Structural analysis of chitosan cross linked membrane by microscopy

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Introduction

Chitosan cross-linked membrane has been developed as an innovative method for oil phase encapsulation in soft conditions. An oil phase containing a cross-linker is dropped in a chitosan solution. The migration of the cross-linker to the aqueous phase provokes a chitosan cross-linking membrane formation around the droplets.

In this article, we will focus on the characterization of the chitosan's crosslinked membrane by two types of microscopies. The scanning electron microscopy (SEM) allows the determination of the size, the capsules sphericity (Weerakody 2008), as well as to observe the surface of the membrane (porosity, fiber) and to measure the thickness of membrane. The confocal laser scanning microscopy (CLSM) gives more information about the membrane's structure. Moreover, this technique makes it possible to visualize the homogeneity thanks to the reconstruction in three dimensions of the images and the coated material or localized substances encapsulated (Lamprecht 2000) .

The objective of this study is to characterize the structure of crosslinked of chitosan's membrane by microscopy. Both the internal and external surface of the membrane were compared for wet and dry membranes. The thicknesses of the membranes are compared using the microscopy.

Materials and methods

Material

Low viscous chitosan (ref: 50494, 15% of acetylation) and acetic acid were purchased from Aldrich Sigma (France). The P-phenylene diisocyanate is used as cross-linker agent and fluorescent dye rhodamine provided by Fluka (France). Marlipal 25-7 (Sasol, France) was added as a non- ionic surfactant in the aqueous phase.

Solutions

Aqueous continuous phase: 20 g/L of chitosan is dissolved in acetic acid solution (0.2 M), adjusted at selected pH with concentrate NaOH, and added with 10g/L of marlipal.
Organic dispersed phase: between 6g/L (37.5 mM) and 20g/L (125mM) of P-phenylene diisocyanate solubilized in tournesol oil.

Capsules formation

Oil containing the cross linker is dropped into the chitosan solution. The chitosan solution was mixed gently during the formation of capsules. The membrane is formed at the interface of the drop. The capsules were filtered through a 40µm mesh nylon filter, washed twice with distilled water and kept in water.

Methods

Scannig electron microscopy

SEM was carried out using a JSM 5800 SEM at 15 kV. The samples were placed on a metal slab using the conductive glue. To obtain a conducting material, samples are covered with a fine layer of gold under vacuum conditions (probably leading to a partial dehydratation). Some of the capsules have been completely dehydrated by the CO_2 critical point method (Hayat 1978)

Confocal laser scanning microscopy

CLSM (LSM510 Zeiss) is assembled on an inverted microscope Zeiss AxioVert200M (Carl Zeiss) equipped with lasers Helium/Neon and Argon. The solution of chitosan is marked using fluorescent dye: rhodamine (adsorption/emission 488nm/520nm).

Results and discussions

Characterization of the membrane by the scanning electron microscopy

Figures 1A and 1B show an external and internal surface of the wet. Both surfaces appear completely smooth (except some folds due to sample preparation). Figures 2A and 2B show an external and internal surface of the dehydrated membrane. A filamentous structure is observed, similar to the native chitosan structure (Wawro 2006). Internal surface (figure 2B) is more homogeneous and organized that external surface (figure 2A).

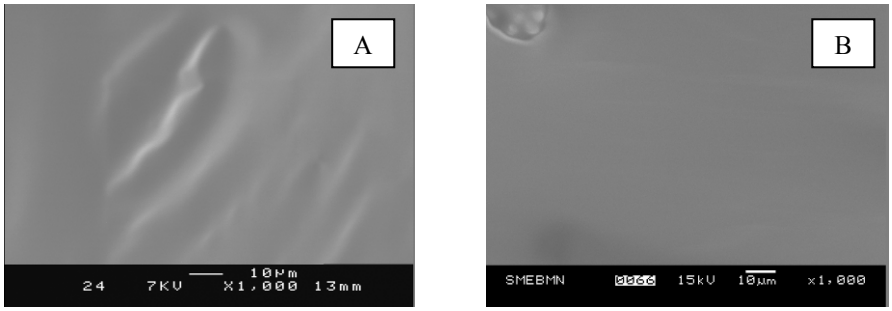

Figure 1: SEM surfaces membranes external wet (A) and internal wet (B)

