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Rheological Characterization of Self-Hardening Hydrogel for Tissue Engineering Applications: Gel Point Determination and Viscoelastic Properties

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Summary: Silated hydroxypropylmethylcellulose (Si-HPMC) is a modified biopolymer used in biomaterial's domain. A hydroxypropylmethylcellulose (HPMC) was functionalised with silane groups. The macromolecular solution of Si-HPMC (pH 12.9) generates an elastic state at physiological pH (~7.4). In this work we present the gel formation of Si-HPMC resulting from the condensation of silanol groups. The crosslinked hydrogel was characterized using rheological techniques. With the scalar percolation model the gelation time was determined as a function of Si-HPMC concentration at 37 °C.

Keywords: biomaterials; cellulose; gelation; hydrogels; rheology

Introduction

Cellulose, the precursor of hydroxypropylmethylcellulose (HPMC), is a polysaccharide composed of hundreds of glucose units linked to a β 1–4 configuration. Cellulose is the main structural ingredient of the cell wall of plants and the most abundant plant carbohydrate.[1] HPMC is a methylcellulose modified with a small amount of propylene glycol ether groups attached to the anhydroglucose of the cellulose (Figure 1). The physicochemical properties of this polymer are strongly affected by: (i) the methoxyl group content, (ii) the hydroxypropyl group content and (iii) the molecular weight. The rheological properties of these biopolymer systems have been studied extensively, both in the sol phase and in the gel phase.

HPMC exhibits appropriate biological properties which make them suitable for biomedical applications. Ten years ago, a new self-hardening hydrogel was developed by Prof. Weiss where a backbone of HPMC was functionalised with silane groups.[2,3] At physiological pH the silated HPMC (Si-HPMC) generates an elastic state[2] and improves biological and biomechanical properties. The self-hardening principle of the hydrogel is based on the silanes grafted along the Si-HPMC chains. Dissolution of this product takes place in strong basic medium (NaOH), which corresponds to the silane ionization into sodium silanolate (SiO^-Na^+). Gel formation is based on the condensation between the silanol groups (SiOH) after decreasing pH (Figure 2). The Si-HPMC solution is transformed into Si-HPMC hydrogel with formation of three-dimensional network.[2] Turczyn et al.[4] were the first to propose biomedical applications and Si-HPMC appeared as a potential scaffold for three-dimensional amplification and transfer of chondrocytes in cartilage tissue engineering.[5] An other application of this hydrogel in the biomaterial's domain is bone regeneration which Si-HPMC associated with biphasic calcium phosphate ceramic particles.[6]

In previous works we showed the dependence of the rheological properties of

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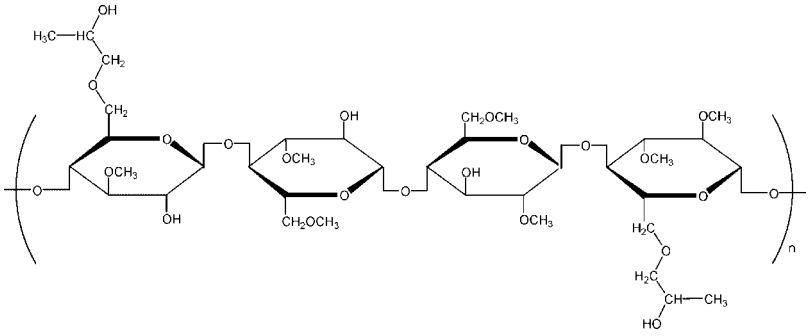

Figure 1.
Idealized chemical structure of hydroxypropylmethylcellulose (HPMC).

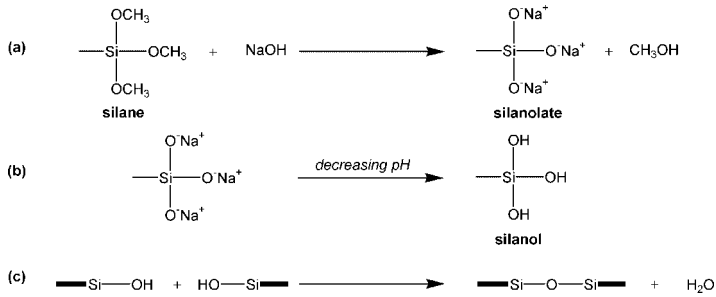

Figure 2.
Silane behaviours: (a) silanolate formation, (b) protonation of silanolate, and (c) silanol condensation.

Si-HPMC hydrogel on pH and temperature.[2] In this work we present a rheological study of the gel formation and gelation time determination using percolation theory[7] as a function of Si-HPMC concentration.

Synthesis of Si-HPMC

Methocel™ E4M from The Dow Chemical Company was kindly supplied by Colorcon-England and used without purification. As specified by producer the methoxyl content is 29% and the hydroxypropyl content is 9.7%, leading to an average DS of 1.9 (Table 1).

