CHITIN and CHITOSAN as sources of biocompatible polymers for microcapsules and membranes production

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ABSTRACT

The microencapsulation process of agents with biological activity, such as, DNA, pharmaceuticals, proteins, probiotics and enzymes could be defined, from the technological point of view, as the coating process of those agents, under a molecular form, as solid particles, or as liquid globules, with materials of different nature, that gives particles of micrometric size. One of the most used natural polymers for the production of microspheres is chitosan (β-1,4-glucosamine).

This polycationic biopolymer, is the chitin soluble form (deacetylated form), a linear polymer constituted by N-acetylglucosamine residues, obtained from the exoskeleton of crustaceans, for example from shrimps.

Various methods have been proposed for the production of microcapsules, microspheres and membranes, divided into three main groups: physical processes (as aspersion drying), chemical processes (as simple or complex coacervation), and physico-chemical processes (as interfacial polymerization).

In this work, different methodologies have been assayed for the production of chitosan microspheres and microcapsules. The morphology of the produced microcapsules was evaluated by optical microscopy, scanning electron microscopy. Stability and liberation studies of the encapsulated agent have been also performed.

Concerning membranes, it was evaluated the production of chitosan membranes and chitosan-collagen membranes. Both types were also studied by scanning electron microscopy (SEM).

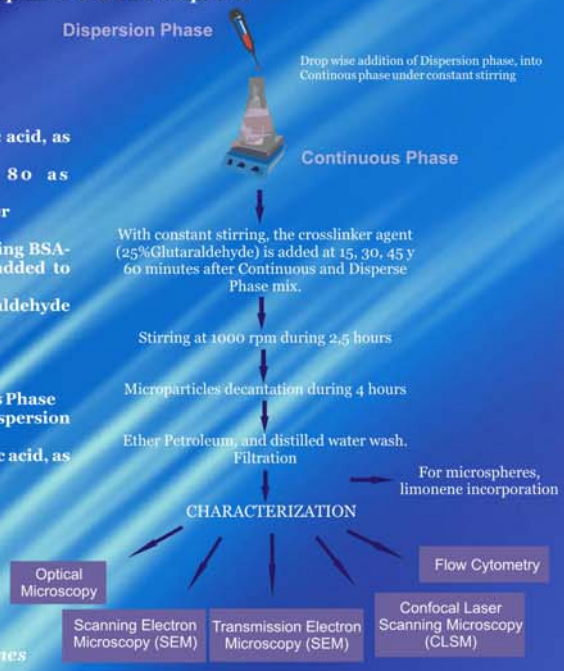
METHODOLOGIES

Preparation of chitosan microspheres and microcapsules

Chitosan Microspheres
- **water-in-oil (W/O) emulsion**
- **3% chitosan solution in 5% acetic acid, as Disperse Phase**
- **Sunflower oil with Span 80 as ContinuousPhase**
- **Glutaraldehyde 25% as Crosslinker**

For chitosan microspheres containing BSA-FITC, a 3% protein solution was added to Dispersion Phase.
As crosslinker, a 30% paraformaldehyde (PFA) solution was used.

Chitosan Microcapsules
- **oil-in-water (O/W) emulsion**
- **1% lecithin solution as Continuous Phase**
- **Pure limonene solution as Dispersion Phase**
- **2% chitosan solution in 10% acetic acid, as Polymer Phase**



Preparation of chitosan membranes

A chitosan solution was prepared, by dissolving purified chitosan 0.5% (w/v) in 0.5 M acetic acid, with moderate agitation at room temperature. The chistosan solution was vacuum filtered and degasified for 60 minutes. The degasified solution was placed in glass casts, properly leveled, and left dry at room temperature.

RESULTS AND DISCUSSION

The result of the procedures described, was the preparation of solid chitosan microspheres, with sizes between 2 and 30 μm. As the stirring speed increased, the size of the particles was reduced. The same pattern was observed when the chitosan microcapsules were analyzed under optical microscopy.

By SEM, it was observed a well defined spherical shape, with compact and homogenous surface. and could be possible to distinguish smooth waves at surface level. The internal structure was compact, and homogenous, as we expected.

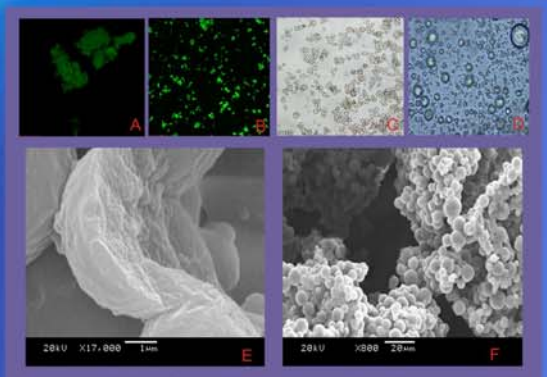


Figure 1: A) CLSM of chitosan-PFA control microspheres - Magnif. x20. B) CLSM of chitosan-PFA microspheres containing BSA-FITC - Magnif. x20 C) Optical Microscopy of chitosan microspheres - Magnif. x10 D) Optical Microscopy of chitosan microspheres- Magnif. x10 E) SEM micrography of chitosan microsphere. - Magnif. x17.000 F) SEM micrography of chitosan microspheres. - Magnif. x800

Furthermore, CLSM was employed to evaluate the BSA-FITC loading of chitosan microspheres. The spheres were efficiently loaded with the labeled protein. The Figure 1B shows increased fluorescence intensity in BSA-FITC loaded microspheres, compared to non-loaded microspheres (Figure 1A), product of the FITC emission.

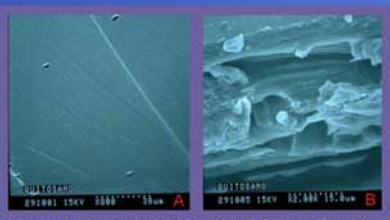


Figure 2: Images obtained by SEM of chitosan films. A) Chitosan film surface. B) Internal structure and layers of the chitosan film

Concerning chitosan-films physical appearance, they have a soft yellow color, with a smooth surface at macroscopic scale. By SEM, films resulted to have homogeneous surfaces at macroscopic and microscopic scales, with a very smooth surface, without roughness (Figure 2A).

In reference to films internal morphology, in Figure 2B can be seen that is composed of several layers or sheets superimposed.

CONCLUSIONS

As far as microcapsules and microspheres preparation is concerned, it could be concluded that these techniques are applicable for such purpose, resulting in interesting microparticles.

Also, it was possible to encapsulate a compound (limonene in this case), either with microspheres or microcapsules. Each form has its own properties and differs from the other especially on its internal structure, resulting in different ways to encapsulate active agents.

Moreover, chitosan spheres are more biocompatible for many purposes than particles made of synthetic polymers. Nevertheless, for the moment, chitosan microparticles seem to have lower mechanical resistance comparatively to their synthetic competitors.

Concerning chitosan films or membranes, these are interesting products that seem to be focus of futures researches. Their biocompatibility is one of the most interesting aspects that make them as possible substitutes of human or animal skin.