



Supramolecular hydrogel based on Poly (ethylene glycol) methyl ether- α -cyclodextrin inclusion complex: a carrier for drug delivery

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Abstract The hydrogel system reported in this paper is a new approach for the development of supramolecular hydrogels. In this system, Folic acid has been conjugated with α -Cyclodextrin and then used as a building block for constructing novel supramolecular hydrogels together with Poly (ethylene glycol) methyl ether in aqueous solutions. To understand and modulate the gelation process, we studied the gelation kinetics, hydrogel strength; shear thinning rheological behavior and thixotropic property by dynamic and steady rheometry give various amounts of MPEG and α -CD-FA. This study is aimed at developing novel, inexpensive hydrogels for containing drug and with the potential for targeting cancer cells *in vitro*.

Keywords gelation kinetics, Folic acid, supramolecular hydrogel, α -Cyclodextrin, Poly(ethylene glycol) methyl ether

Introduction

In recent years, supramolecular hydrogels have attracted considerable attention because of their potential applications in drug delivery and tissue engineering [1-3]. The design and construction of supramolecular hydrogels systems through host-guest interaction have been extensively studied [4-6]. Among all potential hydrogels, the supramolecular hydrogels, as a consequence of the inclusion complexation between α -CD and various guest polymers have sparked growing interest in recent years. For example, Yui et al. synthesized PEG/ α -CD polyrotaxanes capped with amino acids, polypeptides and oligopeptides, which were used as biodegradable drug carriers or stimuli responsive hydrogels [7]. Huh et al. prepared the graft copolymers containing dextran as backbones and PEG or PPG as side chains, while the CDs were threaded into PEG or PPG chains to form hydrogels [8-9]. Zhu et al. [10] used the cisplatin-loaded block copolymer nanoparticles and α -CD to obtain the supramolecular hydrogels with a stepwise delivery property for cancer therapy.

Although supramolecular hydrogels with cyclodextrins as hosts possess interesting properties for the delivery of therapeutics with high pharmacological activity, low therapeutic index, and poor physicochemical properties, they cannot be used for active drug targeting because they are devoid of any specificity for biological structures. To exploit cyclodextrins as targetable drug delivery systems, the oligosaccharide structure should be properly functionalized with targeting moieties such as peptides, hormones, vitamins, antibody fragments, etc.

Folic acid is a small vitamin, which interacts specifically with the folate binding protein (FBP) located in the caveole-like invaginations on the cell surface receptor [11-12]. Upon receptor interaction, the folate acid-FBP complex is taken up by cells and moves through the many organelles involved in endocytotic trafficking, providing for cytosolic deposition [11]. The folic acid receptor is overexpressed by many types of tumor cells, including ovarian, endometrial, colorectal, breast, lung, renal, neuroendocrine carcinomas, and brain metastases [13]. The folate receptor mediated endocytosis has been largely investigated to expand the therapeutic value of drugs, by increasing delivery to the target tissue as well as the target/nontarget tissue ratio. When folic acid (FA) is attached to carboxyl site, the folate retains its normal receptor-binding affinity and therefore, can be



internalized by receptor mediated endocytosis [14]. This principle has been exploited for the selective delivery of imaging agents [15], gene [16], therapeutic agents [17], micelles of block copolymers [18] and other complexes of macromolecular [19] to tumor/cancer cells.

In my previous work, folic acid has been conjugated with the α -CD and then used for constructing supramolecular hydrogels together with Poly(ethylene glycol) methyl ether in aqueous solutions [20]. This strategy can result in the formation of bioactive supramolecular hydrogels with multifunctional characteristics. In this work, to understand and modulate this gelation process, the gelation kinetics, hydrogel strength, shear thinning rheological behavior and thixotropic property have been studied by dynamic and steady rheometry under various amounts of MPEG and α -CD-FA.

Materials and Methods

Materials

Folic acid (FA), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Poly(ethylene glycol) methyl ether (MPEG) with molecular weight of 5000, were purchased from Sigma-Aldrich. α -Cyclodextrin was purchased from Acros. All other solvents and analytical reagents were purchased from commercial suppliers and used as received.

