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pH-dependent stability of scleroglucan borate gels

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Abstract

Scleroglucan, a neutral exopolysaccharide, forms strong physical gels by crosslinking with borate anions, and undergoes a conformational transition at pH > 13 (triple-helix to coil). A study of stability of scleroglucan/borate gels has been carried out over a wide range of pH and we have clearly observed a pH-dependence on the structure of these systems. The gel is dissolved in very alkaline media due to the conformational transition of scleroglucan and in acidic media, in agreement with thermodynamical competition between dissociation constant of boric acid and complexation constant of scleroglucan/borate system. Influence of polymer and borax concentrations have been also studied and it appeared that erosion of these matrices could be easily controlled as a function of the density of the network and pH. The pH-dependence of scleroglucan/borate gels could be used for controlling the release of macromolecular active substances.

Keywords: Polysaccharide; Hydrogel; pH dependent

1. Introduction

Many hydrophilic polymers, and in particular polysaccharides and their derivatives, have been often proposed for the formulation of controlled release systems and in recent years, an ever increasing interest has been focused on the use of hydrogels for these purposes (Chen, Jo, & Park, 1995; Kost & Shefer, 1990; Leonard, De Boisseson, Hubert, Dalençon, & Dellacherie, 2004).

Especially, stimuli-responsive polymeric hydrogels that undergo physical or chemical changes in response to small external modifications in the environmental conditions have been extensively studied. They can provide a variety of materials for various applications, e.g., for the biomedical fields. These hydrogels exhibit dramatic changes in their swelling behavior, network structure, permeability and mechanical strength, in response to a number of chemical or physical external and internal stimuli, including pH (Chen et al., 2004; Nho, Mook Lim, & Moo Lee, 2004), ionic strength (Zhang, Tang, Bowyer, Eisenthal, & Hubble, 2005), temperature (Panayiotou & Freitag, 2005) or applied electrical or magnetic fields (Li et al., 2004). Such polymer systems are termed "intelligent" or "smart" materials and their response to different stimuli are very useful in biomedical applications such as drug delivery.

Various polymeric networks exhibit pH-sensitive bioerodible properties (Heller & Trescony, 1979). An enzyme–substrate reaction (urease–urea reaction) was used to cause pH change that modulated the erosion of a pH-sensitive polymer. In this work, we have focused our attention on pH-sensitive erodible hydrogels based on scleroglucan crosslinked by borate anions.

Scleroglucan is a neutral exopolysaccharide produced by microorganisms, especially by fungi of the genus Sclerotium. Its main chain consists of (1 → 3)-linked β-D-glycopyranosyl units where every third unit bears a (1 → 6)-linked β-D-glycopyranosyl monomer (Fig. 1) (Johnson et al., 1963).

The structure of scleroglucan is chemically identical to that of schizophyllan, a neutral polysaccharide from "Schizophyllan commune" (Tabata, Ito, Kojima, Kawabata, & Misaki, 1981).

In the solid state and in aqueous solution, schizophyllan adopts a stable and very stiff triple-helix conformation,

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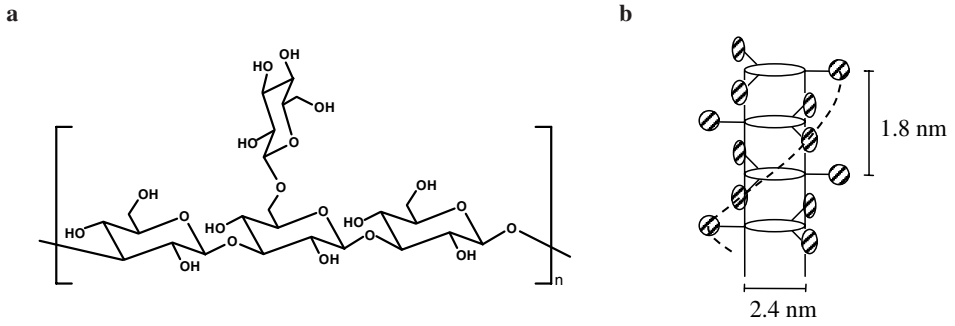

