

Study of the formation of millicapsules by interfacial reticulation

G. Ongmayeb and D. Poncelet

GEPEA, ENITIAA, rue de la Géraudière BP 82225 - 44322 NANTES Cedex 03 France



Introduction

Interfacial polymerisation encapsulation consists to contact droplets containing one monomer with immiscible continuous phase containing a second monomer. Membrane is form at the interface by reaction between the two monomers. This method allows forming microcapsules with fine and strong membrane. However, generally, the production conditions are very strong (pH 13, high concentrations, strong cross-linker, polar solvent).

Several groups (Poncelet 1994 ; Andry 1996) succeed to make capsules in gentler conditions (pH less than 9) by switching to interfacial reticulation, replacing one monomer by a polymer (generally proteins or polysaccharides). However, high initial pH, use of strong cross-linker (dichloride acid) and fast acidification were still limiting use for encapsulation of fragile active.

We then test the production of microcapsules using chitosan (a poly-glucosamine, see fig 1) in combination with an aromatic diisocyanate. The chitosan is biocompatible and biodegradable polysaccharide what is a major asset in the biomedical and environmental application. To get quasi-monodispersed capsules, a dripping method was use to the usual emulsification techniques to contact droplets with continuous phase, allowing more homogenous and reproducible results.

Materials and methods

Material

The chitosan (low viscous) provided by Aldrich Sigma with 15% of acetylation (figure 1). Acetic acid (1M). Diisocyanate aromatic (Aldrich Sigma) is used as cross-linking agent. A non-ionic surfactant was provided by Procter and Gamble is use to facilitate penetration of droplet in the continuous phase and the sodium hydroxide (Panreac) is used to increase the pH.

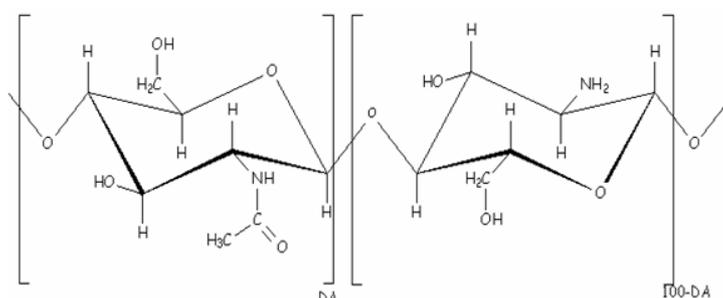


Figure 1: Chitosan

Solutions

Aqueous continuous phase: 20 g/L of chitosan is dissolved in 1% of acetic acid with 10g/L of non ionic surfactant and the pH is adjusted between 4.5 and 6.2 with NaOH (9N).

Organic dispersed phase: between 6g/L (37.5 mM) and 20g/L (125mM) of diisocyanate aromatic solubilized in vegetable oil.

Formation of the millicapsules:

20 ml of organic solution is dropped with help of a needle ($\varnothing = 0,2\text{mm}$) at a flow rate of ml/min in the continuous chitosan solution contained in a beaker (150 ml) agitated by a marine impeller (150 rpm). Reaction is let evolved for 1 to 45 min depending the operating conditions. Capsules are recovered by filtration of $40\ \mu\text{m}$ mesh, rinsed with distilled water, storage in water or dry on the bench. The figure 2 shows the nice beads take by optic microscopy and SEM.

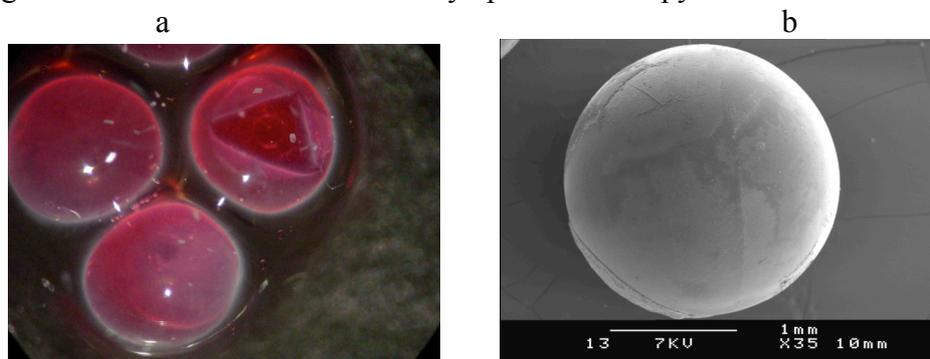


Figure 2: Photos of the beads by optic microscopy (a) and by SEM (b)

Evaluation of the mechanical properties of the beads

The dynamic mechanical analyzer (DMA) is an apparatus, which permits to characterize the properties of capsules (resistance to the deformation, viscoelasticity). One capsule is placed between two plates. The force exerted by the piston on the capsule is recorded in function of the deformation of the capsules with a constant frequency at 1Hz.



Figure 2: Compression of bead (\varnothing : 2mm) between two plates

Characterization of structure membrane

100 mg of pastilles KBr and 2 mg of sample to analyse are mixed and then puts it a disc. Absorbance spectra (Vector 22) were recorded for a wavelength range of $400\text{ to }4000\ \text{cm}^{-1}$ at resolution of $8\ \text{cm}^{-1}$ and 256 scans. Spectra of the pure diisocyanate, pure chitosan and microcapsule membrane were compared.