Methods

Supramolecular hydrogels were prepared by the inclusion complexation of MPEG with α -CD-FA in an aqueous solution. In a typical experiment, the required amount of MPEG (1, 2 or 3 wt %) was dissolved in an aqueous solution and then mixed with an aqueous α -CD-FA solution (6, 7 or 8 wt %) at room temperature.²⁰For the investigation of the gelation kinetics of aqueous MPEG/ α -CD-FA systems, time-sweep rheological analyses were performed by a MCR Rheometer (series MCR 102, Anton Paar Co.) operated in oscillatory mode with a parallel plate geometry (50 mm diameter, 1.0 mm gap) at 25 °C. In this case, the samples were placed on the plate immediately after mixing. The viscoelastic parameters were measured as a function of time within the linear region previously determined by a strain sweep. To investigate the mechanical properties of resultant hydrogels, dynamic frequency sweep tests were conducted. In this case, the hydrogel samples were allowed to consolidate for 1h before the measurements. The frequency applied to the hydrogel sample was increased from 0.1 to 100 rad/s with a strain of 0.05%. In addition, steady rate sweep tests were carried out to investigate the shear thinning and thixotropic properties of the resultant hydrogels. In this case, the hydrogel samples were also allowed to consolidate for 1h before the measurements.

Results and Discussion

To understand the gelation kinetics of the aqueous MPEG/ α -CD-FA system, we carried out time sweep measurements for the viscoelastic properties of each system, in which the storage modulus (G') and loss modulus (G'') were monitored as a function of time. Figure 1 shows the time dependences of G' and G'' for the various MPEG/ α -CD-FA systems. In each case, a crossover point between G' and G'' is observed, which implies that there was a sol-gel transition [21]. After the crossing point, the G' value becomes larger than the G'' value, which demonstrates that the system has become more elastic. The corresponding time of the crossover from a viscous behavior to an elastic response can be regarded as the gelation time [22]. From figure 1, the gelation time is found to decrease with the increase in MPEG or α -CD-FA amount. When the amount of MPEG increased from 1 to 3 wt %, the gelation time decreased from 19.4 to 4.5 min. When the amount of α -CD-FA increased from 6 to 8 wt %, the gelation time decreased from 37.8 to 2.2 min. These results indicate that a higher concentration of MPEG or α -CD-FA would be favorable for the supramolecular gelation, which may be attributed to an enhanced inclusion complexation between MPEG and α -CD-FA. In addition, a “bump” in G' can be observed in Figure 1, where the G' did not increase continuously, but decreased for a few minutes. This phenomenon may be attributed to the syneresis property of the supramolecular hydrogel due to the molecular reorganization of the macromolecular network involving both the disruption and the formation of some supramolecular complex clusters. Similar phenomena have been also observed in some cross-linked polysaccharide systems [23-24].