Fig. 1. (a) Repeating unit of scleroglucan and (b) triple-helix of scleroglucan.

held together by hydrogen bonds, with a persistence length of approximately 150 nm in water, at room temperature (Yanaki, Norisuye, & Fujita, 1980). This triple-helix can dissociate to single disordered chains in function of different effects: (i) in water dimethyl sulfoxide mixture (water weight fraction $W < 0.13$) (Norisuye, Yanaki, & Fujita, 1980), (ii) by increasing the temperature above the triple-helix melting temperature $T_m = 130\ ^\circ C$ (Adachi et al., 1990) (iii) in alkaline medium (pH > 13) (Muller, 1986).

It was reported that when denatured samples of scleroglucan are restored to thermodynamics conditions favoring the triple-helix structure, circular structures (a mixture of linear and macrocyclic topologies and multi-chain clusters) can be observed in the "renatured samples" (Sletmoen, Christensen, & Stokke, 2005). For concentrated solutions ($c > 1.5\%$) gels may even be formed by neutralization of the alkaline solution (Aasprong, Smidsrød, & Stokke, 2003).

Scleroglucan was investigated for modified delivery system (Coviello, Grassi, Lapasin, Marino, & Alhaique, 2003; Coviello et al., 2003) and it was proposed, under its oxidized form, for pH-controlled delivery oral dosage forms (Coviello et al., 2005; Coviello, Grassi, Rambone, & Alhaique, 2001).

Due to its triple-helix conformation, scleroglucan has potential biomedical applications, as antitumour activities (Kikuchi, Iwano, & Numazaki, 1980) and antisense carrier (Ferretti, Stoll, Zhang, & Buffle, 2003).

Grisel and Muller (1998) have reported that, in suitable conditions of concentrations and salinity, schizophyllan was able to form strong physical gels in the presence of borate anions ($B(OH)_4^-$) following the scheme shown in Fig. 2.

At low concentrations, Sodium tetraborate $Na_2B_4O_7$ (borax) is totally dissociated into equal quantities of boric acid and borate ion:

$$Na_2B_4O_7 + 7H_2O \rightarrow 2B(OH)_3 + 2B(OH)_4^- + 2Na^+$$

Both species are in equal quantities and the pH of borax solutions is approximately equal to the pK_a of boric acid ($pK_a = 9.2$).

Borate has been proved to be an effective crosslinking agent for polymers containing hydroxyl groups as poly(vinylalcohol) (PVA) (Shibayama, Sato, Rimura, Fujiwara, & Nomura, 1988) or guar gum. In this latest case, the system is used for a colon delivery formulation based on a swelling-dependent enzymatic degradation (Jasinski, Redwine, & Rose, 1996).

The mechanism of gelation is now well established. Initially neutral, the polysaccharide becomes charged after a first mono-complexation as a result of the complexation of borate ions by the diol sites of the polymer chains and it has been reported that screening of electrostatic repulsion by adding salt is a prerequisite for network formation.

On the other hand the gel properties have been shown to clearly depend on the pH condition (Grisel & Muller, 1998), as alkalinity is necessary for effective crosslinking with borates. It has been established that no gelation occurs for pH lower than 8 (below the $pK_a = 9.2$), as the number of salt crosslinks between pairs of *cis*-OH groups is insufficient.

To our knowledge, there is no previous study on pH-dependence of scleroglucan/borax hydrogels and we have focused our interest on these systems in order to evaluate their ability as a pH-sensitive delivery matrix.

In this paper we report on the behavior of scleroglucan/borate matrices as a function of pH, polymer and/or borax concentrations. We focused our attention on the gel erosion kinetics as a function of time.

