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Synthesis and Mechanical Properties of a Nanocomposite Gel Consisting of a Tetra-PEG/Clay Network

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ABSTRACT: Tetra-poly(ethylene glycol) (PEG)-based nanocomposite hydrogels (tP-NC gels) were prepared by in situ polymerization of two kinds of macromonomer (TN-PEG and TA-PEG), each having four reactive PEG arms, in the presence of clay (Laponite XLG) in aqueous media. By adopting appropriate synthetic conditions such as the use of pyrophosphate-Na buffer and selected homogeneous mixing procedures, tP-NC gels with high transparency and excellent tensile properties, e.g., high elongation (900−1000%) and high tensile strength (300−560 kPa), approximately 2−4 times those of the corresponding original tetra-PEG gel, were obtained at a relatively low clay concentration ($C_{clay} = 2 \times 10^{-2}$ mol/L-H_2O) and a wide range of polymer concentrations ($C_p = 120-240$ mg/mL-H_2O). We investigated the effects of buffer, C_{clay}, and C_p on forming tetra-PEG/clay gels, their tensile properties, and the network structures. Also, we studied interactions between the clay and tetra-PEG segments, by measurements such as viscometry, X-ray fluorescence, transmission electron microscopy, and Fourier transfer infrared spectroscopy. It was revealed that the pyrophosphate-Na buffer plays an important role in dispersing exfoliated clay uniformly in the reaction solution and that the clay platelets incorporated into the tetra-PEG networks interact with a specific ester group in the tetra-PEG arm as well as with amide linkages in the tetra-PEG networks so as to improve their tensile properties.

Introduction

Polymer hydrogels consisting of three-dimensional polymer networks and large amounts of water filling the interstitial space of the network have received extensive attention as transparent, soft, and wet materials,[1,2] since their compositions (nearly 90 wt % or more water) and characteristics (including functions related to biomedical, optical, electrical, analytical fields, etc.) are totally different from those of conventional solid materials. To be utilized in practical applications, it is important that these hydrogels attain the mechanical properties required for various objectives. Most polymer hydrogels used so far, consisting of random arrangements of large numbers of cross-links, are mechanically fragile; the networks are readily broken by small stresses or strains due to the localization of stress in the shorter cross-linking chain.[3] Therefore, an effective way to improve the mechanical properties of hydrogels is to fabricate a network structure in which stress localization is minimized. In the past decade, three different types of such a hydrogel have been reported. "Slide-ring gels"[4] can relax stress localization due to sliding cross-linkers consisting of two cyclodextrin rings, and "nanocomposite gels"[5−7] consist of organic (polymer)/inorganic (clay) networks which cause little stress localization due to plane cross-linking on the surface of clay platelets acting as multifunctional cross-linking agents and can be substantially extended by the entropic effects of flexible polymer chains between clay platelets. Finally, "double network gels"[8] consist of interpenetrating polymer networks which dissipate the energy of stress localization by deforming the flexible network segments. All of these hydrogels achieved high tenacity by fabricating novel networks using different approaches that reduced or nearly eliminated stress localization.

Concerning gels consisting of poly(ethylene glycol) (PEG), the gelation behaviors of various types of copolymer or hybrid systems such as PEG−polycaprolactone,[9] PEG−poly(lactide),[10] PEG−heparin,[11] and PEG−alginate[12] have been studied. Also, a rheological study on a clay−PEG system[13] has been reported. However, the gels obtained were not gels that exhibit high mechanical properties, such as reversible large extensions and high strengths, but they were all very weak gels similar to viscous solutions or microspheres.

Recently, Sakai and Chung developed a new class of high-strength PEG hydrogel with a well-designed homogeneous network structure.[14] The gel was prepared by mixing aqueous solutions of two kinds of symmetrical tetrahedron-like (four-armed) poly(ethylene glycol) macromonomers (TA-PEG and TN-PEG) with a fixed molecular weight (e.g., 20 000 g mol^{-1}) and different end-linking groups (Scheme 1a). As a result of reactions between equimolar proportions of mutually reactive terminal groups, i.e., the propylamine of TA-PEG and the succinimidyl glutarate of TN-PEG, homogeneous three-dimensional network structures with identical molecular weights between cross-links were fabricated. Because of the high homogeneity of the network,[14,15] the resulting tetra-PEG gel showed improved mechanical properties such as high compressive strength (average of 3.3 MPa at polymer concentration (C_p) = 120 mg/mL), although the tensile properties are not very high, as will be shown in the present paper. Tetra-PEG gels have many practical merits including a simple method of synthesis in which gels are prepared by mixing two macromonomer solutions for a few minutes at ambient temperature, without the formation of any toxic substance during synthesis, and the biocompatibility of the constituent

