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# Precise Control and Prediction of Hydrogel Degradation Behavior

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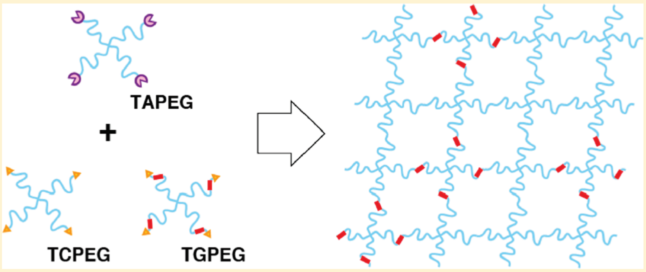

**ABSTRACT:** Hydrogels are promising candidate materials for biomedical applications because of their similarities to human tissue. However, precise and independent control of their physical properties is difficult because hydrogels inevitably have inhomogeneous polymer networks. In particular, precise and independent control of biodegradability, which is one of the most important characteristics for biomaterials, has never been achieved. In order to control the degradability, we invented a novel hydrogel based on the concept of Tetra-PEG gel, which has an extremely homogeneous polymer network. The degradability was precisely controlled, and the degradation time was predicted by a universal equation with one intrinsic parameter: the degradation rate constant.

## ■ INTRODUCTION

Hydrogels are promising candidate materials for use in drug delivery systems and regenerative medicine because of their high biocompatibility that stems from their similarities to human tissue.[1−3] The largely water-filled structure enables the containment and release of drugs and allows biological substances to permeate throughout its body. For practical applications, however, there are major problems to resolve, including mechanical fragility, poor control of elastic modulus, poor control of drug diffusibility, and poor control of degradation time. These problems mainly arise from the inhomogeneities in the structures of hydrogels.

Recently, as a new class of polymer network, we designed and fabricated Tetra-PEG gel. Tetra-PEG gel is formed by A−B type cross-end coupling of two tetra-arm poly(ethylene glycol) (PEG) units of the same size, which have mutually reactive terminal groups (Tetra-PEG units): amine (TAPEG) and activated ester (TCPEG).[4] Although Tetra-PEG gel is fabricated easily by mixing two aqueous solutions, it has an extremely homogeneous network structure, close to the ideal network structure.[5−7] This homogeneity contributes to its high transparency (∼100%), high deformability (∼900%), and high breaking strength (∼30 MPa) and allows for precise control of elastic modulus.[8] In this study, we tried to precisely control and predict the degradation properties of Tetra-PEG gel, taking advantage of its extreme homogeneity. If degradation behavior can be precisely controlled, Tetra-PEG gel will make an excellent material for use in drug delivery system and regenerative medicine.

Currently, there are two general methods for controlling the degradability: radical polymerization using a macro-cross-linker with cleavable sites[9,10] and stepwise polymerization of nondegradable multifunctional polymers forming cleavable bonds.[11] Although these methods allow control of degradability by tuning the initial design of gelling systems, the other important properties such as elastic modulus, mesh size, and water content, which need to be controlled independently, are inevitably varied. Furthermore, degradation behavior has never been fully characterized (e.g., over the full time scale from fabrication to elution).

In order to control the degradability, we have invented a novel molecular design based on the concept of Tetra-PEG gel. In addition to the existing two Tetra-PEG units, we designed a third unit, which contained a degradation site upstream of the terminal activated ester group (TGPEG, Figure 1a,c). TGPEG is expected to react with TAPEG to form an amide bond in the same manner as TCPEG (Figure 1b); the only difference is the coincidental installation of one cleavable site per amide bond. This design enabled us to control the degradability by simply changing the ratio of TCPEG to TGPEG without changing the original reaction system.

## ■ MATERIALS AND METHODS

**Synthesis of THPEG and TAPEG.** Tetraamine-terminated PEG (TAPEG) was prepared from tetrahydroxyl-terminated PEG (THPEG) with equal arm lengths. The fabrication method of tetrahydroxyl-terminated PEG (THPEG) having equal arm lengths has been reported previously.[7]

**Synthesis of TGPEG.** 10 g of THPEG was dissolved in 15 g of toluene. After dehydration, 0.69 g of glutaric anhydride and sodium acetate (50 mg) were added to the solution. The reaction was performed

