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Determination of Crosslinking Density of Hydrogels Prepared from Microcrystalline Cellulose

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ABSTRACT: Hydrogels with four different plant-based microcrystalline cellulose concentrations were prepared using the self-assembly technique. The interaction parameter between cellulose and water was determined by the classical Flory-Huggins theory, and was found to be around 0.44 with weak concentration dependence. The crosslinking density in these hydrogels was measured by both the Mooney-Rivlin equation and the Flory-Rehner theory. Reasonable consistency was found between the two methods albeit results from the Flory-Rehner theory were slightly higher due to the contribution from the physical crosslinks. The crosslinking density values for all four hydrogels determined from both methods were found to range from 19 to 56 mol/m^3. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: cellulose hydrogels; crosslinking density; Mooney-Rivlin; Flory-Rehner; interaction parameter

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INTRODUCTION

Since Wichterle[1] developed the first poly(hydroxyethyl methacrylate) hydrogels in the 1950s, significant progress has been made in synthesizing novel hydrogels from both synthetic and natural polymers. Hydrogels have thus found a wide range of applications including personal hygiene, controlled drug delivery, contact lenses, lubricating surface coatings, wound healing dressings, 3D cell culture substrates, and underwater devices.[2–5] Among the many hydrogels studied, those based on natural cellulose and its derivatives have attracted particular attention due to their adequate biocompatibility and biodegradability.[6] Cellulose based hydrogels have been proven to be useful in tissue engineering, wound healing, and tissue regeneration.[7–10]

Hydrogels based on natural cellulose are typically made through temperature-modulated sol-gel processes.[11] First, a strong solvent is used to dissolve the microcrystals in the cellulose. The dissolved, submicron-size crystallites then fuse together and form a three dimensional network structure via hydrogen bonding upon drying. The structure is hygroscopically stable upon subsequent rehydration.[12,13] Due to their high hydrophilicity, cellulose hydrogels contain >90% water under normal conditions. Depending on the interaction between water and the gel matrix, three types of water, namely free water, bound water, and interstitial water, can be found inside the gel. Free water is in a totally free state, and can be easily removed under moderate temperatures. Bound water is attached to the polymer chains and become an integral part of the gels. These water molecules can only be removed at high temperatures. Finally, interstitial water, which is not attached to the polymer chains, is trapped between the hydrated polymer chains.[14] Due to the amount of water present in the hydrogels, they are normally in a swollen state. Swelling of hydrogels has often been explained by the limited solubility of the matrix polymer,[14] and it depends on several factors, including network parameters, solvent type, and the physical structure of the gels. Without adequate crosslinking, these gels are fully soluble in the solvent and form a uniform solution. The crosslinks effectively "block" the polymer from fully disintegrating in the solvent. The overall, or effective, crosslinks consist of both chemical crosslinks and physical crosslinks. The latter further comprises of chain entanglements, loose chain ends, and tie-chains.[15] Besides maintaining the physical integrity of the hydrogel, the effective crosslinks also play a critical role in controlling the physical and mechanical properties of the hydrogels. However, due to the moisture sensitivity of these hydrogels, accurate determination of the crosslinking density is very challenging via standard crosslinking determination tools, such as dynamic mechanical analysis and rheology.[16]

In this study, hydrogels synthesized from plant-based celluloses with varying cellulose concentrations were prepared, and the crosslinking density values obtained from both Mooney-Rivlin method and the Flory-Rehner theory were compared. The objective of this work is to compare the crosslinking density values obtained from both methods and establish the correlation between hydrogel cellulose content and crosslinking density. Learning from this work will be applied to the drug incorporation and delivery in the hydrogels for ocular injury treatment aspect of this project.

EXPERIMENTAL

Preparation of Cellulose Hydrogels

Micro-crystalline cellulose-Avicel PH101(Sigma-Aldrich) was used for preparing the hydrogels. During the hydrogel synthesis process, 2–5 g of cellulose powder was activated in 100 mL *N, N*-dimethylacetamide (DMAc) with stirring at 350 rpm for 24 h, after which 8 g of LiCl was added with continued stirring at 350 rpm and heating to 95°C until the solution became clear (10–30 min). The resulting solution was then poured into silicone molds, and gelled at 35°C, 73% relative humidity overnight. The resulting gelled samples were gently washed in deionized water until the excess LiCl and DMAc were removed and then the gels were stored in water prior to testing. For simplicity, the hydrogels with different amounts of cellulose are labeled as 2, 3, 4, and 5 wt %.