Silane grafting on HPMC involves a Williamson reaction between the hydroxyl function of HPMC and the epoxy function of 3-glycidoxypropyltrimethoxysilane (GPTMS) (Figure 3). The synthesis of Si-HPMC were described in detail previously.[2,8]

Polymer Solution

The Si-HPMC powder, which is insoluble in water, was dissolved as a 3% solution (w/v) in NaOH 0.2 M (pH 12.9). The solution was stirred for 2 days before dialysis with NaOH

Table 1.
Substitution of Methocel brand of cellulose ethers.*

Polymer	Methoxyl degree of substitution	Methoxyl (%)	Hydroxypropyl molar substitution	Hydroxypropyl (%)
Methocel™ E4M	1.9	28–30	0.23	7–12

*information supplied by Dow Chemical Company®.

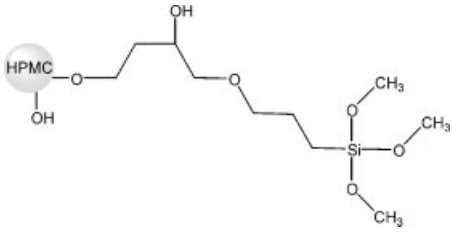

Figure 3.
Chemical structure of silated hydroxypropylmethylcellulose (Si-HPMC).

0.09 M and sterilization by autoclaving (121 °C during 20 min).

Rheological properties of the Si-HPMC solution in oscillatory shear were carried out with a stress-controlled rheometer MARS (ThermoHaake®, Germany) with a cone-plate geometry (60 mm diameter, 1° cone angle). The measurements were performed at 37 °C.

Figure 4 illustrates the frequency (ω) dependence of the storage (G′) and loss (G″) moduli of Si-HPMC solution (3% w/v). In the frequency range studied, the G″ values were found to be higher than G′ values. Such results indicate that Si-HPMC at 3% can be classified as a solution.[9] In the low frequency zone ($\omega < 10$ rad $\cdot$ s^{-1}) the storage modulus G′ increases like ω^2 whereas the loss modulus G″ increases like ω^1, confirming the terminal zone behaviour of macromolecular solutions.[10]

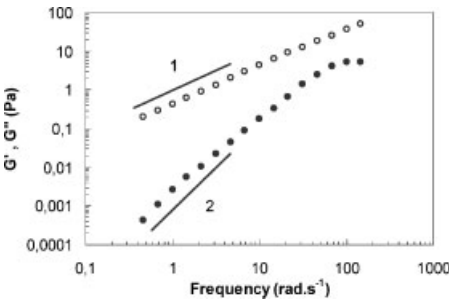

Figure 4.
Mechanical spectra of Si-HPMC solution (3% w/v) at 37 °C: Storage modulus (G′, closed symbol) and loss modulus (G″, open symbol). A shear stress of 1 Pa was applied.

Kinetics of Gelation and Viscoelastic Properties

The Si-HPMC hydrogel used in the present study resulted from the mixing, at 37 °C, of the 3% (w/v) Si-HPMC solution (pH 12.9) with various buffer solutions (pH 3.2 and 3.6) in a 2/1 and 1/1 ratio (v/v), respectively. The buffer solution was composed of NaCl and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid sodium salt 99% (HEPES) (Aldrich, Germany), and the pH (3.2 or 3.6) was adjusted with HCl. The final pH of the Si-HPMC hydrogel was 7.4.

In order to study the frequency dependence of the storage (G′) and loss (G″) moduli during gelation, frequency sweeps between 0.6 and 60 rad $\cdot$ s^{-1} were repeatedly carried out.

Figure 5a is an example of the mechanical spectra obtained during the gelation of

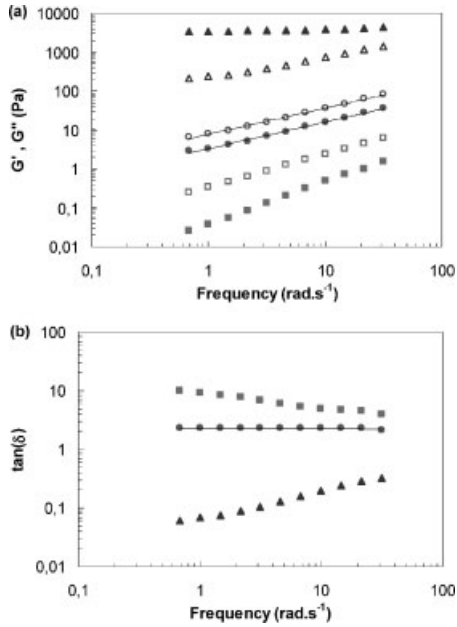

Figure 5.
Gelation process of Si-HPMC (1.5% w/v) at 37 °C. (a) Frequency dependence of the moduli G′ (closed symbol) and G″ (open symbol). The bottom spectrum (■ □), at t = 400 s, is below the gel point; the next one (● ○), at t = 1300 s, is at the gel point; and the following (▲ △), at t = 4800 s, is above the transition. For better visualization each spectrum has been shifted upward vertically by one decade with respect to the previous one. (b) Frequency dependence of tan(δ) (= G″/G′) of the same data.