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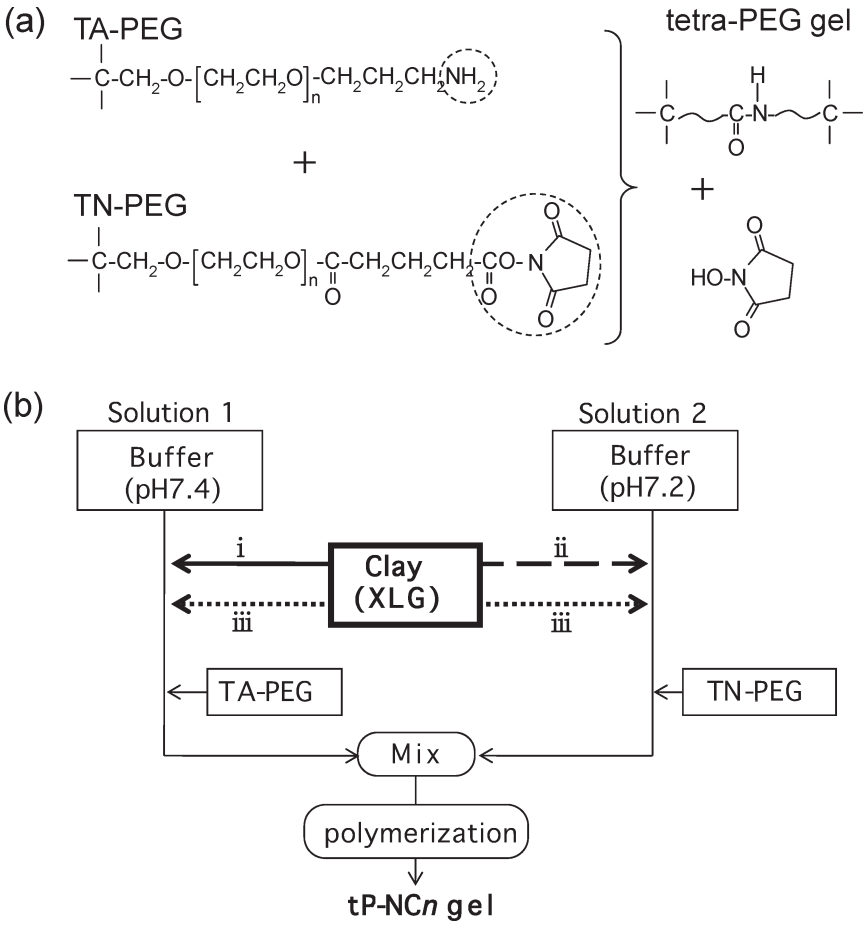
Scheme 1. (a) Reactions between TA-PEG and TN-PEG for the Synthesis of Tetra-PEG Gels; (b) Synthetic Procedures for tP-NC*n* Gels Using Pyrophosphate-Na as a Buffer[a]



[a] Three types of tP-NC*n* gels ((i) tP-NC*n*-A, (ii) tP-NC*n*-N, and (iii) tP-NC*n*-AN gels) were obtained according to three different mixing methods, (i)−(iii).

macromonomers and resulting tetra-PEG gel. Therefore, in order to widen the range of potential applications of tetra-PEG gels, it is desirable to improve their tensile properties while maintaining structural homogeneity (i.e., high transparency) in addition to the advantages cited above.

It has been reported that nanocomposite (NC) gels consisting of poly(*N*-isopropylacrylamide) or poly(*N,N*-dimethylacrylamide) and inorganic clay exhibit extraordinary tensile properties[16,17] such as high elongation at break (≥1000%) and tensile strength (100−1000 kPa) since a unique polymer/clay network structure is formed via interactions between the clay surface and the amide group of the constituent polymer.[18] Here, we expected that NC-type tetra-PEG gels would display improved tensile properties since tetra-PEG gels also have amide linkages due to the reaction between the two kinds of macromonomer. In the present study, we investigated the synthesis of a tetra-PEG based NC gel (tP-NC gel) with excellent tensile properties and high transparency by incorporating clay nanoparticles into the tetra-PEG network. In addition, we discuss how the effective tetra-PEG/clay network is formed via interactions between the clay and the tetra-PEG constituents.