Thermal Analysis of Hydrogels

Water content of all four hydrogels was analyzed by thermal gravimetric analysis (TGA) using the TA Instrument Q5000. Samples with mass of ~30 mg were analyzed for weight loss under N_2 from 25 to 500°C at a heating rate of 20°C/min. The data were analyzed using TA Universal Analysis 2000 Software.

Tensile Testing of the Hydrogels

Stress-strain behavior of the swollen hydrogels was characterized using an Instron 5942 with a 500*N* load cell. Tests were performed according to the ASTM D1708-10 with an extension rate of 2 mm/min. Specimens were gripped with pneumatic grips at 0.14 MPa on cheese cloth tabs that had been embedded in the ends of the dog bones during gelation. Data acquisition and analysis were performed using the BlueHill2 software. A minimum of four repeats were tested for each of hydrogel concentration, and the average test variation was found to be <5% for all four concentrations. Due to the high moisture level of the hydrogels, a certain portion of the moisture was lost during the tensile testing process, which would in turn affect the tensile testing results. To quantify the moisture loss, isothermal TGA tests (at 25°C) were performed on all Avicel 101 hydrogels. Moisture loss of <3.5% was observed for all hydrogels after 6 min. The tensile extension ratio at 6 min corresponds to the lower end of the crosslinking calculation (details will be discussed in the Results and Discussion). As a result, we consider the impact of moisture loss on the crosslinking analysis based on tensile testing to be negligible.

RESULTS AND DISCUSSION

Determination of the Volume Fraction of Hydrogels

The weight fraction of water in hydrogels made with different cellulose concentrations was analyzed by TGA. Prior to the analysis, all hydrogels were soaked in deionized water at 22°C for an extended period of time (3 weeks) to ensure equilibrium swelling. Figure 1 shows a typical TGA trace of a hydrogel containing a nominal cellulose content of 2 wt %. As can be seen in Figure 1, the maximum temperature (*d*W/*d*T) for removing water centered around 127°C and continued to 250°C. Further increase of temperature to 333°C led to thermal decomposition of the cellulose. The fact that water removal temperature being 27°C higher than the boiling point of water indicates the strong interaction between water and hydrogel network structure. These strongly bonded water molecules could exist in the forms of interstitial water and bound water, both of which require high thermal energy for removal than does free water.[14] Water content of hydrogels based on four different runs are reported in Table I along with the calculated hydrogel densities based on the density of water at 22°C (0.9978 g/cm^3) and the density of the amorphous cellulose (1.44 g/cm^3).[17,18]

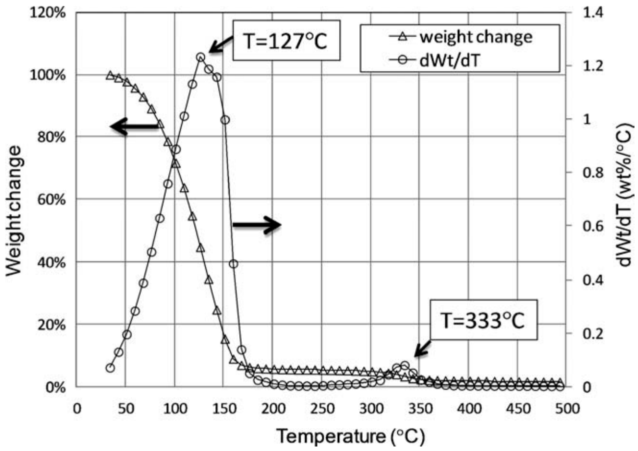

Figure 1. TGA trace for hydrogel with a Avicel 101 nominal concentration of 2 wt % showing the amount of weight loss and the corresponding peak temperatures (*d*Wt/*d*T).

