Characterization of polyelectrolyte interactions of alginate core nanospheres coated with polyelectrolytes and acid-protective biomaterials for oral insulin delivery

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Introduction

Nanoparticles are solid sub-micronic drug carriers of natural, semisynthetic, or synthetic polymeric nature in the nanometer size range. Nanoparticles may or may not be biodegradable (Catarina Reis *et al.*; 2006a) and they have many advantages over traditional formulations and this is why the interest about them has increased in the past years (Catarina Reis *et al.*; 2006b). Insulin, like many other peptidic drugs, is easily degraded in acidic environment in the stomach but also degraded by proteases which are present along the gastrointestinal tract. Nanoparticles appear as a very good alternative system to deliver and to protect peptidic drugs. As well, a promising strategy in nanotechnology field is the use of multifunctional biodegradable polymers exhibiting gastrointestinal permeation enhancing and mucoadhesive properties (Catarina Reis *et al.*; 2006c).

The following work was performed by using polymeric nanospheres with alginate polymer as core polymer and dextran sulphate as adjuvant. As coating biodegradable polymers, nanospheres contain chitosan and polyethyleneglycol (PEG) as first coating material and albumin as second coating material. Nanospheres were prepared by alginate, insulin and sulphate dextran dispersion in a solution followed by *in situ* gelification due to Ca^{2+} release. After alginate gelation, particle core was coated with chitosan-PEG with calcium chloride ($CaCl_2$) and finally with albumin. In order to detect polyelectrolyte interactions between previous materials, nanospheres and solutions of isolated biomaterials were characterized by viscosity measurement and differential scanning calorimetry (DSC).

Materials and Methods

Sodium alginate of low viscosity (2% viscosity in a 25ºC solution, 250 cps), chitosan (50 KDa) and albumin were purchased by Sigma-Aldrich Chimie (L`isle d`Abeau Chesnes, France). The sulphate dextran (5 KDa) was provided by Fluka Biochemika (Buchs, Switzerland). Calcium carbonate was provided by Omya (Orgon, France). The PEG was purchased from Fluka, Chemie GmbH (Buchs, Switzerland). The insulin was kindly donated by *Hospitais da Universidade de Coimbra* (Actrapid Insulin, Novo Nordisk, Bagsvaerd, Denmark).
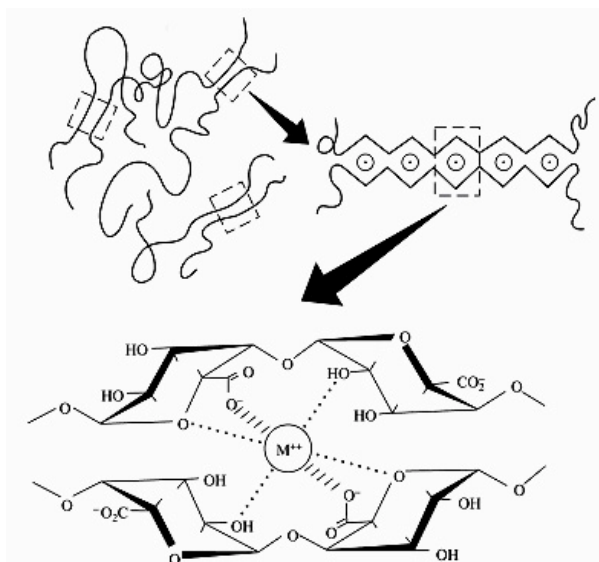
Solutions of isolated compounds were prepared by gentle dissolution in distilled water under magnetic stirring. Solutions which simulate nanospheres core, solutions which contain the first coating material, solutions which contain the second material were prepared by gentle dissolution in distilled water under magnetic stirring. Finally, solutions which simulate coated nanospheres were prepared by gentle and controlled dissolution in distilled water under magnetic stirring. The pH of all solutions was controlled. Viscosity was measured in all solutions. Data were expressed in mean values ± standard deviation. In the DSC technique, samples were lyophilised and each sample gradually heated from 27-28ºC to 450 ºC with a heat flow of 10ºC/minute with a nitrogen atmosphere with the flow of 20 mL/minute. Exothermic and endothermic peaks were analyzed and recorded by using a Shimadzu DSC-50 system (Shimadzu, Kyoto, Japan).

Results and discussion

Viscosity analysis showed following observations:

- Solutions containing alginate had high values of viscosity because alginate itself is the compound with higher viscosity grade.
- It was also possible detect that solutions containing only chitosan and albumin showed a very low value of viscosity.
- Formulations with alginate, calcium carbonate and then acetic acid, demonstrated a considerable viscosity increase.

This last fact may be probably due to alginate gelation. In this method, acetic acid was used to decreased pH of nanoemulsion and then to solubilise calcium salt. Consequently, Ca^{2+} ions were released and interacted with alginate chains. Reticulation of alginate with calcium ions is responsible of the formation of gel compact structure named as egg-box model as illustrated in Figure 1 (Catarina Reis *et al.;* 2006c).

Figure 1. **Egg-box model of alginate gel adapted from Khotimchenko** *et al.* **(Khotimchenko** *et al.***, 2006).**

Isolated compounds solutions were prepared and its thermal behaviour was evaluated. Specifically, it was studied alginate thermal behaviour before and after gelation but also after coating process with chitosan-PEG and albumin. DSC data confirmed alginate gelation with calcium ions but also formation of polyelectrolyte complexes between insulin, calcium carbonate, sulphate dextran, albumin, and chitosan-PEG. Alginate solution was used as reference. Endothermic and exothermic alginate peaks showed a significant deviation after gelation process to 112ºC and to 251.8ºC. Moreover, the coating process changed DSC spectra of gelled and not gelled alginate as seen in Figure 2.

