Structural analysis of chitosan cross linked membrane by microscopies

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Introduction

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

➢ The aim of this work is to characterize the structure of cross-linked of chitosan's membrane by two types of microscopies: the Scanning Electron Microscopy (SEM) and the Confocal Laser Scanning Microscopy (CLSM). Both the internal and external surface of the membrane were compared for wet and dry membranes. The thickness of the membrane was evaluated by using the microscopies.

Materials and methods

➢ *Materials*

Low viscous chitosan (15% of acetylation) and acetic acid were purchased from Aldrich Sigma (France). The P-phenylene diisocyanate was used as cross-linker agent and fluorescent dye rhodamine provided by Fluka (France).

➢ *Capsules formation*

Oil phase, containing the cross linker, is dropped into the chitosan solution. The chitosan solution was agitated gently during the formation of capsules. The capsules were filtered by a 40µm mesh nylon filter, washed twice with distilled water and kept in water

➢ *Analytical methods*

The measurement of SEM was carried out using a JSM 5800 SEM at 15 kV. The measurement of CLSM (LSM510 Zeiss) was assembled on an inverted microscope Zeiss AxioVert200M (Carl Zeiss) equipped with lasers Helium/Neon and Argon.

Results

Scanning Electroscopic Microscopy observation

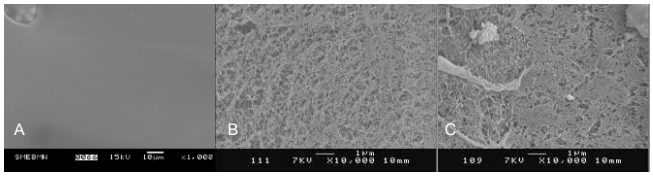
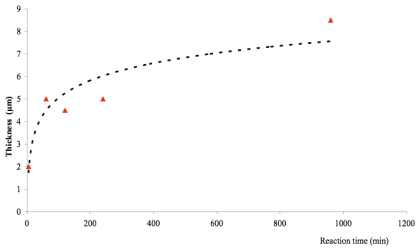
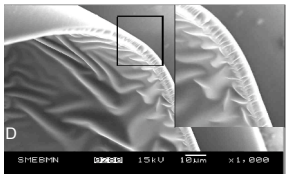


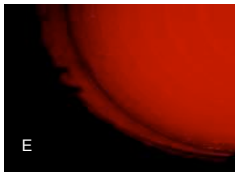
Image A show an internal surface of the wet capsule. The surface appears completely smooth.
Images B and C show an internal and external surface of the dehydrated membrane. Both surface have a filamentous and dense structure. The internal surface is more homogeneous and structured than the external surface.

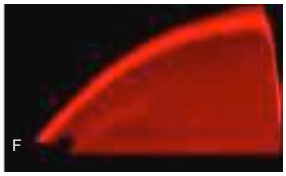




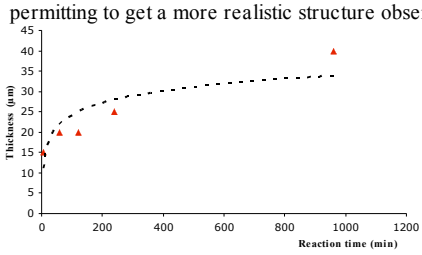
The curve above presents the membrane thickness versus the reaction time and the image D shows the section of the membrane. The membrane thickness of the capsule is very regular. The thickness increases with the reaction time and then reaches a maximum value. For the partially dehydrated membrane the maximum thickness is less than 10 µm.

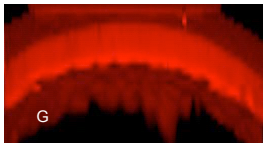
Confocal Laser Scanning Microscopy observation





Images A and B show respectively the external and internal surfaces of the membrane. Both of them show a smooth structure, confirmed by the SEM observations.
Membrane has been made by adding a fluorescent dye (rhodamine) in the chitosan solution. The dye was incorporated in the membrane during its formation. CLSM allows to observe the objects in less intrusive manner, permitting to get a more realistic structure observation.





Membrane thickness has also been deducted from 3D image (figure G). The thickness of the capsule is homogeneous and increases with the reaction time. As a result, the value of the membrane thickness obtained by CLSM is bigger than that of the SEM observations. However, the tendency of the increasing thickness remains the same.

Discussions

Membrane formation

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

The internal phase is formed along the oil surface and is expected to be smooth (images A and B). It may expected that there is a high degree of cross-linking and homogenous structure of the membrane near the oil surface. Moreover, when 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 also due to the slow down of the diffusion. The structure of the external membrane may be then less homogeneous (image C) because the degree of cross-linking is low.

Conclusions & perspectives

➢The results obtained by SEM and CLSM show a homogeneous, dense and smooth surface of the membrane. The membrane thickness is regular and increases with the reaction time until it reaches a maximal value.

➢The preliminary results show that this membrane has interesting release properties since the δ-tocopherol encapsulated does not diffuse through the membrane.