

Thermo-responsive properties of methylcellulose hydrogels for cell sheet engineering

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ABSTRACT

Methylcellulose (MC) hydrogels change their affinity to water depending on their temperature and can thus be used as substrates for cell sheet engineering. In this work, we characterize the thermo-responsive properties of 8% w/v MC hydrogels, produced in two saline solutions (*i.e.*, Na₂SO₄ and phosphate buffered saline) at different concentrations, by investigating the rheological properties and the UV-absorbance in function of temperature. Both rheological and UV-spectroscopy tests showed that the addition of salts to MC hydrogels allowed lowering the LCST of the MC hydrogel; moreover, hydrogels produced in 0.1 M Na₂SO₄ or PBS 20 g/L were proved to be particularly promising for cell sheet engineering application, showing a LCST below 37 °C.

Keywords:

Smart materials

Methylcellulose

Rheology

UV spectroscopy

Cell sheet

engineering

1. Introduction

Smart hydrogels reversibly change their properties when exposed to an external driven force as pH or temperature variation [1]. Methylcellulose (MC) is a polysaccharide derived from cellulose by partial substitution of hydrophilic hydroxyl groups with hydrophobic methoxy groups. MC dissolved in aqueous solvents forms reverse thermo-responsive smart hydrogels that undergo a sol-gel transition when heated, associated with change from hydrophilicity at low temperatures (hydrogen bonds between water and MC hydroxyl groups) to hydrophobicity at higher temperatures (loss of hydration due to methoxy groups exposition and higher interaction among them) [2]. This sol-gel transition is characterised by a Low Critical Solution Temperature (LCST), tuneable by varying MC degree of substitution, concentration [3], and by adding salts in the solution (*i.e.*, salting-in or salting-out) [4]. These features, together with ease of availability, low-cost and biocompatibility, make MC a promising material for regenerative medicine [5] and cell sheet engineering [6]. In the latter case, cells cultured *in vitro* adhere and proliferate on MC at 37 °C (*i.e.*, hydrophobic substrate) while they spontaneously detach at lower temperature (*i.e.*, hydrophilic substrate) preserving the inter-

cellular connections and ECM, thus forming cell sheets to be directly applied on pathological tissues without implanted supporting materials (*i.e.*, scaffold-free tissue engineering). Typical LCST of 1% w/v MC hydrogels in distilled water is >50 °C [7], incompatible with cell culture conditions; thus, tuning the LCST by either increasing MC concentration and/or adding salts to the solvent is a key aspect when producing a substrate for cell sheet engineering.

Here, we propose relatively highly concentrated MC hydrogels (*i.e.*, 8% w/v), produced in differently concentrated ions solutions and we investigate their LCST by rheological tests, proposing an innovative investigation of LCSTs for hydrogels with $G' > G''$, and UV spectroscopy. Moreover, LCST values obtained by the two techniques were compared to verify the possibility to use only one of them for LCST investigation on MC hydrogels.

2. Materials and methods

2.1. Synthesis

METHOCEL powder (A4M, Dow Chemical Company) 8% w/v was dissolved at 55 °C in different distilled water saline solutions (Table 1): phosphate buffered saline (PBS, Sigma Aldrich) at 10 g/L (MC_PBS10) and 20 g/L (MC_PBS20), 0.05 M (MC_Na005) and 0.1 M (MC_Na01) sodium sulfate (Na₂SO₄, Sigma Aldrich) or distilled water as control (MC_water). After complete powder dissolution, solutions were sealed in petri dishes (Ø = 35 mm) and stored at 4 °C for 24 h to allow MC complete hydration [8].

