June 2018
Available online 19 June 2018

Keywords:
High-methoxyl pectin
Gellan gum
Bionanofiller

ABSTRACT

Olive oil-entrapped diethanolamine-modified high-methoxyl pectin (DMP)-gellan gum (GG)-bionanofiller composites were developed for controlled intragastric delivery of metformin HCl (MFM). DMP had a degree of amidation of 48.7% and was characterized further by FTIR, XRD and DSC analyses. MFM-loaded composites were subsequently accomplished by green synthesis *via* ionotropic gelation technique using zinc acetate as cross-linker. The thermal, X-ray and infrared analyses suggested an environment in the composites compatible with the drug, except certain degree of attenuation in drug's crystallinity. Scanning electron microscopy revealed almost spherical shape of the composites. Depending upon the mass ratios of GG:DMP, types of nanofiller (neusilin/bentonite/Florite) and oil inclusion, the composites exhibited variable drug encapsulation efficiency (DEE, 50–85%) and extended drug release behaviours (Q_{8h}, 69–94%) in acetate buffer (pH 4.5). The optimized oil-entrapped Florite R NF/GG: DMP (1:1) composites eluted MFM *via* case-II transport mechanism and its drug release data was best fitted in zero-order kinetic model. The optimized formulation demonstrated excellent gastroretentive properties and substantial hypoglycemic effect in streptozotocin-induced diabetic rats. These novel hybrid matrices were thus found suitable for controlled intragastric delivery of MFM for the management of type 2 diabetes.

1. Introduction

Diabetes encompasses a heterogeneous array of metabolic complications characterized by hyperglycemia, resulting from diminished insulin response and/or impaired insulin secretion [1]. According to the recent update of International Diabetes Federation (IDF), approximately 371 million people worldwide are currently suffering from diabetes [2]. Non-insulin-dependent diabetes mellitus (Type 2) is comprised of approximately 90% of the total diabetes burden. Metformin HCl (MFM), a hypoglycemic agent of biguanide class, is currently being overprescribed for type 2 diabetes management. It exhibits shorter half-life (1.5–1.6 h), dose-dependent and saturable permeability through the narrow absorption window in the upper part of small intestine. These in turn give rise to extremely variable oral bioavailability and inadequate clinical response of MFM. To surmount these drawbacks, the development of gastroretentive sustained-release formulations of MFM is urgently needed. Multi-particulate gastroretentive drug delivery systems (GRDDS) can distribute uniformly over the gastric fluid for prolonged period of time, avoid variable gastric emptying time and release the drug slowly [3, 4]. GRDDS, constituting a variety of devices such as floating, swelling or shape-based, high density and mucoadhesive systems have been exploited [5]. Recently, an array of GRDDS has been developed utilizing Eudragit® RL100, Eudragit® RS100, Carbomer 934P and other co-polymers which displayed excellent gastroretention and sustained drug release behaviour [6–8]. However, the major drawback involved in their development was the usage of synthetic polymers of questionable biodegradation and toxic organic solvent that restricted their further clinical development. Instead, the development of biopolymer-based intragastric drug delivery systems would be appreciable due to their nontoxic, biodegradable nature and easy processing in eco-friendly environment.

In today's pharmaceutical arena, biopolymer-inorganic nanofiller hybrid matrices with complementary features of the distinct constituents have emerged as attractive gastroretentive drug delivery platforms [9–11]. Now-a-days, various silica-based inorganic nanofillers such as magnesium aluminometasilicate [neusilin® US2, $Al_2O_3 \cdot MgO \cdot 1.7SiO_2 \cdot xH_2O$], aluminum silicate [bentonite, $Al_2O_3 \cdot 4SiO \cdot 2H_2O$], calcium silicate [Florite R NF, $2CaO \cdot 3SiO_2 \cdot mSiO_2 \cdot nH_2O$] have been explicitly acknowledged for their diverse drug delivery applications [12]. These nanofillers are characterized by the properties of tuneable pore structure, extremely high surface area and outstanding adsorption capacity. The surface of nanofillers can also be tailored with a wide range of biopolymers like pectin in

* Corresponding author at: Faculty of Pharmacy, AIMST University, Semeling, Kedah 08100, Malaysia.
E-mail address: hriday.bera1@gmail.com (H. Bera).

https://doi.org/10.1016/j.ijbiomac.2018.06.085
0141-8130/

order to improve biocompatibility and targetability [13]. The backbone of pectin consists primarily of linearly connected α-(1–4)-D-galacturonic acid residues repeatedly interspersed with α-(1–2) linked α-L-rhamnopyranose units. The galacturonic acid residues of pectin molecules are partly methylesterified. High-methoxyl (HM) pectins with a degree of methylation above 50% display poor gelling property, which is largely attributed to the presence of inadequate carboxylic acid groups in their structure. The amidation of HM-pectin with diethanolamine could dramatically reduce the hydrophobicity with an increasing tendency to construct gels [14]. Unfortunately, the high swelling characteristics of diethanolamine-modified pectin (DMP) in aqueous fluid could typically lead to poor encapsulation and premature release of the entrapped bioactive molecules [15].

