STUDY OF THE AMOUNT OF CHITOSAN BOUND TO ALGINATE IN POLYELECTROLYTE COMPLEXES

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Abstract

Microcapsules of alginate chitosan can be easily prepared using two different methods. In this study the amount of chitosan bound to alginate was quantified by two different methods. The first method used was based on the determination of chitosan after filtration of the beads using a colorimetric assay described by Muzzarelli in 1998 [1]. The second method was an indirect method based on viscosimetry measurements of chitosan after filtration of the beads. Microcapsules prepared by a two-stage method bind 7% chitosan on the surface, while microcapsules prepared by a one-stage method bind chitosan in a higher extent; up to 11% was bound. This was due to the higher concentration of alginate in the core of the bead in microcapsules prepared by the two-stage method. Results obtained by both methods were in agreement. Some other properties as morphology, size and swelling were also studied.

Introduction

Chitosan is a naturally occurring polymer derived from chitin. The main source of chitin is crustaceans' shells [2].

Alginate- chitosan microcapsules have been widely studied along last years as colonic drug delivery systems [3]. Chitosan has mucoadhesive properties due to the interaction between the positively charged amino groups of the molecule with negatively charged groups of sialic acid present in the surface of the stomach [4, 5]. Alginate has also mucoadhesive properties [6]. Microcapsules of alginate-chitosan can be easily prepared using two different methods. One method is to obtain homogeneous microcapsules and consists in two stages. The other method leads to inhomogeneous microcapsules and has only one stage [7, 8].

A physicochemical characterisation of microcapsules is helpful to better understand the microcapsule behaviour when they are applied for the controlled release of drugs. A characterisation of alginate chitosan polyelectrolyte complexes includes the study of surface morphology, size distribution, swelling, and the amount of chitosan bound to alginate. This last characteristic is not commonly determined, for that reason this work is mainly focused in this study. Some authors have described this binding by a radioactive method in which chitosan fractions were labelled with a radioactive solution [7]. In 1998 Muzzarelli [1] described a colorimetric method to determine chitosan concentration in aqueous solutions. Chitosan in solution can adsorb dyes as Cibacron Brilliant Red 3B-A (Figure 1) that is an anionic compound. They can react ionically thus forming a coloured complex that can be determined at 575nm. The decrease of chitosan concentration after preparation of the beads can be measured by this method. On the other hand, the measurement of the cinematic viscosity is another way to determine the decrease of chitosan concentration, and thus to know the amount of chitosan that has been bound to alginate during the formation of alginate chitosan polylelectrolytes. In this work the quantification of chitosan bound to microcapsules prepared by a one-stage method and a two-stage method was discussed.

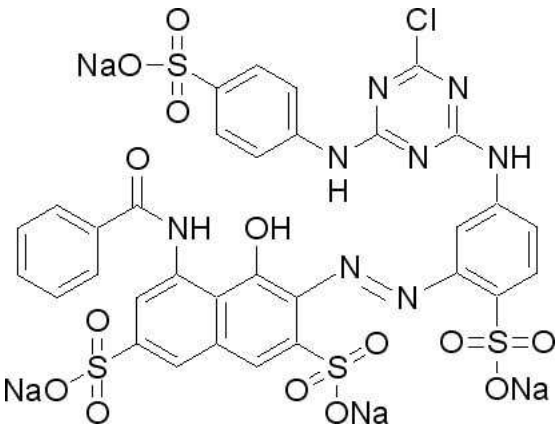

Figure 1 : Molecular structure of Cibacron Brilliant Red 3B-A.

Materials and methods

Materials

Chitosan, from *Palinurus vulgaris*, with deacetilation degree of 90.54% and average molecular weight of 644 kDa, was purchased from Primex (Norway). Sodium alginate, from *Macrocystis pyrifera*, medium molecular weight, and Cibacron Brilliant Red 3B-A (Figure 1), were purchased from Sigma Aldrich (USA). Calcium chloride, acetic acid and sodium acetate were analytical grade reagents and were purchased from Panreac Química S.A. (Spain).