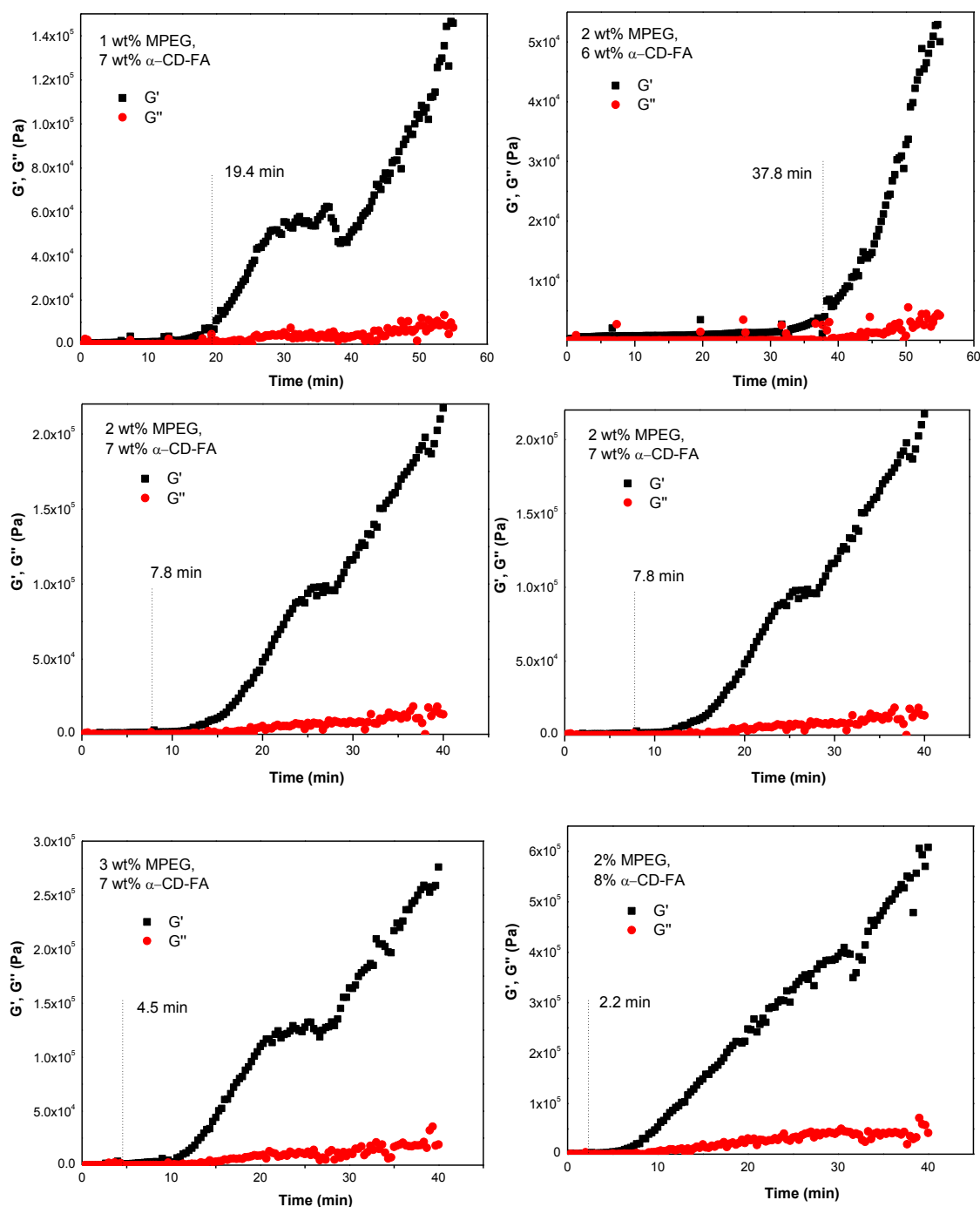


Figure 1: Effects of MPEG and α -CD-FA amounts on the supramolecular gelation kinetics for aqueous MPEG/ α -CD-FA systems. Test conditions: frequency, 1.0 rad/s; strain, 0.05%

The elastic moduli (G'), shear-dependent viscosity, and thixotropic property of the resultant supramolecular hydrogels were also investigated with respect to the effects of amounts of MPEG and α -CD-FA. As shown in Figure 2, G' varied with the amounts of MPEG and α -CD-FA and the G' value of resultant hydrogel increased with the increase in MPEG or α -CD-FA amount. For example, at a frequency of 1.0 rad/s, the G' value increased from 746 to 1640 kPa when the amount of MPEG increased from 1 to 3 wt % and increased from 649 to 1780 kPa when the amount of α -CD-FA increased from 6 to 8 wt %. This fact confirms that the hydrogels were well cross-linked with insignificant sol fraction.