Currently, a flurry of scientific investigations has established that the blending of two naturally occurring polymers could expediently improve the mechanical strength and performance of polysaccharide-based drug carriers [16]. Gellan gum (GG), an anionic exopolysaccharide biosynthesized by the bacterium *Pseudomonas elodea*, is often combined with other polysaccharides to fabricate bioadhesive devices for oral delivery of therapeutic molecules. The backbone of GG is mainly composed of repeating tetrasaccharide unit of glucuronic acid, rhamnose and glucose residues in a molar ratio of 1:1:2. Pristine GG possesses negative charges owing to abundant glucuronic acid units and could be ionotropically crosslinked with divalent metal cations (*e.g.*, calcium, zinc *etc.*) to fabricate rigid GG-based mucoadhesive composite hydrogels [3, 17]. At present, the combined floatation-mucoadhesion approaches for intragastric drug delivery are receiving overwhelming attention. Thus, many pioneering researchers have amalgamated low-density oils with gastroretentive mucoadhesive systems [18]. The inclusion of oils could impart buoyancy and provide a hydrophobic barrier towards drug eluting from the composites, resulting in amplified drug entrapment efficiency with sustained drug release behaviour [19]. To our best knowledge, GG blended DMP-nanofiller composites entrapped with low-density oil have neither been developed nor even their drug delivery properties been explored yet.

The substantial gaps in the pioneering studies led us to hypothesize that oil-entrapped GG-Zn^{+2}-DMP-nanofiller hybrid matrices would execute as reliable intragastric carriers for MFM. It would also portray superior gastroretention by virtue of their floating and bioadhesion properties, modulate drug release in controlled manner and exhibit improved pharmacodynamic response. The zinc (Zn) ions could play an important role in insulin production and the subsequent actions of insulin on glucose metabolism, preventing the pathogenesis and complications of diabetes [20]. To test these hypotheses, a novel oil-entrapped Zn^{+2}-crosslinked organic-inorganic composites that specifically delivers MFM into the intragastric region in a sustained manner was developed and their *in vitro* and *in vivo* performances were evaluated.

2. Materials and methods

2.1. Materials

MFM (Matrix Lab. Ltd., India.), HM-pectin (degree of methylation, 65.7%, Brookfield viscosity of 1% *w/v* aqueous solution at 100 rpm and 25 °C, 10.1 cps, R & M Chemicals, UK), diethanolamine (Merck Lab. Ltd., India), low acetylated GG (Mw, 2×10^5–3×10^5 g/mol, Life Expression Pvt. Ltd., India), Neusilin® US2 (Fuji Chemical Industries Co. Ltd., Japan), Bentonite (Merck Lab. Ltd., India), Florite R NF (Tomita Pharmaceutical Co., Ltd., Japan), olive oil (relative density, 0.91 g/cm^3, Qualigens Fine Chemicals, India) zinc acetate (Merck Lab. Ltd., India) were used. All the reagents and chemicals were of analytical grade.

2.2. Amidation of HM-pectin with diethanolamine

The DMP was synthesized following procedure reported elsewhere [14]. Briefly, an accurately weighed quantity of HM-pectin (5 g) was dispersed in 50 ml methanol. The 50 ml of 20% v/v diethanolamine solution in methanol was then slowly introduced with continuous stirring at 5 °C. The reaction mixture was agitated for another 24 h. The resulting DMP was then washed with 0.1 N HCl in methanol-water mixture (1:1 v/v). The sample was subsequently washed thrice with 40% (v/v) methanol. Finally, the product was treated with 80% (v/v) methanol and dried at 60 °C until constant weight.

2.3. Characterization of DMP

2.3.1. Degree of amidation

The degree of amidation (D_A), mass and molar yields of reaction (Y_M and Y_N, respectively) was estimated based on one time elemental analysis (Elemental analyser EA3000, Japan) results of DMP, according to the following relationships [21]:

$$D_A = \frac{M_N}{M_C}\left[6 + \left(\frac{D_M}{100}\right) + (K-1)\frac{M_N}{14}\right] \times 100$$

$$Y_M = \frac{M_N \times M_A}{14}$$

$$Y_N = \frac{D_A}{D_M} \times 100$$

where, M_N and M_C represent nitrogen and carbon content (%), respectively; M_A and K denote the molar mass ($g \cdot mol^{-1}$) and number of carbons of amine, respectively; and D_M refers to the degree of methylation of native pectin.

2.3.2. FTIR

HM-pectin and DMP were scanned on FTIR spectrophotometer (Perkin Elmer, USA) in the frequency region of 4000–500 cm^{-1}.

2.3.3. DSC

The DSC thermograms of HM-pectin and DMP over a wide temperature range (50–300 °C) were captured on differential scanning calorimeter (DSC) (Pyris 1, Perklin Elmer, USA) at a constant heating rate of 10 °C min^{-1}.

2.3.4. P-XRD

HM-pectin and DMP were analyzed by powder X-ray diffraction (*P*-XRD) (Bruker-AXS D8) coupled with a CuKα radiation detector, operating at an anode voltage of 40 kV and input current of 30 mA.

2.3.5. Viscosity measurement

The viscosity of HM-pectin and DMP was examined using a Brookfield viscometer (model LVDV-E, spindle no. 63, Bruker, Germany) at 100 rpm at room temperature.

2.4. Preparation of MFM-loaded composites

MFM-loaded composite matrices (F-1 to F-5) were prepared by ionotropic gelation technique. Briefly, the required quantities of DMP and GG were dispersed in distilled water by magnetic stirring. To it, MFM (1% *w/v*) and nanofillers [neusiln/bentonite/Florite] (1% w/v) were introduced and the mixture was homogenized at 5000 rpm for 15 min. The drug-polymer ratio of 1:4 was kept constant for all the formulations (Table 1). The resulting dispersions were then extruded through a 21G needle into gently agitated solution of zinc acetate (5% w/v). The hybrid matrices thus formed were allowed to harden in reticulation solution for 30 min, filtered off and copiously washed with distilled water. The composites were subsequently dried at ambient temperature until constant weight and stored in desiccators. The oil-entrapped composites (F-6 to F-8) were prepared in similar manner but with introducing olive oil (2.5% v/v of water) in polymeric dispersions.