

Studies on the Formation of Complex Coacervates between Chitosan and Alginate During Microparticles Preparation

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SUMMARY. The formation of complex coacervates between chitosan and alginate was evaluated during microparticles formation. Mass ratio between polyelectrolytes and calcium chloride concentration were determined by conductimetric analysis and by calcium ions quantification, respectively. Inert microparticles were prepared using a complex coacervation method in W/O emulsion and morphological analyses of microparticles were carried out. This method enabled the production of spherical particles, with slightly rough surface and narrow size distribution with maximal diameter of 10 µm.

RESUMEN. “Estudios en la formación de coacervados complejos entre quitosana y alginato durante la preparación de micropartículas”. La formación de coacervados complejos entre quitosana y alginato fue evaluada durante la formación de micropartículas. La proporción de masa entre polielectrolitos y concentración de cloruro de calcio fue determinada por análisis conductimétrico y por cuantificación de los iones calcio, respectivamente. Las micropartículas inertes fueron preparadas usando un método de coacervación compleja en emulsión de A/O y se llevaron a cabo los análisis morfológicos de las mismas. Este método permitió la producción de partículas esféricas, con superficie ligeramente desigual y distribución de tamaño estrecha, con un diámetro máximo de 10 µm.

INTRODUCTION

The use of natural polymers as drug carriers has received much attention in the pharmaceutical field due to their safety. In particular, polysaccharides such as sodium alginate (AL) and chitosan (CS) has been studied for application in the design of dosage forms for controlled release ¹.

The fascinating properties of chitin, chitosan and derivatives, among them such as antimicrobial agent and gel and film builder ^{2,3}, have stimulated numerous research activities at both industrial and academic levels, which result in an ever-increasing amount of new potential applications. Presently, the major trend is the research on products of high commercial value, such as cosmetics, drug carriers, food additives, semi-permeable membranes and pharmaceutical products.

Capsules of calcium alginate coated with a

membrane constituted by a polyelectrolytic complex have been widely reported in the literature. Polylysine and chitosan are the most commonly used polycations for capsule production ⁴.

This work presents a study on the behaviour of chitosan and alginate during the preparation of microparticles by a complex coacervation method in W/O emulsion.

MATERIALS AND METHODS

Materials

Chitosan of practical grade with desacetylation degree of 63% (molecular weight 1.4.10⁵ as determined by viscosimetry) was purchased from Sigma; sodium alginate (molecular weight 3.27.10⁵ as determined by viscosimetry) was purchased from Henrifarma; calcium chloride and all other chemicals used were of analytical grade.

KEY WORDS: Alginate, Chitosan, Complex coacervation, Microparticles.

PALABRAS CLAVE: Alginato, Coacervación compleja, Micropartículas, Quitosana.

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METHODS

Conductimetric analyses

An initial volume of 50 mL of 0.01% chitosan dispersion (35.7 nmol) in 0.01 M acetate buffer solution, pH 4.8 was placed in a flask in contact with the conductimetric cell and samples of 1ml of 0.1% alginate dispersion (3.0 nmol) in 0.01 M acetate buffer solution pH 4.8 were added with constant stirring. The resulted conductivity was registered after each addition and stabilization. The analysis was accomplished at a temperature of 23 °C. The proportion of mass of the polymers was calculated using the following equation:

$$P = \text{VAL} \times \%AL / \text{VCS} \times \%CS$$

where P = proportion of polymers, VAL = total volume (mL) of sodium alginate dispersion added to the medium, %AL = concentration of alginate dispersion, VCS = total volume (mL) of chitosan dispersion present in the medium, and %CS = concentration of chitosan dispersion.

Analysis of AL-Ca and AL-Ca:CS complexes by titrimetric quantification of calcium ions

The AL-Ca complex was obtained by mixing 4 mL of 0.5% sodium alginate dispersion with 2 mL of calcium chloride solution; the AL-Ca:CS complex by adding 4 mL of 0.25% chitosan dispersion containing calcium chloride to a 0.5% sodium alginate dispersion. The complex formation was carried out under stirring by vortex during 3 min. The mixture was then centrifuged at 3000 rpm during 5 min (Sorvall, TC-6 centrifuge) and washed three times with water. The range of calcium chloride concentration was from 0.1 to 4.0% (21.7 to 720.8 μmol) in both complexes.