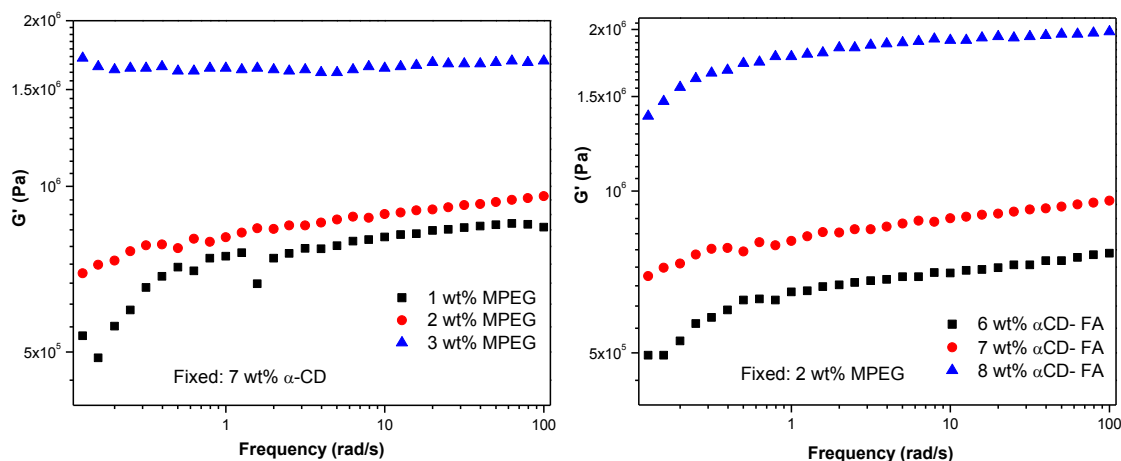


Figure 2: Effects of the amount of MPEG and α -CD-FA on the elastic moduli (G') of the gelled MPEG/ α -CD-FA systems

In order to develop an injectable drug delivery system, remarkable shear thinning and thixotropic properties are required and are important in a hydrogel system and so has recently received much attention [25-26]. To explore the possibility of using the resultant supramolecular hydrogel as an injectable drug carrier, we carried out steady rate sweep tests of the hydrogel samples. Figure 3 gives the steady shear viscosity as a function of the shear rate for the supramolecular hydrogels formed *in situ* under various MPEG and α -CD-FA amounts.

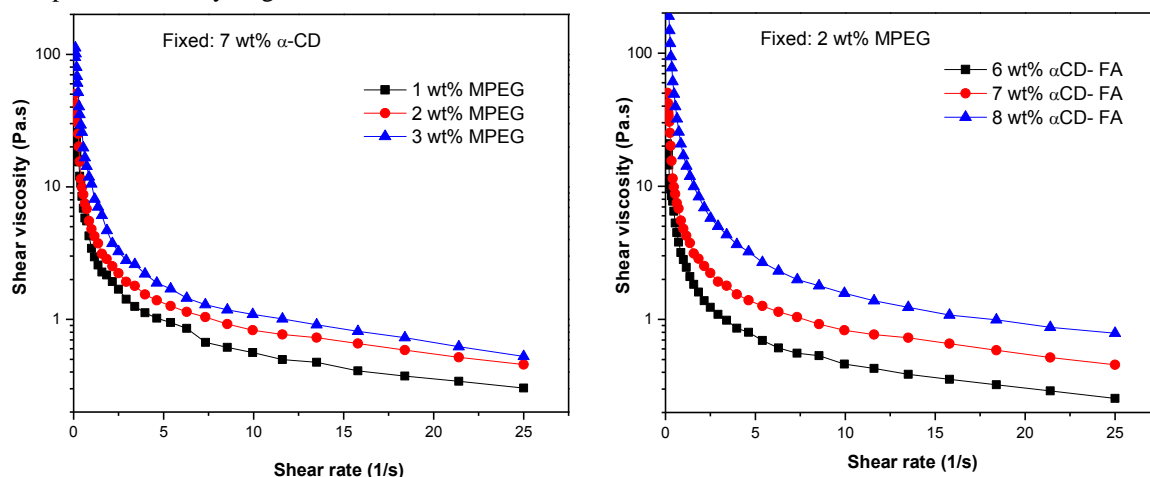


Figure 3: Change of steady shear viscosity as a function of shear rate for the supramolecular hydrogels formed under various MPEG and α -CD-FA amounts. Test conditions: 0.05% strain, 25°C.

