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STUDY OF CHITOSAN MICROCAPSULES DEGRADATION WITH APPLICATION IN THE DEVELOPMENT OF NEW MEDICAL TEXTILES

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Abstract: *Chitosan is a polysaccharide derived from chitin; chitin is the second most abundant polysaccharide in the world, after cellulose. Several remarkable properties of chitosan offered unique opportunities to the development of biomedical and agriculture applications. In addition, chitosan allows obtaining microcapsules with diferrent functionalities according to the core material, but his characteristic allows achieving a control release of the active material. Chitosan microcapsules can be obtained by different encapsulation methods; in this case the extrusion-gelling method has been selected and two kinds of nozzles have been used. The simple nozzle produce a matrix, it can be described as a solid polymer system in wich the core material is distributed more or less uniformly throughout the polymer matrix. The concentric nozzle is a standard nozzle configuration to produce core-shell capsules.*
This study analyses the biodregradability of chitosan microcapsules obtained by co-extrusion/gelling using a single and concentric nozzle, with the aim of defining possible applications in the field of medical textiles.So the weight loss (%) has been calculated in diferent times, in order to compare the weight loss (%) in each type of microcapsules. The difference in degradation time is due to the quantity of chitosan in the microcapsules. The microcapsules obtained by simple coextrusion contain a greater quantity of polymer than those obtained by concentric extrusion

Key words: *Chitosan, biodegradability, microcapsules, co-extrusion and gelling.*

1. INTRODUCTION

Chitosan is a polysaccharide derived from chitin; chitin is the second most abundant polysaccharide in the world, after cellulose. The presence of amino groups in the chitosan structure might be protonated-providing solubility in diluted acidic aqueous solutions, several remarkable properties of chitosan offered unique opportunities to the development of biomedical applications. [1], [2]

In addition, chitosan is applied to crops with the aim of reducing or replacing more costly and environmentally damaging chemical bactericides. With reduced input costs and the potential for increased yields, farmers could gain substantial benefits from these applications of chitosan and its oligosaccharides to crops. [3]

Chitosan has a wide variety of applications in agricultural and biotechnological industries [4], [5], all of them with a direct relationship with the textile sector. So, chitosan allows obtaining microcapsules with diferrent functionalities according to the core material, but his characteristicas allows achieving a control release.

Encapsulation is defined as a process in which an active agent (core) is enveloped by a polymeric membrane (shell) to confer small capsules many useful properties [6].

We can say that encapsulation is a way to protect the substance to be encapsulated, determining its controlled release. Therefore, encapsulation confers added value to a commercial substance. It allows the generation of new application of products for properties that could not be applied so far. Encapsulation can be used to protect the active agents from oxidation caused by heat, light, moisture and contact with other substances and to prevent the evaporation of volatile compounds. An example of very sensitive compounds, to these factors are essential oils, substances responsible for taste, aroma and many functional properties [7],[8].

The obtention of chitosan capsules is realized by means of ionotropic gelation method. In acid solution, the chitosan $-NH_2$ is protonated to be $-NH^{3+}$. This molecule interacts with the Thymidine 5'-triphosphate sodium salt solution (TTP) by ionic interaction to result capsules.

The objective of this research is study the degradation of two kinds of microcapsules using chitosan as a wall material, in order to define new medical applications.The microcapsules were obtained by co-extrusion / gelling using a simple and a concentric nozzle.

2. EXPERIMENTAL

2.1. Materials

Medium molecular weight chitosan with a deacetylation degree of 75-85% (Sigma Aldrich, Spain) was used as shell material. The core material was an essential oil (Ensencias Lozano, Spain). Sodium triphosphate pentabasic (STP) (Sigma Aldrich, Spain) was used as a cross-linker material.

2.2. Microcapsules obtention

Capsules were obtained by BUCHI B-390 encapsulator at room temperature (Fig 1). Two kinds of nozzle were used to obtain different kind of microcapsules (Fig 2). Concentric nozzle configuration produces core-shell microcapsules and single nozzle configuration produces solid microcapsules.

