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Synthesis of modified chitosan and microparticle production by SAA process

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Chitosan has been extensively studied as carrier for drug delivery, but its characteristic molecular structure makes it soluble at the acid pH of the stomach (pH=2) and insoluble at neutral and alkaline pH values. Therefore it cannot be used in colon-targeting formulations. In recent years, chemically modified chitosan derivatives, developed to change the range of solubility of chitosan, have gained increasing attention. Chitosan succinate and chitosan phthalate are two chitosan derivatives, in which carboxylic groups are introduced, leading to changes in the solubility behavior. They are soluble in an alkaline environment and are potential matrices for colon-specific, orally administered drug delivery.

In this work chitosan succinate and chitosan phthalate are synthesized and then micronized using Supercritical Assisted Atomization (SAA). Microparticles with mean diameter in the range 0.5-1.5 μm are obtained, that can be used for controlled release in the gastrointestinal tract. SAA technique is also effective for the simultaneous purification from the reaction residues.

1. INTRODUCTION

The target of a pharmaceutical formulation is to keep the hematic level of active principle inside the therapeutic window, that means between the minimum effective and the minimum toxic concentrations. The controlled release of an active principle can be performed using composite microparticles, in which the drug is incorporated in a polymer matrix that acts as carrier. Chitosan, which is partially deacetylated chitin (poly(N-acetylglucosamine)), has recently attracted great interest as a carrier because of its natural origin, but its applications in controlled release is limited by its solubility characteristics. Chitosan is a weak base with a pK value of the D-glucosamine residue of about 6.2-7.0 and, therefore, it is insoluble at neutral and alkaline pH values. In acidic medium, the amine groups of the polymer are protonated resulting in a soluble, positively charged polysaccharide. Chitosan is very soluble at pH=2, that is typical environment of the stomach, therefore cannot be used in formulations for the gastrointestinal tract that have as release target the colon, where the pH reaches value 7.4, since it would be dissolved before reaching the target. Nevertheless, colon-targeting drug delivery systems find applications in a number of therapeutic areas. For this reason, efforts have been made in the shifting the optimum pH range for chitosan gel-forming capacity from acidic to alkaline values. This shift could be carried out through the covalent insertion of acidic residues as side chain on the polymer backbone. Carboxylic acid residues are expected to interact with the neutral or slightly alkaline pH environment of the terminal ileum and the ileocecal junction, thus yielding potentially suitable matrices for orally administered colon specific drug delivery tablets. The amino groups on the chitosan backbone can be partially replaced with carboxylic acid residues, for example preparing chitosan succinate and phthalate conjugates [1]. The introduction of the carboxylic groups to the polymer, leads to changes in the pH-dependent solubility behavior. Both, chitosan-succinate and chitosan-phthalate are soluble in an alkaline environment [2]. Other studies have demonstrated that

insulin encapsulated in chitosan-succinate microspheres was quickly released in phosphate buffer (pH 7.4), whereas only a small amount of insulin was released at pH 2.0 [3].
Supercritical Assisted Atomization (SAA) is one of the most efficient Supercritical fluid (SCF) micronization techniques proposed in literature [4]. The process is based on the solubilization of controlled quantities of SC-CO_2 in liquid solutions containing a solid solute and on the subsequent atomization of the ternary solution through a nozzle. SC-CO_2 is miscible with the solution to be treated, and in this case it plays both the role of co-solute, , and the atomization agent. SAA process has been applied to the micronization of different kinds of compounds, among them pharmaceutical compounds [5-7], polymers and bio-polymers [8-10]. SAA technique has been also applied to the production of drug–polymer microparticles for controlled drug release [11, 12] in particular, to produce composite micro-particles of chitosan-ampicillin [13].
In this work, chitosan phthalate and succinate are produced starting from chitosan and are then micronized using SAA technique in order to verify the feasibility of the process on these compounds that are not present on the market and to produce microparticles with tunable size for application in controlled drug release for colon-targeting formulations.