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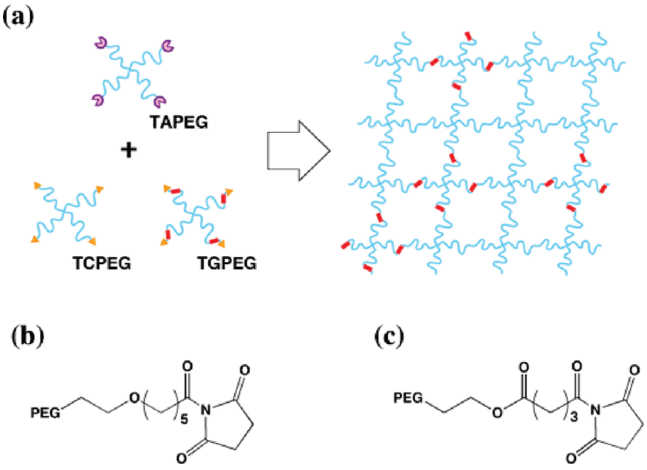

**Figure 1.** (a) Design of degradability-tunable Tetra-PEG gel. The end-group structures of TCPEG (b) and TGPEG (c). TGPEG introduced the cleavable ester site (shown as red square) into the network structure.

under reflux condition (~110 °C) for 12 h. The reaction solution containing tetraglutaric acid-terminated PEG was cooled to 40 °C. Then, 1.38 g of *N*-hydroxysuccinimide and 1.7 g of *N,N*′-2-dicyclohexylcarbodiimide were added and reacted at 40 °C for 3 h. The mixture was purified by a repeated crystallization process from a solvent system comprising toluene, ethyl acetate, and hexane. The crystalline precipitate was dried in a vacuum, and TGPEG was recovered.

**Synthesis of TCPEG.** 10 g of THPEG was oxidized in water (100 mL) with 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO) (200 mg, $1.30 \times 10^{-3}$ mol), NaBr (200 mg, $1.96 \times 10^{-3}$ mol), and 20 mL of aqueous NaClO (available chlorine >5.0%) at pH 10 at room temperature for 15 min. The oxidation was quenched by the addition of 20 mL of ethanol, followed by acidification with HCl to pH < 2 and three extractions with 100 mL aliquots of $CH_2Cl_2$. The combined $CH_2Cl_2$ layers were dried under reduced pressure and dissolved in 250 mL of hot ethanol, followed by precipitation in a freezer overnight. Another recrystallization with ethanol and vacuum-drying gave tetraglutaric acid-terminated PEG. Tetraglutaric acid-terminated PEG obtained thus was dissolved in THF. 1.4 g of *N*-hydroxysuccinimide and 1.7 g of *N,N*′-2-dicyclohexylcarbodiimide were added and reacted at 40 °C for 3 h. The mixture was purified by a repeated crystallization process from a solvent system comprised of toluene, ethyl acetate, and hexane. The crystalline precipitate was dried in a vacuum, and TCPEG was recovered.

**Characterization of Tetra-PEG Units.** The molecular weight and functionality were estimated by $^1$H NMR measurement. The $^1$H NMR spectra were obtained on a JEOL JNM-AL (300 MHz) or JEOL Alpha series (500 MHz) spectrometer with tetramethylsilane (TMS) as the internal standard and $CDCl_3$ as the solvent. The polydispersity was determined using gel permeation chromatography (TOSOH HLC-8220) system equipped with two TSK gel columns (G4000HHR and G3000HHR). The columns were eluted with DMF containing lithium chloride (10 mM) with a flow rate of 0.8 mL/min at 40 °C. The molecular weights were calibrated with poly(ethylene glycol) standards (Polymer Laboratories, Ltd., Church Station, UK).

**Fabrication of Tetra-PEG Gel.** Constant amounts of TAPEG (60 mg/mL) were dissolved in phosphate buffer (pH 7.4, 50 mM). Constant amounts of TGPEG and TCPEG (total amount 60 mg/mL) at a $r_{deg}$ to $(1 - r_{deg})$ ratio where $0 < r_{deg} < 1$ were dissolved in phosphate−citric acid buffer (pH 5.8, 50 mM). The two solutions were mixed, and the resulting solution was poured into the mold. At least 12 h was allowed for the completion of the reaction before the following experiment was performed.

