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Interplay of thermal and covalent gelation of silanized hydroxypropyl methyl cellulose gels

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ABSTRACT

Silanized hydroxypropyl methyl cellulose (Si-HPMC) is a biocompatible polysaccharide that forms a covalently crosslinked hydrogel at all temperatures due to silanol condensation. Unmodified HPMC forms reversible turbid physical gels when heated above 55 °C. The interaction between thermal gelation and covalent crosslinking of Si-HPMC was investigated with rheology, turbidity and microscopy. Thermal gelation of the HPMC backbone was found to reinforce Si-HPMC gels at room temperature. However, simultaneous thermal and covalent crosslinking at higher temperatures led to weaker turbid gels at room temperature. The effect of the pH and the addition of orthophosphate on the elastic modulus and the gelation kinetics was investigated.

1. Introduction

The advancements in tissue engineering have motivated the use of polymer based hydrogels for regenerative medicine (Drury & Mooney, 2003; Van Vlierberghe, Dubruel, & Schacht, 2011; Vinatier, Guicheux, Daculsi, Layrolle, & Weiss, 2006). In order to develop less invasive surgical techniques notably for bone and cartilage tissue engineering, there is an increasing demand for aqueous biocompatible polymer solutions that are injectable and crosslink in situ under physiological conditions (Ashley & Lakshmi, 2012; Fiejdasz, Szczubiałka, Lewandowska-Łańcucka, Osyczka, & Nowakowska, 2013; Overstreet, Dutta, Stabenfeldt, & Vernon, 2012). Once implanted these injectable matrices must acquire the desired form, allow cell diffusion and growth and present mechanical properties in relation to the tissue to be repaired. One widely used biocompatible polymer is hydroxypropyl methyl cellulose (HPMC).

Aqueous HPMC solutions form gels with an elastic modulus (G_{el}) that increases sharply with increasing temperature above a critical temperature (T_c) where a phenomenon of localized phase separation occurs rendering the gels turbid (Allabash, Nicolai, Benyahia, Tassin, & Chassenieux, 2014; Bodvik et al., 2010; Fairclough et al., 2012; Haque & Morris, 1993; Haque, Richardson, Morris, Gidley, & Caswell, 1993; Hussain, Keary, & Craig, 2002; Sarkar, 1979; Silva et al., 2008; Yoo & Um, 2013). In pure water T_c is higher than 50 °C and depends on the degree of substitution of the cellulose chains. The critical temperature T_c can be reduced by addition of salt and in particular orthophosphate (Joshi, 2011). Thermal gelation of HPMC is reversed when the temperature is lowered but the gel melts at a temperature lower than T_c.

The HPMC chains can be covalently crosslinked at physiological conditions by grafting silanes to the HPMC chains (Bourges, Weiss, Daculsi, & Legeay, 2002; Vinatier et al., 2005; Weiss et al., 2008). The silanized hydroxypropyl methyl cellulose (Si-HPMC) is soluble at alkaline condition and transforms into a crosslinked gel after the pH is decreased below 11 due to silanol (SiOH) condensation. (Fatimi, Axelos, Tassin, & Weiss, 2008a; Fatimi, Tassin, Quillard, Axelos, & Weiss, 2008b; Fatimi, Tassin, Turczyn, Axelos, & Weiss, 2009). The rate of gelation increases with increasing temperature and increasing pH between 6 and 10. However the Si-HPMC gels formed in this way have a significantly lower modulus than cartilage.

The objective of the present investigation was to study the effect of reversible thermal gelation of the HPMC backbone on the covalent crosslinking of Si-HPMC. We will compare the case where the covalent gel is made at $T < T_c$ and subsequently heated, with that where the system is heated above T_c so that covalent crosslinking and thermal gelation occur simultaneously. Before discussing the effect of thermal gelation on covalent gelation of Si-HPMC, we show that T_c can be reduced to below 37 °C by addition of orthophosphate rendering this investigation potentially relevant for in vivo

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applications. We have also investigated the effect of varying the pH, because addition of orthophosphate influences the pH.

2. Materials and methods

2.1. Sample preparation

The hydroxypropyl methyl cellulose (Methocel® E4M Premium procured from Dow Chemical) was silanized by grafting 3-glycidoxypropyltrimethoxysilane GPTMS as described elsewhere (Bourges et al., 2002). The desired amount of Si-HPMC powder was dissolved in sodium hydroxide solution (NaOH = 0.2 M) by stirring at room temperature for 24 h, after which the solution was refrigerated for 24 h to ensure complete dissolution of the polymer. Si-HPMC solution was dialyzed (Spectra-por 6000–8000) against 3.8 l of NaOH solution (0.09 M) for 16 h and 4 l of NaOH solution (0.09 M) for 2 h. The final pH value of the basic Si-HPMC solution is 12.7. Pure aqueous HPMC solutions were prepared by dissolving the HPMC powder in water at the desired pH in the same manner.