It was found that all of the hydrogel samples exhibited a shear-thinning behavior, regardless of amount of MPEG or α -CD-FA. In other words, the viscosity of the hydrogel sample greatly diminished as it was sheared, irrespective of the MPEG or α -CD-FA amount. This rheological property may render the resultant supramolecular hydrogel injectable through a hypodermic needle. Further investigation was carried out dealing with the thixotropic properties of these hydrogel samples at 25°C. For this purpose, the flow curves were measured while increasing the shear rate from a minimum of 0.8 s⁻¹ to a maximum of 50 s⁻¹ and then decreasing the shear rate in the same equal steps. The duration of shear at each step was 60s. This method is similar to that adopted by Saunders [27] who studied the thixotropic behavior of thickened polystyrene latexes. If the supramolecular hydrogels investigated exhibit the thixotropy, then a hysteresis loop can be obtained from the “upward” and “downward” curves developed during reversible shear stress-shear rate paths, and the corresponding enclosed area used to evaluate the magnitude of the thixotropy. Figure 4 shows the thixotropic responses of the supramolecular hydrogels formed under various MPEG and α -CD-FA amounts in aqueous systems.

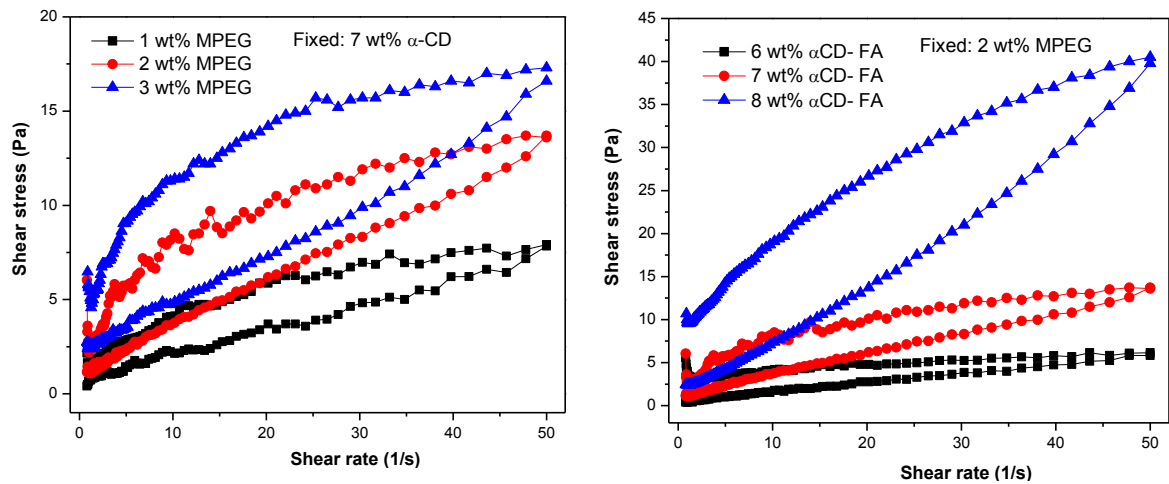
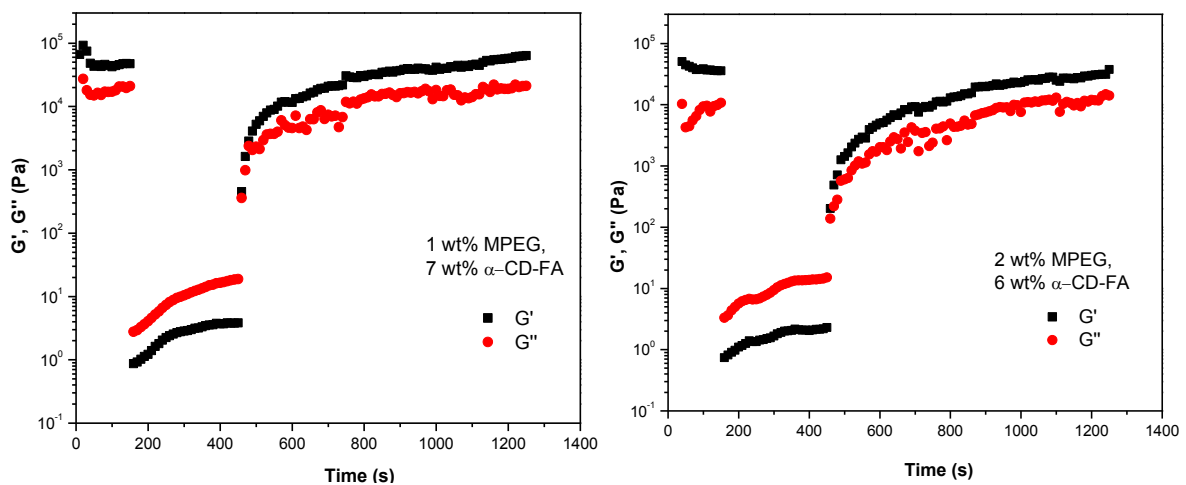


