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Impact of metal oxide nanoparticles on oral release properties of pH-sensitive hydrogel nanocomposites

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ABSTRACT

In this article, modified κ-carrageenan hydrogel nanocomposites were synthesized to increase the release ability of carrageenan hydrogels under gastrointestinal conditions. The effect of MgO nanoparticle loading in a model drug (methylene blue) release is investigated. Characterization of hydrogels were carried out using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM) and Differential Scanning Calorimetry (DSC). Genipin was used to increase the delivery performance in gastrointestinal tract delivery by decreasing release in simulated stomach conditions and increasing release in simulated intestine conditions. It is shown that the amount of methylene blue released from genipin-cross-linked nanocomposites can be 67.5% higher in intestine medium and 56% lower in the stomach compared to κ-carrageenan hydrogel. It was found that by changing the nanoparticle loading and genipin concentration in the composite, the amount of drug released can be monitored. Therefore, applying nanoparticles appears to be a potential strategy to develop controlled drug delivery especially in gastrointestinal tract studies.

1. Introduction

Recently, a great deal of research has focused on the application of nanotechnology in drug delivery as it provides a suitable means for both time and site-specific controlled delivery of drugs and bioactive agents [1–8]. Applying nanoparticles (NPs) in pharmaceutical studies offer many advantages providing targeted delivery of drugs, improving bioavailability and stability of therapeutic agents against degradation, and extending drug effect in target tissue [9]. Since nanoparticles have high specific surface area and unique physicochemical characteristics, they have been employed extensively in medical applications [10–13].

Hydrogels are intelligent materials capable of exhibiting significant volume changes in response to small changes in pH, temperature and other environmental stimuli [13,14]. Hydrogels have a wide range of applications in controlled drug delivery systems, tissue engineering, artificial organs in biotechnology, the recognition of certain bio-molecules and so on [15,16]. These materials can absorb large amounts of water and swell due to the existence of the hydrophilic groups (–OH, –COOH, $–NH_2$, $–CONH_2$, and $–SO_3H$) in their structures [16,17]. In addition, hydrogels can be modified with new functional groups or prepared as composites to increase their chemical/physical properties [18]. Guiseley [19] proposed the synthesis of κ-carrageenan associated with hydroxyalkyl groups. Hydrogels prepared from hydroxyalkyl κ-carrageenan derivatives showed a decrease in syneresis (liquid extraction from a gel) and could be used in a wide range of industrial fields.

Among inorganic materials, metal oxides such as MgO are of particular interest as they are stable under harsh process conditions and are known to be essential minerals for human health [20–23]. Recently, the application of MgO nano- and micro-sized particles has attracted attention due to its biomedical applications [24–27]. However, the toxicity of the MgO nanoparticles to cells and organs remains fairly undiscovered. Ge et al. [28] reported on the cytotoxicity study of MgO nanoparticles on human umbilical vein endothelial cells (HUVECs) in vitro using the transmission electron microscope (TEM) and nanoparticle size analyser. Their results from MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 h-tetrazolium bromide) assay, 4,6-diamidino-2-phenylindole (DAPI) staining analysis, NO release and total antioxidation competence (T-AOC) assay showed that most the MgO nanoparticles significantly enhanced the NO release and T-AOC content of the HUVECs. The testing results indicated that low concentration of MgO nanoparticles (below 200 g/ml) exhibited non-cytotoxicity. However, once the concentration of MgO nanoparticles was higher than 500 g/ml, the relative growth rate was lower than the control. Meanwhile Genipin, a naturally occurring cross-linking agent, is used in many biological research applications [29–31]. It is an

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effective cross-linker for polymers containing amino groups [32] with less cytotoxicity than other conventional cross-linkers such as formaldehyde and glutaraldehyde [33]. Genipin has been widely used in herbal medicine due to its anti-inflammatory, diuretic, choleretic and hemostatic properties [29].

The aim of this study is to improve the performance of carrageenan hydrogels as drug delivery vehicles in gastrointestinal conditions using metal oxide nanoparticles. Characteristics of biomaterials composites are also studied. Genipin is used to control and improve the amount of drug released in the intestine.

2. Materials and methods

2.1. Materials

κ-Carrageenan (κC) (Sigma–Aldrich), sodium carboxymethyl cellulose (NaCMC) (average molecular weight of 250,000, Acros Organic), methylene blue ($C_{16}H_{18}ClN_3S{\cdot}3H_2O$) (Riedel-de-Haën), magnesium oxide nanopowder (MgO) (<50 nm (BET), Sigma–Aldrich), genipin (GN) (Challenge Bioproducts Co., Ltd. Taiwan), HCl (Qrec Grad AR), NaOH (Qrec Grad AR). Distilled water is used in hydrogel synthesis and all chemicals are used as received with no additional purification.