Microcapsule preparation

Microcapsules were prepared by two methods. The first method (VI) was a two stage method in which a solution of sodium alginate (1.5-2% w/v) was dropped through a syringe on a calcium chloride solution (50mM)/sodium chloride (200mM). Resulting beads were gelled for 30 minutes. After that time they were filtered and transferred to a chitosan solution (0.15% w/v) in a buffer solution of acetic acid 0.3M and sodium acetate 0.2M. Beads were maintained in this solution for 30 minutes to 3 hours. After that time beads were filtered, collected and freeze dried. The second method (VII) leaded to inhomogeneous beads. An alginate solution (2% w/v) was dropped though a syringe into a chitosan solution of the same concentration as in the first method, with $CaCl_2$ 50mM. Beads were stirred in this solution between 30 minutes and 3 hours, and then they were filtered and freeze dried.

Morphology. Scanning electron microscopy (SEM)

Resulting microcapsules from both methods after freeze drying were observed by SEM. Samples were mounted directly onto the SEM sample holder using double-sided sticking tape and after that they were sputter coated with Au/Pd using a vacuum evaporator (Balzers SDC 004, Oerlikon, Germany) and observed with a JEOL JSM 3400 (JEOL, Tokyo, Japan) microscope at an accelerating voltage of 10kV for examining surface characteristics.

Size distribution

A hundred randomly chosen microcapsules were taken to measure their individual shape and size. The size of fresh microcapsules was measured with a digital slide calliper (Shaanxi Machinery & Equipment, Xian, China).

Swelling degree

A few number of freeze dried microcapsules were exactly weighed and suspended in a plate with 15 ml of medium (simulated gastric fluid pH 1.2 or simulated intestinal fluid pH 7.4 (USP XXIII)) in an orbital shaker at 100 r.p.m. at room temperature. At fixed times, microcapsules were taken out and the excess of medium was removed carefully with a filter paper. Microcapsules were weighed

and returned to the solution. The assay was stopped after a constant weight was obtained. The swelling ratio (*W*) was calculated form the following equation:

$$W = \frac{M - M_0}{M_0} \qquad (1)$$

where *M* was the weight of microcapsules at time t and M_0 was the initial weight of microcapsules.

Colorimetric assay

A stock solution of Cibacron Brilliant Red 3B-A was prepared in water at a concentration of 1.5mg/ml. A standard curve of chitosan was prepared pouring different volumes of a stock solution of chitosan 0.5% (w/v), glycine hydrochloride buffer 0.1M and dye solution in testing tubes. The absorbance was measured spectrophotometrically (GBC UV/Vis 920, GBC Scientific Equipment Pty. Ltd., Dandenong, Australia) at 575 nm.

Viscosimetric assay

A standard curve was prepared with a stock solution of chitosan 0.15% (w/w) in a buffer solution of acetic acid 0.3 M and sodium acetate 0.2 M. The solution was introduced in an Ubbelohde capilar viscosimeter (Typ. 525 20/ II, Schott AG, Mainz, Germany) and the cinematic viscosity of the solvent and the sample was measured.

After that chitosan solution before and after the preparation of the beads, was tested.

Results and discussion

Morphology. SEM

Microcapsules before freeze drying were spherical in shape, but after the elimination of water by this process they shrank becoming irregular in shape with many pores on the surface (Figure 2). Microcapsules prepared by method VII presented more pores than VI.

These pores increase the surface in contact with dissolution medium, thus promoting the solvent hydratation of the matrix by the solvent, and thus it swells.