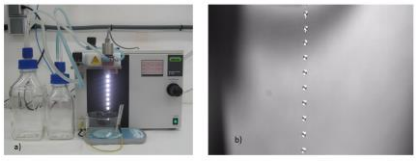

Fig. 1: *(a) Encapsulator BUCHI B-390. (b) Formation string of beads with the chitosan (shell) and core material*

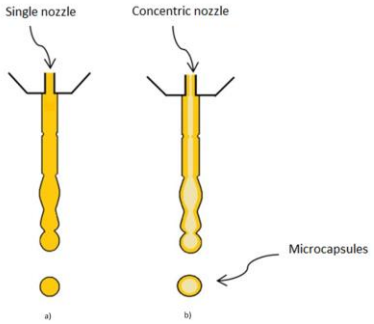

Fig. 2 *(a) single nozzle. (b) concentric nozzle*

Equipment variables are potential (V) and frequency (Hz). These variables were modified until it was perfectly observed the formation of the string of beads. The obtained capsules were filtered and washed three times with distilled water in order to remove any solution of crosslinking agent that could be remaining in the capsule wall. [9, 10]

The following table shows the optimum conditions under which the two types of microcapsules are obtained:

Table 1: *Encapsulation conditions*

	Microcapsules by single nozzle	**Microcapsules by concentric nozzle**
Chitosan Shell material (%)	1	1
Crsolinker agent (g/l)	8	8
Frecuency (Hz)	40	40
Potential (V)	250	250
External nozzle (mm)	0.9	0.9
Internal nozzle (mm)	-	0.45

2.3. Techniques of characterisation

2.3.1. Degradability Test

In order to obtain the samples for the degradability test, the microcapsules were filtered out from the suspensión and were then left exposed to ambient conditions for twenty minutes before being weighed.

The degradability test was performed on each of the two sample types:

- Sample A: microcapsule samples obtained by concentric coextrusion.
- Sample B: microcapsule samples obtained by simple coextrusion.

The degradability test was performed with different samples for each time interval, taken from an initial total of 20.0 g of microcapsules for sample A and 17.3 g microspheres for sample B.

This quantity was used to obtain independent samples for measurement times. Samples were tested in triplicate for each time interval. Thus, degradability tests were performed with an approximate quantity of 0.5 g of microspheres for sample A and 0.4 g of microspheres for sample B for each point of measurement.

The degradability test was performed by immersing the quoted microsphere samples in 10 ml of phosphate buffer solution (PBS, at pH = 7.4), at 37ºC. The degradability tests were performed in the absence of light.

Weight loss for each of the ten time intervals was calculated (t): at 1 day, 3 days, 7 days, 9 days, 11 days, 15 days, 18 days, 21 days, 30 days, 42 days. All tests were performed in triplicate.

The results are presented as averages of the three measurements taken at each point with associated standard deviation, according to the following formula:

$$\% \text{ weight loss} = ((\text{final weight} - \text{initial weight})/\text{initial weight}) * 100 \quad \textbf{(1)}$$

Where: final weight is the weight of the sample at each time interval t, and initial weight is the weight of each sample at the commencement of the experiment (t=0).

3. RESULTS

The following table (**Error! Reference source not found.**) shows the weight-loss results, expressed as a percentage, obtained for both types of chitosan microcapsules. The values obtained correspond to the average weight loss of each of the triplicated samples for each time interval, calculated with standard deviation. The degradability tests were performed under the following physiological conditions (PBS, pH=7.4, 37ºC, in the absence of light).

***Table* 2:** *Degradability of the two types of chitosan microspheres, expressed as a %of the weight loss*

		SAMPLE A	SAMPLE B
$t_{degradation}$ (days)	**$t_{degradation}$ (h)**	**Weight loss (%)**	**Weight loss (%)**
0	0	0	0
1	24	66.85±0.53	33.96±5.15
3	72	69.17±1.96	32.24±2.50
7	168	89.57±0.99	42.37±1.52
9	216	88.90±7.61	49.60±6.41
11	264	96.11±1.91	47.30±5.15
15	360	92.53±11.46	57.98±4.23
18	432	96.65±1.33	59.64±12.49
21	504	98.91±1.78	82.18±2.87
30	720	99.31±0.66	93.64±2.25
42	1008	100±0.00	90.73±5.16

As can be appreciated from Table 2, weight loss during the first 24 hours is considerable, particularly in sample A, and in both cases, the time that the microspheres spend in incubation in the buffer solution at body temperature leads to their progressive degradation: sample A breaks down completely, and sample B practically (90%).

