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Joshi, Sunil Chandrakant; Liang, C. M.; Lam, Yee Cheong

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Effect of Solvent State and Isothermal conditions on Gelation of MC Hydrogels

SUNIL C. JOSHI*, C.M. LIANG, Y.C. LAM
*School of Mechanical and Aerospace Engineering,
Nanyang Technological University, Nanyang Avenue,
Singapore 639 798*

Abstract— Methylcellulose (MC) and its derivative hydrogels are widely used in biomedical and pharmaceutical applications and the process of their gelation at near-body temperature is of research interest. In this studies, the thermal behavior of aqueous MC solutions prepared using cold de-ionized (DI) water was examined at a constant temperature of 50°C using Differential Scanning Calorimetry for two consecutive heating-cooling cycles. The experiments showed that the rate of gelation was faster for the solutions with higher MC concentrations. It was higher during the second heating-cooling cycle than that for the first cycle. The possible reasons behind this are investigated. Various MC solutions prepared using hot DI water were studied to understand the role of solvent state in the isothermal gelation process. The gelation of the MC solutions prepared using hot DI water commenced at lower MC concentrations and resulted in higher gelation rate. The possible gelation mechanism is discussed. Finally, a gel indexing method is proposed to provide a quantitative measure of the gelation states.

Keywords: Methyl cellulose; Hydrogel; Gelation; Micelle-like aggregate; Gel indices

* To whom correspondence should be addressed

Tel: (65)-6790 5954; Fax: (65) 6791 1859

Email: mscjoshi@ntu.edu.sg

INTRODUCTION

Hydrogels are polymer networks that can absorb or imbibe primarily water, thereby forming semi-rigid structures. Hydrogels can be classified into cross-linked gels and physical gels. Physical hydrogels are especially attractive for their use in the biomedical and pharmaceutical areas [1].

Methylcellulose (MC) is a type of thermo-reversible physical hydrogel. It is reported that MC can be favorably used as a viscous ultrasonic coupling medium for transdermal sonophoresis for insulin and vasopressin [1], for cultivation of fresh and frozen mouse bone marrow [2], building a tissue engineering scaffold in injured brain [3], fabricating a microfluidic gel valve for bio-application [4], and producing tablets for the release of drugs [5-7]. It is one of the major constituents in denture adhesive [8]. Also, it is found to help in genetic studies in *Paramecium* species to induce conjugation [9] and reducing drug solution drainage from the eyes of albino rabbits [10]. These various applications highlight the importance of MC as a biomaterial.

MC hydrogels exhibit thermo-reversibility, in that they undergo gelation upon heating and revert to the liquid phase upon cooling. At low temperatures, cage-like structures of water surround the hydrophobic methyl groups, the side groups to the main MC polymer chains. Heating causes the destruction of these water cages that result in the exposure of the hydrophobic regions of MC. In the presence of water, these hydrophobic groups tend to move closer leading to the formation of hydrophobic aggregates of MC. These aggregates join together to form a three-dimensional gel network involving changes in enthalpy, entropy and the Gibb's free energy [11-12]. Thus, the concentration of MC polymer in the solution, the state of solvent, and the temperature play key roles in the gelation process for MC hydrogels.

Different researchers have used different techniques in studying the sol-gel transformation in MC. Desbrieres et al. [13] used only calorimetry whereas Sarkar and Walker [14] used turbidimetric and microcalorimetric methods to study MC gelation. Haque and Morris [15] used differential scanning calorimeter (DSC), light transmission, rheological measurements, nuclear magnetic resonance (NMR). Katsuyoshi and others [16] used only DSC and rheology. In our earlier work, we also used [17] DSC, UV-spectrometry and viscosity measurements. It was noted that the observations based on different techniques were comparable and any one technique could provide pertinent information; the choice of method adopted depended on objective of the work. The DSC was observed to be the most common method used because the MC gelation is essentially a thermally sensitive hydrogel.

None of these studies, however, dealt with the isothermal environment and its part in MC gelation. The upward-looking trends of the rate of energy influx with time at isothermal conditions seen in Fig. 1, obtained from the data presented in our earlier publication [12], indicates the energy intake into MC solution even under hermitically sealed (in an air-tight) conditions in differential scanning calorimeter (DSC). Note that the temperatures 56°C and 64°C were selected intentionally for these DSC measurements because the peak gelation temperature fell within the range bound by these two values. Thus, it is necessary to examine and understand the reasons behind such energy absorption and its use in the gelation at constant temperature. Particularly, behaviour of MC hydrogels at near body temperatures is very important for bio-applications. The effects temperature of water used as solvent and its state on the gelation and thermo-reversibility of MC hydrogel also requires investigation.