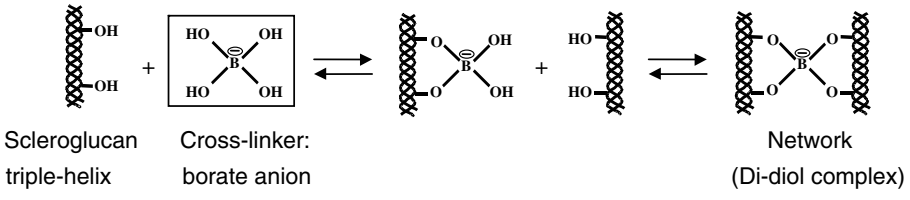

Fig. 2. Crosslinking of scleroglucan with borate anion.

2. Materials and methods

2.1. Materials

Scleroglucan (Actigum CS11) was provided from Degussa (Germany). Its weight average molecular weight, Mw, determined by light scattering, was found to be about 2×10^6 g/mol. Sodium tetraborate $Na_2B_4O_7$ (Borax) and poly(vinylalcohol) PVA ($M_w = 145{,}000$ g/mol) were provided from Merck and Acros Organics, respectively. All other chemicals used (NaCl, NaOH, HCl, formic acid) were of analytical grade. Water was from a Milli-Q water reagent system.

2.2. Hydrogels preparation

Polymer and crosslinker solutions, both in 0.5 M NaCl, were prepared separately at fixed concentrations. Aqueous solutions were prepared under constant stirring for 24 h at 70 °C for scleroglucan and 40 °C for borax.

The hydrogels were then obtained by addition in a beaker of a calculated amount of a solution of borax (of varying concentrations) to a calculated volume of polymer solution under constant stirring for few minutes, to prevent an eventual precipitation due to local variation in concentration. The final volume of borax/polymer solution has always been fixed at 10 mL. The mixture was then left 48 h for gel setting, without any stirring. Final studied polymer concentrations (C_p) were 0.6%, 0.8%, 1%, and 1.5% (w/v). Final studied borax concentrations (C_b) were 8, 11, 14, 20, 30, and 50 mM.

2.3. Measurements of shrinkage

The hydrogels, freshly prepared in a beaker as previously described kept the cylindrical shape of the vessel (height: 1.3 cm; diameter: 2.6 cm). The shrinking measurements are made on around 10 mL of hydrogel at 25 °C.

Erosion was evaluated from the relative decrease of weight of the hydrogels, initially in the swollen form in the studied media. Hydrogels were immersed in 120 mL of aqueous solution set to different pH by adding NaOH, HCl, or acid formic buffer (only used for a specific study at pH 3). For experiment in pH 3 (with or without buffer) and 13.8, the pH has been followed during the time of shrinkage. For both acidic (with or without buffer) and alkaline media, any variation of pH has been observed in the time of shrinking (about 6 h), and in the experimental conditions of measurement (i.e., gel characteristics, especially borax concentration of gel elaboration, and volume of aqueous media used for the measurement). At given time intervals, the hydrogels were removed from the medium and were blotted with a piece of paper to absorb excess water on the surface. The shrinking (or erosion) rates of the samples were calculated from the following expression:

$$\text{Weightloss } (\%) = [(W_o - W_{sh})/W_o] \times 100,$$

where W_{sh} is the weight of the shrinked hydrogel and W_o is the initial weight of the freshly swollen hydrogel.

All reported data are the average values of three measurements (uncertainly 5%).

3. Results and discussion

As documented by Grisel and Muller (1998), two types of pH-dependent crosslinks are involved in the sol/gel transition of scleroglucan/borate system: entanglements of polymer chains and salt crosslinks between pairs of *cis*-OH group on different chains. Thus, in this work we have studied stability of these gels as a function of pH (very acidic to very alkaline), polymer and borax concentrations. These three parameters were varied in order to evaluate the ability of these hydrogels to resist in different pH conditions and as a function of their structure.