The free calcium ions present in the supernatant solution were titrated using a calcon carboxylic acid mixture as indicator and EDTA disodium solution as titrimetric agent ⁵.

Preparation of inert AL-Ca:CS microparticles

After the optimal concentrations of polyelectrolytes and calcium ions were determined, microparticles were prepared by means of a new approach of the complex coacervation method using a W/O emulsion. The 0.5% alginate dispersion (4 mL) in 0.01 M acetate buffer pH 4.8, was emulsified in liquid paraffin (20 mL) containing 0.5% of sorbitan monooleate. A 0.25% chitosan dispersion (4 mL) in 0.01 M acetate buffer pH 4.8 containing calcium chloride was added dropwise into the emulsion under contin-

uous stirring provided by sonication (Sonicator® - Model XL2020). After reaction, microparticles were isolated by phase separation in n-butanol, centrifuged at 3000 rpm during 8 min, washed twice with n-butanol and three times with water, and finally freeze-dried (Edwards®, Modulyo Pirani 10) during 24 h.

Morphological analyses of the microparticles

Morphological analyses were performed by scanning electron microscopy (Jeol JSM T330A).

Size distribution analyses

For size distribution determination photomicrographs obtained by scanning electron microscopy were used. With relief of a calliper, the diameters of the particles were registered according to Feret ⁶.

RESULTS AND DISCUSSION

As shown in Figure 1, the best chitosan:alginate proportion for achieve the higher yield of polyelectrolytic complex is 1.6 (weight) or 0.67 (nmol). As the polyanion dispersion was added, the amino groups of chitosan were neutralised by complexation with carboxylic groups of sodium alginate, leading to a decrease on the conductivity caused by decreasing concentration of overall ions present in the dispersion. After achieving the equivalence point, an increase of conductivity was observed due to the excess of added alginate ions in the dispersion.

The polymeric dispersions of alginate and of chitosan were prepared with buffer solutions of acetate pH 4.8. In previous analysis of the complexation of these polyelectrolytes by viscosimetry, a larger complex formation was observed at pH approximately 5 ⁷; at lower or higher pH values a decrease of complexation happened.

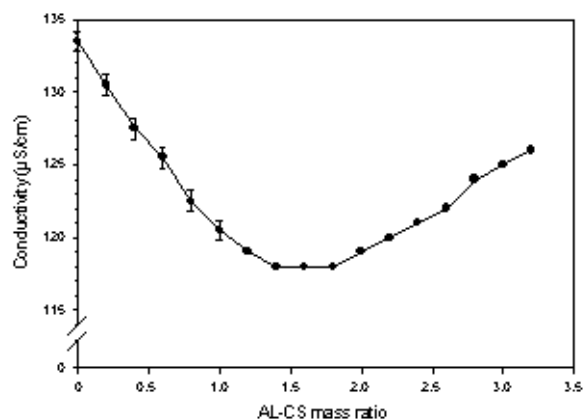


Figure 1. Conductimetric curve of sodium alginate and chitosan mixed at different ratios.

Due to the dissociation characteristics inherent to each polymer, with the increase of pH the number of dissociated carboxylic groups in the alginate increases and the number of protonate amino groups in the chitosan decreases. At extremely acid pH, most of the carboxylic groups of the alginate are in the non ionised form (-COOH) and in compensation, at high pH values, most of the amino groups of the chitosan are in the -NH₂ form.. At pH approximately 5, as much the amino groups as the carboxylic groups in the polyelectrolytes are almost totally dissociated. These results are in agreement with those described by Lee *et al.* ⁸.