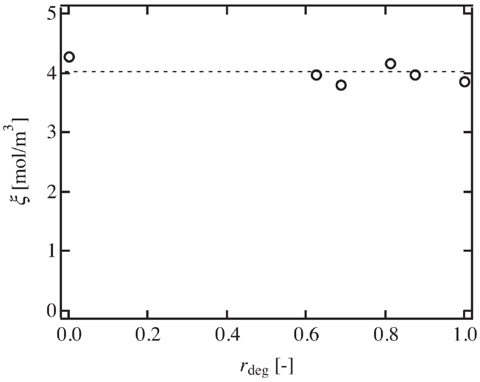

**Figure 2.** Cycle rank (ξ) of Tetra-PEG gels having different degradable unit fraction ($r_{deg}$).

**Stretching Measurement.** The stretching measurements were carried out on rectangular films (30 mm high, 5 mm wide, 2 mm thick) using a mechanical testing apparatus (CR-500DX-SII, Sun Scientific Co.) at a crosshead speed of 0.1 mm/s. The gel samples were used in the as-prepared state. Each specimen was stretched and released repeatedly two times, and the reproducibility was thus confirmed. More than 10 samples were tested, and the observed moduli were arithmetically averaged.

**Degradation Test.** The degradation measurements were carried out on cylinder-shaped samples (10 mm length, 1.9 mm diameter). Each specimen was immersed in the phosphate buffer (pH 7.4, 50 mM) at 37 °C. The diameter was measured using an optical microscope (M165C, Leica) once a day. More than three samples were tested, and the observed diameters were arithmetically averaged.

## ■ RESULTS AND DISCUSSION

**Elastic Modulus.** We formed the Tetra-PEG gel by mixing three kinds of tetra-PEG units, i.e., TAPEG, TGPEG, and TCPEG, at a 1 to $r_{deg}$ to $(1 - r_{deg})$ ratio (Figure 1). In order to investigate the effect of $r_{deg}$ on the physical structure of polymer network in as prepared state, we measured the elastic moduli ($G$) of Tetra-PEG gels with different $r_{deg}$. The mole density of cycle rank (ξ), which is the maximum number of independent loops contributing to the elasticity of polymer network, is related to $G$ as follows:

$$G = \xi RT \tag{1}$$

where $R$ is the gas constant and $T$ is the absolute temperature. The values of ξ are shown in Figure 2 against $r_{deg}$. The values of ξ were almost constant, suggesting that $r_{deg}$ does not affect the physical structure of polymer network. In other words, TGPEG and TCPEG have practically the same reactivity to TAPEG, and obtained hydrogels have the same elastic moduli, mesh size, and water content.

**Degradation Behavior.** We immersed the gel samples in the phosphate buffer (pH 7.4) at 37 °C and measured the degradation behavior. The variation of swelling ratio ($Q$), which is the ratio of the gel volume in the equilibrium-swollen state to that in the as-prepared state, of the Tetra-PEG gels with various $r_{deg}$ is shown in Figure 3. The gel samples ($r_{deg} \geq 0.69$) swelled with time and finally disintegrated, showing bulk degradation behavior. The degradation time ($t_{deg}$) was determined as the point at which no gel could be observed.

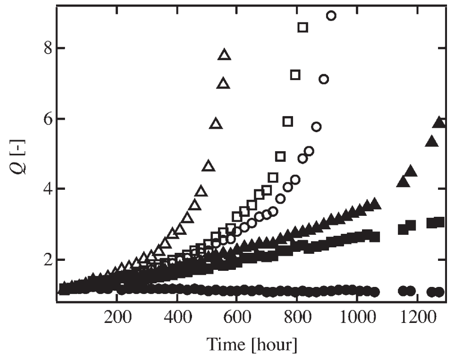

**Figure 3.** Time course of swelling ratio ($Q$) of Tetra-PEG gel with different $r_{deg}$: solid circle, $r_{deg} = 0$; solid square, $r_{deg} = 0.63$; solid triangle, $r_{deg} = 0.69$; open circle, $r_{deg} = 0.81$; open square, $r_{deg} = 0.88$; open triangle, $r_{deg} = 1.0$.

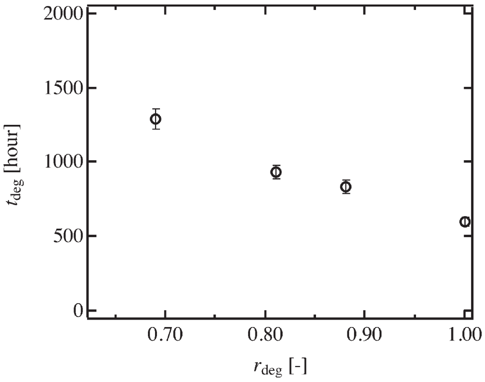

**Figure 4.** Degradation time ($t_{deg}$) as a function of $r_{deg}$.

As shown in Figure 4, $t_{deg}$ shortened with an increase in $r_{deg}$. The swelling ratio of Tetra-PEG gel with no degradation unit ($r_{deg} = 0$) was constant for over 50 days; i.e., the TCPEG unit was not cleaved. The gel sample ($r_{deg} = 0.63$) kept swelling, but did not elute, suggesting that this sample either does not elute or has extremely large $t_{deg}$.