3.1. Stability of hydrogels as a function of boric acid/borate equilibrium

Behavior of gels was first studied in a range of pH below the pK_a of boric acid (p$K_a = 9.2$), i.e., in a pH range wherein any gelation is possible as the predominant specie is boric acid (Grisel & Muller, 1998).

As illustrated in Fig. 3, the gels were rapidly and totally eroded in approximately two hours at pH 2. This disruption is attributed to the displacement of equilibrium between the both species ($B(OH)_3$ and $B(OH)_4^-$), and therefore to the destruction of network crosslinks. However, at a fixed polymer concentration ($C_p = 1\%$, w/v), erosion kinetics was not dependent on borax concentrations, i.e., on the number of cross-links (number of di-diol complexes). This could be explained by the very fast displacement from borate anion to boric acid at pH 2 and therefore the junctions are all rapidly disrupted. As a consequence it seems difficult in very acidic media to control erosion kinetics by varying borax concentrations.

The same experiments (same polymer and borax concentrations) were performed in a slightly less acidic medium

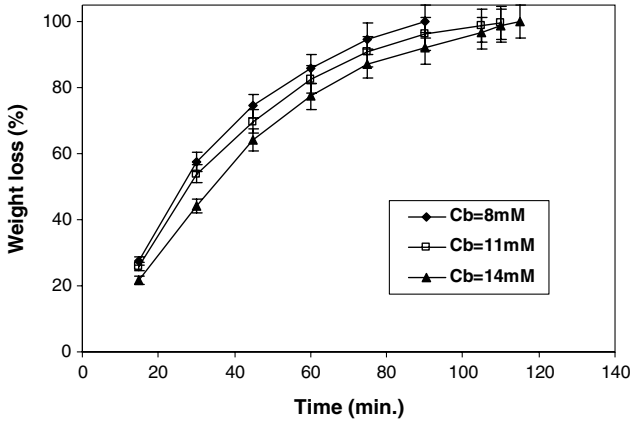

Fig. 3. Erosion kinetics of scleroglucan/borate hydrogels ($C_p = 1\%$, w/v), as a function of time and borax concentration at pH 2.

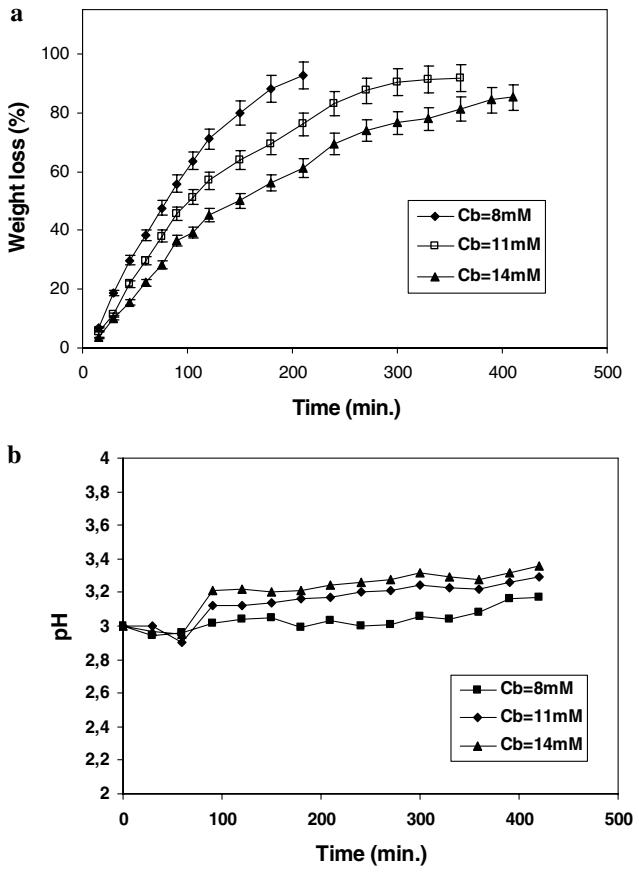

Fig. 4. (a) Erosion kinetics of scleroglucan/borate hydrogels ($C_p = 1\%$, w/v) as a function of time and borax concentration at pH 3. (b) pH evolution of scleroglucan/borate hydrogels ($C_p = 1\%$, w/v) during erosion, as function of time and borax concentration (conditions of Fig. 4a).

