Biopolymers with Medical Applications

Optimized method for obtaining chitosan-based delivery systems

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The aim of this study was to optimize the method used for obtaining microparticles based on chitosan – a biocompatible, biodegradable, and nontoxic polymer, and to characterize the developed systems. Chitosan microparticles, as drug delivery systems were obtained by inotropic gelation method using pentasodiumtripolyphosphate (TPP) as cross-linking agent. Chitosan with low molecular weight (CSLMW) in concentration which ranged between 0.5 and 5 %, was used while the concentration of cross-linking agent ranged between 1 and 5%. The characterization of the microparticles in terms of shape, uniformity and adhesion was performed in solution and dried state. The size of the microparticles and the degree of swelling were also determined. The structure and the morphology of the developed polymeric systems were analyzed by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).The average diameter of the chitosan microparticles was around 522 µm. The most stable microparticles were obtained using CSLMW 1% and TPP 2% or CSLMW 0.75%and TPP 1%. The micropaticles were spherical, uniform and without flattening. Using CSLMW in concentration of 0.5 % poorly cross-linked and crushed microparticles have been obtained at all TPP concentrations. By optimization of the method, stable chitosan-based micropaticles were obtained which will be used to develop controlled release systems for drug delivery.

Keywords: biopolymers; chitosan; microparticles

Over the last few decades, researchers' interest in using polymeric systems as carriers for oral drug delivery increased significantly, a large number of both biodegradable and non-biodegradable polymers being investigated [1, 2]. In contrast to the non-biodegradable polymers, which are characterized by increased toxicity and difficulty in removal from the body, biodegradable polymers protect the embedded drug from the external medium, being uptaken by enterocytes, depending on the nature of the polymer [3]. After absorption, they will be degraded with a specific kinetic profile, providing a controlled release of the drug [2].

The most important characteristics for the clinical use of biopolymers is to have non-toxicity, low immunogenicity, good biocompatibility and accessibility. These criteria are fulfilled by natural polymers including proteins (such as albumin and gelatin) and polysaccharides (such as alginate, chitosan, starch, dextran, cyclodextrin) [4, 5].

Chitosan is the deacetylated form of its parent polymer chitin, a naturally occurring polymer, which is the second most abundant polysaccharide in nature, after cellulose [6].

Chitosan is a liner copolymer composed of two units, D-glucosamine and N-acetyl-D-glucosamine, linked by (1→4)-β-glycosidic bonds, in different ratio depending on the degree of deacetylation [7, 8]. It is a polycationic polymer, having in every glucosidic unit one -NH_2 and two -OH groups, exhibiting special chemical and biological properties, making it suitable for drug carrier [9, 10] (fig. 1).

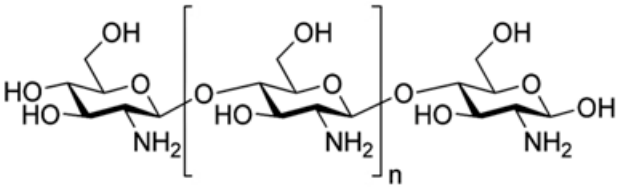

Fig. 1. Chemical stucture of chitosan

Experimental part

Materials and methods

Chitosan with low molecular weight (CSLMW) and deacetylation degree of 80%, pentasodium tripolyphosphate (TPP) and glacial acid acetic (minimum 99.84%) were purchased from Sigma-Aldrich (Iceland).

Preparation of chitosan microparticles

Chitosan microparticles were obtained by inotropic gelation using pentasodiumtripolyphosphate (TPP) as cross-linking agent. It was used chitosan with low molecular weight (CSLMW) in concentration which ranged between 0.5 and 1 %, while the concentration of cross-linking agent ranged between 1% and 5%. Briefly, chitosan solution was prepared by dissolving the desiredamount of chitosan in 1% (v/v) acetic acid, under stirring. The particles were formed by adding dropwise of 3 mL of chitosan solution into 20 mL of TPP solution, using a syringe with a 27-gauge needle. The dropping rate was 15 drops/min, the falling distance was 10 cm and the stirring rate was 250 rotations per minute. To increase their stability the particles formed were kept under stirring for some hours and then were separated, washed with distilled water and dried at room temperature [11, 12].

Characterization of the chitosan microparticles

The characterization of the microparticles in terms of shape, uniformity and adhesion was performed in solution and in dried state. The data obtained at all the concentrations of chitosan and TPP used were analyzed and compared.

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Surface morphology and particle size

The surface morphology of the particles was analysed by Scanning Electron Microscopy (SEM) using a Quanta 450 (SUA) microscope. Samples were placed on a metal support covered with a carbon layer and images were taken by applying an electron beam with an accelerating voltage of 20 kV. The particle diameters were calculated using SEM software.

Spectral characteristics

The IR spectra of the chitosan microparticles were recorded using a Fourier transform infrared spectrophotometer ABB-MB3000 FT-IR MIRacle TM Single Bounce ATR, operating from 4000 to 650 cm^{-1}, the resolution being 4 cm^{-1}. The IR spectra were processed with Horizon MBTM FT-IR Software.