The photographs which appear below were taken of the appearance of the chitosan microcapsules at each degradation time interval. The images show the turbidity present in the solution containing samples from the first stage: this turbidity is due to the breakdown of the chitosan itself coupled with the presence of micelles of oil deriving from the release of the functional oil from within the microcapsule. This study does not include trials to determine whether or not the micelles observed derive only from the functional oils released during degradation or if a percentage may derive from diffusion of the oil through the membrane.

The following images compare the appearance of the microcapsule suspension with the appearance once filtered at the different time intervals used for analysis.

Fig 3: *Appearance of the microcapsules at rest for SAMPLE A after 24 h at 37ºC.*


Fig 4: *Appearance of the microcapsules at rest for SAMPLE B after 24 h at 37ºC.*


Fig 5: *Appearance of the microcapsules at rest for SAMPLE A after 15 days at 37ºC.*


Fig 6: *Appearance of the microcapsules at rest for SAMPLE B after 15 days at 37ºC.*


Fig 7: *Appearance of the microcapsules at rest for SAMPLE A after 42 days at 37ºC.*


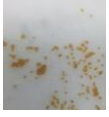

Fig 8: *Appearance of the microcapsules at rest for SAMPLE B after 42 days at 37ºC.*

As can be seen in the above images, the type B samples maintained in the buffer solution for 15 days at 37ºC gradually darken, which may be an additional indication of the degradation process acting upon the biopolymer. Plotting a graph for the values obtained in Table 1 clearly shows that type A samples present faster degradation than type B (Figure 9).

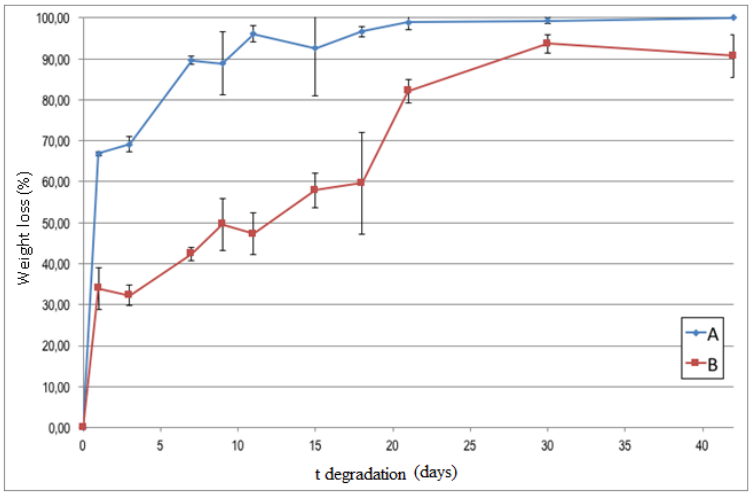

Fig 9: *A comparison of the degradation of the two samples analyased´*

5. CONCLUSIONS

The chitosan microcapsules analysed display a progressive rate of degradation under physiological conditions. The chitosan samples obtained by concentric extrusion (reference A) display a faster degradation rate at shorter time intervals and achieve almost total degradation at 21 days.

However, the samples obtained by simple extrusion are much more resilient to degradtion under the test conditions and display a linear rate of degradation to 30 days at which point degradation stabilises at around 90% weight loss.

This difference in degradation time is predictable as the quantity of chitosan differs: the microcapsules obtained by simple coextrusion contain a greater quantity of polymer than those obtained by concentric extrusion, as can be seen in figure 10:

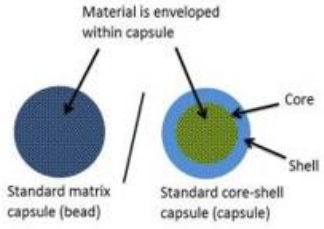

Fig 10: *a) Micropaculas por co-extrusión simple; b) microcápsulas por co-extrusión concéntrica.*

The release time for the core material can be defined based upon the results of the study, and the results can then be applied to the development of medical textiles which have been functionalised with microcapsules for use in the treatment of a range of conditions and pathologies.

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