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Thermogelling behaviour of PEG-enclatherated Methylcellulose/Alginate sols

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Abstract

Thermogelling systems offer several advantages over conventional cross-linked hydrogels with respect to showing stimuli responsiveness and reversibility. They also offer functional and fabrication flexibility in terms of the design, injectability, and performance control offered by the in situ phase transition at various temperatures. Once gelled at the site of application or after injection, thermogels provide passive targeting with increased efficacy and decreased toxicity of the incorporated drugs. In this research, we describe methylcellulose-based thermogels blended with sodium alginate and enclatherated with poly(ethylene glycol) 400 (PEG400). The concentration of methylcellulose (MC) was kept constant at 4.0% w/v, while that of sodium alginate (ALG) varied from 0.5 to 2.0% w/v. Clear sols were obtained and PEG400 were added (1 ml or 2 ml/10 ml of sol) to obtain final thermogels. The triplymeric blend displayed hydrogen bonded O–H…C=O which was not evident in the bipolymeric systems (FTIR analysis). The DSC analysis corroborated the above with the absence of MC exotherm in triplymeric blend formulations and the hijacking of MC-ALG interaction by the enclatherated PEG chains. The temperature ramp rheological profiling of the thermogels (20 °C–50 °C) revealed an increase in elastic modulus of M4A20P2 (sol with highest weight content) due to PEG400 interactions with ALG which in turn interacted with MC forming a self-assembled repeating network structure: PEG400-ALG{hydrophilicMChydrophobic}{hydrophobicMChydrophilic}-ALG-PEG400. With MC forming the hydrophobic or dehydrated part of the thermogel, ALG and PEG chains concentrated in the aqueous medium and were compressed from both side by the stiff MC architecture creating ‘intra- and inter-network stress’ induced polymer-polymer interactions. In conclusion, MC-ALG-PEG formed as complex thermoresponsive system showing physical restructuring induced chemical stabilization (PRICS) and can be employed in various biomedical applications.

1. Introduction

Stimuli responsive polymers or composite hydrogels form an inherent part of intelligent drug delivery and biomaterial classification systems [1]. They are unique in their ability to respond to the surrounding biological environment or induced external physical stimuli; and may effectively perform their biomedical function in response to pH, temperature, glucose, ions, electric or magnetic fields [1, 2]. Their response time and matrix strength vary with the ‘extent’ of applied stimuli and hence may show an on-off mechanism (responsiveness and reversibility) thereby leading to variability in response [3–5]. Temperature sensitive or thermoresponsive systems are usually devoid of this on-off functionality under in vivo conditions as the sol-to-gel or gel-to-sol transitions are initiated almost immediately after injection or insertion into the body [6, 7].

The thermo-responsive, -sensitive, and -reversible sol-to-gel transitioning systems (from here on referred to as thermogels) have been extensively investigated in the past with polymers and polymeric composites such as pluronics or ploxamers, methycellulose, chitosan-β-glycerophosphate, poly(N-isopropylacrylamide), and
amphiphilic poly(ethylene glycol)/polyhydroxy acids block copolymers; as well as their hybrids being widely explored and used [6, 7]. There are several mechanisms that have been proposed for the thermoresponsive or thermosensitive behaviour of these complex system such as, but not limited to, dehydration of the sol leading to solidification of the dispersion, interchain interactions arising from attraction and repulsion, reduced mobility of the polymeric chains, formation and concentration of hydrophobic but stable and reversible cross-linked networks, entropy changes and lattice restructuring, and chain collapse followed by polymer aggregation and scattering [6–8]. There are several approaches that can be employed to customize and design a Thermointelligent Matrix System (TIMS) and essentially involve varying the material composition by blending with another thermosensitive polymer or simply a matrix strengthening polymer; increasing the molecular weight of the polymer thereby increasing the gel strength, or increasing the polymer or crosslinker concentration [9, 10].

Methylcellulose (MC), a United States Food and Drug Administration’s Generally Regarded As Safe (US FDA GRAS) listed excipient, is a cellulose derivative synthesized by heterogeneous and partial substitution of hydroxyl functional group of the anhydroglucose repeat units (AGU) at C2/C3/C6 positions with methyl (−CH3) moieties [6, 8]. This substitution converts the otherwise insoluble cellulose biomacromolecule to an aqueous soluble polymer with low viscosity and thermoreversibility [11]. Since the discovery of thermoresponsive behaviour of MC in 1936 [12], several thermogelation mechanisms have been proposed [6, 8] such as heat induced dehydration-co-precipitation of the sol, glucose units with methyl functionality forming ‘reversible crosslinking loci’ leading to crystalline sequences [13], hydrophobic clustering at low temperatures converted into hydrophobically cross-linked networks at high temperatures [14], lattice restructuring at high temperatures causing phase separation [15, 16], and fibriller aggregation minimizing the water-polymer interactions [8, 17]. However, the exact thermogelling mechanisms are still unclear, particularly at high concentrations [18].