By titrimetric analyses of free calcium ions it was observed that the amount of calcium ions bound to the polymer decreases at concentrations higher or smaller than 1.5% (Fig. 2), which can be related with the non participation of some uronic acid molecules and/or monomer unit sequences such as mannuronic (M-M) or mannuronic-guluronic (M-G) blocks of alginate in the complexation process ⁹. It also shows that the linkage between divalent cations and alginate occurs mainly at the guluronic (G-G) sequences due to the favourable orientation of the carboxilate groups in such chains. The specific interchain bounds of G blocks with calcium resulted in the formation of alginate gel net structure ^{4,10,11}.

By further addition of chitosan dispersion to build AL-Ca:CS complex, the amount of calcium ions bound to alginate decreases by approxi-

mately 13.5% (Fig. 2). This confirms that calcium ions are displaced during formation of alginate-chitosan polyelectrolytic complexes, and also confirms the formation of the polyelectrolytic complex of alginate-chitosan; in other words, the reduction of calcium ions linked to the alginate indirectly demonstrates the complexation of the polymers.

The shape and size of microparticles obtained by complex coacervation method between alginate and chitosan in the presence of calcium chloride in a W/O emulsion system were defined by the internal phase of the system. The emulsion was submitted to sonication prior to the complexation process between polyelectrolytes occurred, which provided the reduction of internal phase size and consequent reduction of particles' diameter. The intensity of this method turns it more effective than the use of mechanical agitation that provides the formation of larger particles and with larger agglomeration.

During microparticles formation, due to molecular weight difference, the calcium ions diffuse into the internal phase of the emulsion (alginate dispersion) faster than chitosan can do, building a gel core. The membrane consisting of alginate-chitosan complex is then built at the interface of the emulsion, coating the gel core (Fig. 3) ^{8,12}.

The presence of calcium ions in the chitosan dispersion during the complexation affects the chitosan linkage capacity to the AL-Ca gel, due

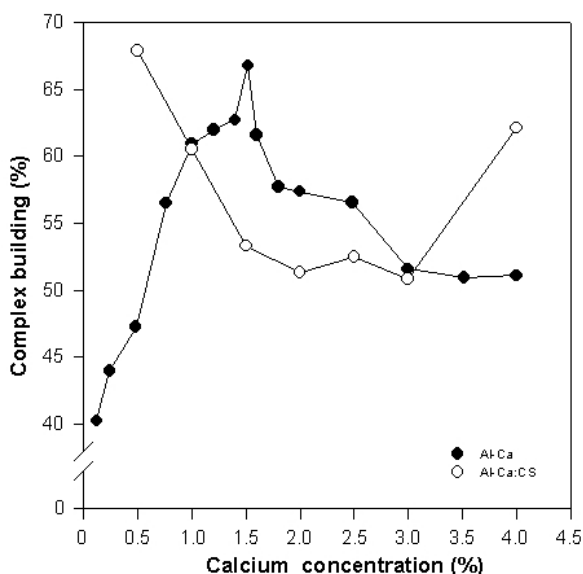


Figure 2. Effect of calcium concentration on the building of AL-Ca and AL-Ca:CS complexes.

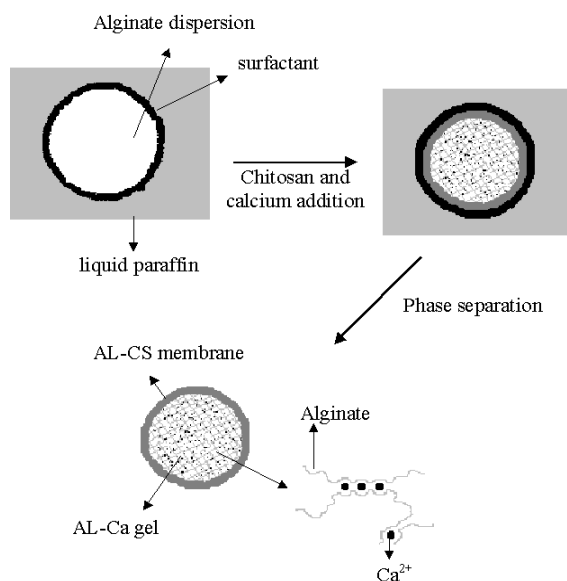


Figure 3. Preparation and probable structure of AL-Ca:CS microspheres by complex coacervation method using a W/O emulsion.