Swelling degree (SD)

In order to study the swelling characteristics, a weighted amount of microparticles was introduced in distilled water and in simulated gastric fluid (SGF) and at different times the swelled microparticles were removed from the solution, dried on a filter paper and weighed [13]. The swelling degree (%) was calculated using the following formula:

$$SD\,(\%) = \frac{Ws - Wd}{Wd} \text{X } 100, \text{ where} \qquad (1)$$

W_s = weight of swollen microparticles, W_d = weight of dried microparticles.

Results and discussions

Characterization of chitosan microparticles

The characteristics of microparticles in solution and in dried state, obtained in different experimental conditions, in terms of concentration of CSLMW and TPP, are summarzed in table 1. Microparticles obtained using CSLMW in concentration of 0.5% were poorly cross-linked nd crushed at all TPP concentrations used, which made impssible their drying and analysis in dried state. At 0.75% CSLMW and 1 and 2 % TPP small uniform particles ~~at~~were obtained in solution, but after drying, they becoming more or less flattened. The most stable microparticles, both in solution and in dried statewere obtained using CSLMW in concentration of 1% and TPP in concentration of 2%. These stable micropaticles were small, spherical, uniforms and ithout flattening, making possible subsequent analyses in dried state.

Surface morphology and particle size

The diameter of the chitosan particles ranged between 499.3 µm and 579.7 µm, with an average of 522.3 ± 16.26 µm. The morphology of the polymeric matrix at different resoltions (200, 50 and 20 µm, respectively) is presented in figure 2. The particles obtained were spherical, with the surface slightly rough and uniform structure.

Table 1

CHARACTERISTICS OF CHITOSAN MICROPARTICLES OBTAINED AT DIFFERENT CONCENTRATION OF CHITOSAN AND TPP

Chitosan concentration	TPP concentration	Characteristic of chitosan microparticles	
		Solution	Dried state
0.5%	1%	weakly cross-linked, crushed	-
	2%	weakly cross-linked, crushed	-
	3%	weakly cross-linked, crushed	-
	4%	weakly cross-linked, crushed	-
	5%	weakly cross-linked, crushed	-
0.75%	1%	small uniform particles	slightly flattened, uniform
	2%	small uniform particles	flattened
	3%	larger uniform particles	flattened
	4%	weakly cross-linked, filamentous	flattened and adherent particles
	5%	weakly cross-linked, crushed	flattened and adherent particles
1%	1%	small uniform particles	flattened and adherent particles
	2%	small uniform particles	small, spherical and uniform particles
	3%	small uniform particles	small, spherical particles; slightly adherent
	4%	large particles, few filaments	flattened and adherent particles
	5%	large particles, filamentous	flattened and adherent particles