The gel strength of MC thermogels can be increased with an increase in polymer concentration (2%–12% w/v) as well as by addition of salts (salting-out effect), further chemical functionalization, and addition of chemical crosslinkers [8, 19]. Most of these strategies involve chemical interventions and physical approaches for gelation modification are still outstanding. This research work explores the potential chemical stabilization of a thermogelling system through physical restructuring with the addition of well-established polymers. In this study, a three-component gelling system comprising methylcellulose, alginate and PEG400 was systematically studied to elucidate the possible mechanisms of thermogelation, and the role of alginate and PEG400 in modifying the gel structure of methylcellulose. It is envisaged that the addition of sodium alginate (a watersoluble biomacromolecule obtained from brown seaweed) to clear MC thermogels (10 °C; 4% w/v) may increase its gel strength as well as lower the gelation temperature. Further to this, the effect of PEG400 enclatheration on the gelation strength and temperature of the MC-ALG bipolymeric blend has been explored. The physicochemical profiling of the tripolymeric blends was performed using FTIR and DSC while the physicomechanical properties were determined using rheological analyses under various temperature and stress conditions.

2. Materials and methods

2.1. Materials
Alginic acid sodium salt (ALG; viscosity 15–20,000 cps), methylcellulose (MC; Methocel® MC; 27.5%–32% as methoxyl), and polyethylene glycol 400 (PEG400) were supplied by Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade and were used as received.

2.2. Preparation of methylcellulose-alginate-poly(ethylene glycol) (MAP) thermogel
The MAP thermogel was prepared by simply dissolving specified quantities of methylcellulose and sodium alginate in deionized water followed by the addition of poly(ethylene glycol) as per the concentrations and codes provided in table 1. Methylcellulose concentration was kept constant at 4% w/v, to obtain clear sols at 10 °C and as employed in previous studies [19, 20]. Sodium alginate concentration varied from 0.5 to 2% w/v, with the sols in upper concentration values retaining some degree of optical clarity. PEG was added at quantities of 1 and 2 ml per 10 ml of the polymer solution. Molecular complexation and interaction profile of the three component system in close geometrical placment was determined using molecular mechanics simulations (static lattice atomistic simulation) using a default MM+ force field and a Polak–Ribiere Conjugate Gradient (RMS gradient of 0.001 kcal mol−1) as described elsewhere [21].

2.3. Polymeric structural variation analysis
The structural interactions and variations within the developed thermogels were assessed using Attenuated Total Reflectance–FTIR (ATR-FTIR) analysis. The FTIR analysis was performed on the native as procured polymers
and thermogels were lyophilized to compare the structural transformations. The thermogels were heated to their
gelling temperature in glass vials followed by snap freezing in liquid nitrogen. The frozen samples were then
lyophilized (FreeZone 2.5, Labconco, Kansas City, MS, USA) at 25 mtorr for 24 h at −42 °C. ATR-FTIR spectra
were recorded on a Perkin Elmer Spectrum 2000 FTIR spectrometer with a MIRTGS detector (PerkinElmer
Spectrum 100, Llantrisant, Wales, UK) within wavenumber range of 650–4000 cm\(^{-1}\) and a resolution of 4 cm\(^{-1}\).

2.4. Thermal mapping of the lyophilized thermogels
Differential Scanning Calorimetry (DSC) analysis was performed on the native as procured polymers and the
lyophilized thermogels on a Mettler Toledo, DSC1, STARe System (Schwerzenback, Switzerland) at a heating
rate of 10 °C min\(^{-1}\) from 10 to 300 °C under a constant flow of N\(_2\) gas. Indium metal (99.99%) was used to
calebrate the DSC modulus in relation to temperature and enthalpy while an empty sample holder was used as
reference.