According to the Flory–Rehner equation with phantom model,[12] $\xi$ is related to $Q$ as

$$\xi = \frac{\ln\left(1 - \frac{\phi_0}{Q}\right) + \frac{\phi_0}{Q} + \chi\left(\frac{\phi_0}{Q}\right)^2}{-V_1 Q^{-1/3}} \tag{2}$$

where $V_1$ is the molar volume of solvent (18 cm$^3$/mol), $\phi_0$ is the polymer volume fraction in the as-prepared state (0.053), and $\chi$ is Flory's interaction parameter. The value of $\chi$ was estimated to be 0.45 using the values of $\xi$ and $Q$ for nondegradable Tetra-PEG gel ($r_{deg} = 0$). The value of $\chi$ corresponded well with the value obtained by other authors.[11,13] The time course of cycle rank ($\xi$) estimated by eq 2 for all samples is shown in Figure 5. Here, it should be noted that the use of eq 2 must be restricted in the high $\xi$ region because it does not take into account the sol dissociated from the bulk gel with degradation. The dissociated sol fraction released from gel ($P(X_0)$: eq A2 in the Appendix) decreases the polymer fraction and the mixing energy term (numerator in eq 2). In the range $\xi > 2$, $P(X_0)$ was negligibly small ($\sim 10^{-2}$) and the estimated $\xi$ seemed valid.

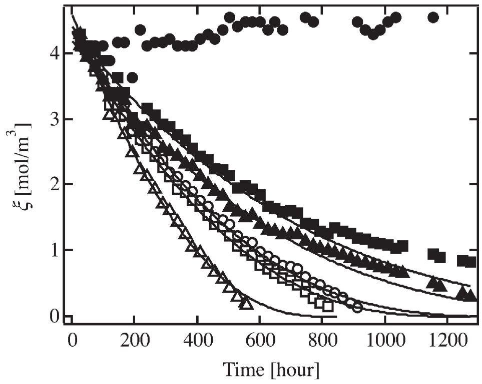

**Figure 5.** Variation of cycle rank ($\xi$) of Tetra-PEG gel having different degradable unit fraction ($r_{deg}$). The symbols show the same samples with Figure 3.

**Modeling of Degradation.** Following this, we set up a new model predicting the degradation behavior of Tetra-PEG gel. The theory of treelike structures is a powerful tool that describes the gelation threthold and postgelation properties of network structures.[14] According to the theory of treelike structures for tetrafunctional networks, $\xi$ is represented as

$$\xi = U\left(\frac{1}{2} + \left(\frac{1}{p} - \frac{3}{4}\right)^{1/2}\right)\left(\frac{3}{2} - \left(\frac{1}{p} - \frac{3}{4}\right)^{1/2}\right)^3 \tag{3}$$

where $U$ is the molar concentration of the tetra-arm polymer in a unit volume of material and $p$ is reaction conversion (fraction of connected bond). Here, we assumed that the reactivities of TCPEG and TGPEG to the TAPEG were the same and that the degradable units dispersed homogeneously and were hydrolyzed over time according to pseudo-first-order kinetics. Considering the case where Tetra-PEG gel has the constant fraction of degradable unit ($r_{deg}$), $p$ is represented as

$$p = p_0((1 - r_{deg}) + r_{deg}\exp(-k_{deg}t)) \tag{4}$$

where $p_0$, $k_{deg}$, and $t$ are the initial fraction of connected bonds, degradation rate constant, and time, respectively. By substituting eq 4 into eq 3, the time variation of $\xi$ is represented as

$$\xi = U\left(\frac{1}{2} + \left(\frac{1}{p_0((1-r_{deg}) + r_{deg}\exp(-k_{deg}t))} - \frac{3}{4}\right)^{1/2}\right)\left(\frac{3}{2} - \left(\frac{1}{p_0((1-r_{deg}) + r_{deg}\exp(-k_{deg}t))} - \frac{3}{4}\right)^{1/2}\right)^3 \tag{5}$$

