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CHITOSAN COMPOSITE FILMS: BIOLOGICAL AND PHYSICOCHEMICAL CHARACTERIZATION

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Because of its high molecular weight and its positive charge in acidic solutions, chitosan shows the ability to form films and gels this cationic property makes it to interact with the negative residues of the cellular wall and alter the permeability causing the cell to loss proteins and intracellular electrolytes; lisozyme hydrolyzes the β (1-4) glycosidic links of cellular walls of bacteria constituted by poly (β-N-acetyl-muramil (1–4)-N-acetyl-glucosamine) and chitin, although it could hydrolyzes chitosans partially acetylated [1].

The purpose of this study was to characterize the composite films and investigate the biodegradation of chitosan composite films by lisozyme and the antimicrobial properties of the films over some microorganisms. Chitosan composite films obtained by casting solutions with organic acid solutions and different additives, following a experimental design have been used for the assays [2].

The antimicrobial activity of the films were measured first by diffusion disc method or the Kirby – Bauer Method in order to find the leaching or some active antimicrobial additive, the second antimicrobial assay was made following the dynamic method described by ASTM E 2149-01 with small pieces of films immersed in bacterial culture with 10^5 – 10^6 CFU/mL.

The *in vitro* degradation of the films was measured at physiological conditions in a phosphate buffered solutions at pH 7.4, discs of chitosan composite films previously weighted were immersed during 28 days in the buffered solution containing lisozyme at body fluids concentration at 37℃.

The IR spectra of chitosan composite films (fig. 1) showed the presence of the characteristic band corresponding to the chitosan acetate or chitosan lactate showing the salt formation according to the results obtained by Bhattarai et al. (2006)[3].

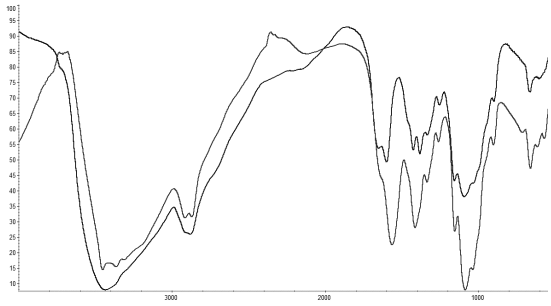

Fig. 1. IR spectra of chitosan powder compared to chitosan acetate composite film

First antimicrobial assays showed that films have not leaching of any additive on the agar plate and there was not important hale of inhibition, but dynamic method showed that almost all the microorganisms did not survive after 24 hours incubation of the films in the bacterial culture (table 1).

Table 1. Number of Microorganism after incubation (CFU/mL)

Sample	***S. aureus***	***S. epidermidis***	***P. aeruginosa***
Control	$1{,}6 \times 10^6$	$5{,}5 \times 10^5$	Uncount.
2	10	Uncount.	100
3	100	0	60
4	0	80	0
5	150	0	30
8	0	30	10

The biodegradation of the films carried out at physiological conditions showed that the films lost only around 10% of the standardized weight after 28 days.

It is possible to conclude that the chitosan composite films are in the form of salts of the organic acid used as solvent and the films have antimicrobial effect over a wide spectrum of bacteria under dynamic conditions. We observed that the films are slowly degraded by the lisozyme.

Finally the chitosan composite films show good biological properties that could be applied in biomedical materials.

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REFERENCES:

[1] Je J., Kim S., *Biochem. Biophys. Acta,* 2006, **1760,** 104–109.
[2] Cárdenas G., Anaya P., von Plessing C., Rojas C. and Sepúlveda J., J. Mater. Sci.: Mater. Med., 2008, **19,** 2397–2405
[3] Bhattarai N., Ramay H., Chou S.-H. and Zhang M., Int. J. Nanomedicine, 2006, **1(2),** 181–187.