2.5. Rheological measurements of constituent polymer blend solutions
Rheometric analyses involving oscillatory temperature and frequency sweeps were conducted for the thermogels
in order to assess their thermoresponsive behaviour over a temperature range as well as the mechanical
transitions inherent within the polymeric solution phase over a frequency range \([22]\). A cone-and-plate Haake
MARS (Modular Advanced Rheometer System) rheometer (Thermo Electron Corporation, Karlsruhe, Germany)
with cone diameter of 35 mm, cone angle = 1° (sensor C35/1°) Ti, and cone/plate distance of 0.59
mm was employed. For MAP thermogels, the temperature sweeps were conducted over a temperature range of
20 °C–50 °C at constant stress and frequency values of 1 Pa and 1 Hz, respectively (as calculated from the yield
stress test). The oscillation frequency sweep analyses were conducted over a frequency range of 10–0.01 Hz with
a constant stress value of 1 Pa at 25 °C, 37 °C, and 45 °C. RheoWin PC Software.v3 was employed for data
collection and analysis, and directly plotted from the equipment.

3. Results and discussion
Thermo-sensitive, -responsive, or -reversible polymeric systems provide a unique opporuntity for biomedical
applications given their flexibility with fabrication and function \([6]\). Once an appropriate polymeric platform is
obtained, the function of thermogels can be effectively controlled by varying the concentration of the solid
contents, the phase transition temperature, and even employing an external stimulation to affect the gelation
in situ and in vivo. The flexibility of design is however restricted by the scarcity of such systems as well as the
limitations in achieving the desired mechanical properties and hence requiring combination strategies \([7]\). This
research is focused on strengthening a methylcellulose based thermo-intelligent platform by employing several
strategies such as varying polymer/biomial material composition by the addition of alginate and PEG400 in blend.

| Formulation Code | Methyelcellulose (%w/v) | Sodium Alginate (%w/v) | PEG400 (per 10 ml of polymer solution) |
|------------------|-------------------------|------------------------|----------------------------------------|
| M4A00P0          | 4.0                     | 0.0                    | 0.0                                    |
| M4A05P0          | 4.0                     | 0.5                    | 0.0                                    |
| M4A10P0          | 4.0                     | 1.0                    | 0.0                                    |
| M4A15P0          | 4.0                     | 1.5                    | 0.0                                    |
| M4A20P0          | 4.0                     | 2.0                    | 0.0                                    |
| M4A00P1          | 4.0                     | 0.0                    | 1.0                                    |
| M4A05P1          | 4.0                     | 0.5                    | 1.0                                    |
| M4A10P1          | 4.0                     | 1.0                    | 1.0                                    |
| M4A15P1          | 4.0                     | 1.5                    | 1.0                                    |
| M4A20P1          | 4.0                     | 2.0                    | 1.0                                    |
| M4A00P2          | 4.0                     | 0.0                    | 2.0                                    |
| M4A05P2          | 4.0                     | 0.5                    | 2.0                                    |
| M4A10P2          | 4.0                     | 1.0                    | 2.0                                    |
| M4A15P2          | 4.0                     | 1.5                    | 2.0                                    |
| M4A20P2          | 4.0                     | 2.0                    | 2.0                                    |
form, using a solid (alginate) and a liquid (PEG) adjuvant, and varying added polymer concentrations to essentially lower the critical solution temperature and to potentially elucidate a mechanism for the same.

3.1. Investigation of polymeric structural transitions

3.1.1. FTIR analysis of pristine polymer components

Sodium alginate showed major representative peaks and bands at 3700–3000 cm$^{-1}$ (broad) O–H stretch, 3000–2850 cm$^{-1}$ C–H stretch, 1081–1027 cm$^{-1}$ antisymmetric C–O–C stretch, 1620 cm$^{-1}$ asymmetric – COOH stretch, and 1416 cm$^{-1}$ asymmetric –COOH stretch. In addition, the bands around 1320 cm$^{-1}$ (C–O stretching), 1130 cm$^{-1}$ (C–C stretching), 1090 cm$^{-1}$ (C–O stretching), 1020 cm$^{-1}$ (C–O–C stretching), and 950 cm$^{-1}$ (C–O stretching) were attributed to the skeletal vibrations of the saccharide structure [23, 24]. For the MC molecule, the major peaks and bands appeared at 3469 cm$^{-1}$ –OH stretching vibration), 2921 cm$^{-1}$ (symmetric C–H stretch corresponding to the cellulose pyranoid ring), 2837 cm$^{-1}$ (C–H stretch in methyl ether), 1641 cm$^{-1}$ (C–H bending), 1081 cm$^{-1}$ (C–O–C stretch of glucosidic units), and 951 cm$^{-1}$ (–OCH3 group) [18]. The major peaks and bands in the case of PEG were attributed to C–O–C ether antisymmetric stretch (1100 and 1300 cm$^{-1}$), –CH$_2$ stretching vibrations (2960 and 2869 cm$^{-1}$), alkyl CH stretching (2882 cm$^{-1}$), and alkyl CH deformation (1457, 1352 1298, and 1250 cm$^{-1}$) [25] (scans not shown).