Figure 4: Thixotropic responses of the supramolecular hydrogels formed under various MPEG and α -CD-FA amounts. Test conditions: 0.05% strain, 25 °C.

It was found that the thixotropic property of the resultant supramolecular hydrogel would build with an increase in the amount of MPEG or α -CD-FA. As shown in Table 1, the area of the hysteresis loop increased from 85.84 to 251.17 kPa s⁻¹ as the amount of MPEG increased from 1 to 3 wt%, and increased from 84.27 to 488.07 kPa s⁻¹ when the amount of α -CD-FA increased from 6 to 8 wt %. It is known [28] that the thixotropy of a material can be quantified by its ability to regain its gel structure after being allowed to rest for a period of time after the sol phase is attained. Therefore, it can be seen in this study, that the structure of the supramolecular hydrogel is build up from sol to gel, especially in the case of higher MPEG and α -CD-FA amounts.

Table 1: Effects of MPEG and α -CD-FA amounts on the hysteresis loop area for the resultant supramolecular hydrogel

Hydrogel compositions	Intergrating area for up curve	Intergrating area for down curve	Hysteresis loop area (kPa.s ⁻¹)
Effect of MPEG amount			
1 wt% MPEG + 7 wt% α -CD-FA	286.12	200.28	85.84
2 wt% MPEG + 7 wt% α -CD-FA	507.6	357.95	149.65
3 wt% MPEG + 7 wt% α -CD-FA	689.58	438.41	251.17
Effect of α -CD-FA amount			
6 wt% α -CD-FA + 2 wt% MPE	243.09	158.82	84.27
7 wt% α -CD-FA + 2 wt% MPE	507.6	357.95	149.65
8 wt% α -CD-FA + 2 wt% MPE	1397.91	909.84	488.07



