

Chitosan thermogels: The synergistic effect of mixing gelation agents

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Statement of Purpose: Chitosan thermosensitive hydrogels have been proposed for multiple biomedical applications during the last decades. However, the simultaneous optimization of gelation time, porosity, mechanical resistance and biocompatibility of hydrogels is still a challenge. In the present work, we show that the combination of weak bases used as gelation agents may provide a solution to this challenge and allows to obtain injectable hydrogels with high mechanical resistances (storage modulus, G') despite using relatively low concentrations of salts.

Methods: Chitosan hydrogels (2% w/v) were prepared at room temperature by mixing a chitosan acidic solution with a solution containing one of the gelation agent(s), namely β -glycerol phosphate (BGP), sodium hydrogen carbonate (SHC), phosphate buffer (PB) or their combination (SHC:PB or SHC:BGP). The rheological properties of the hydrogels and the morphology of the freeze-dried hydrogels were studied using a rheometer equipped with a co-axial cylinder geometry and a scanning electron microscopy, respectively. The cytotoxicity of hydrogels was evaluated *in vitro* on hydrogel extracts using L929 fibroblast cells.

In the following text, hydrogels are named according to the gelation agent and its concentration. e.g. HC005:PB004pH7 represents a hydrogel containing 0.05 M SHC and 0.04 M PB at pH 7.

Results: Combining SHC with PB had an important synergistic effect on the gelation and mechanical properties of the hydrogels (Fig.1). The values of G' for SHC:PB were considerably higher than those corresponding to the sum of G' obtained with SHC and PB used individually at the same total concentration, even if the pH values of the hydrogels were not necessarily higher. This synergistic effect of SHC and PB may partly be explained by the presence of PB, which reduces SHC decomposition before and after the mixing with the acidic chitosan solution.

Adding SHC to BGP also had a synergistic effect on chitosan gelation (Fig.1). This synergistic effect with SHC:BGP, as well as with SHC:PB, may possibly be explained by a slower reaction of one salt than the other with the ammonium groups of chitosan. This effect led to a progressive gelation, and may have allowed the chitosan chains more time rearrange themselves and to get stronger interactions.

The gelation rate and the values of G' of the hydrogels increased considerably with temperature from 22 °C to 37 °C (e.g. Fig. 2), confirming that the hydrogels are thermosensitive (thermogels). This behavior is of great importance, allowing easier injection of the hydrogel at room temperature and rapid *in situ* gelation at body temperature. The hydrogels were flowable after preparation and had almost neutral pHs.

No cytotoxicity on L929 cells was noticed with any of the hydrogel extracts, except for extracts from hydrogels prepared with BGP at 0.4 M and higher.

The chitosan hydrogels showed porous structures which should be favorable to cell invasion, tissue regeneration or drug release.

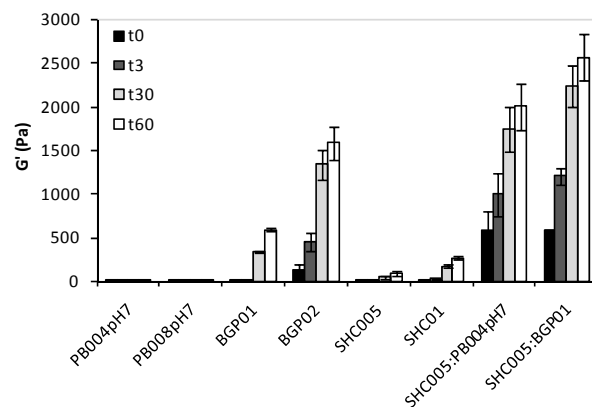


Figure 1. Storage modulus (G') of chitosan hydrogels at 37 °C at 0, 3, 30 and 60 min ($n = 3$, mean \pm SD).

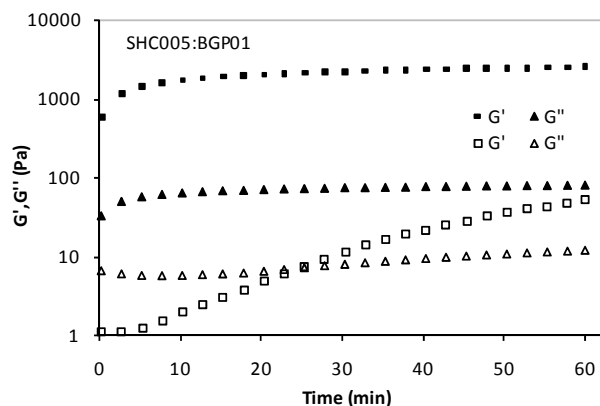


Figure 2. Storage modulus (G') and loss modulus (G'') of chitosan hydrogel prepared with SHC005:BGP01 as a function of time at 22 °C (G' \square , G'' \triangle) and at 37 °C (G' \blacksquare , G'' \blacktriangle) ($n = 3$, mean values).

Conclusions: The use of the SHC:PB and SHC:BGP combinations for the preparation of injectable chitosan hydrogels showed many advantages over that of using each salt individually. These advantages primarily include low salt concentrations, the absence of cytotoxicity, the control of gelation time, the possibility of obtaining a flowable hydrogel after preparation and the rapid increase in the mechanical resistance of the hydrogels. These hydrogels may be particularly useful for the embolization of blood vessels and for drug delivery applications.

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