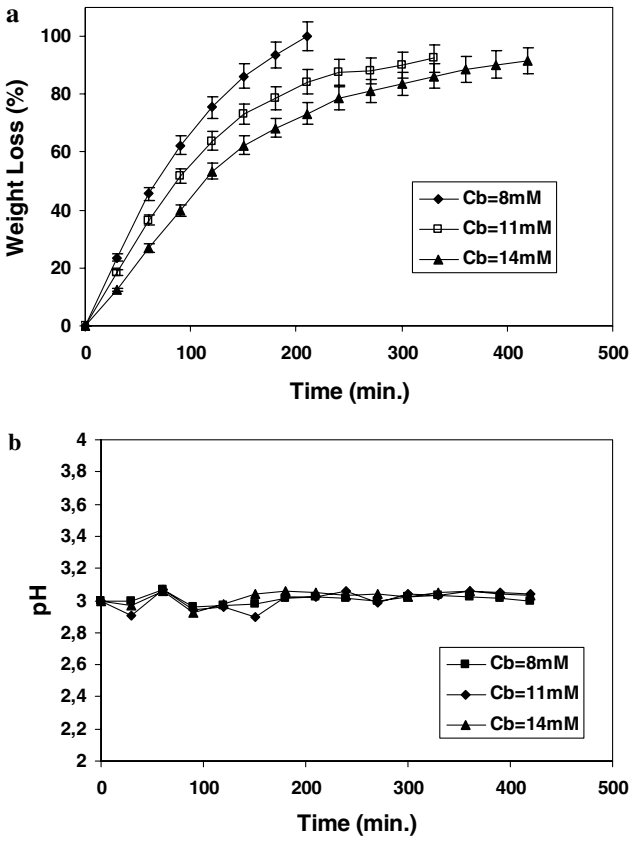

Fig. 5. (a) Erosion kinetics of scleroglucan/borate hydrogels ($C_p = 1\%$, w/v), as function of time and borax concentration at pH 3 (formic acid buffer). (b) pH evolution of scleroglucan/borate hydrogels ($C_p = 1\%$, w/v) during erosion, as function of time and borax concentration (conditions of Fig. 5a, formic acid buffer).

(pH 3). The obtained erosion kinetics was slower than those observed at pH 2. Moreover, contrary to what happens at pH 2, the higher the borax concentration (i.e., the cross linking density) the slower the erosion kinetics (Fig. 4a). For this shrinking conditions, we have followed the pH during the first 6 h of gel erosion which represent a quite total erosion (Fig. 4b). It appears that any variation of pH is observed in these conditions. In order to confirm this result, we have followed both erosion and pH in the same experimental conditions excepted for the pH which has been fixed to pH3 thanks to a formic acid buffer (Fig. 5a and b). Both erosion (weight loss) and pH profiles appear quite similar in buffer pH 3 as that is obtained for initial pH 3. This result indicates that in these experimental conditions the disruption of the gel does not modify the pH of erosion aqueous media.

The effect of polymer concentration on the erosion kinetics, at fixed borax concentration (14 mM) and pH 3, is shown in Fig. 6. It appears that the kinetic of erosion is slowed down when polymer concentration increases in the hydrogel. As the polymer concentration increases, the degree of overlap among polymer chains increases and entanglements could participate in the network formation as physical crosslinks in addition to borate formation.