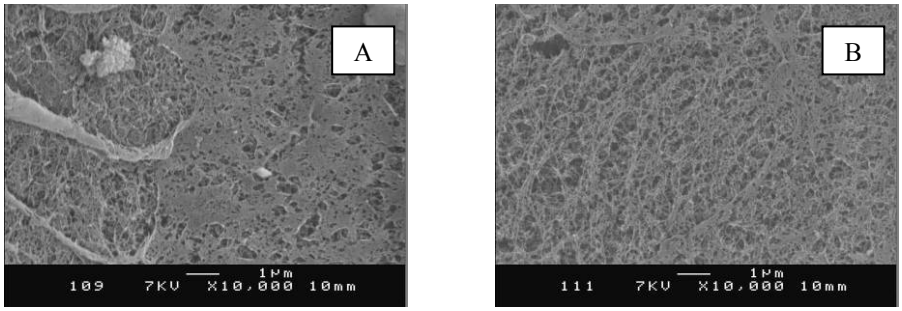

Figure 2: SEM surfaces membranes external dehydrated (C) and internal dehydrated (D).

Figure 3 shows that the membrane thickness is very regular over one capsule. The thicknesses were measured as the average of 5 capsules of the same batch to compensate the irreproducibility of the sample preparation. The membrane thickness increases with the reaction time to reach an

asymptotic value (Fig 4). For the partially dehydrated membrane (sample preparation) the maximum thickness is less than 10 µm.

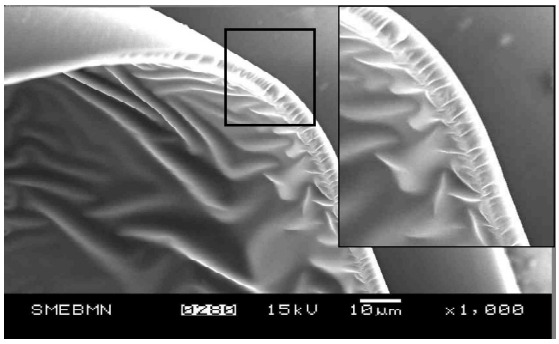

Figure 3: SEM section of the membrane

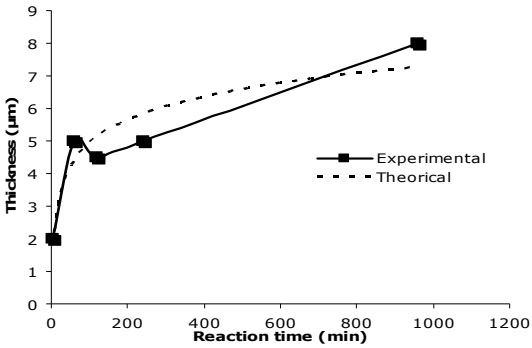

Figure 4: Membrane thickness

Characterization of the membrane by the confocal laser scanning microscopy

Membrane has made fluorescent by adding a dye (rhodamine) in the chitosan solution. The dye is incorporated in the membrane during its formation. CLSM allows to observe object in less intrusive manner, allowing to get a more realistic structure observation. CLSM from a series of image allows also a 3D reconstruction of the object.

Figure 5A and B shows respectively the external and internal surfaces of the membrane. Both of them show a smooth structure, confirming the SEM observations. Membrane thickness has also been deducted from 3D image (figure 6). The thickness is homogeneous for one capsules and increases asymptotically according to the reaction time (Figure 7). No dehydration has been applied for CLSM sample preparation. As a result, the value of the membrane thickness obtained by CLSM (Fig 7) is larger than for SEM observations (Fig 4). However, tendencies are the same in function of the reaction time.