In order to evaluate our model, we fitted the experimental data ($\xi > 2$) using eq 5 (solid lines in Figure 5). The value of $k_{deg}$ was determined from the least-squares method. The curve fit worked well for all samples; the slight upward deviation of points was attributed to the dissociation of the sol mentioned above. The value of $k_{deg}$, which is the fitting parameter, is plotted against $r_{deg}$ in Figure 6. The values of $k_{deg}$ were almost constant, confirming the validity of our model. The average value of $k_{deg}$ was 9.76 × $10^{-4}$ (1/h) and is shown as a dashed line in Figure 6.

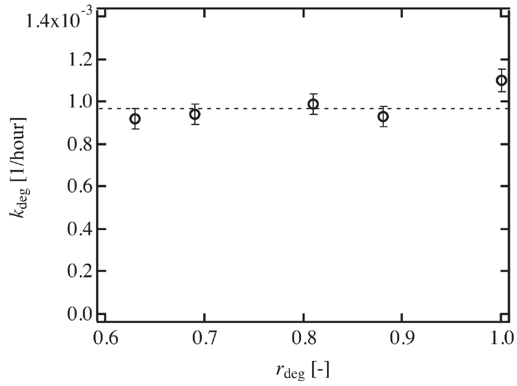

**Figure 6.** Degradation rate constant ($k_{gel}$) as a function of $r_{deg}$.

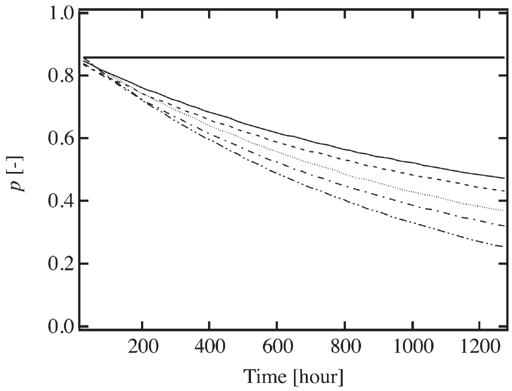

**Figure 7.** Time course of the fraction of connected bond ($p$) of Tetra-PEG gels having different degradable unit fraction ($r_{deg}$): thick line, $r_{deg} = 0$; thin line, $r_{deg} = 0.63$; dashed line, $r_{deg} = 0.69$; dotted line, $r_{deg} = 0.81$; long dashed dotted line, $r_{deg} = 0.88$; long dashed double-dotted line, $r_{deg} = 1.0$.

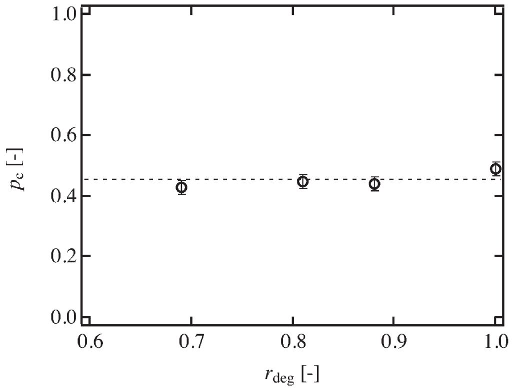

**Figure 8.** Fraction of connected bond at degradation ($p_c$) as a function $r_{deg}$.

Because it was confirmed that the degradation behavior of Tetra-PEG gel could be modeled using the universal parameter $k_{deg}$, we can now predict the time course of $p$. The time course of $p$ was estimated using eq 4 for different $r_{deg}$ values (Figure 7). We then estimated the fraction of connected bonds at degradation ($p_c$) using $t_{deg}$ and time course of $p$ estimated using eq 4. As shown in Figure 8, all gel samples had identical values of $p_c$ ($p_c = 0.46$); i.e., all gel samples sustained their 3D continuous structure above the $p_c$ and then immediately degraded into soluble clusters below the $p_c$. Although this value is different from the $p_c$ calculated by the theory of treelike structures ($p_c = 1/3$), the deviation is conceivable, considering that the real network structure is not "treelike".[15] This deviation had been predicted by using the spanning-tree model, which is a more realistic model than the theory of treelike structure.[16] This value is also similar to the value predicted by site percolation model ($p_c = 0.43$) and bond percolation model ($p_c = 0.39$) for diamond lattice.[17] This slight discrepancy between the experimental value and the theoretical prediction is probably because the network of Tetra-PEG gel is not a perfect diamond lattice and/or degradation sites do not distribute homogeneously in the network; i.e., degradation sites are only on TGPEG.