Experimental Section

Raw Materials. Tetraamine-terminated PEG (TA-PEG), tetra-NHS-glutarate-terminated PEG (TN-PEG), and tetra- NHS-carboxypentyl-terminated PEG (TC-PEG), all with a molecular weight of ca. 20 000 g mol^{-1}, were purchased from NOF Co. Japan and used without further purification. Here, NHS denotes *N*-hydroxysuccinimide. Actual molecular weight is 20 444 (TA-PEG), 19 389 (TN-PEG), and 21 060 g mol^{-1} (TC-PEG). For TA-PEG and TN-PEG, a different molecular weight (ca. 5000 g mol^{-1}) was also used. Actual molecular weights were 5328 (TA-PEG) and 5341 g mol^{-1} (TN-PEG). As for the buffer, phosphate-Na ($H_2NaPO_4 + HNa_2PO_4$) and pyrophosphate-Na ($Na_4P_2O_7$) were used. As an inorganic clay, the synthetic hectorite "Laponite XLG" (Rockwood Ltd., UK; $[Mg_{5.34}Li_{0.66}Si_8O_{20}(OH)_4]Na^+_{0.66}$: a layer size ~30 nm in diameter and 1 nm thick) was used after purification by washing and freeze-drying. The water used for all experiments was ultrapure water supplied by a Puric-Mx system (Organo Co., Japan).

Synthesis of tP-NC Gels and Sample Nomenclature. tP-NC gels were synthesized by reacting equimolar quantities of two kinds of macromonomer, TA-PEG and TN-PEG, in the presence of clay in aqueous media, using a buffer solution. Before reacting TA-PEG and TN-PEG together, the inorganic clay (Laponite XLG) was mixed with macromonomer in aqueous solution by one of the following three methods: (i) clay first mixed with TA-PEG and then with TN-PEG, (ii) clay with TN-PEG and then with TA-PEG, and (iii) half the amount of clay with each of TA-PEG and TN-PEG and the resulting mixtures combined. For reactions using pyrophosphate-Na buffer, the synthetic procedures for tP-NC*n* gels are shown schematically in Scheme 1b. For example, for route i, a transparent aqueous clay suspension consisting of a 100 mM buffer (pH 7.4) (15 mL) and inorganic clay (Laponite XLG) (0.3 g) was first prepared. Next, TA-PEG (1.2 g) was added to the clay suspension, and the mixture was stirred at room temperature for 15 min (solution 1). TN-PEG (1.2 g) was separately dissolved in 5 mL of 100 mM

buffer (pH 7.2) (solution 2); because of the similarity of molecular weights of TA-PEG and TN-PEG, equal weights were used. Then, solutions 1 and 2 were mixed at 4 °C, and polymerization proceeded at room temperature (~25 °C) for 2 h. The resulting gels were named (i) tP-NCn_{py}-A, (ii) tP-NCn_{py}-N, and (iii) tP-NCn_{py}-AN gels depending on the mixing procedure (i)−(iii) described above and in Scheme 1b. Here, n and the suffix respectively denote the clay concentration ($C_{clay} = n \times 10^{-2}$ mol/L-H_2O) and the kind of buffer (e.g., py for pyrophosphate-Na and ph for phosphate-Na) in the reaction solution. C_{clay} was varied in the range of $1 \le n \le 20$. The weight ratio of TA-PEG/TN-PEG was always fixed at 1:1. The polymer concentration (C_p) was normally 120 mg/mL but was varied within the range of 80−240 mg/mL to study the effect of C_p on tensile properties.

Measurements. *Viscosity*. Viscosity measurements were performed for two purposes. One was to reveal the effects of various kinds of buffer on (time-dependent) changes in the viscosity of aqueous clay suspensions. The other was to assess the effect of adding macromonomer on the viscosity of aqueous clay suspensions. Aqueous clay suspensions were stirred for 15 min at 35 °C, and viscosities were measured at 20 °C using a vibration digital viscometer VM-100A (Yamaichi Electronics, Japan). The sensor was fixed at the center of the suspension (20 mL) contained in a glass vessel.