In the work presented in this paper, gelation of MC by varying its concentration under isothermal conditions is studied. The MC solution was allowed to undergo two

cycles of heating and cooling. The physical appearance of the MC solutions was captured at various stages of gelation at constant temperature and subsequently used for the comparison of the thermal behaviour of MC gel. The effects of hot de-ionized (DI) water as solvent on the gelation are examined. The possible underlying mechanism is discussed. Subsequently, a gelation index ranging from 1 to 10 was proposed to assess the degree and state of gelation quantitatively.

MATERIALS AND METHODS

Materials

Methylcellulose (MC), purchased from Sigma-Aldrich as a white fine powder, was used in this work. It had a weight-average molecular weight of 40,000. The degree of substitution ranged from 1.6-1.9 and the viscosity for a 2% MC solution is 400 cps at 20°C. The MC powder was dried overnight at 60°C and stored in desiccator before use. Cold DI water was obtained from Millipore Alpha-Q water purifying system.

Preparation of MC hydrogels

The cold DI water was added to the pre-determined amount of dry MC powder until a 20-gram mixture was obtained. For MC solutions with hot DI water as solvent, the cold DI water was first heated to 95°C before adding to the MC powder. The hot-water/MC mix was allowed to cool down to some extent with the cap of the bottle on, but not fully tightened to provide escape route for gases. Subsequently the mixtures prepared with cold and hot water were stirred using magnetic stirrer (with the bottle lids still on) for a minimum of 24 hours at the controlled laboratory temperature of 22°C and the atmospheric pressure. Following that, the solutions were kept in a refrigerator at 4 °C for a minimum period of 1 day until a clear and transparent solution was obtained. Nine different concentrations (0.5, 0.75, 1.0, 1.25, 1.5, 1.75,

2.0, 2.25 and 2.5 wt %) of MC gel solutions were prepared using both, cold and hot DI water to obtain a representative spread and wide coverage in experimental observations. It was noticed that the gels get very thick beyond 2.5 wt% of MC.

Monitoring the gelation process

The steps shown in Fig. 2 were followed sequentially for monitoring the gelation process where each MC solution was taken through two gelation cycles. MC solutions with different concentrations in cold DI water were transferred to an oven with the bottle lids removed and their temperature was monitored continuously. The temperature was allowed to stabilize at a desired value. Digital images signifying the state of each MC solution were captured every ten-minute interval using a digital camera, once the set temperatures, i.e. 40, 50 and 60°C, as desired, were reached. The gelation process for each sample was visualized for two heating-cooling cycles. The MC gels after the first heating process were allowed to cool down naturally and kept in the refrigerator at 4 °C for 24 hours. The next heating-cooling cycle was followed after this under the same conditions as the first gelation cycle.

Microscopic analysis of gelation

Micro-structural changes during and after the gelation were examined using a confocal microscope (Axiotron 2, Zeiss) coupled with a Linkam hot plate (CSS 450, Linkam Scientific Instruments Ltd). The temperature of the hot plate was allowed to stabilize first at 60°C before loading the MC sample on to the plate. A glass slide was used to cover the sample so as to avoid water loss due to evaporation. The sol-gel transition was then continuously monitored until no further change in the microstructure could be observed.

Indexing for state of gelation

The images of the sol-gel transformations obtained at different time intervals were analyzed using Adobe Photoshop for examining the state of gelation under the chosen isothermal conditions. A care was taken to keep the sample bottles a similar position to minimize fluctuations in brightness and contrast. MC samples that attained the different gel states were indexed on the scale of 1 to 10. A grid of 1 mm size squares was created on the image of the gel container so that the same spot on image could be selected for consistency in analysis. The spot chosen for the analysis was in the middle at the coordinates (8mm, -3mm). The Adobe Photoshop software was used as the tool for analyzing the colour of the digital images of the MC solutions. The primary colour of the MC solutions was analyzed by breaking it into three basic colour components - red, blue and green. Each colour component had a value between 0 and 255. The value for each of the basic components is 0 for pure black while pure white yields a value of 255.

RESULTS AND DISCUSSION

Selection of temperature for isothermal gelation

To determine the range of near-body temperature for isothermal gelation of MC to occur, aqueous MC solutions were kept at constant temperatures of 40°C, 50°C, and 60°C. No gelation occurred at 40°C, even though the solutions were kept in the oven for more than an hour. For the samples at 60°C, gelation occurred to a high degree before the MC solutions reached the set temperature. The thermal gelation of MC under the isothermal conditions at 50°C was found to be occurring at the pace required for experimental observations. It may be noted that the gelation temperature can be brought down with the addition of certain human-body-compatible salts [11].