3.1.2. FTIR analysis of the MAP thermogels

To closely assess the inherent polymeric transitions among the blend components, the formulations were snap frozen in liquid nitrogen after equilibration at the respective thermogelation temperatures for 10 min and then subjected to lyophilization. Figure 1 represents the FTIR spectra of all 15 formulations and are classified as follows:

MC-ALG blends (M4A05P0, M4A10P0, M4A15P0, and M4A20P0): The MC-ALG blends demonstrated characteristic properties of a polysaccharide blend. The characteristic MC peak at 1642 cm$^{-1}$ merged with the much sharper peak of alginate forming a common peak at 1610 cm$^{-1}$ (M4A05P0) to 1603 cm$^{-1}$ (M4A20P0), moving closer to 1594 cm$^{-1}$ ALG peak with an increase in ALG concentration. Similar results were obtained for other overlapping bands such as O–H band wherein an increase in ALG concentration lead to peak band shift towards corresponding ALG wavenumber [26–28].

MC-PEG blends (M4A00P1 and M4A00P2): The addition of PEG to MC showed no major changes in terms of vibrational transitions except the C–O–C stretching at 1048 cm$^{-1}$ for MC and 1094 cm$^{-1}$ for PEG merged together to form a common band around 1058 cm$^{-1}$. No significant evidence related to H-bonding within M4A00P1 and M4A00P2 was located as explained in an earlier study by Turhan et al., 2001 where the shape of O–H band showed no significant distortion [29]. Thus, it can be concluded that the PEG chains do not essentially show physicochemical interactions with the MC polymeric chains. There have been conflicting reports with respect to the compatibility of PEG and methylcellulose wherein both incompatibilities (presence of two phases) and compatibilities (transparent systems up to 30% concentration of PEG in MC) [30–32]. In the present study, no solid–liquid phase separation was observed for the formulations tested at 10°C. However, cloudiness of the samples increased with an increase in PEG content as well as with an increase in temperature.

MC-ALG-PEG blends: The MC-ALG-PEG blends demonstrated all FTIR peaks and bands corresponding to MC-ALG and MC-PEG blends. However, the most striking feature of MC-ALG-PEG blends was the appearance of a new band at 1737 cm$^{-1}$ which was not present in either of the pristine polymer scans and the polymer blends. This band can be assigned to the protonation of sodium alginate to alginic acid or to an ester formation or to the hydrogen bonded –C = O. The possibility of formation of an ester can be ruled out, as no acidic or basic conditions were present for esterification. The possibility of protonation of sodium alginate can also be discarded as the blend was not acidic in nature as well as due to the fact that the sodium alginate peaks around 1600 and 1400 cm$^{-1}$ (asymmetric and symmetric stretching peaks of carboxylate salt groups) were still present in the spectrum. In fact, the intensity of these bands decreased with an increase in PEG content at a constant sodium alginate concentration.

Interestingly, the corresponding band at 1610 cm$^{-1}$ in M4A05P0 (MC-PEG blend) shifted to 1634 cm$^{-1}$ in M4A05P1 and to 1642 cm$^{-1}$ in M4A05P2—moving closer to the 1643 cm$^{-1}$ C–H band of MC which was otherwise overlapped by the 1594 cm$^{-1}$ band of alginate in MC-ALG only formulations. This further provides confirmation of the involvement of PEG and sodium alginate in the appearance of the band at 1737 cm$^{-1}$. Additionally, this implied that PEG acted as a plasticizer breaking the intra- and intermolecular interactions in and between the polysaccharide chains, respectively.

From the above discussion, it can be speculated that the PEG chains and sodium alginate together formed a hydrogen bonded O–H…C = O giving a sharp band at 1737 cm$^{-1}$. In order to prove the ALG-PEG interaction, FTIR analysis was performed on lyophilized M0A20P2 (sample formulation with no methylcellulose). Surprisingly, no 1737 cm$^{-1}$ band appeared in the spectrum of ALG-PEG blend (scan not shown). This implied...
that the hydrogen bonded O-H...C = O only formed in the triopolymeric blend while the bipolymeric blends showing no such interaction. This meant that there is a possibility of hydrogen bonded O-H...C = O between MC and ALG. The appearance of the MC-ALG vibrational bands were observed again with further addition of sodium alginate - the 1594 cm⁻¹ peak of alginate overlapping the MC peak at 1642 forming a common intermediate peak. Furthermore, at a given PEG content, the intensity of the band at 1737 cm⁻¹ increased with an increase in sodium alginate concentration and vice versa confirming the potential involvement of MC, PEG.