Analysis Based on the Mooney-Rivlin Equation

Mooney-Rivlin equation has been widely used to correlate the network deformation behavior with the crosslinking properties of the polymer network.[6,19–24] This theory was derived from elastic strain energy in ideal rubbers. In comparison to ideal rubber theory, which works only at very low strain levels, the Mooney-Rivlin theory works well from low to moderate strain levels. The general form of the Mooney-Rivlin is shown in eq. (1) below.

$$\frac{\sigma_e \upsilon_p^{1/3}}{\left(\lambda - \frac{1}{\lambda^2}\right)} = 2C_1 + 2C_2\frac{1}{\lambda} \tag{1}$$

where σ_e is the engineering stress applied during a tensile test, υ_p is the polymer volume fraction, and λ is the corresponding extension ratio. A plot of $\frac{\sigma_e \upsilon_p^{1/3}}{\left(\lambda - \frac{1}{\lambda^2}\right)}$ as a function of $1/\lambda$ yields a straight line with a slope of $2C_2$ and an intercept of $2C_1$. The C_1 constant is correlated to the polymer network structure via eqs. (2) and (3), and is analogous to the crosslinking density of the network structure as in rubber elasticity. The constant C_2 is a measure of the deviation from the ideal rubber behavior. A number of factors including non-Gaussian chain, internal

Table I. Volume Fraction of Cellulose and Water in the Swollen Hydrogels

Avicel 101 nominal concentration (%)	Average cellulose content (wt %)	Average water content based on TGA (wt %)	Calculated hydrogel density (g/cm^3)	Calculated cellulose volume fraction (υ_p)	Calculated volume fraction of water (υ_s)
2	6.3	93.7	1.017	0.044	0.956
3	8.1	91.9	1.023	0.057	0.943
4	9.0	91.0	1.026	0.064	0.936
5	9.4	90.6	1.028	0.067	0.933

energy, and chain entanglements can contribute to the magnitude of C_2. However, there appears to be no consensus on its real origin.[19]

$$\rho RT/M_c = 2C_1 + 2C_2 \tag{2}$$

$$N = 2C_1/kT \tag{3}$$

where ρ is the density of the swollen polymer, R is gas constant (8.314 J/mol/K), k is the Boltzman constant (1.38×10^{-23} J/K), N is the crosslinking density (mol/m^3), T is the absolute temperature (K), and M_c is the molecular weight between crosslinks.

Figure 2(a) shows the true stress-true strain curves for all four hydrogel concentrations. There are no significant features in the "toe region" of the curve, indicating that the uncrimping of the network structure is insignificant. However as the deformation continues and true strain exceeds 40%, noticeable strain hardening was observed. Although the strain hardening effect has commonly been attributed to strain-induced crystallization as in conventional butadiene rubbers, the strain hardening observed in this study is likely due to the limited chain mobility upon stretching in the hydrogels given the low probability of strain induced crystallization due to the presence of large amount of water in the matrix. Figure 2(b) shows the Mooney-Rivlin modeling results based on the reduced stress versus reciprocal of extension ratio. With the increase in cellulose concentration, the C_1 values were found to increase, illustrating higher crosslinking density was achieved at higher cellulose concentrations. Combining the results shown in Figure 2 and eqs. (1) and (3), the tabulated crosslinking density values of the hydrogels at four different concentrations are reported in Table II. It is worth noting that C_2 for all four cellulose concentrations dropped to zero indicating the hydrogels synthesized in this work, all of which had polymer volume fraction (υ_p) of <0.1, followed the ideal rubber behavior. This phenomenon has also been reported by Gumbrell et al.[25] for swollen polymers with a polymer volume fraction <0.2.

Determination of the Interaction Parameter

A key parameter in analyzing the crosslinking density of the hydrogels is the interaction parameter (χ) between the solute and the base solvent. This unitless parameter quantifies the exchange interaction between the pure solvent and polymer in the lattice, and represents the tendency for the polymer to be dissolved in the solvent.[26] Based on the Flory-Huggins theory, the mixing free energy (ΔG) is a function of the volume fraction of the polymer (υ_p) and χ:

$$\Delta G = RT\left\{\ln\left(1-\upsilon_p\right)+\upsilon_p+\chi\upsilon_p^2\right\} \tag{4}$$

Upon expansion of the natural log term in eq. (4) and ignoring the terms with orders >2, one can show that $\Delta G < 0$ when $\chi < 0.5$ for all values of the $\upsilon_{p,}$ indicating the polymer and the liquid are miscible in all proportions, that is, polymer is soluble. However, when $\chi > 0.5$, there is a particular value of υ_p for which $\Delta G = 0$, which represents the condition for limited or equilibrium swelling.[19] For highly crosslinked systems, the contribution from the configurational entropy of the network during swelling needs to be taken into consideration. As such, the overall free energy is comprised of both the free energy of