2. MATERIALS AND METHODS

Low molecular weight chitosan with a degree of deacetylation of 84.0%, phtalic anhydride and succinic anhydride were supplied by Sigma-Aldrich (Milan, Italy). Distilled water and glacial acetic acid 96% purity were supplied by Carlo Erba reagents (Milan, Italy).
For both the syntheses we followed a modified procedure of the one reported by Aiedeh et al [1]. In particular: 1.0g of Low Chitosan were dissolved in an acid solution (0,5ml of HCl 37% w/w in 49,5ml H_2O) at room temperature. Separately, 6.25mol of phtalic anhydride (0.93g) or succinic anhydride (0.63g) were dissolved in 5ml of pyridine. This solution was slowly added to the first, under stirring at room temperature. The resulting acid solution was neutralized to pH=7 with NaOH 1M. The resulting neutral solution was stirred for 1h at 70°C, and then stirred overnight at room temperature. The final white solution underwent to SAA process. The solid product was recovered as white powder. The FT-IR analysis confirms the presence of phthalate moieties on the chitosan backbone (see **Figure 5**).
The SAA laboratory apparatus consisted of two high-pressure pumps delivering the liquid solution and liquid CO_2 to a saturator. The saturator is a high pressure vessel (25 cm^3 IV) loaded with stainless steel perforated saddles which assure a large contact surface between liquid solution and CO_2. The solution obtained in the saturator is sprayed through a thin wall (80 μm ID) injection nozzle into the precipitator (3 dm^3 internal volume) operating at atmospheric pressure. A controlled flow of N_2 is taken from a cylinder, heated in an electric heat exchanger and sent to the precipitator to assist liquid droplet evaporation. The saturator and the precipitator are electrically heated using thin band heaters. A stainless steel filter located at the bottom of the precipitator allowed powder collection and the gaseous stream flow out. The plant was completed by a condenser, that separates the solvent from the gas stream. SAA apparatus layout and further details on the experimental procedures were published elsewhere [4, 14].
The morphology of the powder was observed by a field emission-scanning electron microscope (FESEM, mod. LEO 1525, Carl Zeiss). Particle size (PS) and the particle size distribution (PSD) of the microparticles were measured from SEM photomicrographs using the Sigma Scan Pro Software (release 5.0). Approximately 500 particles were measured for each particle size distribution calculation. Histograms representing the particle size distribution were fitted using Microcal Origin Software (release 8.0, Microcal Software).

Solid state analysis of the samples (XRPD=X-ray powder diffraction) was performed using an X-ray diffractometer (mod. D8 Discover, Bruker AXS) with a Cu sealed tube source. Samples were placed in the holder and flattened with a glass slide to assure a good surface texture. The measuring conditions were as follows: Ni-filtered CuKα radiation, λ=1.54 Å, 2θ angle ranging from 2 to 90° with a scan rate of 3 s/step and a step size of 0.02°. Fourier transform infrared (FT-IR) spectra were obtained via M2000 FTIR (MIDAC Co, Costa Mesa, CA), at a resolution of 0.5 cm^{-1}. The scan wavenumber range was 4,000–400 cm^{-1}, and 16 scan signals were averaged to reduce the noise. Powder samples were analysed in KBr discs.

3. RESULTS AND DISCUSSION

Chitosan has been micronized in the past years by Reverchon and Antonacci using SAA technique and spherical particles were obtained [9]. The same optimized operating conditions have been used here to micronize chitosan phthalate and chitosan succinate obtained by the synthesis.

Chitosan Phthalate

The crude product obtained from the synthesis was a solution containing chitosan phthalate, some unreacted reagents and side products. To recover the chitosan phthalate as a solid, the solution was treated with acetic acid and the resulting precipitate was filtered and dried. The product was collected as amber color bug crystals, that were then dissolved in water in order to perform the SAA micronization. The preliminary tests were conducted at concentration of 25 mg/mL, CO_2/water w/w ratio 1.8, temperature at the mixer 85°C, pressure in the mixer 100 bar, precipitation temperature 100°C After SAA process the polymer was collected as white powder. The SEM analysis showed that the product had a spherical morphology (see **Figure 1**). The difference in color of the product compared to the raw product of the synthesis suggested that during the SAA precipitation also a purification mechanism took place, therefore, the next tests were performed directly using the final reaction solution and skipping the steps of precipitation, drying and re-dissolution in water.

Several solutions were prepared, with different reaction times and temperature conditions. Increasing the reaction time, the final concentration of chitosan phthalate in the solution increased, but increasing the temperature for more than one hour, the final yield decreased. The synthetic procedure reported in section 2 was the one that lead to the best results, as will be discussed in the following sections. The SAA tests on the solutions at different concentrations were performed with the same conditions described for the preliminary tests.