Figure 1. FTIR spectra of lyophilized MAP thermogels.
and sodium alginate in the appearance of this band. The mechanism of formation of this bond is explained under the rheological analysis of the various formulations.

3.2. DSC analysis
The DSC thermograms related to various blend formulations are presented in figure 2. The endothermic peak appearing between 89 °C and 120 °C can be assigned to evaporation of water from the polysaccharide molecules. To assess the thermal properties of the thermoresponsive formulations, one major exothermic peak each in MC and ALG was focused on and following observations were made:

1. MC exotherm at 194 °C: The exotherm observed in MC only formulation (M4A00P0) can be assigned to the glass transition temperature of MC. Park and Ruckenstein (2001) reported that due to the plasticization effect of PEG400 on MC, the exothermic peak around 200 °C shifted to lower temperatures with an increase in wt% of PEG400 in MC films with the peak almost flattened at 24 wt% [33]. In the current study, 1.13 g and 2.26 g of PEG400 was added to 400 mg of MC, which is much more than the already reported studies on

![Figure 2. DSC thermograms of lyophilized MAP thermogels.](image)
MC plasticization employing PEG. Therefore, the exotherm completely disappeared from the thermal profile of MC after addition of PEG400 at all reported concentrations. In the case of MC-PEG blends, with an increase in ALG concentration, the MC exotherm shifted towards higher temperatures from 194 °C to 197 °C with a decrease in enthalpy from 3.87 to 1.70 J g⁻¹, respectively. This increased thermal stability of MC after ALG addition could be attributed to the formation of an H-bonding interaction between MC and ALG, as explained under FTIR and rheological analyses of the blend formulations. No MC exotherm was reported in MC-ALG-PEG blend formulations.

2. ALG exotherm at 238 °C: The exotherm observed in ALG at 238 °C was assigned to its decomposition temperature (scan not shown). In the case of MC-ALG formulations, this exotherm appeared at higher temperatures reaching 243 °C in M4A05P0. However, further increase in ALG concentration showed no significant increase in the peak value with (244 °C for M4A20P0). This increased thermal stability of ALG in presence of MC could be attributed to the formation of an H-bonding interaction between MC and ALG, as explained under FTIR and rheological analyses of the blend formulations. The addition of PEG to MC-ALG blends led to a lowering of the decomposition temperature due to potential plasticization by PEG400 thereby decreasing the interchain interactions and increasing the flexibility of the ALG network. Interestingly, in the case of M4A05P2, the ALG exotherm temperature was observed at 238 °C, which was equal to native ALG exotherm temperature—nullifying the role of MC interactions in raising the exotherm temperature. This observation corroborates perfectly with the FTIR discussion related to PEG hijacking the MC-ALG interaction in M4A05P2. However, the MC-ALG interaction appeared again in further formulations with increased amount of ALG forming H-bonding with MC and hence a relative increase in exotherm temperature was reported. This further is in line with the FTIR analysis wherein the essentiality of all the three polymers (involvement of MC, PEG and ALG) in formation of this complex thermoresponsive system was proposed.

3.3. Rheological analysis

The photomicrographs of the thermogelling behaviour of the MAP formulations are depicted in figure 3. The sol-gel phase transition profiles for the various thermogels are described under the subsections below and are referred to figure 3 where applicable.

3.3.1. Temperature sweep analysis

The temperature sweep analysis for the 15 thermoresponsive is presented in figures 4–8 and were classified into following formulations groups:

- MC only: M4A00P0
  - The MC only formulation containing 4% methylcellulose showed a G″/G′ crossover at 47 °C (figure 4). When kept at the body temperature (37 °C), M4A00P0 formed a very weak, fairly transparent gel unable to hold its weight and appeared to flow when tilted to one side (figure 3).