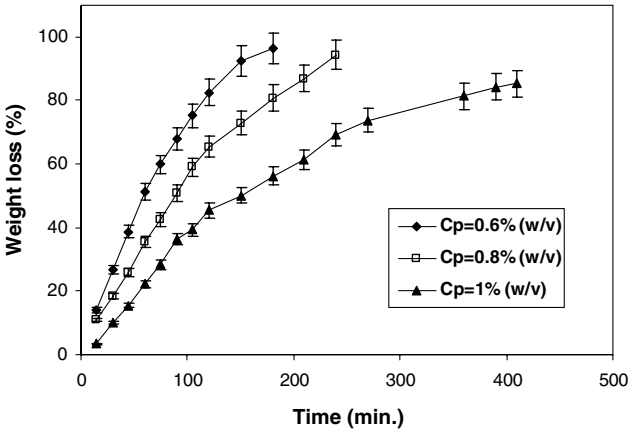

Fig. 6. Erosion kinetics of scleroglucan/borate hydrogels ($C_b = 14$ mM) as a function of time and polymer concentration at pH 3.

Data displayed in Fig. 7 illustrate the effect of pH on the erosion of hydrogels prepared from scleroglucan (0.6%, w/v) and borax (8 mM). The improved stability of gels as pH increases seems clearly related to the equilibrium between the two species boric acid/borate, which is displaced in favor of borate form for higher pH.

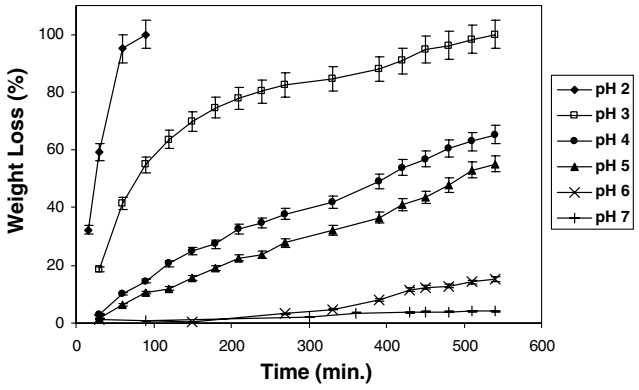

Fig. 7. Erosion kinetics of scleroglucan/borate hydrogels ($C_p = 0.6\%$ (w/v) and $C_b = 8$ mM) as a function of time at $2 < pH < 7$.

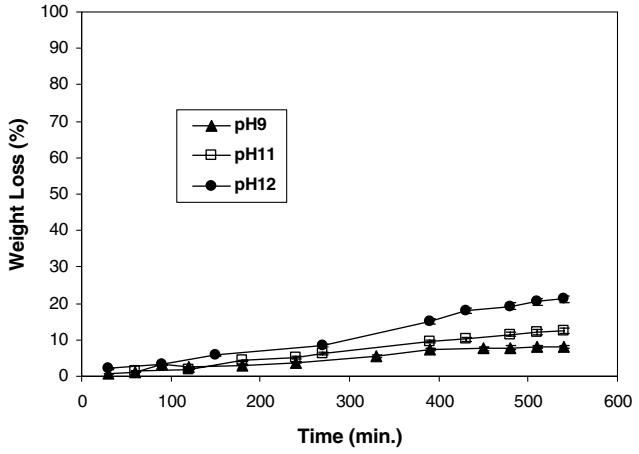

Fig. 8. Syneresis phenomena of scleroglucan/borate hydrogels ($C_p = 0.6\%$ (w/v) and $C_b = 8$ mM) as a function of time at pH 9, 11, and 12.

Consequently, pH appears to be a suitable chemical stimulus in order to control the erosion properties.

At pH above the pK_a, as borate anion is the predominant form, gels are expected to be stable. However a weight loss (<25%) was observed (Fig. 8) probably not due to disruption of crosslinks but rather to syneresis as a consequence of over crosslinking. This hypothesis is reinforced by the observed increase of weight loss as pH is higher than 9.

