Preparation and Evaluation of Chitosan-Treated Alginate Microparticles for Sustained Release of All-trans Retinoic Acid

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Introduction

Recently, efforts have been focused on microparticles prepared with natural polymers such as alginates and chitosans. Sodium alginate in particular has been commercially applied and investigated due to its low cost and minimal processing requirements (Murata et al., 1993; Gombotz & Wee, 1998; Sezer & Akbuga, 1999a and b). The use of sodium alginate for microencapsulation has been extensively studied for a long time.

However, gel erosion is an important problem of alginate microparticles since it accelerates drug release (Murata et al., 1993). Therefore, many attempts have been made towards controlling the disintegration of alginate microspheres and extending drug release. Coating alginate microparticles with polycationic polymers such as chitosan has been shown to suppress gel matrix erosion of alginate microparticles containing drugs with different physical and chemical properties, thus reducing its release rate (Murata et al. 1993; Gombotz & Wee, 1998; Sezer & Akbuga 1999a and b; Lee et al., 2003)

In this paper, chitosan-treated alginate microparticles were prepared with the purpose of incorporating all-trans retinoic acid (ATRA), which is an insoluble drug with low molecular weight. The effect of different processing factors on microparticles properties was also investigated.

Material and Methods

Materials

ATRA acid was acquired from RJR Nutrientes e Farmoquímicos LTDA. (Brazil). Chitosan (Hydagen® HCMF Type, MW 50.000 - 1.000.000 g/mol) with min. 80% deacetylation degree was donated by Cognis, Brazil. Solvents were obtained from HPLC grade (Merck & Co. Inc, USA). Other reagents were all analytical grade reagents.

Preparation of drug-loaded chitosan microparticles

In general, ATRA (0.5% w/v) was dissolved in 1.5mL of chloroform. Alginate solution (150mg) was prepared by dissolving it in water, and Tween 80 (2% w/v) was added to the solution as a surfactant. Then, the organic phase (drug solution) was mixed with the aqueous phase (alginate solution) using an Ultraturrax® at 19000rpm for 20min. The ratio of the organic and aqueous phases was 1:10. O/W emulsion was pumped (2mL/min pump speed) toward a 0.7mm nozzle (air flow rate, 20mL/min; pressure, 3KgF), sprayed downward into 150mL of $CaCl_2$ (0.5%) and chitosan (0.2 or 0.4%) solution and stirred for 5 minutes. After the cross-linking time, microparticles were filtered, washed with distilled water repeatedly and then freeze-dried.

Microparticles Characterization

The surface morphology of microparticles was observed under a scanning electron microscope (Leica Model Stereoscan 440). The samples were attached to the plate surfaces with double-sided adhesive tapes and then coated with gold. Scanning electron photomicrographs were taken at appropriate magnification. The size and size distribution of microparticles were evaluated by a Light Scattering particle size analyzer (LS™ 13 320, Beckman Coulter).

Assessment of Encapsulation efficiency

5mg of microparticles were dissolved in 10mL of phosphate buffer (pH 7.4). Then, the polymer was precipitated with the addition of 15mL of ethanol. The supernatant was filtered and analyzed (n= 3) by HPLC Shimadzu® model SPD 10AVP equipped with a pump LC-10 ADVP, Reodyne injector, integrator model CR6-A and UV detector (λ =348), model SPD-10A VP. A column RP-18 shim-pack (125x4mm, 5µm) provided by Merck® and coupled to a guard column was used in controlled room temperature (25ºC). The mobile phase consisted of methanol: 1.2% solution of acetic acid: acetonitrile: alcohol isopropyl (10:30:30:30, v/v), 1.2mL.min^{-1} of flow.

Drug- Polymer Interaction

To examine the interaction of chitosan with ATRA, differential scanning calorimetry (DSC) and FTIR spectroscopy were employed. DSC thermograms of the pure drug, chitosan, binary mixtures (1:1) and microparticles loaded with ATRA were obtained by a differential scanning calorimeter Netzsch thermal analysis, model 200, under a nitrogen flow of 50 mL.min^{-1}, at a heating rate of 10 ºC.min^{-1}, up to 300ºC. Samples (20 mg) were weighed and open aluminum pans were used. FTIR spectra of the same samples were also measured using KBr pressed disks with a Nicolet FTIR spectrophotometer (Model PROTEGÉ ™ 460).

In-Vitro Drug Release Study

The release rate of the microparticles was evaluated using a Hanson dissolutor, model SR-8 Plus, with paddle assembly (USP Apparatus 2 or BP Apparatus II). 10mg of microparticles were suspended in 300µl of 30% ethanol in pH 7.4 phosphate buffer solution (PBS), and then placed within a dialysis membrane with closed extremities. The bags were immersed into 100ml of 30% ethanol in PBS solution as a dissolution medium and shaken at a rate of 100rpm at 37°C. Dissolution medium (1mL) was periodically drawn for analyzing all-trans retinoic acid by HPLC. The same volume of fresh medium was simultaneously replaced to the vials. The samples was analyzed by HPLC (n = 5).