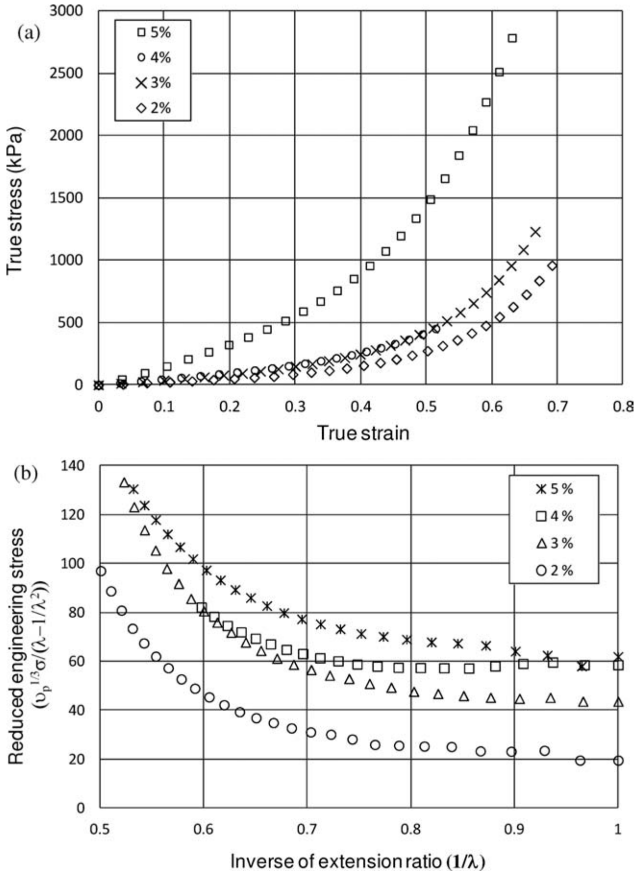

Figure 2. (a) True stress-true strain curves for hydrogels with 2–5 wt % nominal cellulose concentrations and (b) reduced engineering stress (corrected by the volume fraction of the polymer) as a function of inverse of extension ratio ($1/\lambda$).

Table II. Hydrogel Crosslinking Densities Calculated Based on the Mooney-Rivlin Equation

Avicel 101 nominal concentration (%)	C_1 (kPa)	Molecular weight between crosslinks (kg/mol)	Crosslinking density (mol/m^3)
2	24	52	19
3	43	29	35
4	60	21	48
5	67	19	54

Please note C_1 was determined from the inverse extension ratio between 0.8 and 1.

dilution (ΔG) for the polymer in the state before crosslinking, and the free energy due to associated elastic expansion of the Gaussian network $\left(\Delta G_e = \frac{\rho RT}{M_c} V_s \upsilon_p^{1/3}\right)$, where V_s is the molar volume (m^3/mol) of the solvent.

Combining the contribution from Flory-Huggins and the network configurational entropy, the overall free energy becomes:

$$\Delta G = \mathrm{RT}\left[\ln(1-\upsilon_\mathrm{p}) + \upsilon_p + \chi\upsilon_p^2 + \frac{\rho V_1}{M_c}\upsilon_p^{1/3}\right] \quad (5)$$

where M_c is the molecular weight between crosslinks. At equilibrium, $\Delta G = 0$, and eq. (5) becomes:

$$\ln(1-\upsilon_\mathrm{p}) + \upsilon_p + \chi\upsilon_p^2 + \frac{\rho V_1}{M_c}\upsilon_p^{1/3} = 0 \quad (6)$$

As a result, with the polymer volume fraction, density, and crosslinking density known, the interaction parameter can be calculated by combing the correlation between C_1 and M_c based on the Mooney-Rivlin theory as follows:

$$\frac{\rho \mathrm{RT}}{\mathrm{M_c}} = 2C_1 \quad (7)$$

The interaction parameter can then obtained via the Flory-Huggins equation:

$$\chi = -\frac{\ln(1-\upsilon_\mathrm{p})}{\upsilon_p^2} - \frac{1}{\upsilon_p} - \frac{2C_1 V_1}{RT}\upsilon_p^{-5/3} \quad (8)$$