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Table 1Methylcellulose hydrogels and their characteristic gelation temperatures (LCST) measured by rheology (G' , η^*) and UV spectroscopy.

| Sample | MC concentration [%w/v] | Salt concentration | LCST/rheology G' [°C] | LCST/rheology η^* [°C] | LCST/UV [°C] |
|----------|-------------------------|--|-------------------------|-----------------------------|--------------|
| MC_water | 8 | – | 38.7 ± 0.5 | 39.1 ± 0.4 | 42.5 ± 0.3 |
| MC_PBS10 | 8 | PBS 10 g/L | 39.9 ± 0.8 | 40.1 ± 0.7 | 39.4 ± 0.3 |
| MC_PBS20 | 8 | PBS 20 g/L | 37.9 ± 0.3 | 38.1 ± 0.4 | 35.4 ± 0.3 |
| MC_Na005 | 8 | Na ₂ SO ₄ 0.05 M | 36.9 ± 0.3 | 37.2 ± 0.5 | 33.4 ± 0.2 |
| MC_Na01 | 8 | Na ₂ SO ₄ 0.1 M | 37.2 ± 0.5 | 37.6 ± 0.6 | 31.9 ± 0.3 |

2.2. Weight variation

Weight variation tests were performed in distilled water at 37 °C. Hydrogels ($n = 3$) after 24 h of hydration were weighted (w_0) and immersed in distilled water at 37 °C; at established time points, up to 7 days, hydrogels were weighted (w_t) and the percentage weight variation ($\Delta W\%$) was calculated as (1)

$$\Delta W\% = \frac{W_t - W_0}{W_0} \cdot 100 \quad (1)$$

2.3. Rheological properties

Rheological tests were performed using a rotational rheometer (AR-1500, TA instruments) equipped with cone-plate geometry (SN982907, TA instrument, angle = 1.023°, $\emptyset = 20$ mm, geometry gap = 32 μ m). First, the linear viscoelastic region (LVR) at 20 and 37 °C was identified by applying a strain sweep between 0.1 and 100%, frequency 1 Hz. Subsequently, temperature sweep tests were performed on hydrogels ($n = 3$) applying a temperature ramp at 2 °C min⁻¹ between 10 and 60 °C, 0.5% strain (*i.e.*, LVR), frequency 1 Hz. The LCST was identified from storage modulus (G') and complex viscosity (η^*) curves as the intersection between the interpolant of the initial T range, characterized by a slight decrease of the considered parameter, and the interpolant of the following T range, characterized by an increase of the parameter (Fig. 2b).

2.4. UV spectroscopy

UV–VIS spectroscopy was performed using a spectrophotometer (6705 UV–vis Spectrophotometer, Jenway), by measuring the hydrogels ($n = 3$) absorbance (A) at 500 nm and by increasing the temperature between 20 and 50 °C, at 5 °C discrete steps. The percentage of transmittance ($T\%$) was reported in function of temperature following (2), being A the measured absorbance at 500 nm, and the LCST was identified as the intersection between the interpolation in the initial T region, with stable $T\%$ values, and the interpolant of the following range, characterized by a decrease of $T\%$ (Fig. 3b)

$$T\% = \frac{1}{10^A} \cdot 100 \quad (2)$$

All data are reported as mean ± standard deviation. Statistical analysis was performed by ANOVA, considering $p < 0.05$ statistically significant.

3. Results and discussion

Hydrogels $\Delta W\%$ ranged from 150% for hydrogels produced with lower salts concentration (*i.e.*, MC_Na01 and MC_PBS20) to more than 200% for hydrogels produced with higher salts concentration (*i.e.*, MC_Na005 and MC_PBS10); all hydrogels showed stable $\Delta W\%$ values for more than 5 days. Macroscopic images of MC hydrogels are shown in Fig. 1. Hydrogels showed no macroscopic differences at 20 °C (Fig. 1a–c–e–g–i); in fact, MC is soluble in water at $T < \text{LCST}$, due to the formation of hydrogen bonds between water molecules

