STUDY OF CHITOSAN ENZYMATIC GELATION KINETICS FOR MEMBRANE PREPARATION

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Key Words: Enzymatic gelation; advanced gelation time; reaction kinetics

Chitosan is a natural biopolymer with excellent sorption properties that can be used to prepare flat-sheet "greener" filtration/sorption membranes without using organic solvent. The first step in chitosan membrane preparation, before drying and cross-linking steps, lies in the formation of a chitosan gel. Generally, chitosan is initially dissolved in acidic water then gelation is induced by basic media intake (usually ammonia) from liquid or vapor phase (Equation 1). However, the gelation process is initiated on one face of the polymer solution, which induces a non-solvent concentration gradient leading to a heterogeneous structure of the gel. An original enzymatic gelation process has been recently proposed (Chenite et al. 2006, Yan et al. 2014), based on the in-situ production of ammonia in the chitosan solution by the enzymatic hydrolysis of urea by urease (Equation 2).

$Chit - NH_3^+ + NH_3 = Chit - NH_2 + NH_4^+$ (Equation 1)
$(NH_2)_2CO + H_2O \xrightarrow{urease} 2NH_3 + CO_2$ (Equation 2)

The aim of this study is to determine the key operating parameters required to control the enzymatic gelation reaction kinetics and subsequently the gel final morphology. Urease from *Canavalia ensiformis* (Jack bean) and chitosan (degree of deacetylation = 80%, mean MW = 180 000 g/mol) were used in this study. Four different and complementary methods were assessed to determine enzymatic gelation kinetics:

- pH monitoring using Hanna Instruments pH-meter (Hi 2214 pH/ORB)
- pH colorimetric monitoring using Bromothymol Blue
- Qualitative flowing tests.
- Rheological measurements (Anon Paar Rheometer).

Influences of various operating conditions on chitosan enzymatic gelation kinetics were investigated: temperature (from 5 to 37°C), urea concentration (from 25 to 200 mM), urease activity (from 0.5 to 10.5 u/mL) and chitosan concentration (from 2.5 to 3 %w/v). Comparison with enzymatic reaction kinetics without chitosan was performed to approach mechanisms occurring during the chitosan gelation

In water media without chitosan, pH variations can be directly related to the ammonia production by urea hydrolysis and thus the enzymatic reaction kinetics (Reaction 2) while in chitosan media, pH variations are now related to the ammonia presence in solution and thus to both the enzymatic reaction kinetics and chitosan gelation kinetics, i.e. to the chitosan enzymatic gelation kinetics. Experiments have allowed defining the gelation time and its related gelation pH of around 6.5 when the gelation point is reached (determined by the rheological measurements) and the so-called advanced gelation time and its related advanced gelation pH of 7.35 when a gel suitable for future membrane preparation is obtained (determined thanks to the flowing test). Colorimetric monitoring has shown that the gelation occurred homogeneously in the chitosan media, which has been confirmed by MEB observations of chitosan gels.
Results have shown that the enzymatic kinetics seem no to be modified by the presence of chitosan and an ANOVA demonstrated that the more significant influent parameters on chitosan gelation kinetics and advanced gelation time are the urease concentration and the temperature. On the contrary, urea concentration has nearly no influence on the chitosan enzymatic gelation kinetics but must be higher than 75 mM to prevent depletion of urea before a complete gelation of the chitosan (for a 2.5% w/v chitosan concentration).
Thanks to this multi-parametric study, the key parameters in the control of chitosan gel preparation were optimized for future membrane preparation.

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