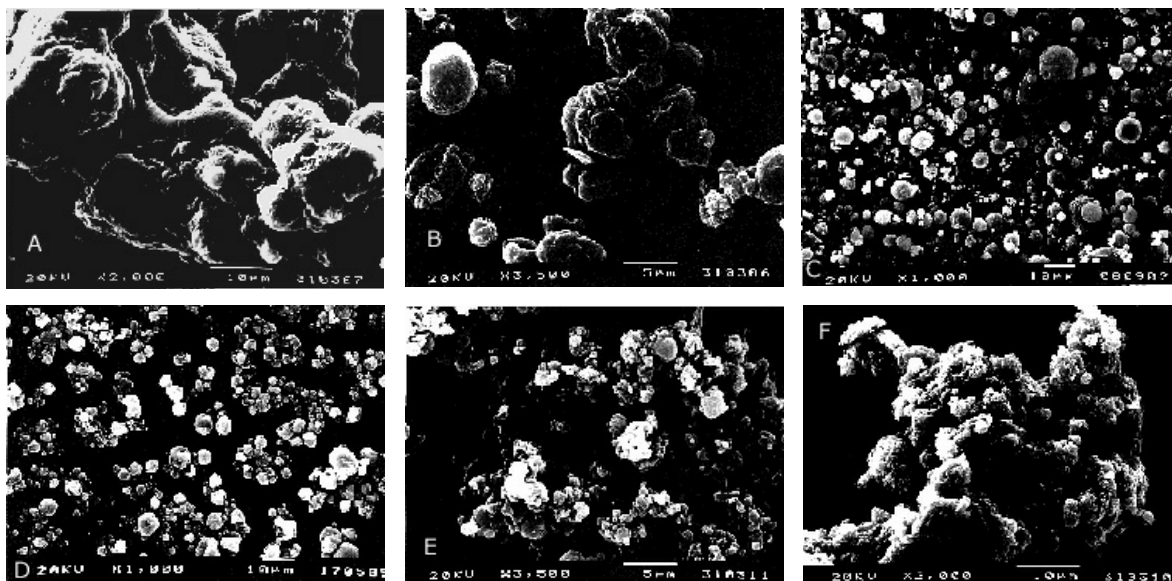


Figure 4. Photomicrographs of AL-CA:CS microparticles containing 0.25 (A), 0.5% (B), 1.0% (C), 1.5% (D), 2.0% (E) and 3.0% (F) calcium chloride.

to the increase of ionic strength in the reaction. When a chitosan dispersion containing calcium is added to alginate, the calcium ions initiate the gelification process, building a porous matrix that allows the diffusion and the bounding of chitosan molecules to the gel net ⁴.

Calcium is the cation more used for the reticulation of alginate due to its capacity to be linked to two adjacent monomers of guluronic acid in the internal parts of the polymeric chain. This ion possesses a greater affinity with alginate than other divalent ions such as magnesium ¹⁰.

The addition of calcium is necessary for the formation of the capsules, because the calcium alginate gel functions as structure for the capsule, since the complexation of the polyelectrolytes probably occurs only at the surface. During the development step it was observed that without the gelified nucleus the particles broken when separated from the emulsified system.

Preparing microparticles with different concentrations of calcium chloride and keeping the alginate/chitosan ratio constant allowed to observe the behaviour and morphology of the microparticles obtained.

In formulations containing only 0.25% of calcium, no microparticles formation was observed but only amorphous polymeric agglomerates. As Figure 4-A shows, the product obtained has undefined shape, probably related with an amorphous complexation of AL-CS due to the absence of a better structured AL-Ca gel. These re-

sults are in accordance with those of Lim & Wan ¹¹, when they evaluated alginate-calcium capsules containing propranolol.