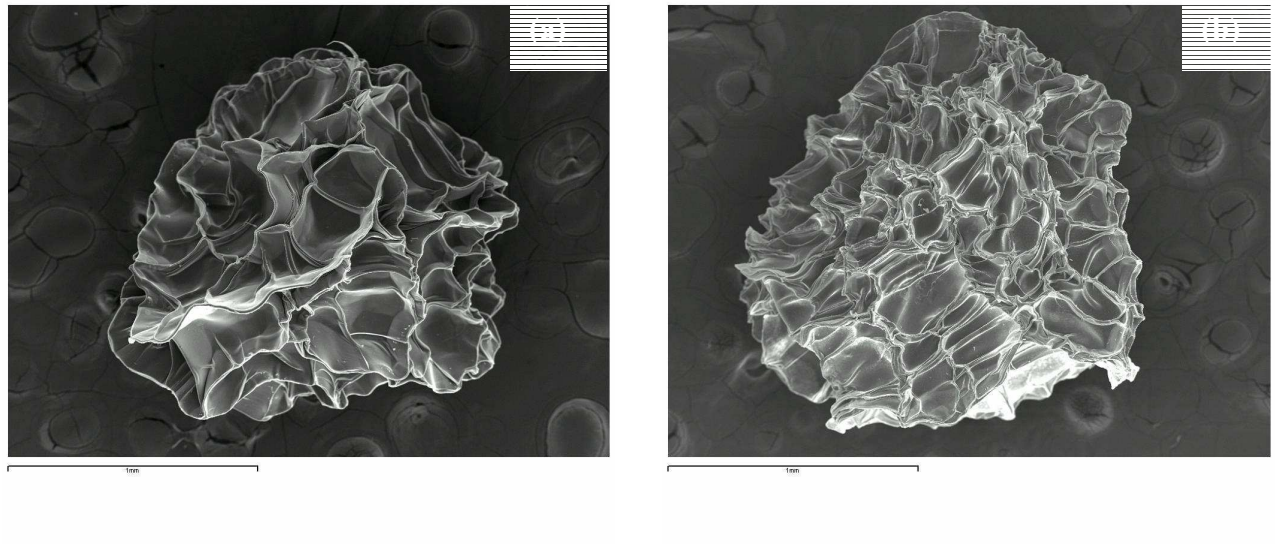

Figure 2 : Scanning electron micrographs of alginate chitosan microcapsules prepared by method VI (a) and VII (b).

Size distribution

The diameter of fresh microcapsules prepared by both methods was in the range of 1.7 to 2.3 mm (Figure 3). The size of the beads was homogeneous as well as the shape. Microcapsules were

slightly bigger when the alginate concentration was increased to 2% (w/v) (data not shown). It was observed during the assay that microcapsules of 1.5% (w/v) alginate were weaker than the ones prepared with 2% (w/v) alginate.

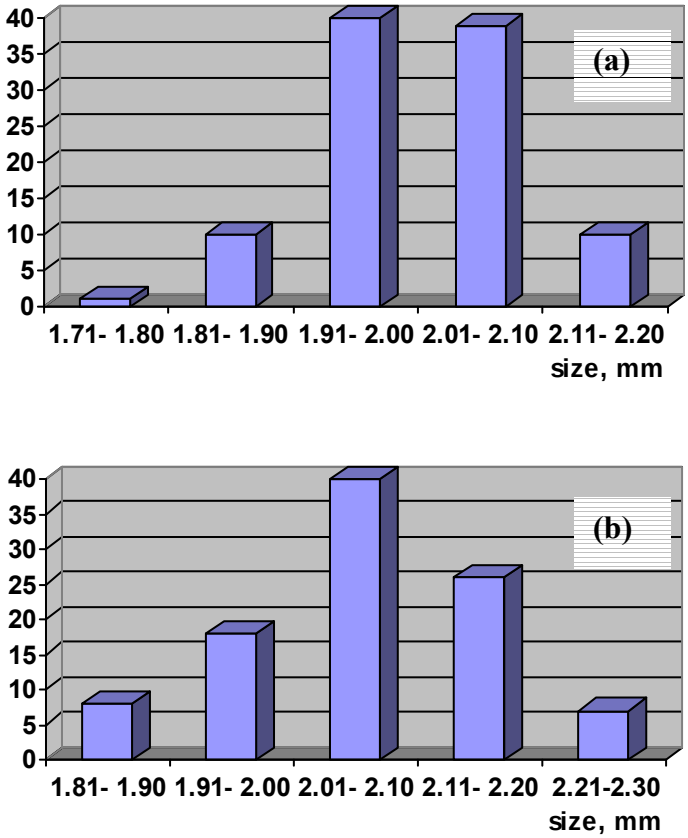

Figure 3 : Size distribution of alginate-chitosan microcapsules with alginate 2% (w/v) prepared by method VI (a) and VII (b).

Swelling degree of microcapsules

The study of swelling in polymers is interesting to know the diffusion properties of the gels. After the swelling experiments performed, it was observed that swelling in these microcapsules was pH dependent. This property is important to apply these systems for the controlled release of active molecules. Microcapsules swelled in a low extent in acidic media (only up to 3 times its initial weight) while in basic media they increased their weight up to 50 times its initial weight (Figure 4). In acidic medium there is at the beginning a slight swelling due to the neutralisation of alginate by the protonated amino groups of chitosan and the repulsion of the chains promotes the water uptake and the swelling. But when the contact with water is prolonged, mobility of the chains leads to the total reaction of both polyelectrolytes and the complex do not swell. At a basic pH swelling occurs due to the negative charges of the alginate. Alginate pK_a is 3.3-4.4, and at values of pH near 5 its carboxylic groups are completely ionized. For this reason there is a chain repulsion that promotes water uptake and swelling. Results were similar in microcapsules prepared by both methods (data of microcapsules prepared by method VII not shown).