The acidic buffer solution used to neutralize the basic Si-HPMC solution was prepared using 0.1 M hydrochloric acid and HEPES (4-2-hydroxyethyl piperazine-1-ethanesulfonic acid). The buffer solution was prepared by dissolving 6.2 g of HEPES and 1.8 g of NaCl in 60 ml HCl and the final volume was adjusted to 100 ml. The pH value of the final acidic buffer solution was 3.6. Si-HPMC hydrogels were prepared by rapidly mixing the polymer and buffer (HEPES) in the ratio of 2:1. This led to a reduction of the pH to 7.2 and initiated the silanol condensation. In some cases, the pH was varied by varying the amount of HCl in the buffer. The pH increased slightly during the reaction.

2.2. Methods

The rheological properties of the hydrogels were studied by carrying out oscillatory shear experiments on a stress controlled rheometer (AR G2, TA Instrument) using a cone and plate geometry (40 mm, 2°). The oscillatory stress was set sufficiently low to remain well within the linear response regime. Gelation was monitored by measuring the shear modulus at 1 rad/s. In order to avoid drying, a small amount of low viscosity mineral oil was added at the periphery of the cone.

The turbidity was determined as a function of the temperature using a UV-Visible spectrometer Varian Cary-50 Bio (Les Ulis, France). The path length through the cell was 10 mm and the temperature was controlled using a thermostatic bath. An optical microscope (Leica DMLP) coupled to a temperature controller was used to obtain the images of the gel.

3. Results and discussion

3.1. Thermal gelation of HPMC

As was mentioned in Section 1, thermal gelation of HPMC has been studied in detail in the past. Our aim here is to show that the critical temperature above which the elastic modulus of HPMC increases rapidly can be decreased by addition of sodium orthophosphate. The storage modulus at 1 rad/s for a solution of 2 wt% HPMC during a heating ramp is shown in Fig. 1a. Initially, G' decreased progressively with increasing temperature until it reached a minimum and started to increase with increasing temperature. At a critical temperature (T_c) G' dropped sharply before it increased again. As was discussed in detail by Allabash et al. (2014), this characteristic behavior of HPMC can be explained by a combination of three distinct phenomena. Disordered HPMC chains form a transient network with a terminal relaxation time that

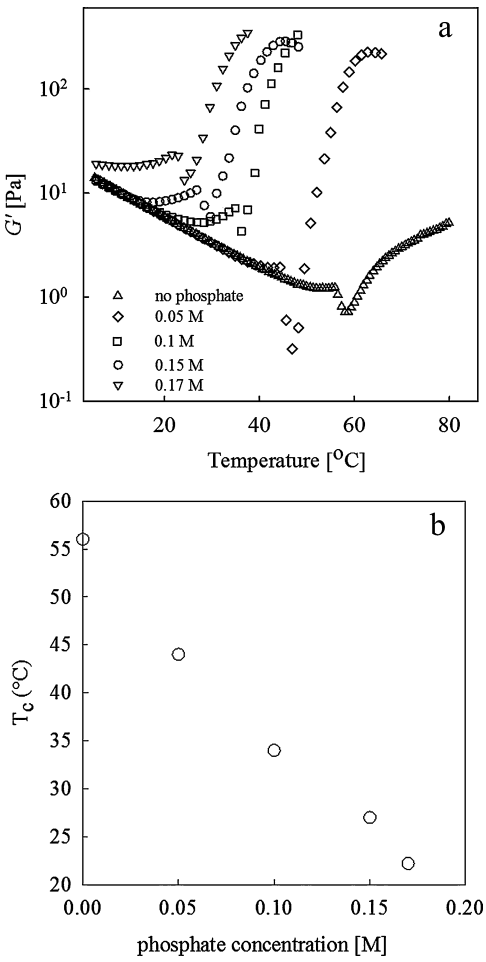

Fig. 1. (a) Evolution of G' at 1 rad/s during a heating ramp at a rate of 5 °C/min for 2 wt% HPMC in the presence of varying amounts of Na_3PO_4. (b) Dependence of the critical temperature on the Na_3PO_4 concentration.

decreases with increasing temperature. With increasing temperature an increasing fraction of the HPMC chains associates into long rigid fibrils that form a permanent network. At lower temperature this permanent network is very weak and G' is dominated by the transient network so that it decreases with increasing temperature. But with increasing temperature the permanent network becomes increasingly stronger and causes an increase of G'. At T_c the HPMC chains are no longer soluble and phase separation sets in, which leads to a collapse of the transient network and causes a sharp drop of G' at T_c. Macroscopic phase separation is inhibited by the permanent network but microphase separation causes the system to become turbid at T_c. The increase in the shear modulus of the permanent network becomes strong for $T > T_c$.

T_c depends on the content of propylene and methylene groups, but for commercially available HPMC it is higher than 50 °C. It can however be decreased by addition of large amounts of salt that reduce hydration of HPMC. Orthophosphate was reported to be particularly effective in reducing T_c (Joshi, 2011). The effect of addition of different amount of Na_3PO_4 is shown in Fig. 1a. Clearly addition of more phosphate induced both gelation and phase separation at lower temperatures. The critical temperature decreased approximately linearly with increasing phosphate concentration, see Fig. 1b. Notice that addition of phosphate changed the pH, but we checked that varying the pH in itself did not modify the thermal gelation of HPMC contrary to the covalent gelation of Si-HPMC that will be discussed in the following section.