Optical Transmittance. Optical transmittances were measured at 600 nm using a UV/vis spectrophotometer (V-530, JASCO Co., Japan) at room temperature for gel samples 10 mm thick and for reaction solutions in a polystyrene cuvette (10 × 10 × 30 mm).

Analytical Measurements. Thermogravimetric (TG), X-ray fluorescence (XRF), and transmission electron microscopy (TEM) measurements were carried out for washed and dried tP-NC gels to analyze their compositions and structures. Washing and drying processes were as follows: the gel was soaked in excess amount water at 20 °C for 24 h, changing the water several times. After washing, the gel was dried at room temperature and subsequently dried in vacuum at 25 °C for 6 h. TG measurements were conducted with a TG/DTA 220 (Seiko Denshi Ind. Inc., Japan) heated from 30 to 800 °C at rate of 10 °C/min^{-1} in an air flow. The clay content of the dried gel was evaluated from the residual weight at 800 °C. XRF measurements were carried out for washed and dried gels using an X-ray fluorescence analyzer (Rigaku). TEM measurements were performed with a JEM-2200FS (Nihon Denshi Co.) at 200 kV for washed and dried tP-NC gels. Ultrathin films (ca. 70 nm thick) of dried tP-NC gels were prepared by cutting the dried gels using an ultramicrotome.

Fourier Transfer Infrared (FTIR) Spectroscopy. To prepare macromonomer/clay samples for FTIR measurements, macromonomer (e.g., TA-PEG, TN-PEG, TC-PEG) was added to the transparent aqueous clay suspension (10 mL). The mixture was lyophilized immediately after mixing at room temperature for 2 min. FTIR spectra were obtained using FTIR 4200 (JASCO Co., Japan) with KBr pellets containing tetra-PEG, freeze-dried tetra-PEG/clay, and freeze-dried tP-NC gel. Values of C_{clay} were in the range of $1 \le n \le 5$, while C_p was either 120 or 60 mg/mL.

Tensile Properties. Tensile properties were measured for rectangular films (30 mm long, 10 mm wide, and 1 mm thick) of tP-NCn gels using a Autograph AGS-H (Shimadzu, Japan), at a cross-head speed of 100 mm/min. The tensile strengths and moduli were calculated on the basis of the initial cross-sectional areas. Average values from three experiments were determined.

Results and Discussion

Synthesis of tP-NC gels in Phosphate Buffer Solution. Tetra-PEG gels were synthesized by reacting two kinds of macromonomer (TA-PEG and TN-PEG) in aqueous media, using a buffer solution to maintain appropriate pH conditions for the reaction of the end-linking groups [NH_2 of TA-PEG and activated ester (*N*-hydroxysuccinimide-glutarate) of TN-PEG],[19] as shown in Scheme 1a. In a previous paper,[14] we used phosphate-Na as a buffer to synthesize tetra-PEG gels. The synthesis of tP-NC gels consisting of a tetra-PEG/clay network requires the reaction of equimolar quantities of the two macromonomers in the presence of exfoliated clay platelets in aqueous media. Then, it is important to prepare uniform macromonomer/clay solutions devoid of heterogeneous aggregations due to inadequate exfoliation of clay and clay−macromonomer aggregates, since aqueous clay suspensions often become viscous or form aggregates on adding salt.[18] First, in order to synthesize tP-NC gel in the present study, a phosphate-Na buffer was used in a manner similar that used to synthesize tetra-PEG gels.[14] The resulting gels were named tP-NCn_{ph}-A, tP-NCn_{ph}-N, and tP-NCn_{ph}-AN gels according to the mixing methods employed, as described in the Experimental Section. Here, suffix ph denotes the kind of buffer (phosphate-Na). C_p was fixed at 120 mg/mL, since this is a well-balanced polymer concentration from the perspective of good mechanical (compressive) properties of tetra-PEG gels and low viscosity of macromonomer solutions.[14]