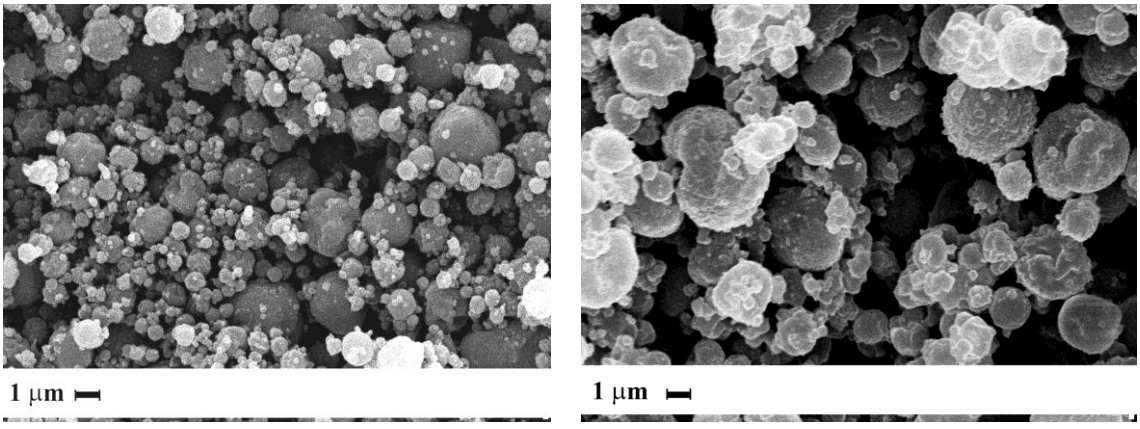

Figure 1. SEM photomicrographs of chitosan phthalate microparticles at concentrations of 20 mg/mL (left) and 37 mg/mL (right).

The PSD is shown in **Figure 2**, in which the frequency of the number of particles vs the particle diameter is reported. It is evident that increasing the concentration of the product in the starting solution, the size of the particles increases. In particular, the mean diameter moves from 0.5 μm to about 2 μm.

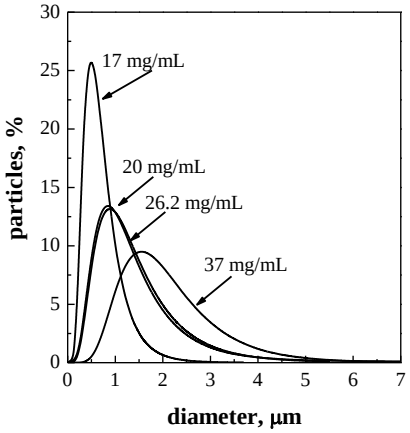

Figure 2. PSDs in terms of number of particles percentage of chitosan phthalate at different concentrations

Chitosan Succinate

The solution obtained from the reaction to produce the chitosan succinate has been filtered in order to separate suspended material and unreacted components and directly processed by SAA. The operating conditions were chosen accordingly to the results obtained for the chitosan phthalate: CO_2/water w/w ratio 1.8, temperature at the mixer 85°C, pressure in the mixer 100 bar, precipitation temperature 90-100°C. In all the cases using a precipitation temperature of 100°C, spherical particles were obtained, as shown in the SEM photomicrographs in **Figure 2**. When the precipitation temperature was 90°C, non-spherical and collapsed particles were obtained.