- MC-ALG blends: M4A05P0, M4A10P0, M4A15P0 and M4A20P0
  - With the addition of alginate to M4A00P0, the gelation temperature decreased considerably reaching a minimum of 40 °C in the case of M4A20P0 (figure 8). The strength of the gel also increased significantly with G′ reaching a maximum of 5 Pa for M4A20P0. Additionally, the elastic modulus in the case of M4A20P0 appeared at the beginning of the rheogram at 20 °C. None of the gels among the MC-ALG blends were able to hold their own weight at 37 °C and were fluid to semi-solid at 37 °C (figure 3). The increase in the gel strength for the MC-ALG blends as compared to MC alone can be attributed to increased polysaccharide content in the gel as well as due to the H-bonding formation between the hydrophilic moieties of MC and ALG as predicted under FTIR and DSC analyses. The relative decrease in the gelation temperature with the addition of alginate can be attributed to the salting-out effect of the Na⁺ ions as well as the anionic salt-like nature of the alginate moiety. These results are in line with the earlier reports wherein both anionic and cationic salts decreased MC’s gelation temperature due to their comparatively higher and competitive water absorbing characteristics as compared to MC thereby enhancing the dehydration of the MC network [26, 34, 35].

- MC-PEG blends: M4A00P1 and M4A00P2.
  - The addition of PEG400 at 1 ml and 2 ml to 10 ml M4A00P0 produced gels with significantly lowered gelation temperature with an approximate reduction of 6 °C per 1 ml addition of PEG400. M4A00P1 and M4A00P2 demonstrated G′/G″ crossover at 41 °C and 35 °C, respectively (figure 4), and M4A00P2 was able to hold its weight at 37 °C. The lowering of gelation temperature due to addition of PEG400 can be based on the theory that at very high concentrations, plasticizers significantly disrupt the polymer-water interactions leading to the formation of concentrated hydrophobic regions within MC network and hence lowering the temperature required for phase transition [36]. PEG400, being a superhydrophillic polymer with a partition coefficient of...
1.5 × 10⁻³ [37], was capable of absorbing large amount of water molecules forming, causing and enhancing the phase separation between MC and the aqueous system. Since the polymers were concentrated in their respective mediums, such super-separated system ruled out the formation of an interaction between the MC and PEG400 chains as predicted in the FTIR analysis. However, the possibility of the PEG400 chains in between MC hydrophobic fibriller structure cannot be ruled out as with the disappearance of Tg exotherm of MC after PEG400 addition reported earlier in this study. This further implied that while the methyl domains of MC formed the hydrophobic phase, the hydrophilic -OH functionalities concentrated within the PEG400-water layer thereby maintaining an intact thermogel system. It is noteworthy that the value of elastic modulus at the G''/G' crossover in the MC-PEG blends remained within the MC crossover range confirming the non-involvement of PEG400 in the solid-like network formation (but rather promote the formation of the MC thermogel at lower temperatures by efficiently absorbing the water and providing flexibility to the network) and hence an interaction possibility can be ruled out as observed in FTIR analysis.

**Figure 3.** Photographs depicting the gelation profile of Methylcellulose–Alginate–Poly(ethylene glycol) injectable system at 10 °C and 37 °C.
MC-ALG-PEG blends: M4A05P1, M4A05P2, M4A10P1, M4A10P2, M4A15P1, M4A10P2, M4A20P1 and M4A20P2.

In this study, eight (08) formulations constituting the tripolymeric architecture were tested (figures 5–8). With MC being constant, the rheological data were discussed w.r.t. addition of the third component keeping the second component constant.

At a constant PEG amount, the addition of sodium alginate led to a decrease in gelation temperature as observed in the case of MC-ALG blends except for M4A05P1 and M4A05P2 (figure 5) which were the odd-ones-out again (see FTIR and DSC results). The gelation temperature in the case of M4A05P1 was equivalent to MC Figure 4.

**Figure 4.** Rheograms representing the viscoelastic sol-to-gel conversion of (a) M4A00P0; (b) M4A00P1; and (c) M4A00P2 under a temperature range of 20°C–50°C. Color codes: $G'$ (storage modulus)—blue; $G''$ (viscous modulus)—red; $f^*$ (complex viscosity)—green.

MC-ALG-PEG blends: M4A05P1, M4A05P2, M4A10P1, M4A10P2, M4A15P1, M4A10P2, M4A20P1 and M4A20P2.

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At a constant PEG amount, the addition of sodium alginate led to a decrease in gelation temperature as observed in the case of MC-ALG blends except for M4A05P1 and M4A05P2 (figure 5) which were the odd-ones-out again (see FTIR and DSC results). The gelation temperature in the case of M4A05P1 was equivalent to MC
only thermogel while M4A05P2 showed no effect of addition of alginate to the MC-PEG blend (M4A00P2). This means that in the case of M4A05P1, the functional groups in PEG (1 ml/10 ml) and ALG (0.5%w/v) interact with each other at perfect molar ratios making their addition to MC sol negligible. This observation was further proven in M4A05P2 (figure 5), where the effect of ALG (0.5%w/v) and 1 ml PEG were nullified leaving 1 ml PEG with in the MC sol and hence rheological data equivalent to the formulation M4A00P1 was obtained. This validated the perfect corroborations of our physicochemical and physicomechanical results.