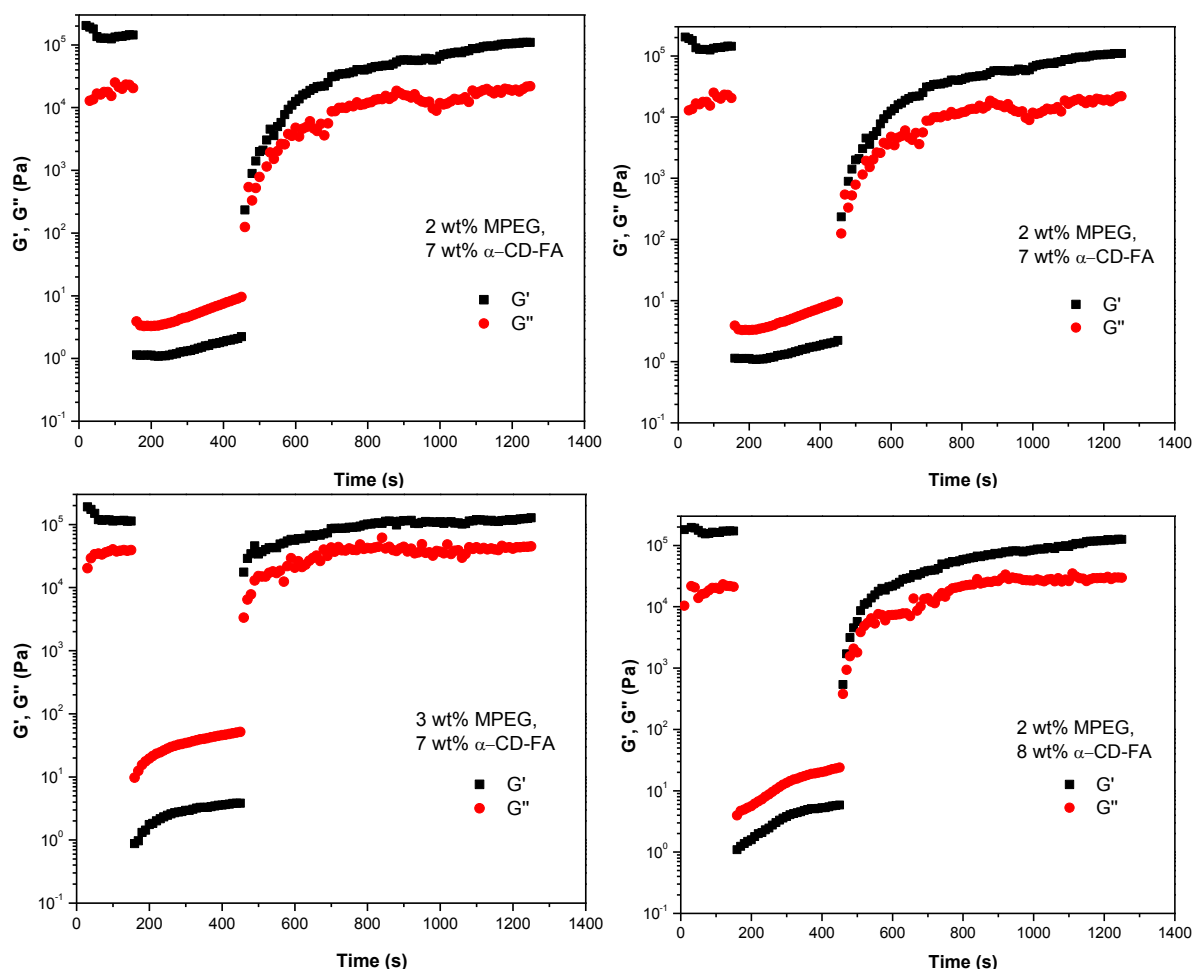


Figure 5: Dynamic step strain amplitude test ($\gamma = 0.05\%$ or 100%) for the supramolecular hydrogels formed under various MPEG and α -CD-FA amounts ($25\text{ }^{\circ}\text{C}$)

In addition, it was observed that a disrupted sol phase could be reversed into a gel after shearing and then standing for a particular period of time. To confirm this recovery property, the supramolecular hydrogels formed from 1 to 3 wt% MPEG and 6 to 8 wt % α -CD-FA were subjected to dynamic step strain amplitude testing ($\gamma = 0.05$ or 100%). In the recovery measurements shown in Figure 5, the initial G' is observed to be higher than 10^4 Pa for the small strain ($\gamma = 0.05\%$). Under large strain ($\gamma = 100\%$) to the supramolecular hydrogels, there was an immediate decrease in the value of G' by more than 10^4 times to less than 10 Pa. When the strain again returned to the small value ($\gamma = 0.05\%$) after 300 s of the continuous stress, the G' rapidly recovered within 800 s to greater than 10^4 Pa, estimated to be about 80% of the initial G' .

Conclusion

A bioactive supramolecular hydrogel with multifunctional characteristics was prepared by the conjugation of the FA with α -CD and the interaction with MPEG in an aqueous solution. The gelation time, mechanical strength, shear thinning, and thixotropic properties of the resultant supramolecular hydrogels depend on the amount of MPEG or α -CD-FA used for their preparation. From the viewpoint of applications, such a rheological behavior of the resultant supramolecular hydrogel would be advantageous for use as an injectable hydrogel matrix for drug delivery and cell encapsulation. This study provides a novel drug-entrapment strategy for hydrophilic hydrogel-based carriers.

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