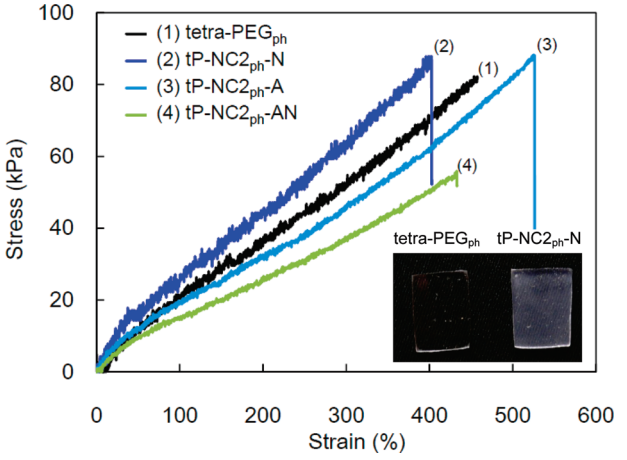
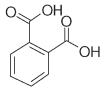
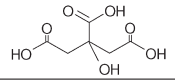
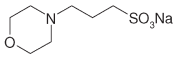
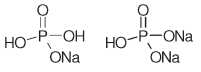
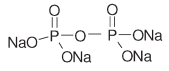


Figure 1. Stress−strain curves for (1) tetra-PEG$_{ph}$ gel and three types of tP-NC2$_{ph}$ ((2) tP-NC2$_{ph}$-N, (3) tP-NC2$_{ph}$-A, and (4) tP-NC2$_{ph}$-AN) gels synthesized using phosphate-Na. Inset photos show tetra-PEG$_{ph}$ and tP-NC2$_{ph}$-N gels.

In tP-NC gel syntheses using phosphate-Na buffer, it was found that the macromonomer/clay suspensions showed very high viscosities and turbidities due to interactions between clay and phosphate-Na. In order to synthesize tP-NC gels with as high a uniformity as possible, we finally selected a lower concentration (50 mM) of phosphate-Na buffer, although the resulting TN-PEG/clay and TA-PEG/clay suspensions still developed a high viscosity of about 70−80 cps and a low transparency of 10−20%. We thus prepared three types of tP-NC2 gels by mixing two aqueous solutions containing macromonomer, phosphate-Na buffer, and/or clay at room temperature (25 °C) for 3 min while stirring, after which the mixture was kept for 2 h without stirring. As shown in the inset photographs in Figure 1, the resulting tP-NC2$_{ph}$ gels were all fairly opaque almost regardless of mixing procedure, although tetra-PEG gel without clay was transparent. The opacity of tP-NC2$_{ph}$ gels indicates that the clay aggregated during their synthesis.

The tensile stress−strain curves for the three kinds of tP-NC2$_{ph}$ gels and tetra-PEG gel are shown in Figure 1. In general, tP-NC2$_{ph}$ gels showed tensile properties very similar to those of tetra-PEG gel. For tP-NC2$_{ph}$-N and tP-NC2$_{ph}$-A gels, the tensile strengths (TS) were the same as that of tetra-PEG gel, although the modulus (E) was slightly

Table 1. Effects of Buffer on the Transparency and Viscosity of the Aqueous Clay Suspension[a]

Buffer	Composition	Clay suspension	
		Transmittance (%)	Viscosity (cps)
None	-	96	1.6
Boric acid	H_3BO_4, $Na_2B_4O_7$	47	36
Carbonic acid	$NaHCO_3$, Na_2CO_3	88	60
Phthalic acid		2	2
Citric acid		30	11
MOPS-Na		74	96
Phosphate-Na		55	50
Pyrophosphate-Na		96	1.6

[a] $C_{clay} = 2 \times 10^{-2}$ mol/L-H_2O (1.6 wt %). Buffer concentration = 10 mM. Viscosity was measured after maintaining the suspension at 20 °C for 40 min.

different: tP-NC2$_{ph}$-N > tP-NC2$_{ph}$-A > tP-NC2$_{ph}$-AN. These data suggest that clay interacts with TN-PEG more strongly than with TA-PEG. On the other hand, tP-NC2$_{ph}$-AN gel showed the lowest values for both E and TS probably because homogeneous mixing was most difficult for this gel due to the high viscosities of each of the aqueous solutions, each containing clay, prior to reaction. Furthermore, we observed that tP-NCn_{ph} gels prepared using higher C_{clay} showed inferior mechanical properties. Thus, simply adding clay to the reaction solutions does not produce the desired tP-NC gels with excellent optical and mechanical properties. The opacity in all gels indicates that uniform dispersion of exfoliated clay, a decisive factor for forming polymer/clay networks, was not achieved in tP-NC2$_{ph}$ gels. Therefore, in order to prepare uniform and transparent tP-NC gels with excellent mechanical properties, we sought to clarify the effects of the buffers used in the syntheses—used to maintain appropriate pH conditions of the system[19,20]—on the transparency and viscosity of aqueous clay suspensions.