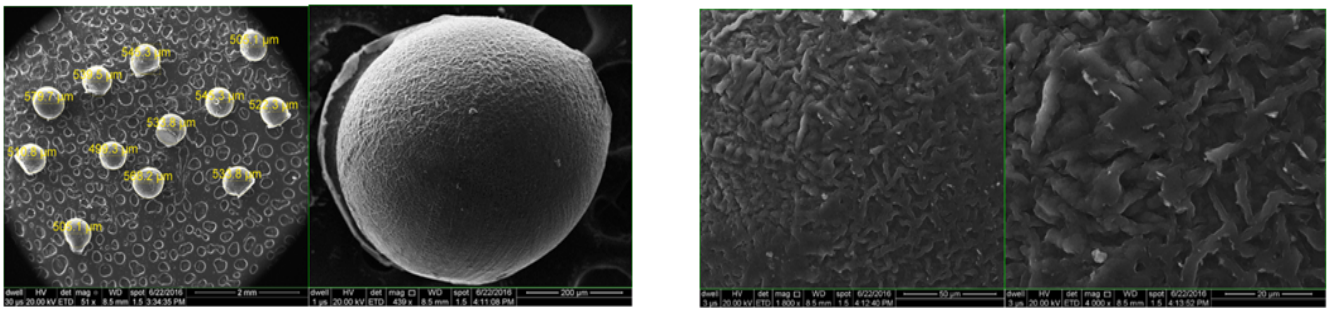

Fig. 2. SEM images of chitosan microparticles

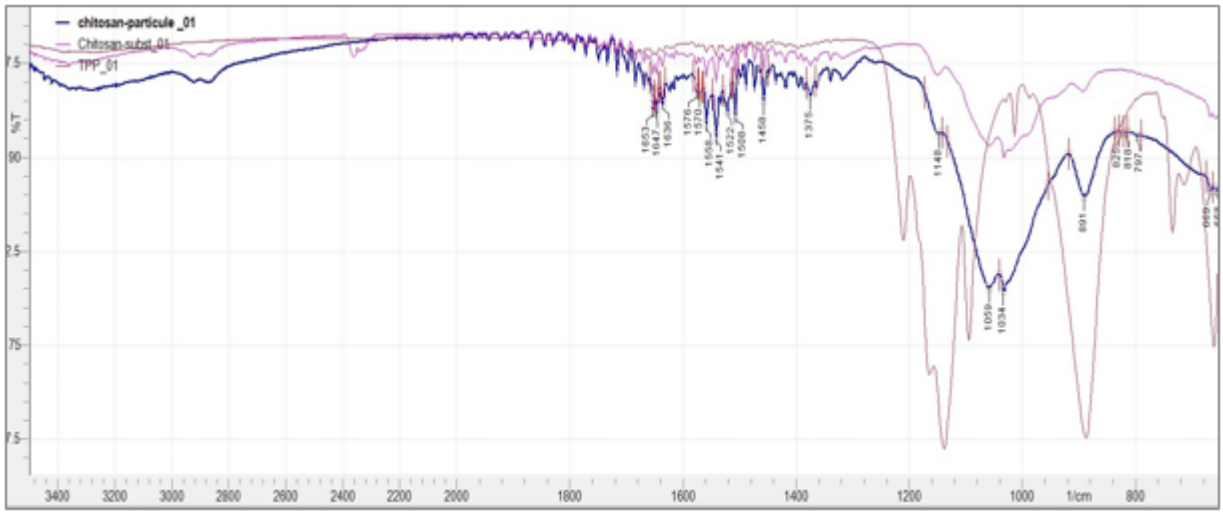

Fig. 3. IR spectra of chitosan microparticles in reference with chitosan and TPP

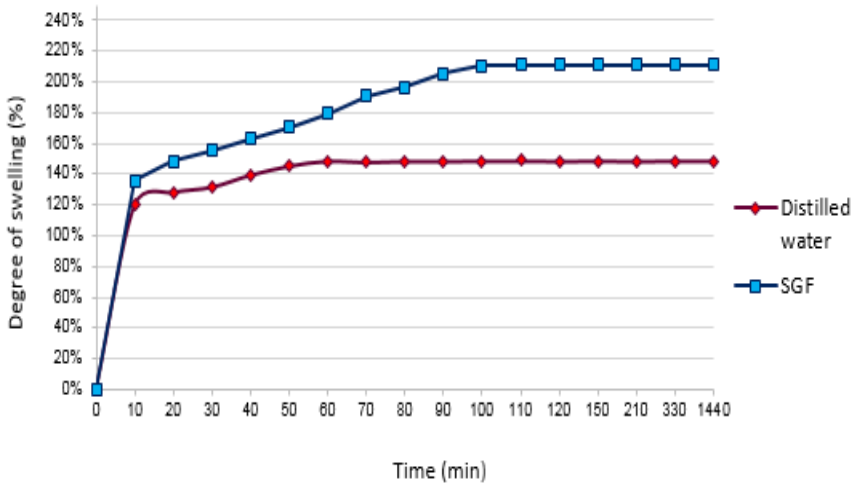

Fig. 4. The swelling degree (%) of the chitosan microparticles as function of time

Spectral characteristics

The IR specta of chitosan, TPP and chitosanmicroparticles are presented in figure 3. The analysis of IR spectrum of chitosan microparticles, revealed the presence of both the polymer and the cross-linking agent. TPP was identified through its characteristic absorption bands in areas of 1151-1148 cm^{-1} (P=O) and 895-891 cm^{-1} (P-O-P). The presence of chitosan was proved through the absorption bands specific to its functional groups: the stretching vibrations of the O-H and N-H bonds could be identified in the 3362-3285 cm^{-1} area, the $-CH_3$ and $-CH_2$ groups were evidenced through their valence vibrations in 2981-2872 cm^{-1} area and through their deformation vibrations in 1431-1373 cm^{-1} area. The -CO-NH- groups were identified in the region of 1600 cm^{-1} (C=O) and 1200 cm^{-1} (C-N). The etheric bond was proved by a high intensity absorption band identified at 1030 cm^{-1}.

Swelling degree

Chitosan microspheres showed good swelling properties, which is an important characteristic for the release of the active substance embedded in polymer matrix. It is known that the porosity of the polymer network is increasing with the increase the number of available charged amino groups [14]. The swelling degree was determined every 10 min, during the first 2 h , then the time of determination was increase to 30 min, 60 min and 120 min, and finally the swelling degree was determined after 24 h from the beginning of the experiment. The results showed for distilled water, a fast increase in swelling degree during the first 10 min (120.27%), followed by a slight increase within the next 70 min (from 120.27% to 148.6%). After that the swelling degree remained constant until the end of the experiment. In comparison, the swelling degree in SGF was higher than in distilled water, the highest value being 211% that was reached after 110 min. This fact suggests the possibility of a better release of the embedded drug in the gastric medium in comparison with distilled water. In addition the hydration process was slower in SGF than in distilled water (fig. 4).

Conclusions

The method of preparing chitosan-based microparticles as drug delivery systems was optimized. As result, stable microparticles were obtained which were characterized in terms of morphology and particles size, IR spectroscopy and swelling degree. The developed chitosan microspheres are spherical, with slightly rough surface and uniform structure. In SGF the chitosan micropraticles showed a higher swelling degree than in distilled water. The optimized method will be used to develop new chitosan-antidiabetic drug delivery systems.

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