At a constant ALG concentration, given the observations made in MC-PEG blends, one would expect a decrease in gelation temperature with the addition of PEG while the value of elastic modulus remaining
unchanged. As expected, the gelation temperature of MC-ALG decreased with the addition of PEG reaching 32 °C in the case of M4A20P2 (figure 8). However, the magnitude of elastic modulus significantly increased with addition of 1 ml PEG and further increased with further addition of PEG (2 ml). This is in contrast with the elastic modulus observations made in MC-PEG blends. This increase in modulus reaching more than 10Pa in M4A20P2 can be attributed to PEG400 interactions with ALG which in turn interacted with MC forming following self-assembled repeating network structure:

\[ \text{PEG400-ALG-} \{ \text{hydrophilicMC-hydrophobic} \} - \{ \text{hydrophobicMC-hydrophilic} \} - \text{ALG-PEG400}. \]

Since MC formed the hydrophobic/dehydrated part of the thermogel, the alginate and PEG chains concentrated in the aqueous

![Figure 6](image_url)

**Figure 6.** Rheograms representing the viscoelastic sol-to-gel conversion of (a) M4A10P0; (b) M4A10P1; and (c) M4A10P2 under a temperature range of 20 °C–50 °C. Color codes: \( G' \) (storage modulus) — blue; \( G'' \) (viscous modulus) — red; \( f' \) (complex viscosity) — green.
medium were compressed from both side by the stiff MC architecture and release of the aqueous medium on the periphery (figure 9). This might have caused the overlapping of van der Waals radii of PEG400/ALG and ALG/MC leading to the formation of hydrogen bonded OH…C = O within MC-ALG-PEG blends. These ‘intra- and inter-network stress’ induced polymer-polymer interaction explained the appearance of a strong vibrational peak at 1737 cm$^{-1}$ in FTIR spectra of triplymeric formulations and not among the bipolymeric formulations. These interactions were further evident from the fact that the decrease in gelation temperature was 6 °C per 1 ml addition of PEG400 in the respective formulations. To further visualize the molecular complexation and geometry, molecular mechanics simulations were conducted and are shown in figure 9 (ChemLite 3.0 Molecular

Figure 7. Rheograms representing the viscoelastic sol-to-gel conversion of (a) M4A15P0; (b) M4A15P1; and (c) M4A15P2 under a temperature range of 20°C–50 °C. Color codes: G’ (storage modulus)—blue; G″ (viscous modulus)—red; $\gamma$ (complex viscosity)—green.
Modelling Software, Gainsville, USA) [38]. The tripolymeric structure was stabilized with an energy minimization of $-32.572 \text{ kcal mol}^{-1}$ ($\Delta E$; total energy) with van der Waals contributions ($\Delta E = -13.874$) and electrostatic interactions ($\Delta E = -15.277$) majorly contributing to the energetic and geometric stabilization of the matrix (table 2). A close look at the proposed schematic in figure 9 depicts the potential van der Walls overlaps and the preferential placement of ALG and PEG within the MC chains.

It is evident from rheograms for formulations M4A00P0, M4A00P1, and M4A00P2 that addition of polyethylene glycol enhanced the thermogelling capacity of methylcellulose solution by decreasing the thermogelling temperature from 47 °C to 35 °C—other formulation variables being constant. Additionally, the
rheograms for formulations M4A00P2 (figure 4), M4A05P2 (figure 5), M4A10P2 (figure 6), M4A15P2 (figure 7), and M4A20P2 (figure 8) demonstrated the effect of addition of sodium alginate on the gel strength of methylcellulose gel by increasing the elastic modulus \( \log G' \) from 0.2 Pa to 6.0 Pa—other formulation variables being constant. This confirms the physiological thermogelling capability of methylcellulose-alginate-poly(ethylene glycol) at a temperature as low as 30°C while increasing the gel strength by a factor of 50.

### 3.3.2. Oscillation frequency sweep

Oscillation frequency sweep analysis compares and characterizes the material properties and structures within the tested sol-gel systems. The elastic (or storage, \( G' \)) or viscous (or loss, \( G'' \)) moduli can be used to determine the structure of the inherent three-dimensional network within the MAP thermogels as: liquid (sol), solid (gel), or even colloidal state. The rheograms depicted in figure 10 display the elastic and loss moduli in response to increasing frequency under a constant stress.