A

B

C
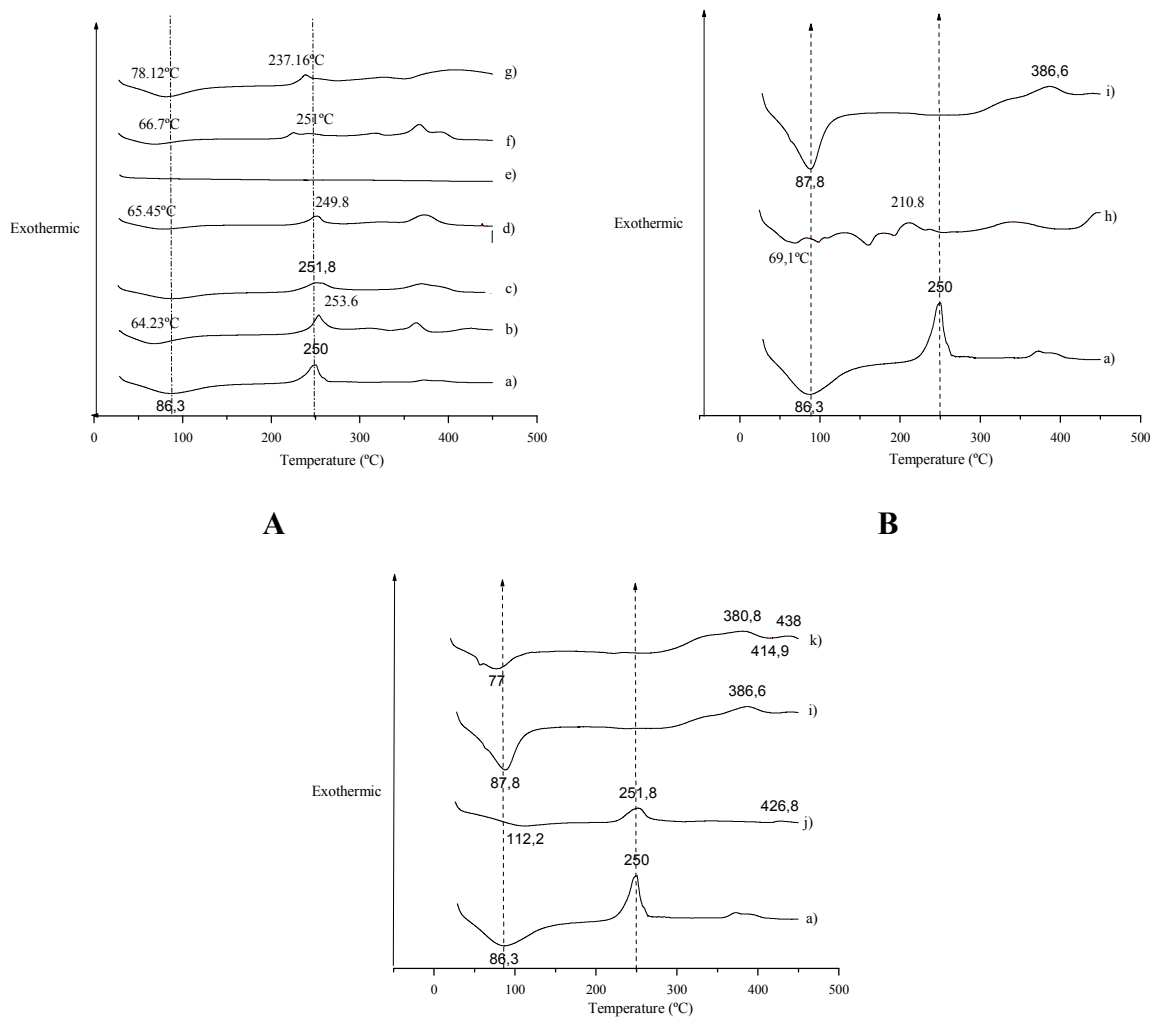
Figure 2. A) Simulated solution of nanospheres core without alginate gelation where a) Alginate, b) Alginate + calcium carbonate, c) Alginate + sulphate dextran, d) Alginate + sulphate dextran + calcium carbonate, e) Alginate + sulphate dextran + calcium carbonate + insulin, f) Alginate + insulin, g) Alginate + sulphate dextran + insulin. B) First coating process without alginate gelation where a) Alginate, h) Chitosan + PEG + CaCl₂ + Alginate + sulphate dextran + calcium carbonate + insulin and second coating process where i) Chitosan + PEG + CaCl₂ + alginate + albumin + sulphate dextran + calcium carbonate + insulin, C) Alginate gelation where a) Alginate, j) Alginate + calcium carbonate + CH₃COOH, i) Chitosan + PEG + CaCl₂ + alginate + albumin + sulphate dextran + calcium carbonate + insulin but without alginate gelation and finally alginate gelation and coating process where k) Chitosan + PEG + CaCl₂ + alginate + albumin + sulphate dextran + calcium carbonate + CH₃COOH + insulin.

Conclusions

We may conclude that viscosity and thermal analysis are very reliable and effective methods to detect polyelectrolyte interactions. Both analyses also showed the occurrence of alginate gelation but also interactions between alginate, calcium carbonate, insulin, sulphate dextran, chitosan, PEG and albumin. However, in this study we did not evaluate whether these chemical interactions may cause or not damage to insulin. Further *in vivo* assays are required in order to evaluate insulin stability when these chemical interactions occur.

References

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