It is known that the viscosities of aqueous clay suspensions are strongly affected by the presence of ionic salts or polar compounds.[18] Table 1 shows changes in the optical transmittance and viscosity of an aqueous clay suspension ($C_{clay} = 2 \times 10^{-2}$ mol/L-H_2O) on adding various kinds of buffer (10 mM). In buffer-free water, clay (Laponite XLG) is exfoliated into disklike particles (about 30 nm in diameter and 1 nm thick) with a strongly negative face charge and a weakly positive rim charge. A uniform and transparent aqueous clay suspension is obtained (transmittance = 96%, viscosity = 1.6 cps) because the disklike particles are stabilized by electrostatic repulsions. When acidic buffers or their salts such as boric acid, carbonic acid, phthalic acid, citric acid, and 3-morpholinopropanesulfonic acid (MOPS) were added, clay platelets tended to aggregate, and the viscosity of the suspension became high (36−60 cps) due to the presence of clay−acid interactions. Also, phosphate-Na buffer, which has been used for preparing tetra-PEG gels, caused low

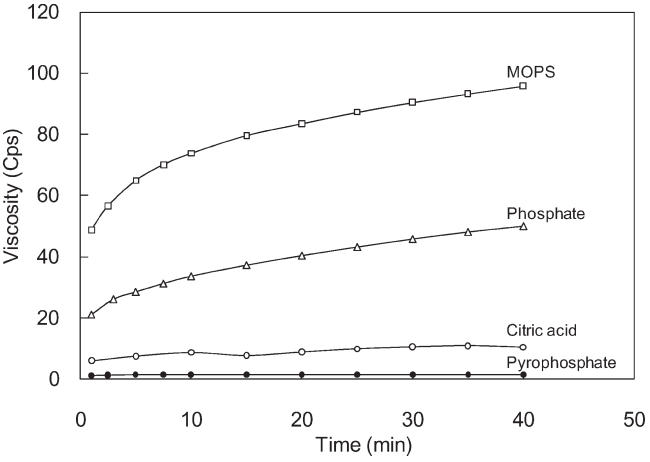


Figure 2. Effects of buffer on the viscosity of the aqueous clay suspension. $C_{clay} = 2 \times 10^{-2}$ mol/L-H_2O (1.6 wt %). Buffer concentration = 10 mM. Temperature = 20 °C.

transparency (55%) and high viscosity (50 cps) simultaneously (Table 1). As tP-NC2 gels with inadequate mechanical and optical properties were obtained by using phosphate-Na buffer, we conclude that phosphate-Na buffer and other buffers described above are unsuitable for preparing reaction solutions with high transparency and low viscosity.

In contrast, the effect of pyrophosphate-Na buffer on viscosity and transparency was quite different. As shown in Figure 2, an aqueous clay suspension containing pyrophosphate-Na displayed a reliably low viscosity (1.6 cps) and high transparency (96%). Pyrophosphate-Na, which is a tetravalent negatively charged ion, may adsorb onto the positively charged rims of platelets and effectively screen the rim charge.[21] This effect prevents development of the rim−face interaction of clay platelets (i.e., preventing the formation of the house-of-cards structure[22]) and causes the reliably low viscosity and high transparency of aqueous clay suspensions. In fact, pyrophosphate-Na is used commercially as an additive to reduce the rate of Laponite aggregation.

Before studying tP-NC gel synthesis using pyrophosphate-Na, it was necessary to investigate the effect of pyrophosphate-Na on the synthesis and properties of a tetra-PEG gel (≡ tP-NC0 gel). We observed that tetra-PEG gel prepared with a 100 mM pyrophosphate-Na buffer was uniform and transparent. (1) and (2) in Figure 3 show the tensile stress−strain curves for tetra-PEG gels prepared with phosphate-Na (tetra-PEG$_{ph}$ gel) and pyrophosphate-Na (tetra-PEG$_{py}$ gel), respectively. Here, the tensile properties of tetra-PEG$_{py}$ gel are almost identical to those of tetra-PEG$_{ph}$ gel. We therefore surmised that the use of pyrophosphate-Na buffer might be suitable for preparing tP-NC gels. In the following section, we describe results obtained using pyrophosphate-Na buffer and a C_p of 120 mg/mL (unless otherwise noted) for preparing tP-NC gels.