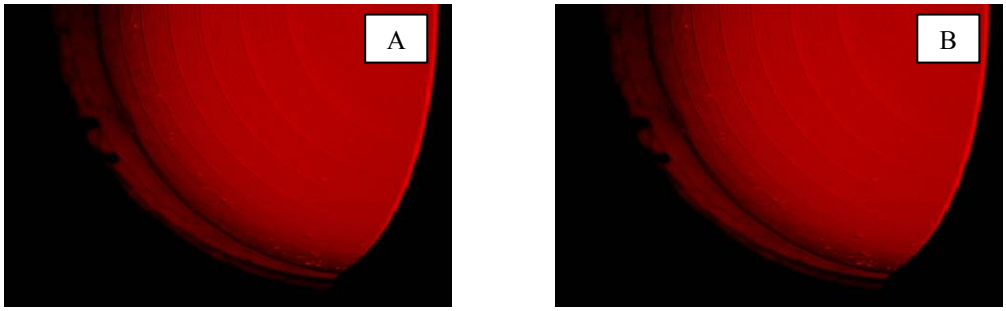

Figure 5: CLSM observation of external (A) and internal (B) membrane surface

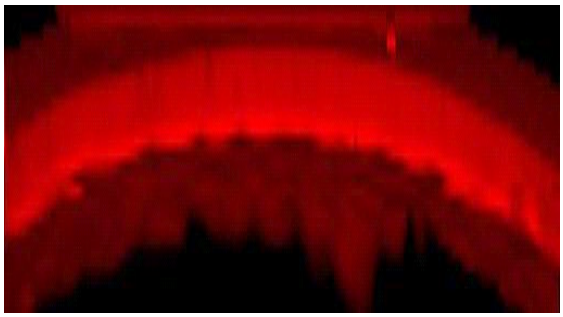

Figure 6: CLSM image of membrane section

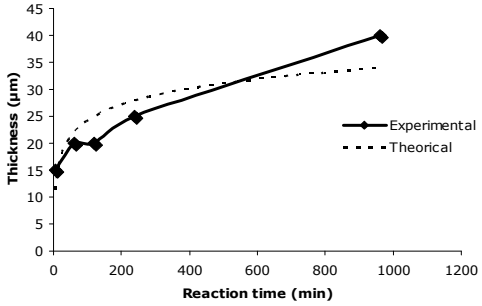

Figure 7: Membrane thickness

Membrane formation

Chitosan is insoluble in oil phase (charged polymer) and the membrane constitutes a barrier for the chitosan diffusion. However, the low molecular cross-linkers have an (even low) solubility in water and could diffuse through the membrane. In the present process, membrane is then formed from the oil/water interface and extends to the aqueous phase.

The internal phase is build along the oil surface and is expected to be smooth (Fig 1A and 2A). One could expect a high degree of cross-linking and homogenous structure of the membrane near to the oil surface. On other hand, as the membrane growths, the quantity of cross-linker reaching the external side decreases due to the decrease of its concentration in the oil phase and but also the slow down of the diffusion. The structure of the external membrane may be then less homogeneous (Fig 2B) and weaker as the degree of cross-linking is low and the membrane is stabilized by mainly other interactions.

As the diffusion of the cross-linker is homogenous in all direction, the membrane thickness is itself homogenous. Chitosan cross-linked membrane seems to have a dense and homogeneous membrane in opposition to what has been observed for interfacial polymerisation membrane (Janssen 1992). In order to verify the permeability properties of the membrane, the diffusion experiments of the δ-tocopherol with the molecular weight of 500 g/mol have been tested. The preliminary results indicate that the tracer does not diffuse through the membrane for the capsules formed with reaction times higher than 30 minutes.

The membrane rate formation decrease over time as the quantity of cross-linker reaching the surface decrease due to internal concentration decrease and membrane thickness increases (Groboillot 1993) This is in agreement with our observations (Fig 4 and 7).

Conclusion

The results obtained by SEM and CLSM show a homogeneous and smooth structure of the membrane. The membrane thickness is regular and increases with a reaction time to reach asymptotic value. The preliminary results show that this membrane has interesting release properties because the δ-tocopherol encapsulated does not diffuse through the membrane.

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