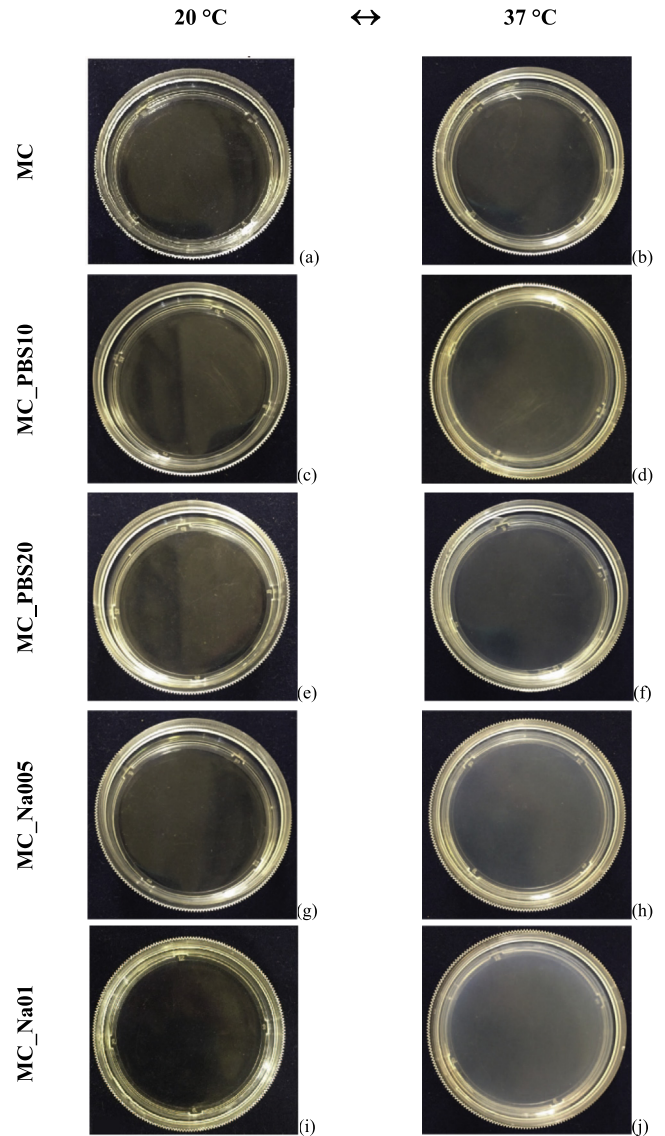


Fig. 1. Macroscopic observation of methylcellulose hydrogels (a–b), MC_PBS10 (c–d), MC_PBS20 (e–f), MC_Na005 (g–h) and MC_Na01 (i–j) at 20 and 37 °C respectively.

and the MC polymer chains, thus giving a transparent hydrated low viscous hydrogel. Oppositely, hydrogels conditioned for 1 h at 37 °C showed macroscopic differences when observing MC_PBS20, MC_Na005 and MC_Na01 (Fig. 1f–h–j, respectively), which appear opaque due to the sol-gel transition of the hydrogel (*i.e.*, $T > \text{LCST}$) and the hydrophobicity caused by interactions between the MC polymer chains and separation from the water molecules.

The LVR was identified until 50% strain at 20 °C and until 1% strain at 37 °C (data not shown). Temperature sweep tests showed similar rheological trends for all the tested hydrogels (Fig. 2a): in the initial temperature interval, G' and η^* slightly decrease until