Microparticles prepared with 0.5% calcium chloride showed amorphous particles and agglomerates, but with small size and better sphericity (Fig. 4-B) if compared with those prepared with 0.25% calcium chloride. This is probably related with the building of an AL-Ca gel.

Increasing of calcium ions concentration to 1.0 and 1.5% provided spherical particles, free of agglomeration and with slightly rough surface (Figs. 4-C and D).

AL-Ca:CS microparticles prepared with 2.0 and 3.0% calcium chloride appeared agglomerated, with poor sphericity and greater size. The higher the concentration of calcium chloride was, the bigger the particle deformation (Figs. 4-E and F). Therefore, by using concentrations higher or smaller than 1.0 to 1.5% it was not possible to obtain microparticles with good morphology.

The calcium concentration that allows the formation of microparticles with greater yield, better sphericity and absence of agglomerates, as indicated by morphological analyses, confirms the results obtained by the formation of AL-Ca and AL-Ca:CS complexes (1.5%). A decrease on the amount of calcium ions bound to alginate occurs in both higher or smaller concentrations of calcium.

The microparticles obtained showed spherical with a slightly rough surface and with small morphological and size variation, which denotes

the efficacy of the method in the preparation of AL-Ca:CS microparticles.

Particles show a narrow size distribution, the diameters going from less than 1 μm up to 9 μm , approximately 50% of them showing 1-2 μm . Such size uniformity is related with the use of an emulsion system for obtaining microparticles, as well as the use of sonication as the stirring source for the system, providing simultaneously stirring and size reduction of the emulsion's internal phase, which directly decreases the size of the final solid particles.

The use of emulsion for microparticles formation allowed the building of particles with narrow size distribution and diameters up to 9 μm , which is almost fifty times smaller than those of microparticles of the same type usually described in the literature ⁸.

CONCLUSIONS

The use of a W/O emulsion, as primary environment for microparticles formation, allowed the preparation of spherical particles, with slightly rough surface and with few agglomerations. By means of microscopic analyses it was observed that the size distribution was narrow, with approximately 50% of the particles showing diameters in the range of 1-2 μm .

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REFERENCES

1. Miyazaki, S., A. Nakayama, M. Oda, M. Takada & D. Attwood (1995) *Int. J. Pharm.* **118**: 257-63.
2. Rathke, T. D. & S. M. Hudson (1994) *J. Macromol. Sci. - Rev. Macromol. Chem. Phys.* **C34**: 375-437.
3. Peter, M. G. (1995) *J. Macromol. Sci. - Pure Appl. Chem.* **A32**: 629-40.
4. Gåserød, O., O. Smidsrød & G. Skjåk-Bræk (1998) *Biomaterials* **19**: 1815-25.
5. British Pharmacopoeia (1988) Her Majesty's Stationery, London, pág. A115.
6. Barber, T.A. (1993) "*Pharmaceutical particulate matter. Analysis and control*", Interpharma, Buffalo Grove, p. 266-303.
7. Lucinda-Silva, R.M., K.C.M. Monteiro & R.C. Evangelista (2001) VI Pharmatech International Conference on Pharmaceutics and Pharmaceutical Technology, Recife, Brazil, p. 56
8. Lee, K. Y., W.H. Park & W.S. Ha (1997) *J. Appl. Polymer Sci.* **63**: 425-32.
9. Takahashi, T., K. Takayama, Y. Machida & T. Nagai (1990) *Int. J. Pharm.* **61**: 35-41.
10. Skaugrud, O. (1995) in: "*Excipients and delivery systems for pharmaceutical formulations*" (D.R. Karsa & R. A. Stephenson, eds.). Royal Society of Chemistry, Cambridge.
11. Lim, L.Y. & L.S.C. Wan (1997) *Drug Develop. Ind. Pharm.* **23**: 973-980.
12. Daly, M. M. & D. Knorr (1988) *Biotechnol. Progr.* **4**: 76-81.7