Results and Discussion

Scanning electron micrographs of microparticles are shown in Figure 1. Microparticles presented regular and spherical surface. Their average diameter was of 148.2µm with $d_{50\%}$ of 79.2µm and $d_{90\%}$ of 335.9µm.

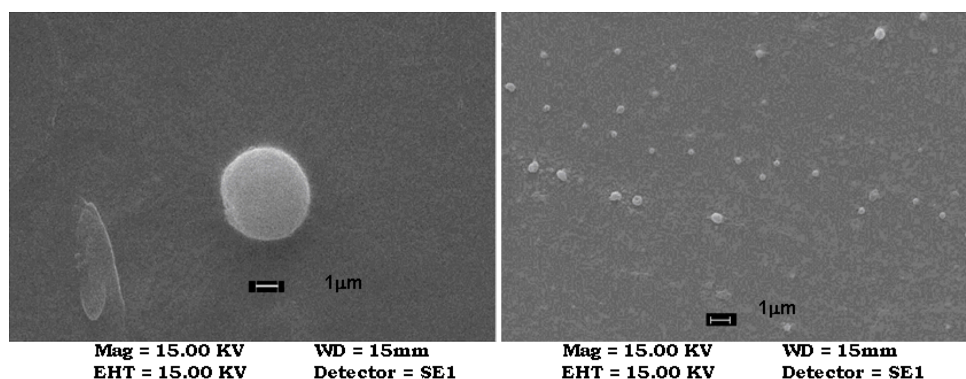

Figure 1. Scanning electronic photomicrographs of alginate microparticles loaded with ATRA.

Encapsulation efficiencies of ATRA were greater than 40% for both preparations. Microparticles prepared with 0.4% chitosan presented higher encapsulation efficiency (47.46 ± 4.47) than microparticles prepared with 0.2% chitosan (43.72 ± 1.61). These results are according to findings for alginate particles by Sezer & Akbuga (1999a and b) in which they found that a decrease of chitosan concentration resulted in lower encapsulation efficiency.

Figure 2 shows DSC thermograms of pure drug, isolated alginate, physical mixtures and alginate microparticles loaded with ATRA. Alginate exhibited no endothermic peak owing to its amorphous form. In the case of ATRA and the physical mixture, an endothermic peak due to ATRA melting was observed at 185ºC and 179.6ºC, respectively. That slight shift observed in the physical mixture may be due to an interaction between drug and polymer or drug solubilization in the polymer. In contrast, in the case of the microparticles, no ATRA peak was observable.

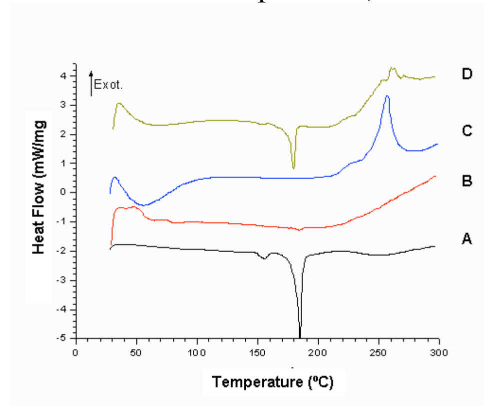

Figure 2 – DSC thermograms of pure ATRA (A), alginate (B), alginate microparticles loaded with ATRA (C) and (1:1) drug-polymer physical mixture (D).