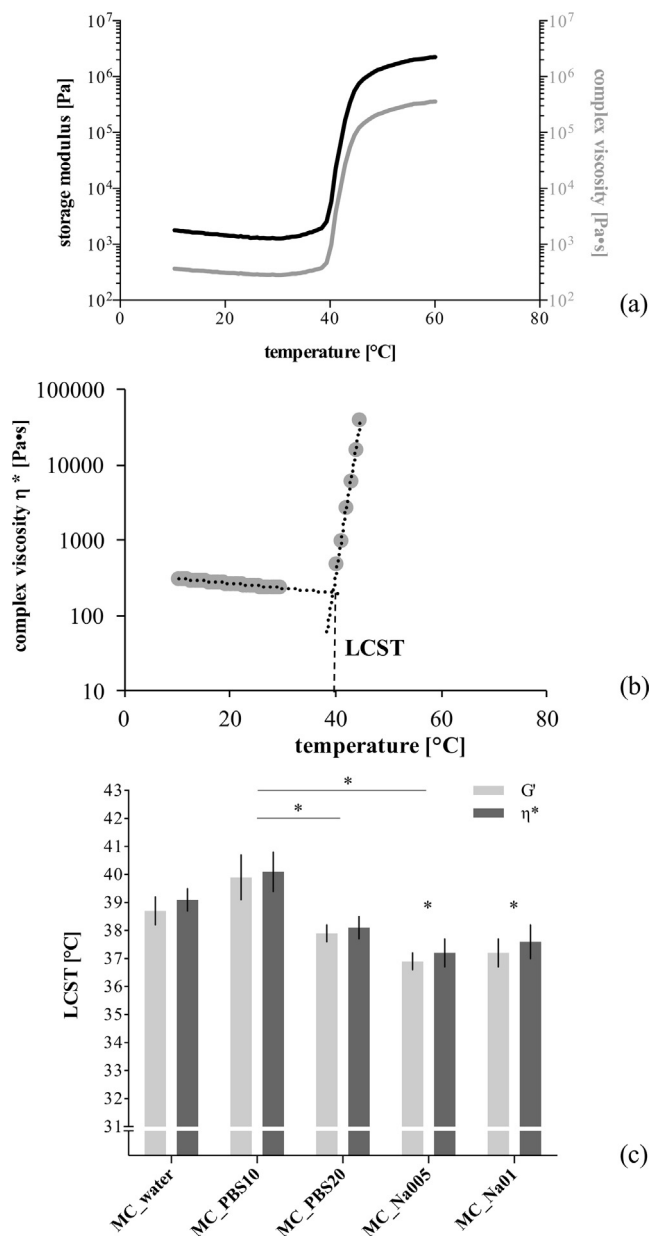


Fig. 2. (a) Representative temperature dependence of storage modulus, G' , and complex viscosity, η^* , of methylcellulose hydrogel (i.e., MC_water), (b) calculation of the LCST from the rheological curve and (c) transition temperatures (LCST) measured for hydrogels produced in different ions solutions ($p < 0.05$).

a temperature (specific for each formulation) at which both parameters dramatically increase, and finally reach a second temperature range where parameters are stable, as confirmed for lower concentrated MC hydrogels [9]. For all the formulations, G' was higher than G'' over all the tested T ranges, because of the relatively high MC concentration; thus, the LCST couldn't be identified, as commonly performed, as the intersection between G' and G'' [10]. Then, an alternative, more appropriate method was here proposed and used. LCST of MC hydrogels identified by rheology with this method (Table1) are shown in Fig. 2b. No statistical differences ($p > 0.05$) were detected comparing the LCST obtained from G' and η^* curves, considering the same hydrogel formulation. The addition of low salt concentrations (i.e., MC_PBS10 and MC_Na005) did not influence the LCST compared to the LCST of MC_water. Instead, the addition of higher salt concentrations decreased the LCST; in fact, statistical differences ($p < 0.05$) were detected

comparing the LCST obtained from η^* curves of MC_PBS20 and MC_Na01 vs. MC_water (i.e., 38.1 ± 0.4 and 37.6 ± 0.6 vs. 39.1 ± 0.4 °C, respectively). No differences ($p > 0.05$) were identified comparing the LCST of the two hydrogels produced with the higher salt concentrations (i.e., MC_PBS20 vs. MC_Na01) one to the other.

UV tests showed an increase in the MC hydrogel absorbance with increasing temperature (Fig. 3a), due to the formation of hydrophobic agglomerates (gel-state) promoting light scattering [7]. The trends of T% with increasing temperature of all hydrogels (Fig. 3a) are characterized by stable values at low temperature followed by a dramatic decrease at higher temperatures. The LCST identified for the MC hydrogels (Table1) by transmittance tests