The pH-sensitivity could be very useful to control release of high molecular weight active compounds (as protein or polymer). For a slightly alkaline pH, the matrices will not be eroded and the release of high molecular weight active compounds will only depends on their diffusion through the pores of the network and therefore on their steric hindrance (Rousseau, Le Cerf, Picton, Argillier, & Muller, 2004). On the other hand, at very acidic pH the matrices will be rapidly and totally disrupted and the active species will be rapidly released.

3.2. Stability of hydrogels at pH above the order/disorder transition

In the above conditions, scleroglucan was under ordered conformation. As is well known the scleroglucan triple-helix is denatured at $pH > 13$. This conformational transition is usually attributed to a change in the local structure of the side chain glucose residues and water organization within the triple-helix structure (Sato, Norisuye, & Fujita, 1983). By size exclusion chromatography and light scattering, it has been shown that no degradation of the polymeric backbone occurs at this pH (Zentz, 1991; Grisel, 1996).

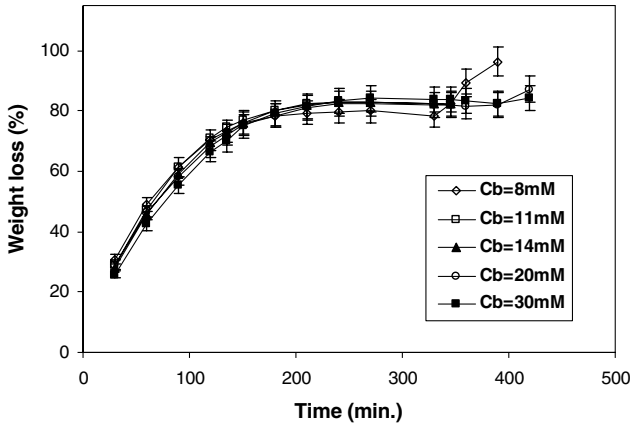

Fig. 9. Erosion kinetics of scleroglucan/borate hydrogels ($C_p = 0.6\%$, w/v) as a function of time at pH 13.8.

The stability of hydrogels at pH above the pH of transition (pH 13.8) was evaluated. The polymer concentration was $C_p = 0.6\%$ (w/v) and borax concentration varied from 8 to 30 mM. It has been verified that any variation of pH occurs during the time of erosion experiment. As shown in Fig. 9, whatever the borax concentration hydrogels were totally disrupted (about 80%) in approximately 6 h. This agrees with data from Coviello et al. (2003) who reported that for $pH > 13$ (pH 14), a model drug (theophylline) was immediately released from a scleroglucan/borate hydrogel by a process leading to a complete dissolution of the matrix in the first 30 min. However the authors gave no explanation.

Shrinkage could be due to disruption of cross-links, but more likely to the denaturation of scleroglucan triple-helix. To confirm this hypothesis we have studied the behavior of a PVA/borate gel in the same pH condition. As well established the gelation of PVA with borate ions is similar to that of scleroglucan/borate.

A PVA/Borate gel was prepared ($C_{PVA} = 3\%$ (w/v), $C_b = 15$ mM, $C_{NaCl} = 0.5$ M, $T = 25$ °C) then immersed in an alkaline medium at pH 13.8. No weight loss was observed during 24 h. Thus indicating that the di-diol complex sites resist (for 24 h) to hydrolysis by hydroxide ions. Therefore, destruction of scleroglucan/borax hydrogels, at very alkaline pH, was only due to the denaturation of scleroglucan triple-helix.