a 1.5% (w/v) Si-HPMC. Three data series for G′ and G″ below, at and above the transition are shown. For better visualisation, each series was shifted vertically by one decade with respect the previous one. The first spectrum (t = 400 s) was taken at the beginning of the gelation process. G′ and G″ behave as an macromolecular solution; their low frequency behaviour approaches the expected limiting power law, with slopes of ~2 for G′ and ~1 for G″.[9,10] The next spectrum (t = 1300 s) in the series was recorded at gel point (t_{gel}). As predicted by theory, both G′ and G″ exhibit a power law behaviour with an exponent ($\Delta = 0.66$) which extends over the entire frequency range ($G' \sim G'' \sim \omega^{\Delta}$). An universal exponent of 0.7 is already found for weak gels.[7] The last spectrum (t = 4800 s) is characteristic of a gel. The material behaves like an elastic medium with a storage modulus G′ which is frequency independent and a loss modulus G″ which decreases with decreasing ω. The same data have been plotted as tan(δ) (= G″/G′). According to the theory, at sufficiently low frequency tan(δ) vs ω should be frequency independent at the gel point (t_{gel}), it should decrease with increasing frequency below t_{gel} and increase above t_{gel} (Figure 5b). The validity of the percolation theory was confirmed. The measured value of tan(δ) ($G''/G' = \tan(\Delta\pi/2)$) at the gel point yields $\Delta = 0.66$ for the critical exponent value Δ, very close to the expected universal value $\Delta = 0.7$.[11]

The same results were obtained during the gelation of a 2% (w/v) Si-HPMC (not shown here). The gel point was observed at 300 s, when both G′ and G″ exhibit a power law behaviour with an exponent ($\Delta = 0.67$) which extends over the entire frequency range ($G' \sim G'' \sim \omega^{\Delta}$).

The viscoelastic properties of Si-HPMC hydrogel were examined as a function of time and polymer concentration. Figure 6 shows the storage (G′) and loss (G″) moduli and damping factor tan(δ) (= G″/G) values obtained after three weeks of crosslinking for Si-HPMC hydrogels at 37 °C. It can be considered that the gel formation has reached the equilibrium. The storage

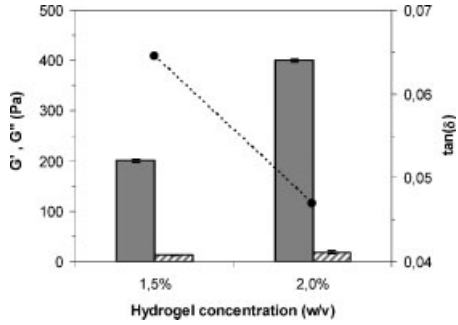

Figure 6.
The storage modulus (G′, solid bar), loss modulus (G″, slashed bar) and damping factor (tan(δ), ●) as a function of Si-HPMC hydrogel concentration after three weeks of crosslinking. A frequency of 1 Hz and a shear stress of 1 Pa at 37 °C were applied.

modulus of hydrogel increases with increasing polymer concentration. The hydrogel showed storage moduli of 200 and 400 Pa at 1.5 and 2% (w/v), respectively. Under this conditions, the loss modulus only increases from 12 to 18 Pa when increasing concentration. Therefore, the damping factor tan(δ) of hydrogel decreased with increasing polymer concentration, showing the formation of higher network densities at higher polymer concentration.

Conclusion

We have synthesised the Si-HPMC polymer and confirmed the gelation properties.[2,8] Si-HPMC solution exhibits a viscous behaviour and can be classified as a macromolecular solution. Percolation theory was applied to determine the gel point and to discuss the dependence of G′ and G″ on frequency during sol-gel transition. The gelation depends on buffer volume used to neutralize the Si-HPMC solution. Gel point ranging from 300 to 1300 s, depending on the buffer solution ratio (2/1 and 1/1 (v/v), respectively). After three weeks of crosslinking, the Si-HPMC hydrogels were elastic with a storage modulus ranging from 200 to 400 Pa. In conclusion, the rheological characterisations of Si-HPMC hydrogels illustrated the ability to control these

gelation properties, such as gel point and gel strength, by altering the concentration of the polymer as a function of the buffered acid volume.

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