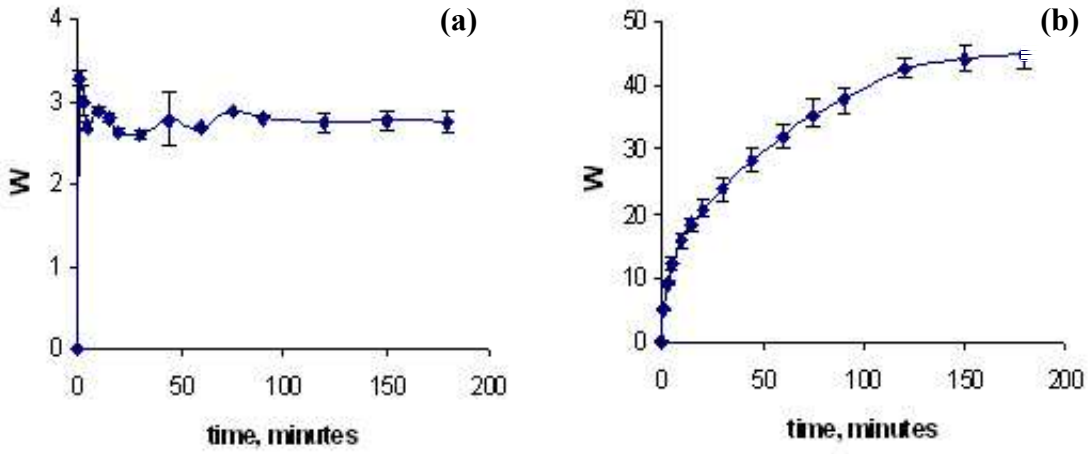

Figure 4 : Swelling degree of microcapsules prepared by method VI in SGF (a) and SIF (b).

Determination of chitosan bound to alginate

Results of the colorimetric assay show that microcapsules prepared with both concentrations of alginate bind a higher amount of chitosan in method VII. In Figure 5 the amount of chitosan bound to alginate in microcapsules prepared with alginate 1.5% (w/v) is represented. Alginate bound up to 12% chitosan in microcapsules prepared by method VII, while in those prepared by method VI they bound only up to 8%. In addition the binding increased with time. With three hours of reaction the greatest union was obtained. In the first 30 minutes the binding was also quite high, but with one hour it was found that the amount bound was lower. This can be explained because of a transient binding at the beginning of the reaction. After the first period of time, the reaction was stronger; it indicates that alginate could even bind more chitosan due to the presence of free L-guluronic units. In microcapsules prepared with a concentration of alginate of 2% (w/v) the behaviour was quite similar (Figure 6). Microcapsules prepared by the method VII had more chitosan than the ones prepared by the method VI. In this case the binding increased with time in all cases, due to the higher concentration of alginate that allowed a more irreversible union between both polyelectrolytes. The reason because alginate bound more chitosan when microcapsules were prepared by the one- stage method is the homogeneity of the beads [9]. The concentration of alginate in the outer layer of the bead in microcapsules prepared by the method VI is higher than in the method VII. As mentioned above, method VI leads to homogeneous beads, while method VII leads to inhomogeneous beads, that have a gradient of alginate concentration from the core, in which alginate is in liquid phase, to the outer layer where the concentration of alginate is higher. For that reason there are more L-guluronic units available to react with chitosan.

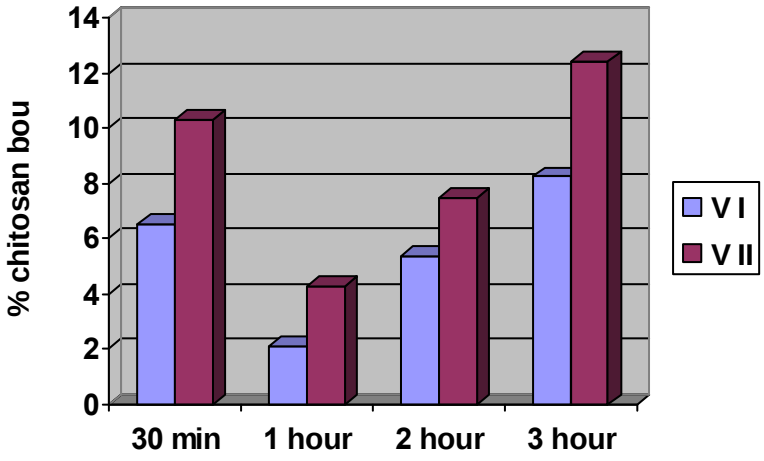

Figure 5 : Percentage of chitosan bound to microcapsules prepared with 1.5% alginate. Results presented are media of experiments made in triplicate.