Synthesis of tP-NC*n* Gels in Pyrophosphate Buffer Solution. Scheme 1b shows the synthetic procedures for tP-NCn gels using pyrophosphate-Na buffer (100 mM). The resulting tP-NC2-A, tP-NC2-N, and tP-NC2-AN gels, where the suffix py was omitted except for comparisons with phosphate-Na (ph), were all uniform and transparent regardless of the mixing procedure, as shown in the inset photo of Figure 3, although the mechanical properties differed with the method. The tensile stress−strain curves for tP-NC2-A, tP-NC2-N, and tP-NC2-AN gels prepared with pyrophosphate-Na buffer are shown in (3), (4), and (5) of Figure 3,

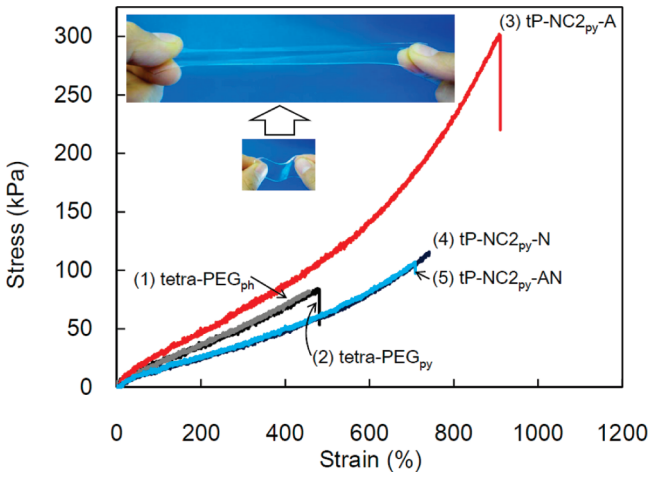


Figure 3. Tensile stress−strain curves for tetra-PEG gels and tP-NC2 gels synthesized with different buffers and mixing procedures. The suffixes ph and py respectively indicate phosphate and pyrophosphate buffer. Inset photograph shows a transparent and stretchable tP-NC2$_{py}$-A gel film. C_p = 120 mg/mL.

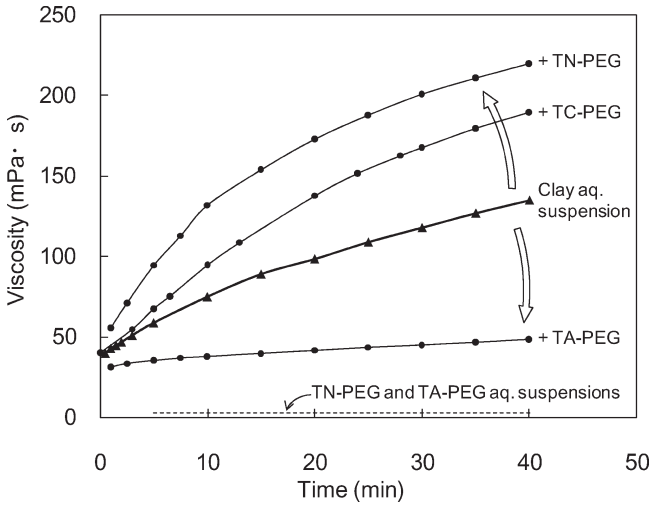


Figure 4. Effects of adding macromonomer (TN-PEG, TA-PEG, and TC-PEG) on the viscosity of the aqueous clay suspension. $C_{clay} = 5 \times 10^{-2}$ mol/L-H_2O (4 wt %). C_p = 2.2 mmol/L-H_2O (4.4 wt %). The viscosity of each macromonomer solution (TN-PEG and TA-PEG) is about 3 cps as shown by a dotted line.

respectively. tP-NC2-A gel in particular displayed mechanical properties (for E, TS, and ε_b) superior to those for the other gels (tP-NC2-N, tP-NC2-AN, and tetra-PEG gels). These results indicate that adding clay to the TN-PEG solution, as used in the syntheses of tP-NC2-N and tP-NC2-AN gels, is unsuitable for synthesizing tP-NC gels with excellent mechanical properties. In addition, tP-NC2-N and tP-NC2-AN gels exhibited lower moduli than pristine tetra-PEG gel (≡ tP-NC0 gel) (Figure 3). This implies that adding clay to the TN-PEG solution interferes with the polymerization (i.e., cross-linking) reaction of TA-PEG and TN-PEG, presumably due to the strong interaction between clay and TN-PEG molecules in solution. Therefore, the cross-link density (∝ E) in the resulting tP-NC2-N and tP-NC2-AN gels is lower than that of tetra-PEG gel.