2.2. Preparation of κ-carrageenan nanocomposites

2.2.1. Preparation of modified κ-carrageenan hydrogel

Clearly, when hydrogel swelling is increased, its ability to release higher amounts of encapsulated drug will also increase. Therefore κ-carrageenan hydrogel was blended with NaCMC to improve the swelling properties of carrageenan. Different concentrations of NaCMC were prepared and their swelling ability was tested to find the most suitable blend to be loaded with MgO nanofillers. Briefly, for a typical hydrogel synthesis, 0.48 g of κC was dissolved in 20 ml of distillated water at 80 °C before mixing with 0.12 g NaCMC dissolved in 10 ml distillated water. One hour gentle stirring provided a clear, viscous and homogenous solution with no bubbles and the reflux kept the water content of the system constant. The resultant hot solution was poured into ceramic moulds to form the hardened hydrogel of a desired shape. Samples were equilibrated with ambient temperature (25 °C) for 24 h prior to drying at 37 °C in an oven over night. Swelling test was carried out immediately after the drying process. Non-modified κC hydrogel was also prepared using the same method. Hydrogels will be referenced using their carrageenan concentration in the blend. For example 90:10 (kC:NaCMC) will be referred as kC90 and so on.

2.2.2. Preparation of methylene blue loaded MgO/κ-carrageenan nanocomposites

MgO/κ-carrageenan nanocomposites were prepared by blending the MgO NPs with the polymer matrix as follows: 0.1 ml of methylene blue (MB) 1% containing MgO NPs in different concentrations were prepared using ultra sonication and rigorous stirring. Then 10 ml of well-dispersed solution was added to 20 ml of hot modified κC solution under gentle stirring after further intense sonication of hot solution in a Brandson (USA) ultrasonic processor at 80 °C. Afterwards, hydrogel nanocomposite was poured into ceramic moulds and immediately put in a fridge to be cooled at −10 °C for 10 min. The final disc was approximately 3.5 cm in diameter and a thickness of 1.0 cm. Table 1 lists the synthesis conditions for the different hydrogels synthesized in this experiment.

2.3. Measuring the swelling ratio

To study the swelling behavior of hydrogels, modified and non-modified gels were immersed in different pH buffer solutions of pH 4, pH 6.5 and pH 8 at room temperature (25 °C). Synthesized gels were placed in a Petri dish filled with 50 ml of each buffer solution. Prior to weighting, filter paper was used to remove the surface water of swollen hydrogels. The swelling ratio (%) was then determined using Eq. (1).

$$\text{Swelling ratio}\,(\%) = \left[\frac{w_t - w_0}{w_0}\right] \times 100 \quad (1)$$

where W_0 is the initial weight of samples and W_t is the weight of swollen gels at predetermined time t. To allow hydrogels to reach their highest swelling ability, they were immersed in fresh buffer solution after weighting. The test was conducted in triplicate and reported as mean values to maximize accuracy.

2.4. Instrumentation

2.4.1. FTIR analysis of hydrogel

Blank and nanocomposite hydrogels were dried and 3 mg quantities were grounded and then mixed with 10 times as much KBr powder. 500 kg/cm^2 pressure with hydraulic press formed the sample pellets. Prepared samples were analyzed using a Fourier transform infrared spectroscope (FTIR) (Nicolet 670 FTIR, USA) with 16 scan per sample in the region of 370–4000 cm^{-1} at 1.0 cm^{-1} intervals and a resolution of 4.

2.4.2. X-ray diffraction (XRD)

Nanocomposites X-ray diffraction measurements were conducted at room temperature using a Siemens D5000 X-ray diffractometer with Cu Kα at 40 keV and 40 mA and step length of 0.05° with step time of 1 s. The diffraction angle (2θ) was set between 20 and 80. Nanocomposites were dried in an oven before experimentation to reach constant weight. Disc thickness was approximately 0.55 ± 0.05 mm.

2.4.3. Hydrogel nanocomposites microstructure

Field emission scanning electron microscope (FESEM) (Gemini Supra 35VP) was used to investigate the micro-structural changes in nanocomposite hydrogels. Prior to observation, samples were coated with gold using gold sputter coater Bio Rad Polaran Division (E6700, USA) under vacuum. Samples were studied at accelerating voltage of 10 kV and magnification of 1000× and 5000×. Samples were lyophilized before measurement and were left in liquid nitrogen to keep the hydrogels pores intact for imaging.

2.4.4. Differential Scanning Calorimetry (DSC)

Thermal properties of hydrogels were determined using Differential Scanning Calorimetry (DSC822 METTLER TOLEDO, Switzerland) of the dried hydrogel samples. 5 ± 0.5 mg of each hydrogel sample was sealed in an aluminum pan and then heated from room temperature to 350 °C at a heating rate of 5 °C/min. The flow rate of N_2 was maintained at 50 ml/min.

2.5. Release study of encapsulated methylene blue in gastrointestinal conditions

As a model drug, methylene blue (MB) was used to study the gastrointestinal tract (GIT) release behavior of nanocomposite hydrogels. The MB loading process into the nanocomposites is as described in Section 2.2.2. Prepared samples were exposed to 60 ml of the medium solutions in Petri dishes placed in a thermostatic Kuhner Climo-Shaker (ISF1-X) at 37 °C and 120 rpm. For the first 10 min the pH of the medium was kept at 0.1 N HCl then changed to buffer solution pH 6.6 for another 10 min and finally pH 7.4 up to 30 min. At regular intervals, 1.0 ml aliquot of the buffer solutions was removed to determine its concentration using Shelton UV/Vis spectroscopy (ct 06484 USA). The same amount of fresh solution