However, this approach was avoided so as help in understanding the solvent effects clearly and independently.

Study during two consecutive gelation cycles

Fig. 3 shows a typical temperature cycle used in the present studies. The observations were carried out only upon reaching the stable isothermal conditions. Each MC solution was allowed to go through two consecutive cycles. Some of our studies showed that behaviour of MC gels is different during the first two heating-cooling cycles. Beyond the 2nd cycle, the behaviour is consistent and the results are reproducible. For the first cycle, when the MC solutions reached 50°C, gelation was obvious only for MC concentrations of 1.75 wt % and above. However, gelation was seen for MC concentrations of 1.25 wt % and higher during the second cycle. Thus, the sol-gel transformations were noticeable for solutions with the MC concentration higher than 1 wt %. The rate of gelation (i.e. the speed at which the sol-gel transformation progressed and completed) for the second cycle was higher than the first cycle for the same MC solutions; refer Fig. 4.

In order to ascertain the reasons for such increase in the rate of gelation during the second cycle, a sample of 2.5 wt % MC solution was observed under the confocal microscope after it underwent the first gelation cycle. Interestingly, fine structures as seen in Fig. 5(a), which could not be found in a MC solution that had never been through any gelation cycle, were present within the transparent MC solution.

Subsequently, the 2.5 wt % MC solution was allowed to undergo second cycle of gelation by keeping it on the hot plate maintained at 60°C. The changes in the morphology at different time intervals during the heating process are shown in Fig. 5(b) to 5(h). Fig. 5(b) shows the initial state at 0 minute for the second cycle. Thus, it shows minimal microstructural features that became more conspicuous as time

progressed. After keeping the sample at 60°C for 10, 15, and 20 minutes, hump-like projections with different geometry and sizes, seen in Fig. 5(c)-5(e), developed from the surface of the sample. As time passed, at 25, 30, and 35 minutes, the surface of the sample became flat and the hump-like projections were replaced by many irregularly-shaped micro-structures; refer Fig. 5(f)-5(h).

It has been reported that both inter-molecular association and intra-molecular aggregation take place simultaneously in gels [18-19]. Tanaka and Koga presented their analysis by considering telechelic polymer chains with hydrophobes at each of its two ends [18].

It is believed that the fine structures observed in Fig. 5(a) were formed by intra-molecular association and were almost similar to the flower micelles mentioned in [18]. As a MC polymer consists of more than two hydrophobes in a single chain compared to a telechelic associating polymer, the macromolecules of MC are considerably longer, thus forming a bigger isolated loop of MC. Such loop, as shown in Fig. 6a, is formed by the hydrophobic association of the methoxyl groups on the same side of an MC chain. It is expected that a substantial amount of energy is required to facilitate the movement of such isolated loops of MC in the solution. Such 2-3 loops form a micelle-like aggregate as shown in Fig. 6b. It is suggested that such MC aggregate is made up of a small number of isolated loops of MC that are held in place by many surrounding water molecules. This could explain why these MC aggregates could only be observed after heating during the first gelation cycle. This also shows that the MC gelation is not fully reversible at the test temperature.

These aggregates s probably play a part in the early gelation observed during the second gelation cycle. This is due to the early exposure of the hydrophobic groups associated with the isolated loops of MC when the water cages surrounding them

break. The presence of micelle-like structures also facilitates the quick formation of the gel network. It should be noted that these aggregates could be observed even after refrigeration of the sample; it reflects how stable their structures were. Another reason for the increased gelation rate during the second gelation cycle could be that after the first gelation cycle, water molecules within the MC solution gain internal energy due to heating and become more dispersed. Even though the MC solution was refrigerated for minimum one day after it underwent the first gelation cycle, the water cages formed around the hydrophobic methoxyl groups might not have been as compact as they originally were before any gelation took place. As a result, it did not require exposing the MC solution to the desired temperature for a long time in order to absorb enough energy to melt the water cages. This, in turn, caused the early exposure of the hydrophobic groups and encouraged the hydrophobic association to take place early leading to gelation.

As discussed in [20], hydrophobic association of exposed methoxyl groups results in three-dimensional network of MC chains upon heating. Some water molecules then move into the network and get trapped inside. As this process of water uptake proceeds, the network cells are gradually enlarged. A fully developed and stabilized gel is obtained once equilibrium is reached between the pressure exerted by the trapped water molecules and the elastic retraction force of the network.