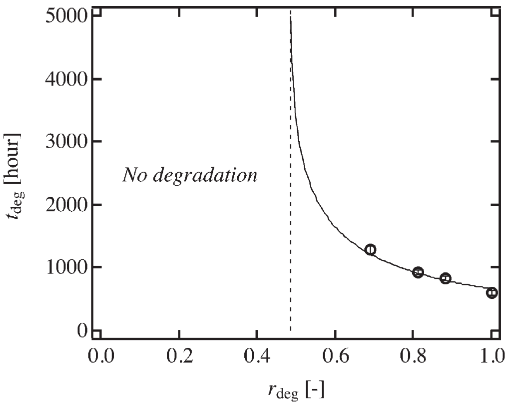

**Figure 9.** Degradation time ($t_{deg}$) as a function of $r_{deg}$.

In the above discussion, we confirmed the validity of our assumptions and estimated the degradation criterion, $p_c$. By substituting $p = p_c$ into eq 4, we can set up the equation describing the relationship between $r_{deg}$ and $t_{deg}$ as

$$t_{deg} = \frac{1}{k_{deg}} \ln \frac{r_{deg}}{\frac{p_c}{p_0} - (1 - r_{deg})} \tag{6}$$

The theoretical curve and experimental data of $t_{deg}$ as a function of $r_{deg}$ are shown in Figure 9. Here, we substituted $k_{deg} = 9.76 \times 10^{-4}$, $p_0 = 0.86$, and $p_c = 0.46$. As clearly shown in Figure 9, our model precisely described the degradation behavior of Tetra-PEG gel; we are now able to precisely predict and control the $t_{deg}$ by tuning the $r_{deg}$.

## ■ CONCLUSIONS

The ability to control the degradability of hydrogels has been sought after for many years. In previous attempts, the methods to control the degradation rate affected the other important properties that need to be controlled independently. In addition, the precise prediction of degradation behavior over full time scale using an equation with intrinsic parameters has never been achieved; there were only scaling arguments and kinetic costatistical models predicting initial degradation.[9,11]

In contrast, we have invented a novel system by which we can tune the degradation behavior without affecting other properties. Using this system, the degradation behavior over the full time scale of a hydrogel has been, for the first time, precisely predicted by a universal equation with one intrinsic parameter, $k_{\mathrm{deg}}$. Thus, we are able to predict the degradation behavior in any media by knowing the $k_{\mathrm{deg}}$. Because the precise prediction and control of degradation behavior is an essential property for materials used in drug delivery systems and regenerative medicine, Tetra-PEG gel will make a promising candidate material for these applications.

## APPENDIX. MODELING OF DEGRADATION

The theory of treelike structure is based on the recursive nature of the branching process and on elementary probability laws under three assumptions including (1) all functional groups of the same type are equally reactive, (2) all groups react independently of one another, and (3) no intramolecular reactions occur in finite species. For the tetrafunctional network, we correlate the fraction of connected bonds ($p$) and the probability that 1 of 4 arms leads out to a finite chain ($P(F^{\mathrm{out}})$)

$$P(F^{\mathrm{out}}) = \left(\frac{1}{p} - \frac{3}{4}\right)^{1/2} - \frac{1}{2} \tag{A1}$$

Using $P(F^{\mathrm{out}})$, the probability that a given tetra-arm polymer was a connection degree of $n$ ($P(X_n)$) can be calculated as

$$P(X_n) = {}_4C_n P(F^{\mathrm{out}})^{(4-n)}[1 - P(F^{\mathrm{out}})]^n \tag{A2}$$

Because the connections become cross-links only when the $n$ is equal to or greater than 3, the concentrations of active cross-links ($\mu$) and of elastically effective chains ($\nu$) are represented as follows:

$$\mu = U(P(X_3) + P(X_4)) \tag{A3}$$

$$\nu = U\left(\frac{3}{2}P(X_3) + \frac{4}{2}P(X_4)\right) \tag{A4}$$

Here, $U$ represents the molar concentration of the tetra-arm polymer in the unit volume of material. Therefore, the cycle rank ($\xi$) is given by

$$\xi = \nu - \mu = U\left(\frac{1}{2}P(X_3) + P(X_4)\right) \tag{A5}$$

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