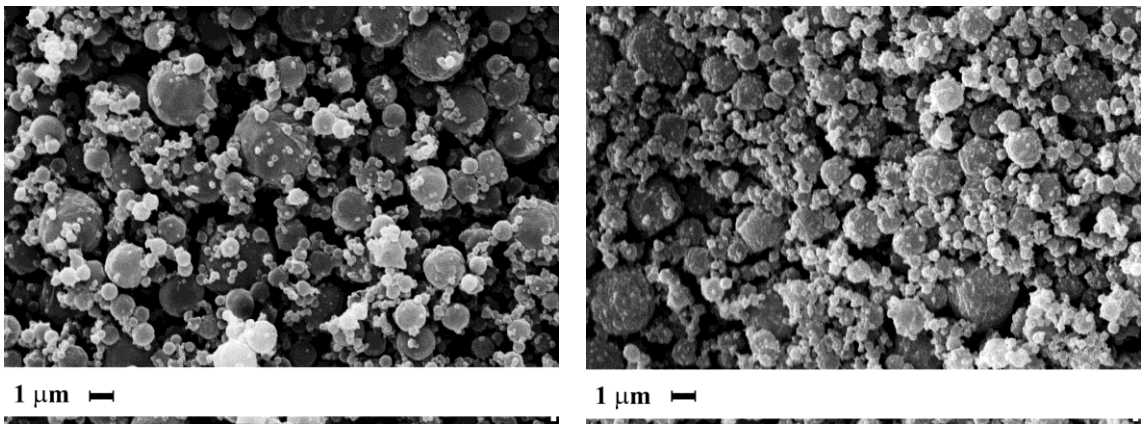

Figure 3. SEM photomicrographs of chitosan succinate microparticles at concentrations of 15 mg/mL (left) and 29.4 mg/mL (right).

Also in the case of chitosan succinate, the size of the particles increases when the concentration of the product in the starting solution increases. Indeed, the PSD shown in the graphic in **Figure 3** shows that the mean diameter moves from 0.5 μm to 1.5 μm.

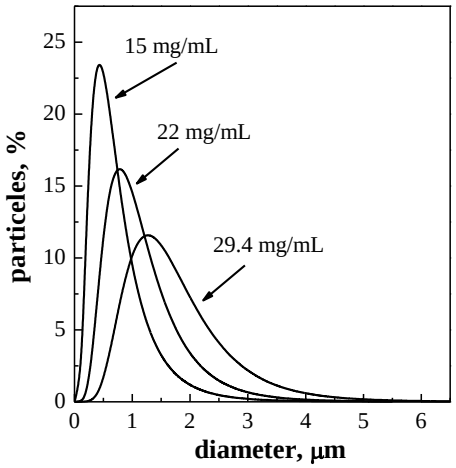

Figure 4. PSDs in terms of number of particles percentage of chitosan succinate at different concentrations

The FTIR analyses performed on: untreated chitosan, micronized chitosan, micronized chitosan phthalate and micronized chitosan succinate (**Figure 5**) show that the SAA process does not modify the structure of the chitosan. Moreover, the spectra of the chitosan phthalate and chitosan succinate show the peaks typical of chitosan and the carboxylic stretching at 1730-1700 cm^{-1}, attesting the substitution introduced by the reaction.
Furthermore, in **Figure 5** the IR spectrum of pyridine, the solvent used in the reaction process is reported. From a comparison with the chitosan curves, it is clear that the characteristic peak at 2900-3100 cm^{-1} is absent in both the substituted chitosan spectra, confirming the purification action operated by SAA process during the micronization process. Since the pyridine is toxic, it is important to remove it from the final product.

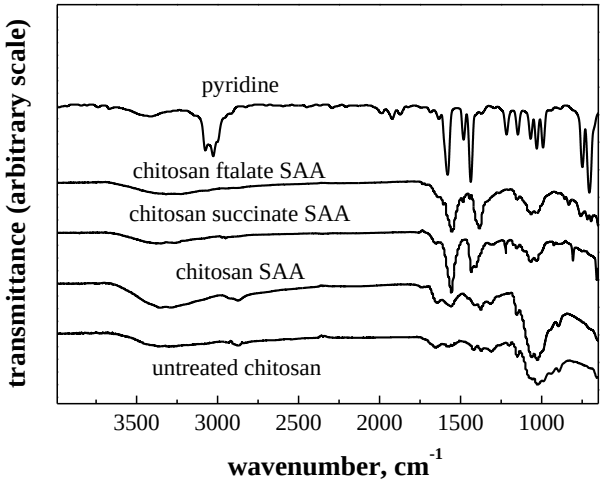

Figure 5. FTIR spectra comparison of different modified chitosan

4. CONCLUSIONS AND PERSPECTIVES

Chitosan phthalate and succinate have been successfully produced via modification of natural chitosan. A new approach has been validated for the micronization and simultaneous purification of the substituted chitosan using SAA technique, in a single step. The size of the particles produced can be modulated changing the concentration of the reaction product in the liquid of reaction and spherical particles with a mean diameter of 500 nm can be obtained.
Some tests are currently under study to attest that the chitosan phthalate and succinate microparticles produced by SAA have the required solubility at different pH. Furthermore the coprecipitation of these modified polymers with a pharmaceutical compound and controlled release studies are necessary to propose the studied systems as suitable materials for application at different levels of the administration route.

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