The appearance of the hump-like projections seen in Fig. 5(c)-5(e) could be due to enlargement of the network cells during the water uptake and the entrapment of the water molecules into these network cells. The relatively flat surface seen in Fig. 5(f)-5(h) could mean that the flexibility of the network structure was largely reduced leading to the stabilization of the gel.

Effect of water state

When hot DI water was used for preparing the MC solutions, gelation started for lower MC concentrations as compared to when cold DI water was used as solvent. Gelation was observed for 1.25 wt % during the first gelation cycle and for 1.0 wt % and higher during the second gelation cycle without much isothermal hold at 50°C, as compared to the samples prepared using cold DI water. The rate of gelation seen in both cycles was much faster with hot DI water solvent than the ones for the MC solutions prepared using cold DI water.

As a general trend, a sample prepared in cold water and then made to undergo one thermal cycle showed improved gelation similar to the case of a sample in a hot water with the same MC wt%. It is as expected, because in both conditions we subject the solutions to heating.

The primary reasons as to why hot water has a greater impact than cold water may be due to the difference in water density and the ability to remove dissolved air bubbles within the solutions. Hot water has a lower density than cold water, as the space between the water molecules is greater. A comparison of orientation of water molecules with respect to MC polymer chains for MC solutions prepared using hot and cold DI water before the first and the second gelation cycles is depicted in Fig. 7. The ability of the hot water to degas or to cause any dissolved air within the MC solution to rise to the surface helps to create fewer obstacles in the networking of the hydrophobic bonds, resulting in a faster gelation.

Indexing of state of gelation

The colour of the images of the MC solutions was analyzed using the Adobe Photoshop software. To establish the gel index of 1 to 10, two bottles of MC were chosen as reference. The image of the sample with 0.5 wt % of MC prepared using cold DI water at time zero of the first gelation cycle was used for describing index 1

and the image of the sample with 2.5 wt % of MC prepared using hot DI water at time 75 minutes of the second gelation cycle was used for index 10. The gel with index 1 had 96 red, 82 blue and 97 green components whereas the gel with index 10 had 197 red, 207 blue and 214 green components. The values of the 3 basic colour components were subsequently interpolated for arriving at the remaining gel indices. Gel indices of 1 to 3 were considered to be indicating the solution state. Index 3 to 6 represented the sol-gel state, and indices 6 to 10 were allocated to the gel state. Fig. 8 is the graphical representation showing the distribution of gel state for various MC samples. The MC gel must be able to display the ability to undergo transition from solution state to the gel state for useful bio-applications. It is important to determine the final state of the gel desired when subjected to isothermal conditions, before deciding on the correct concentration of MC required. From the graphs in Fig. 8, it can be seen that by using hot DI water as solvent and by allowing the aqueous solution with 1.5 wt % and above of MC to undergo a few (at least one) gelation cycles, MC is able to attain a steady state of gelation within a shorter period of time under isothermal conditions.

CONCLUSIONS

MC displays the ability to gel under isothermal conditions with time. The initial state of the solvent is seen to affect the gelation process. Hot DI water has favourable impact on the rate and degree of gelation as compared to the cold DI water as solvent. This can be attributed to the reduced water density and increased ease with which dissolved gases can come out with heat-treated water solvent. Conditioning of aqueous solutions of MC during sol-gel through gel-sol cycles facilitates the initial

formation of micelle-like aggregates, which further favours accelerated gelation. These aggregates could also have some significant role in drug delivery.

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REFERENCES

1. N.A. Peppas, P. Buresa, W. Leobandunga, H. Ichikawaba, *Euro. Jr. of Pharmaceutics and Biopharmaceutics*, **50**, 27 (2000).
2. E. Hermanova and L. Viktora, *Folia Haematol Int Mag Klin Morphol Blutforsch*, **105**, 733 (1978).
3. M. C. Tate, D. A. Shear, S. W. Hoffman, D. G. Stein and M. C. LaPlaca, *Biomaterials* **22**, 1113 (2001).
4. D. S. Yoon, Y. K. Cho, K. W. Oh, S. Kim, Y. A. Kim, J. I. Han and G. Lim, *Microsyst. Technol.*, **12**, 238 (2006).
5. W. J. Hodgson, *Br. J. Surg.* **59**, 909 (1972).
6. B. Daggy, N. I Mehta and P. Nayak, *United States Patent* **7125562**.
7. N. Hirasawa, S. Ishise, H. Miyata and K. Danjo, *Chem. Pharm. Bull.* **52**, 244 (2004).
8. A. Yadav, S. Yadav, *J Indian Prosthodont Soc.*, **5**, 62 (2005).
9. A. Yanagi, N. Haga, *The Jr. of Eukaryotic Microbiology*, **45**, 87 (1998).
10. S. S. Chrai, J. R. Robinson, *Jr. of Pharma. Sci.* **63**, 1218 (2006).
11. S. C. Joshi and Y. C. Lam, *J. App. Polym. Sci.*, **101**, 1620 (2006).
12. Y. C. Lam, S. C. Joshi and B. K. Tan, *J. Therm. Anal. Cal.* **87**, 475 (2007).

