P-016	**Synthesis and characterization of antibacterial chitosan microspheres for textile applications**	

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INTRODUCTION AND OBJECTIVES

Chitosan, [(1,3,4)-2-amino-2-deoxy-b-D-glucan] is a natural polysaccharide normally obtained by alkaline deacetylation of chitin, which is widely spread among marine and terrestrial invertebrates. Chitosan, known for its antibacterial properties and low toxicity toward mammalian cells, not only possesses a wide inhibition spectrum against Gram-positive and Gram-negative bacteria, but also against some yeasts and moulds. Its antimicrobial properties originate from its polycationic nature (Kong M. et al. 2010). However, the main problems associated with its application on textiles include the high add-ons required to ensure efficient functionality, which leads to inferior mechanical and comfort properties, as well as the relatively low durability of the resulting antibacterial properties to washing.

To address these limitations, the SAFEPROTEX project has contemplated the development of chitosan microspheres for the design of proper antimicrobial medical garments in emergency situations. Three different techniques have been used: simple coacervation, suspension crosslinking and spray drying. The crosslinking reaction has been studied extensively because of its positive relationship to the mechanical properties of the synthesised microspheres and its effect on the antimicrobial properties. Crosslinkers, and especially covalent ones, can react with free amine groups of chitosan and impair its antibacterial nature. To overcome this problem, different crosslinking methods, crosslinker agents and degrees of crosslinking have been studied.

In this paper, we present the results of the spray-drying method taking into account that it has been considered the best procedure for several reasons discuss below.

MATERIALS AND METHODS

Materials

For the preparation of chitosan microspheres, the reactants used were: chitosan low-molecular weight (Sigma Aldrich), pure acetic acid (Fluka Analytical), glutaric dialdehyde 25 % wt solution in water (Acros Organics), sodium tripolyphosphate (TPP, Acros organics).

For the microbiological analyses, the reactants used were: triptone soy broth (TSB) (Sigma Aldrich) as a growth media. *S.aureus* ATCC 6538 was used to evaluate the antibacterial activity *in vitro*.

Preparation of chitosan microspheres

Chitosan microspheres by spray-drying were obtained according to the experimental procedures describes by Desai (2005). Different crosslinkers and polymer/crosslinker ratios have been used. They have been prepared using a Mini Spray-dryer B-290 Buchi, a laboratory mixer Dispermat CV3® and a laboratory mixer IKA ® EUROSTAR 6000.

Characterization of chitosan microspheres

Optical microscopy

The morphology and size of chitosan microspheres were evaluated by an optical microscopy Zeiss Axioplan coupled to a digital camera DP300. The optical images were analysed by Dpx View Pro.

Thermogravimetric analysis (TGA)

Thermograms of crosslinked chitosan were obtained using the TGA Q500 (TA instrument). The samples were heated at a constant rate of 10 ºC/min, over a temperature range of 25 to 850 ºC. Inert atmosphere was maintained by purging nitrogen at the flow rate of 100 mL/min.

Antibacterial assay (in vitro assay)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of chitosan microspheres required to inhibit bacterial growth and it was determined by the contact turbidimetric method. Different types of chitosan microspheres were dispersed in distilled water and then added to TSB to have a final concentration of microspheres of 0,008%, 0.01%, 0.03%, 0.06%, 0.09%, 0.1% and 0.3% (w / v).These tubes were inoculated with *S. aureus*, at a starting bacterial concentration of 3-5x10^5 cfu /ml. Inoculated tubes containing test microspheres were incubated during 24 hours at 37ºC with constant agitation. After incubation, tubes were evaluated for their turbidity. The MIC is the first concentration at which no growth is visible by.

RESULTS AND DISCUSSION

Spray-drying is extensively applied in the pharmaceutical and food industry to produce raw drugs, excipients and microcapsules. This technique transforms liquid feed into dry powder in one step and is feasible for the scaling-up of the microencapsulation in a continuous particle processing operation which can we used for a wide variety of materials.

