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Radiation grafting of *N*-vinylcaprolactam onto nano and macrogels of chitosan: Synthesis and characterization

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ABSTRACT

The aim of this study was to synthesize chitosan hydrogels, in macro- and nano-size, grafted with N-vinylcaprolactam (NVCL) using gamma radiation, and evaluate their potential application as a drug delivery system, using 5-fluorouracil (5-FU) as a model drug. The effect of dose and monomer concentration in the grafting process was studied, and the materials were characterized by FTIR, TGA, DLS, SEM and AFM. Higher grafting percentages were observed for the nanogels system. Although both the grafted macro- and nanogels, (*net*-CS)-g-NVCL, showed a response to pH (4.75) and temperature (31–33 °C), the nanogels showed a better swelling response to both stimuli because of their higher surface area. Both systems were able to load 5-FU in small amounts (2–3.5 mg g^{-1}) and the release was sustained for more than 12 h, showing that the modified macro and nanogels can be a potential alternative for the administration of drugs.

1. Introduction

Chitosan (CS) is a linear polysaccharide composed of randomly distributed β-(1–4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl D-glucosamine (acetylated unit). As a polysaccharide, CS exhibits attractive properties such as biocompatibility and biodegradability (Kumar, 2000), and thus it is extensively used in the pharmaceutics, cosmetics, biomedical, agriculture, biotechnological, paper, and textile fields (Mourya & Inamdar, 2008), as well as in water treatment. In addition, its degradation products are non-toxic, non-immunogenic and non-carcinogenic, so CS has also been found to be a good candidate as a supporting material for gene delivery, cell culture, and gene tissue engineering. Through the use of graft copolymerization, CS can be endowed with new desired properties that will enlarge its potential applications in the drug delivery, tissue engineering, antibacterial, biomedical, and dye removal fields (Chmielewski, 2010; Majeti & Kumar, 2000). In this regard, poly(*N*-vinylcaprolactam), PNVCL, is a non-ionic, biocompatible, thermoresponsive polymer, that is water soluble at room temperature, and has a lower critical solution temperature (LCST) in the 32 – 35 °C temperature range, which is near physiological temperature. Moreover, PNVCL has a relatively high resistance to hydrolysis and it does not produce toxic amide compounds (Vihola, Laukkanen, Valtola, Tenhu, & Hirvonen, 2005), making it attractive for biomedical and pharmaceutical applications (De las Heras, Pennadam, & Alexander, 2005 ; Zdyrko, Klep, & Luzinov, 2003).

Hydrogels can be size-tuned into macroscopic networks, or into gels with smaller dimensions such as microgels. When the size of microgels is in the submicron range, they are known as nanogels. Some of the features of using microgels and nanogels for biomedical purposes are that they offer a large surface area for multivalent conjugation, and they have an interior network that can be used for the incorporation/retention of bioactive molecules such as drugs, proteins, carbohydrates, and DNA (Oh, Drumright, Siegwart, & Matyjaszewski, 2008). In addition, compared with CS macrogels and microgels, the adsorption performance could be greatly improved with nanogels (Jia, Yujun, & Guangsheng, 2005). When it comes to drug delivery vehicles, nanoparticles, which can be composed of natural or artificial polymers ranging in size between 10

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and 100 nm (Hans & Lowman, 2002), have several advantages over micro and macroparticles such as site specific targeting, prevention of dose dumping via sustained and controlled release, high surface to volume ratio, long shelf life, easy transport to different body sites, and a high drug entrapment efficacy. All of the aforementioned advantages can lead to a reduction in drug dose and frequency of administration (Gulati, Nagaich, & Saraf, 2013).

Although there are many methods to carry out graft-polymerizations, it is known that ionizing radiation, such as gamma rays, is a powerful technique for grafting reactions because it does not require the use of initiators, or any other additives, and the reaction can be carried out at any temperature and in the solid or in solution state. For the synthesis of biomaterials, the above mentioned are important factors (Burillo, Díaz, & Bucio, 2006; Chapiro, 1962; Kudryavtsev, Kabanov, Yanul, & Kedik, 2003; Melendez-Ortiz, Alvarez-Lorenzo, Concheiro, & Bucio, 2015).

In this paper, macro and nano chitosan gels were prepared using glutaraldehyde as a crosslinking agent, and afterwards grafted with N-vinylcaprolactam (NVCL) by gamma radiation. The effect of dose and NVCL concentration on the grafting process was studied, and the response of the modified gels to changes in pH and temperature was evaluated. Also, the capacity of the synthesized materials to load and release 5-FU was measured by UV-spectroscopy. A comparison of the properties of both sized gels is discussed.

2. Experimental

2.1. Materials

CS powder with an average molecular weight of 1.46×10^5 and deacetylation grade of 79%, glutaraldehyde (GA, solution 50% v/v), n-hexanol, cyclohexane, Triton-X-100, and fluorouracil, were obtained from Sigma-Aldrich (México). Acetic acid, acetone, and ethanol from Reproquifin, México; boric acid, citric acid and trisodium orthophosphate from J.T. Baker (México), were utilized without treatment. *N*-vinylcaprolactam (NVCL) from Sigma-Aldrich was vacuum distilled before being used.