13. J. Desbrieres, M. Hirrien, M. Rinaudo, *Carbohydrate Polym.* **37**, 145 (1998).
14. N. Sarkar and L.C. Walker, *Carbohydrate Polym.* **27**, 177 (1995).
15. A. Haque and E.R. Morris, *Carbohydrate Polym.*, **22**, 161 (1993).
16. N. Katsuyoshi, E.H. Kate, M. Hatsue, K. Kaoru, *Macromol. Chem. Phys.*, **198**,1217 (1997).
17. S.Q. Liu, S.C. Joshi, Y.C. Lam and K.C. Tam, *Carbohydrate Polym.* (2007),
in press.
18. F. Tanaka and T. Koga, *Comput. Theoret. Polym. Sci.*,**10**, 259 (2000).
19. M. Hirrien, C. Chevillard, J. Desbrieres, M.A.V. Alexos and M. Rinaudo,
Polymer **39**, 6251 (1998).
20. J. M. Rosiak, and F. Yoshii, *Nucl. Instr. Meth. Phys. Res. B* **151**, 56 (1999).

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Figure 1. DSC measurements under different isothermal conditions for MC solutions as a function of time [12].

Figure 2. Procedure used for monitoring the gelation process for one gelation cycle.

Figure 3. A typical heating cycle followed for MC gelation in convection oven.

Figure 4. Different degree of gelation observed for 1.5 wt % MC solution with cold DI water as solvent, at stabilized 50°C isothermal conditions.

Figure 5. Morphological characteristics of 2.5 wt % MC hydrogel at 60°C temperature. (a) micelle-like structures present in MC at the end of the first gelation cycle. Gelation after (b) 0; (c) 10; (d) 15; (e) 20; (f) 25; (g) 30; (h) 35 minutes during second gelation cycle.

Figure 6. Schematic illustration of MC aggregates formation (Black dots represent the water molecules. Light lines are the MC chains and the dark lines are the methoxyl side groups).

Figure 7. Schematic illustration of orientation of water molecules surrounding methoxyl groups in MC solutions at room temperature (a) cold DI water as solvent, before first gelation cycle; (b) cold DI water as solvent, before second gelation cycle; (c) hot DI water as solvent, before first gelation cycle; (d) hot DI water as solvent, before second gelation cycle.

Figure 8. Gelation index variations showing distribution of gel state for MC hydrogel with hot DI water as solvent at 50°C isothermal hold: **(a)** for the 1st gelation cycle; **(b)** for the 2nd gelation cycle.

Figure 1. DSC measurements under different isothermal conditions for MC solutions as a function of time [12].

Figure 2. Procedure used for monitoring the gelation process for one gelation cycle.

Figure 3. A typical heating cycle followed for MC gelation in convection oven.

Time	1 st gelation cycle	2 nd gelation cycle
35 min
45 min
55 min
65 min
75 min
85 min

Figure 4. Different degree of gelation observed for 1.5 wt % MC solution with cold DI water as solvent, at stabilized 50°C isothermal conditions.

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Figure 6. Schematic illustration of MC aggregates formation: (a) MC isolated loop formed by intramolecular association; (b) MC micelle-like aggregate formed when a small number of MC isolated loops associate together. (Black dots represent the water molecules, and light lines are the MC chains and the dark lines are the methoxyl side groups)

Figure 7. Schematic illustration of orientation of water molecules surrounding methoxyl groups in MC solutions at room temperature (a) cold DI water as solvent, before first gelation cycle; (b) cold DI water as solvent, before second gelation cycle; (c) hot DI water as solvent, before first gelation cycle; (d) hot DI water as solvent, before second gelation cycle.

(a) Time from the isothermal gelation (min)

(b) Time from the isothermal gelation (min)

Figure 8. Gelation index variations showing distribution of gel state for MC hydrogel with hot DI water as solvent at 50°C isothermal hold: (a) for the 1st gelation cycle; (b) for the 2nd gelation cycle.