### MAP thermogels at 25°C

At 25°C, the thermogels with no PEG or lower PEG (1 ml) showed primarily viscous behaviour with \( G'' > G' \) in all cases and no crossover observed at all frequency values. At higher PEG (2 ml) values; the rheograms showed appearance of a viscoelastic phase with a \( G'/G'' \) crossover at low frequency values in all cases (figure 10). This is the first appearance of a sol-gel transition within the MAP thermogels, which might be due to the formation of a colloidal system [39].

### MAP thermogels at 37°C

The MAP thermogels showed varied viscoelastic profiles at this temperature. The thermogels with no PEG continued to show viscous behaviour with \( G'' > G' \) for all sols at all frequency values. With the addition on 1 ml PEG, a \( G'/G'' \) crossover was observed for all the five thermogels at higher frequency values. An increase in alginate concentration however, led to an earlier crossover which may be attributed to due to polymer chain

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**Table 2.** Molecular attributes and energy components inherent to the three component polymeric system.

|          | ALG | MC  | PEG | MAP | \( \Delta E \) |
|----------|-----|-----|-----|-----|----------------|
| Total energy | −8.896 | 18.907 | 12.722 | −9.839 | −32.572 |
| Bond energy | 1.450 | 0.842 | 0.344 | 2.68 | 0.044 |
| Angle energy | 12.841 | 5.803 | 4.108 | 23.316 | 0.564 |
| Dihedral energy | 19.232 | 10.300 | 5.473 | 29.272 | −5.733 |
| van der Waals energy | 7.331 | 1.959 | 2.796 | −1.788 | −13.874 |
| Electrostatic energy | −46.515 | 0.000 | 0.000 | −61.792 | −15.277 |

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Figure 9. Schematic visualization of the tripolymeric structure consisting of MC, ALG, and PEG.
relaxation. In formulations with 2 ml PEG, a complete gel system was observed with $G'' > G'$ for all five formulations with no crossovers across the entire frequency range (figure 10). This is in line with the data obtained from the temperature ramp analyses discussed earlier.

**MAP thermogels at 45°C**

The viscoelastic profile of the MAP thermogels at this temperature (higher than that of the body temperature) was conducted to elucidate the performance of the viscoelastic system under increased liquid loss. All 15 formulations showed gel-like structure with $G'' > G'$ with no crossovers under the entire frequency range (figure 10). The addition of PEG in these thermogels displayed irregular rheograms, which may be attributed to collapse of gel structure, but still no flow was observed [40].

### 4. Conclusion and future prospects

A very interesting tri-component thermogel has been reported here as a simple blend comprising methylcellulose, sodium alginate and poly(ethylene glycol). The thermogel solidified at physiological temperature confining its potential applicability in vivo. The outstanding feature of MAP thermogels was the formation of hydrogen bonded O–H…C = O which only formed in the triopolymeric blend while the bipolymeric blends showing no such interaction. It is proposed that the MAP thermogel self-assembled into a repeating network structure represented by $\text{PEG400-ALG-}_{\text{hydrophillicMC}}_{\text{hydrophobic}}-\text{hydrophobicMC}_{\text{hydrophillic}}$-$\text{ALG-PEG400}$ and the physical ‘compression’ might have led to the formation of hydrogen bonded O–H…C = O among MC/alginate or PEG/alginate in the presence of PEG or MC, respectively. Based on the above observations, a new theory is proposed: Physical Restructuring Induced Chemical Stabilization (PRICS) theory, and will be tested in further studies. In terms of its biomedical applications, the MC-ALG-PEG thermogel can be employed for non-invasive tissue engineering applications such as in neurotrauma. The thermoreversible nature of the thermogel will allow for shape conformation, sodium alginate can further be cross-linked to customise the soft-to-hard mechanical properties, and PEG may act as a neuronal cell-membrane sealant. The tripolymeric thermogel presented in this

![Figure 10. Rheograms representing the oscillatory frequency ramp for various MC/ALG/PEG thermogels at various temperatures and under a frequency range of 10–0.01 Hz. Color codes: $G'$ (storage modulus)—blue; $G''$ (viscous modulus)—red; $\eta^*$ (complex viscosity)—green.](image-url)
study has potential to be used as a bioink for stem cell 3D bioprinting and soft tissue engineering with implications reaching to clinical applicability for neurotrauma interventions.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Conflict of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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