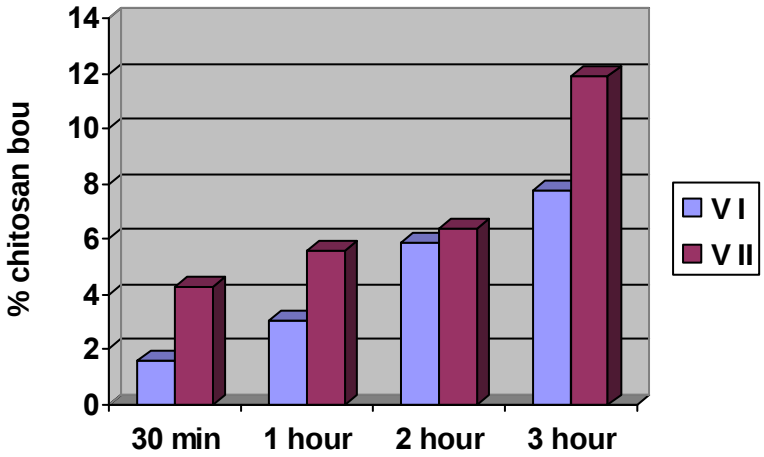

Figure 6 : Percentage of chitosan bound to microcapsules prepared with 2% alginate. Results presented are media of experiments made in triplicate.

The decrease of viscosity of the chitosan solution after preparing microcapsules is indicative of its binding to the alginate. A lower viscosity means that there are less chitosan chains, because they have previously bound to alginate forming a polyelectrolyte complex. Results of the viscosimetric assay are in agreement with the method described before, and show that microcapsules prepared by the one-stage method bind more chitosan than the ones prepared by the two-stage method (Figure 7).

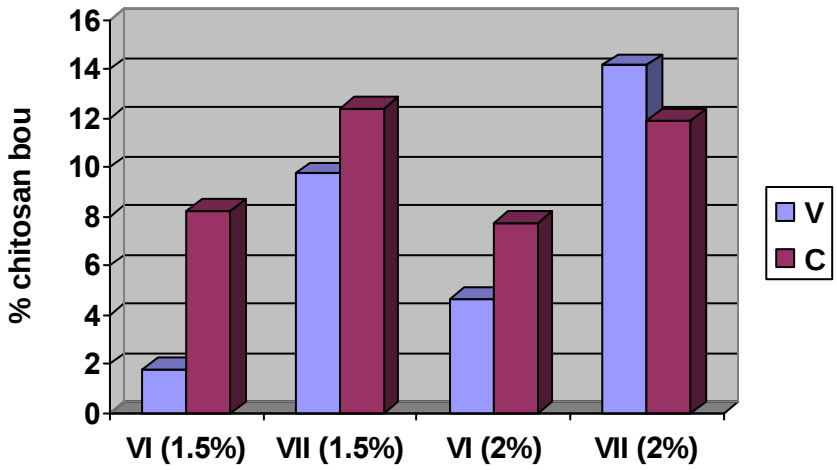

Figure 7 : Comparison between chitosan bound to microcapsules at 3 hours of curing time determined by the two methods: viscosimetric method (V), and colorimetric method (C), in the 4 kinds of microcapsules. Results presented are media of experiments made in triplicate.

Conclusions

Both methods are useful to determine the amount of chitosan bound to alginate in polyelectrolite complexes, but the colorimetric method is more reliable due to the sensibility of it. This quantification that usually is not taken into account when preparing alginate chitosan microcapsules is important because the higher the amount of chitosan bound to alginate, the stronger the microcapsule will be, and it could better control the release of active molecules included into these systems. So we recommend determining this property, in addition to other kinds of characterisation as presented here.

Acknowledgements

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