Alternatively, using the simplified Flory formula, the interaction parameter can also be obtained via the modified Flory-Huggins equations below:

$$\chi = -\frac{\ln(1-\upsilon_\mathrm{p})}{\upsilon_p^2} - \frac{1}{\upsilon_p} - \frac{2C_1 V_1}{RT}\left(\upsilon_p^{-5/3} - \frac{1}{2\upsilon_p}\right) \quad (9)$$

Using the C_1 constant obtained from Mooney-Rivlin analysis together with the volume fraction and density at each cellulose concentration, χ for hydrogels at all four concentrations levels are tabulated in Table III. The interaction parameter was found to be around 0.44, although its value decreases slightly with cellulose concentration. More work is thus needed to understand this effect. It is worth noting that the interaction parameter value of 0.44 is in agreement with the literature reported value for cellulose materials in water.[28]

Table III. The Interaction Parameter (χ) based on Flory-Huggins and modified Flory-Huggins theories

Avicel 101 nominal concentration (%)	C_1 Based on Mooney-Rivlin equation (kPa)	χ Based on Flory-Huggins theory	χ Based on modified Flory-Huggins theory
2	24	0.45	0.46
3	43	0.45	0.45
4	60	0.44	0.44
5	67	0.44	0.44

Analysis Based on Flory-Rehner Equation

Compared with the Mooney-Rivlin theory, the Flory-Rehner equation was derived from three dimensional networks of randomly coiled chains, and was based on the equilibrium swelling of polymer in solvent.[6,22,28–31] The Flory-Rehner equation is given as:

$$-\left[\ln(1-\upsilon_p) + \upsilon_p + \chi\upsilon_p^2\right] = NV_s\left[\upsilon_p^{1/3} - \frac{\upsilon_p}{2}\right] \quad (10)$$

where N is the crosslinking density (mol/m^3) and all other parameters bear the same meaning as before. Using the interaction parameter of 0.44 and the equilibrium volume fraction of polymer in the hydrogels determined earlier, the crosslinking density for hydrogels at different cellulose concentration was obtained and listed in Table IV. Table IV also summarizes the crosslinking density values obtained utilizing both the Flory-Rehner and the Mooney-Rivlin analyses. In general, very good agreement was found between the two methods. However, a closer look at the results showed that the crosslinking density values based on Mooney-Rivlin equation are lower than those based on the Flory-Rehner equation. This trend has also been noted by Sombatsompop when studying natural rubber systems,[32] and the differences were attributed to the conformation of the polymer chain involved in the two cases. That is the Mooney-Rivlin theory requires low levels of stretching of the polymer chain[23]

Table IV. Comparison of the Crosslinking Density Determination Based on Different Methods at 298 K

Avicel 101 nominal concentration (%)	Crosslinking density based on Flory-Rhener equation with $\chi = 0.44$ (mol/m^3)	Crosslinking density based on Mooney-Rivlin equation (mol/m^3)
2	25	19
3	41	35
4	51	48
5	56	54

whereas the Flory-Rehner does not require the alignment of the chains. As a result, not only the chemical crosslinks but also a certain fraction of the physical crosslinks (chain entanglement) will also be accounted for with the Flory-Rehner thoery. It is also worth noting there seems to be a jump in the true stress-strain response when comparing 4–5 wt % cellulose concentration, as shown in Figure 2(a). Although the exact nature of this effect is not fully understood, it is possible this is due to the intrinsic test variation associated with tensile testing.

CONCLUSION

The interaction parameter between water and Avicel 101 is found to be 0.44 and is in general agreement with the literature result of 0.4. The detailed Mooney-Rivlin analysis of the tensile data indicates these hydrogels can be well characterized by the ideal rubber theory with the C_2 term dropped to zero. Both the Mooney-Rivlin and the Flory-Rehner analyses generated similar level of crosslinking density, and the levels increase with Avicel concentration. However, the values obtained using the Flory-Rehner method are somewhat higher due to the fact that this theory does not require chain alignment, and thus the crosslink density includes not only chemical, but also physical crosslinks. While the determination of interaction parameter and crosslinking density based on Mooney-Rivlin and Flory theory remains to be classical techniques in polymer science field; in this study, we have successfully compared the pros and cons of both techniques in the quantification of the crosslinking density and interaction parameters of plant based cellulose hydrogels. Learning from this study will be used for drug incorporation into these gels for ocular injury treatment, and details will be reported separately.

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