The fact that the erosion kinetics is slowed down with increasing polymer concentration is in favor of this hypothesis (Fig. 10). The resistance to erosion with increasing polymer concentration can be related to the degree of overlap

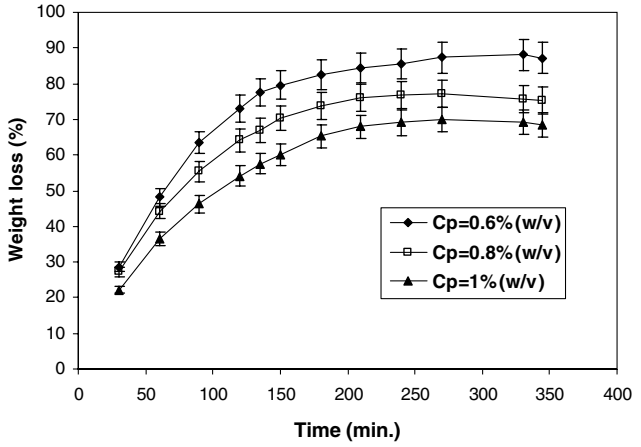

Fig. 10. Erosion kinetics of scleroglucan/borate hydrogels ($C_b = 11$ mM) as a function of time at pH 13.8.

among the polymer chain above a critical concentration, which can be estimated from the intrinsic viscosity of scleroglucan: in water at 25 °C under coil form ($[\eta] = 0.7$ L g^{-1}). C^* is around 0.2% (w/v) and under helix conformation ($[\eta] = 2.7$ L g^{-1}) C^* is around 0.04% (w/v).

On the another hand, it is known (Lack, Picton, Le Cerf, Argillier, & Muller, 2004) that gel formation often need polymer concentration much higher than C^*. Consequently, one can imply the level of entanglement (higher when scleroglucan concentration increases) to explain the resistance observed for hydrogels obtained with higher polymer concentration. However, a 1% (w/v) of scleroglucan (coil) solution (pH 13.8) doesn't lead to gel formation at the same borax concentration.

For a pH 13.4, slightly higher than pH of denaturation but lower than the above studied pH of 13.8, a kind of "time lag" to erosion was observed (Fig. 11). On the other hand, for a pH of 13.8, the erosion starts according to a "burst effect". This behavior difference could be explained by the denaturation kinetics that is slower at 13.4 than at 13.8. This particularity could be exploited to modulate the release of active molecules.

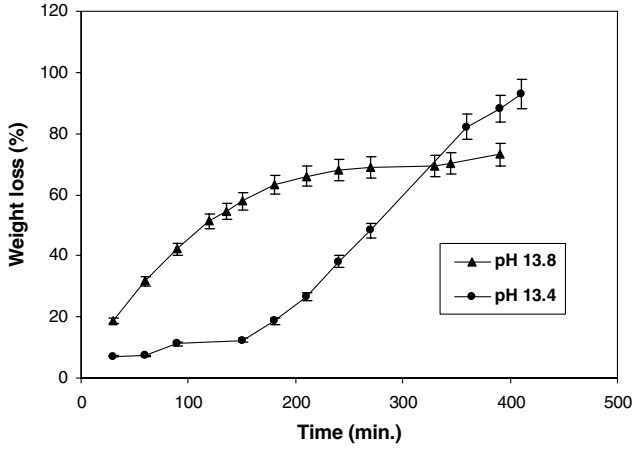

Fig. 11. Erosion kinetics of scleroglucan/borate hydrogels ($C_p = 1\%$ (w/v), $C_b = 14$ mM) as a function of time at pH 13.4 ($T = 25$ °C) (full symbols) and pH 13.8 (empty symbols).

4. Conclusion

We have studied stability of scleroglucan/borate hydrogels as a function of pH, polymer and borax concentrations. This system appears to be pH-sensitive and hydrogels underwent erosion with controlled kinetics by these three parameters. It has been demonstrated that erosion was due to the conformational transition of scleroglucan in very alkaline media and to the dissociation equilibrium of boric acid in acidic media. We have shown that it would be easy to modulate the time of disruption of such hydrogels, and therefore to control the release kinetics of macromolecular active species, by varying network structure and pH according to the application needs.

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