Results and discussions

a) Preliminary experiments

By dropping the diisocyanate solution in chitosan solution, 2 mm diameter beads were formed with 20g/L of chitosan with diisocyanate concentrations between 6g/L and 20g/L. pH Seems one of the essential parameters in defining type of membrane.

b) Influence pH on the formation of millicapsules by dripping

pH of the chitosan solution was varied between 4.2 and 6.2. The aspect of membrane is observed by the optical microscopy (table 1). Thick membranes were obtained for pH lower than 5.5, whereas for pH higher than 6, fine membranes were obtained.

pH of the chitosan solution	Time of formation of the capsules (min)	Membrane thickness (μm)	Membrane aspect
4,2	45	200	Thick
5	35	200	Thick
5,5	4-5	20	Fine
6,2	2-3	20	Fine

Table 1: Influence of the pH on the on the formation of membrane beads

Chitosan is polymer supporting many amino groups. It is soluble in low pH (acid) and positively charged. When pH approach its pKa (6.5) the charge diminishes and polymer becomes insoluble, then incompatible with the actual process. At low pH, chitosan is largely dispersed in water and most amino groups are protonated (a low reactive form). The diisocyanate has to cross the interface and diffuse in the water phase to react. Formation of the membrane is slow. Membrane is formed by coacervation leading to thick and fragile structure.

When pH approach the pKa, chitosan has tendency to accumulate at the interface of the droplet and the majority of amino groups are non-protonated it why reaction is fast. We may then observe a real interfacial reaction leading to fine and rigid membrane.

Evaluation of the mechanical properties of the capsules

Figure 3 shows the resistance of a capsule to the pressure of the piston. Most curves show a good elasticity of the capsules (relatively linear curve) that confirmed by similar experiments at variable frequency (data not shown). For a same deformation, the higher the concentration of diisocyanate is the more the capsules break and the more the force measured is high. It was assumed that increasing cross-linking lead to a more rigid membrane. A higher slope in Figure 3 confirms this higher rigidity. Chitosan beads seems more rigid than the alginate beads (David 2006).

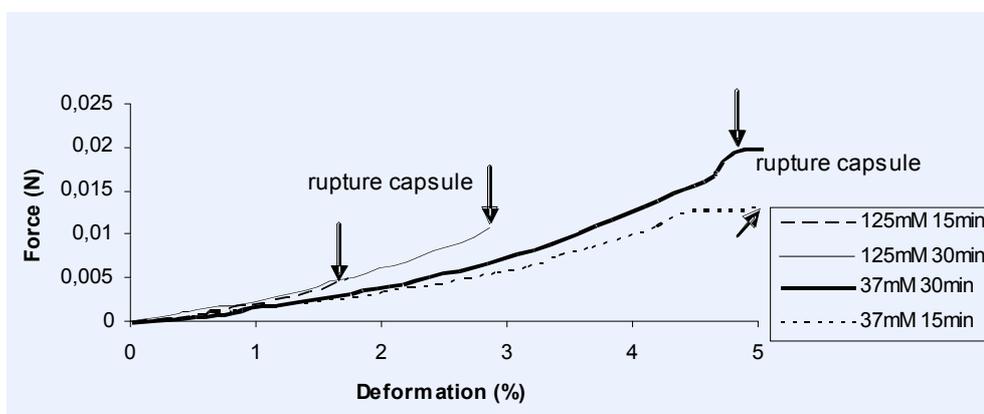


Figure 3: Effect of the diisocyanate concentration on the rigidity of beads

Structure of the membrane by Red Infra spectrophotometry

The wavenumber of the characteristic groups of chitosan (NH and C=O) and group diisocyanate (N=C=O) are resumed in the table 2. On the spectra of the membrane, the isocyanate peak disappeared whereas the intensity of the absorbance peaks of NH and CO increased. The increase results of the formation of urea functions NH-CO-NH (reaction between NH₂ chitosan and isocyanate) and urethan functions O-C-ONH (reaction between OH chitosan and isocyanate). It is why the intensity of groups (NH and CO) increases.

	Functions	Wavenumber (cm ⁻¹)	Intensity of the peak
chitosan	NH	1550	++
	C=O	1652	++
diisocyanate	N=C=O	2266	+++
Membrane	NH	1550	++++
	C=O	1652	++++
	N=C=O	2266	No peak

Table 2: Wavenumber and intensity of the peak with functional groups

Conclusions and perspectives

Microcapsules of chitosan was produced by interfacial reticulation is gentle conditions allowing encapsulation of fragile material. Membrane properties could be modulated by controlling the concentrations, the pH and reaction time. In the future work, the permeability of the membrane would be study because the aspect of the membrane by SEM shows a smooth membrane (figure 5).

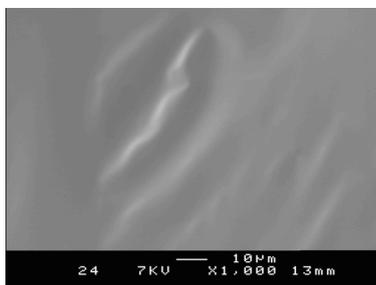


Figure 5: External face of the membrane by SEM

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