The advantages of spray-drying over the emulsion or simple coacervation methods to obtain microspheres are: the large amounts of chitosan microspheres produced and the particle size, they are smaller, as it can be noticed in Table 1. For that reason, an extensive research based on this method has been performed.

Table 1. Average diameter of chitosan microspheres obtained by the spray-drying technique

Crosslinking agent	mmol crosslinker/g chitosan	Average particle diameter (μm)
Glutaraldehyde (GA)	0.6	4.2
	1.3	5.0
Sodium Tripolyphosphate (TPP)	0.6	6.2
	1.3	1.4

By optical microscopy, it was observed that GA crosslinked-microspheres prepared by spray-drying were spherical with a regular shape whereas the TPP-microspheres had an irregular shape.

The antibacterial activity of chitosan microspheres obtained by spray-drying was measured according to the experimental procedure describe in the experimental part and the obtained results are shown in Table 2.

Table 2. Antibacterial activity of chitosan micro spheres obtained by the spray-drying technique

Crosslinking agent	mmol crosslinker/ g chitosan	Microorganism	MIC (ppm)
Glutaraldehyde (GA)	0.6	S. aureus ATCC 6538 (TSB)	300-600
	1.3		600
Sodium Tripolyphosphate (TPP)	0.6	S. aureus ATCC 6538 (TSB)	100
	1.3		600

For both crosslinkers, it can be observed that the lower the crosslinker concentration, the lower the minimum inhibitory concentration (MIC) is. These results agree with the expected behavior since more free amino groups are available when less crosslinker is used. Comparing MIC values for GA and TPP crosslinked microspheres it is possible to conclude that at a low crosslinker concentration, sodium tripolyphosphate produces a lower crosslinking degree and a better antibacterial activity than glutaraldehyde. It should also be pointed out that this improvement in the antibacterial properties leads to a more instable chitosan-TPP microspheres. When a high crosslinker concentration is used, no differences between the MIC values for TPP-chitosan and GA-chitosan microspheres are found.

Thermogravimetric analyses of these samples have been done in order to know the degradation temperature of the produced microspheres which it is important to select the textile application method. Usually, microspheres or microcapsules are applied onto textile surface by an exhausting method, impregnation by foulard or printing. During most of these processes a temperature curing step is needed and therefore it is important to establish at which temperature chitosan microspheres suffer from a degradation process. Thermogravimetric analysis of low molecular weight chitosan have been performed to compare with the crosslinked chitosan microspheres. The TG curve of LMW show a first weight loss around 50 ºC and a second one around 295 ºC, associated with the degradation of the polymer. When the crosslinked chitosan microspheres were analyzed some differences were found. GA-chitosan microspheres TG curves show three different weight losses at an average temperatures of 45 ºC, 142 ºC and 270 ºC while TPP-chitosan microspheres TG curves (Figure 4) show two weight losses around 42 ºC and 220 ºC. It can be seen that the crosslinking reaction decreases the polymer degradation temperature.

CONCLUSIONS

Chitosan microspheres have been obtained by spray-drying method and they have been characterized by optical microscopy, thermogravimetric analysis and antibacterial properties.

Two crosslinking agents have been used: TPP which causes an ionic interaction with the protonated amino groups of chitosan chains and glutaraldehyde which reacts covalently with the amino groups of chitosan. As the results show TPP-chitosan microspheres have a better antibacterial character than GA-chitosan, but the weakness of the TPP-chitosan interaction causes the swelling of the TPP-chitosan microspheres when they are used in aqueous solution and a lower degradation temperature of the polymer. Spray-drying is a simple and quick way to obtain the desired product with good antibacterial and physical properties. These microspheres show an average diameter particle of 6-1.5 μm and a minimum inhibitory concentration between 100-600 ppm, depending on the crosslinking degree.

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