2.2. Preparation of CS macrogels

To obtain the CS macrogels, 15 mL of a 2% w/v CS solution in aqueous acetic acid (2% v/v) were placed in a Petri dish (5 cm diameter), and then 0.15 mL of the GA solution were added and left to react for24 h. Afterwards, the Petri dish solution was left to evaporate in a hood until a crosslinked CS dried film was obtained. The synthesized macrogels were extracted with a 1% v/v aqueous acetic acid solution to remove any uncrosslinked CS, and then with water for 24 h, until a neutral pH was reached. The CS network was vacuum dried and weighed to determine the crosslinking percentage (gel) as follows,

$$\%\text{gel} = (W_f/W_i) \times 100 \quad (1)$$

where W_f and W_i are the weight of crosslinked and initial CS, respectively.

2.3. Preparation of CS nanogels

Although the size distribution of CS nanoparticles is very difficult to control, several studies have shown that reverse microemulsion (w/o) is a good method for the preparation of low-sized particles with uniform particle size distribution. Several authors have employed the well established microemulsion formulation based on cyclohexane/Triton X-100/n-hexane/water to obtain nanosized CS particles (Arteche Pujana, Pérez-Alvarez, Cesteros Iturbe, & Katime, 2012; Jia et al., 2005).

The crosslinked CS nanogels were obtained by mixing two separately prepared CS and crosslinker (GA) microemulsions. First, the CS microemulsion was prepared according to Jia et al. (2005), with minor modifications. Briefly, 4 mL of CS solution (2% w/v) in aqueous acetic acid (2% v/v) were mixed with 4 mL of *n*-hexanol and 11 mL of cyclohexane under vigorous stirring (700 rpm), to result in a proportion of 1:1:2.75 parts in volume. Finally, Triton X-100 (surfactant) was added drop by drop into the mixture, under vigorous stirring, until a transparent solution was obtained, which is an indication that the particles reached nanometric size. The GA microemulsion was prepared with the same procedure. Then, the GA microemulsion was added, drop-by-drop, to the CS microemulsion under mild stirring. Lastly, the solution was left stirring for 24 h, and a progressive color change was observed to happen, where the solution went from being transparent into attaining a dark amber color as the crosslinking reaction took place.

After the crosslinking reaction, the nanogels were slowly precipitated in ethanol/acetone (1:1) under magnetic stirring. The nanogels were then separated by centrifugation (7 min at 3000 rpm), washed with acetic acid (1% v/v), water, and methanol to eliminate the surfactant and excess GA, and vacuum dried.

2.4. Radiation grafting of NVCL onto CS gels

In glass ampoules, CS macrogels samples (0.08–0.1 g) were immersed in 5 mL of an NVCL solution in acetic acid (10% v/v) at concentrations of 20, 30 and 50% v/v. The ampoules were bubbled with argon to eliminate oxygen, and sealed. The samples in the ampoules were left to swell at room temperature for 24 h, and afterwards they were irradiated using a ^{60}Co gamma source (Gamma beam 651 PT, Nordion Co., Canada) at a dose rate of 5.7 kGy h^{-1},and different dose from 1 to 20 kGy. After radiation, the grafted samples were extracted with acetic acid (1% v/v) to eliminate monomer and ungrafted homopolymer, and then with water to eliminate residual acetic acid. The grafted samples were vacuum dried and weighed (Pérez-Calixto, Ortega, García-Uriostegui, & Burillo, 2016).

The procedure to graft NVCL onto nanogels is as follows: 0.05 g of CS nanogels samples were placed in glass ampoules with 3.5 mL of an NVCL solution in acetic acid (10% v/v) at different concentrations (20–50%v/v), and allowed to swell for 24 h. The samples were then bubbled with argon, sealed, and irradiated at different doses at a dose rate of 4.6 kGy h^{-1}. After radiation, the grafted samples were separated by centrifugation, rinsed with distilled water to remove ungrafted monomer and homopolymer, separated by centrifugation, and dried by lyophilization. The grafting yield of nano and macrogels was calculated as follows:

$$G(\%) = [(W_g - W_o)/W_o] \times 100 \quad (2)$$

where W_o and W_g are the weight of initial and grafted CS, respectively.

2.5. FTIR characterization

The attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of the systems were recorded with a Perkin-Elmer Spectrum 100 spectrometer (Perkin Elmer Cetus Instruments, Norwalk CT) with a diamond tip, over 16 scans.

2.6. Scanning electron microscopy (SEM)

The morphology of the macrogels was studied using a JSM6330F (JEOL Ltd., Japan) with an acceleration voltage of 5 kV and a magnification factor of ×500. The samples were coated with gold using a magnetron sputtering coater.