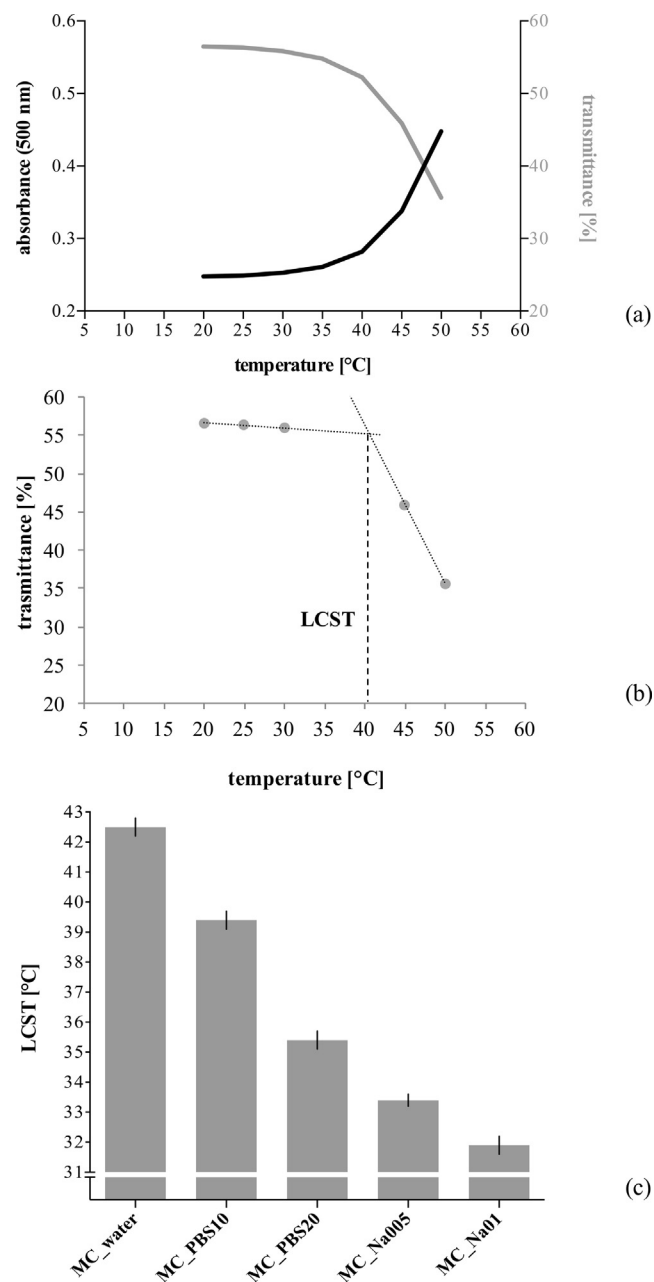


Fig. 3. (a) Representative temperature dependence of absorbance and transmittance of methylcellulose hydrogel at 500 nm (i.e., MC_water), (b) calculation of the LCST extrapolated from transmittance curves and (c) transition temperatures (LCST) measured for hydrogels produced in different ions solutions ($p < 0.05$ for all comparisons).

are shown in Fig. 3c. Statistical difference ($p < 0.05$) was detected comparing the LCST of all the hydrogels. All hydrogels produced with salt ions showed lower LCST compared to MC_water, thus confirming the involvement of salts in decreasing LCST. Moreover, comparing the LCST of hydrogels produced with the same salt at different concentration (*i.e.*, MC_PBS10 vs. MC_PBS20, MC_Na005 vs. MC_Na01), it is clear how higher salt concentrations cause a major decrease in the LCST, according to data obtained for less concentrated MC hydrogels [11]. Finally, hydrogels produced with sodium sulfate (*i.e.*, MC_Na005 and MC_Na01) showed lower LCST compared to hydrogels produced with PBS (*i.e.*, MC_PBS10 and MC_PBS20). LCST obtained from UV-spectroscopy are lower than those obtained from rheological analysis; in fact, during MC gelation process, reduction of hydrogels transmittance (caused by the formation of hydrophobic aggregates) begins before the increase of G' and η^* (caused by the interconnection of these aggregates).

4. Conclusion

The thermo-responsive properties of methylcellulose hydrogels 8% w/v were characterized by investigating the effects of two ion solutions (*i.e.*, sodium sulfate and PBS) at different concentrations. The LCST of the hydrogel formulations were identified both by UV spectroscopy and rheological tests; in particular, we propose an efficient method to determine the LCST by rheological measurements for hydrogels with predominant storage modulus (*i.e.*, $G' > G''$), when the intersection between G' and G'' is not detectable. The salts addition clearly lowered the LCST of the MC hydrogel formulations; MC_PBS20, MC_Na005 and MC_Na01 showed a LCST around or below 37 °C, optimal for culturing cells at 37 °C and

switching the substrate hydrophilicity by lowering the temperature to obtain cell sheets.

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