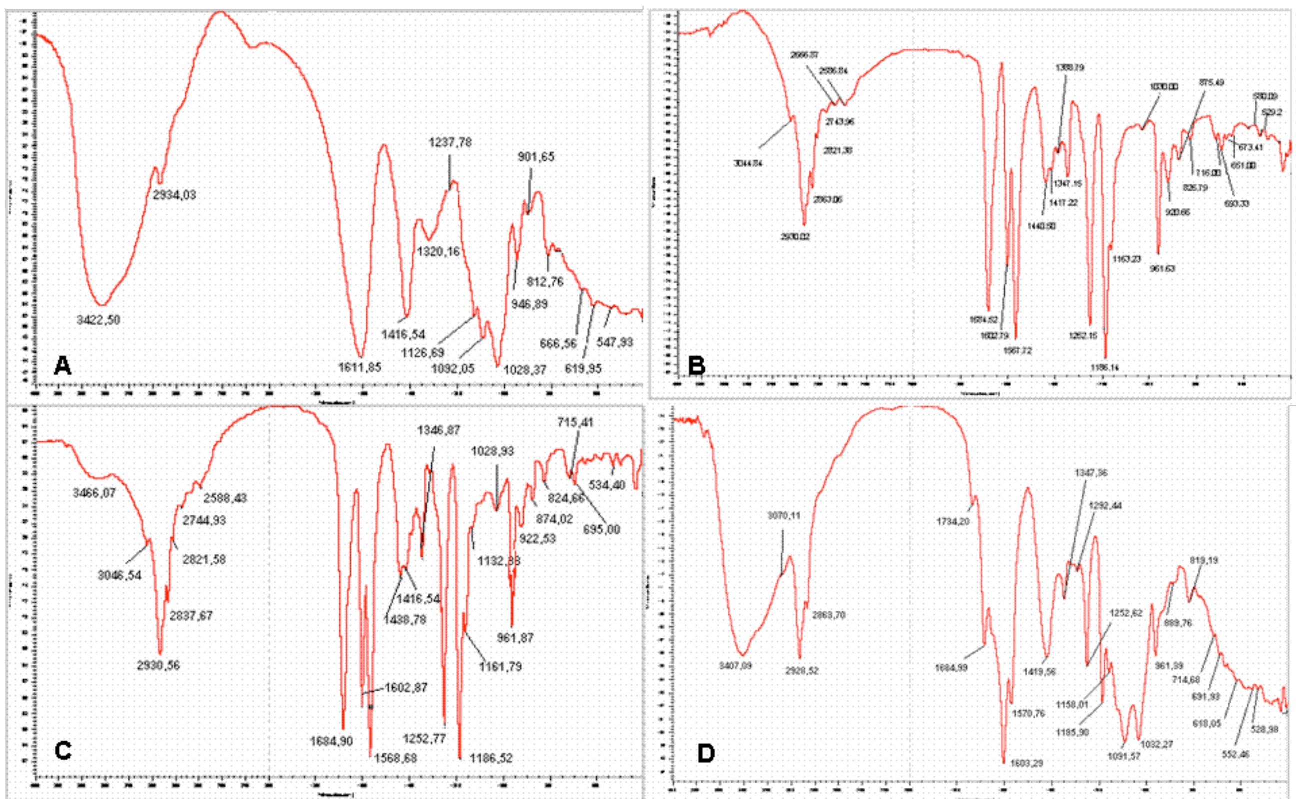

Figure 3. FTIR spectrum of (A) alginate, (B) ATRA, (C) drug-polymer physical mixture (1:1) and (D) alginate microparticles loaded with ATRA.

FTIR spectra of chitosan microparticles, ATRA and physical mixture are shown in Figure 3. Changes in the physical mixture and microparticles were not observed when they were compared with the ATRA spectrum, suggesting no drug-polymer interaction. Then, it can be stated that the slight shift observed in DSC of the physical mixture was more probably due to the solubilization of drug into polymer.

A protein or a molecule with an overall net positive charge can potentially interact with the negatively charged alginate polymer, thus inhibiting diffusion from the gel (Gombotz & Wee, 1998; Stockwell et al, 1986). In some cases interactions of a cationic drug can lead to drastic changes in particles morphology (Segi et al., 1989). In fact, it is expected that ATRA, an ionic drug, interacts with basic polysaccharides, such as chitosan, rather than with acidic polysaccharides, such as alginates.

The release profiles of microparticles are given in figure 4. Microparticles prepared with a higher amount of chitosan significantly delayed the release of ATRA ($p < 0.05$). These results are also according to the findings for alginate particles by Sezer & Akbuga (1999a and b) and by Lee at al. (2003), in which they found, when the concentration of chitosan-coating solution increased, that drug release was significantly reduced due to increased chitosan interaction on the surface of alginate microparticles.

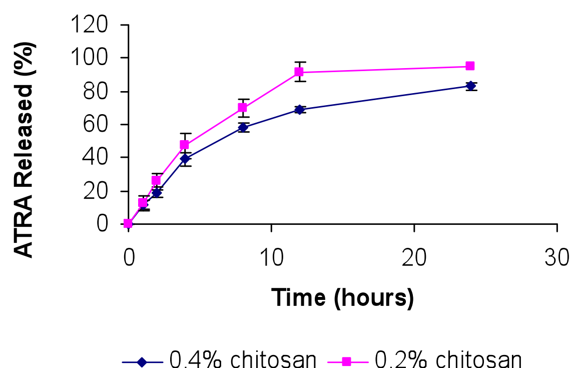

Figure 4. Release profile of ATRA from chitosan microparticles. Mean values and their standard deviations (bars), n = 5 determinations.

Conclusion

Chitosan coating provided extended drug release. Besides, chitosan-treated alginate microparticles can easily be made and used for controlled drug delivery systems due to a convenient process and sustained drug release.

References

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