To reveal the interactions between clay and two macromonomers (TN-PEG and TA-PEG), we investigated changes in the viscosity of aqueous clay suspensions with a relatively high clay concentration ($C_{clay} = 5 \times 10^{-2}$ mol/L-H_2O) upon adding the macromonomers (Figure 4). TN-PEG and TA-PEG displayed diametrically opposite tendencies; the viscosity of the clay suspension significantly increased on adding TN-PEG, whereas the increase in viscosity of an aqueous clay suspension with retention time was markedly depressed by adding TA-PEG. These results suggest that the end-linking group of TN-PEG strongly interacts with clay platelets in aqueous media, although the resulting TN-PEG/clay suspension is not a gel with a fixed form and high mechanical properties, but a very weak gel similar to aqueous clay suspension with a little higher clay concentration. On the other hand, TA-PEG molecules prevent the clay platelets from forming a house-of-cards structure by surrounding each platelet with a mildly interacting macromonomer that effectively screens the electrostatic clay−clay interaction. Thus, by retaining the molecular dispersion of clay platelets, the TA-PEG/clay aqueous solution shows high transparency. The effects of adding TN-PEG and TA-PEG are quite similar to those of adding ionic salts (e.g., KCl, $CaCl_2$, potassium persulfate) and nonionic monomers (e.g., *N*-isopropylacrylamide), respectively.[18]

Thus, the use of a clay/TN-PEG solution appears to disturb the ensuing polymerization (cross-linking) reaction with TA-PEG due to the strong interaction between TN-PEG and clay. Consistent with this view, the E values of tP-NC2-N and tP-NC2-AN gels are lower than that of tetra-PEG (≡ tP-NC0) gel (Figure 3). On the other hand, with a clay/TA-PEG solution, it is possible to prepare a homogeneously mixed reaction solution since both TN-PEG and clay/TA-PEG solutions have low viscosities (29 and 9 cps) and high transparencies (78% and 83%) in 100 mM pyrophosphate-Na buffer. This enables the clay platelets, molecularly dispersed in the reaction solution, to be fixed through the cross-linking reaction. Thus, clay platelets can be effectively incorporated into tetra-PEG networks by using method i and pyrophosphate-Na buffer, i.e., in tP-NC2$_{py}$-A gel.

Effects of Clay and Polymer Content on the Mechanical Properties of tP-NC Gels. tP-NCn-A gels with widely different C_{clay} (n = 1−20) were prepared using pyrophosphate-Na buffer solutions and constant C_p (= 120 mg/mL). All tP-NCn-A gels obtained were uniform and transparent, although their transparencies slightly decreased with increasing n, becoming translucent at n = 20. Figure 5a shows typical tensile stress−strain curves for tP-NCn-A gels. Here, both ε_b and TS increased markedly with increasing C_{clay} in the range of NC0 to NC2. With further increases in C_{clay} above NC2, TS decreased quite steeply while ε_b decreased slightly and then again increased. The detailed changes in E caused by altering C_{clay} are shown in Figure 5b. The tensile moduli E_{10-50} and $E_{100-200}$, which correspond to the slopes between 10−50% and 100−200% elongation, respectively, showed similar changes as a function of C_{clay}. That is, both E_{10-50} and $E_{100-200}$ increased with increasing C_{clay} in the range of NC0 to NC2 and, after reaching a maximum at around NC2, decreased with further increases in C_{clay}. These changes in the tensile properties of tP-NC gels are different from those of normal NC gels[16,17] in which E and TS increase nearly in proportion to n within the wide range of C_{clay} values examined here. The results for tP-NCn gels indicate that only small amounts of clay ($C_{clay} \leq 2 \times 10^{-2}$ mol/L) can be effectively incorporated into the tetra-PEG networks to act as cross-linking agents and thereby improve their mechanical properties. Further addition of clay causes decreases in E and TS, presumably because tetra-PEG network formation is disturbed by an excess amount of clay platelets which strongly interact with TN-PEG.

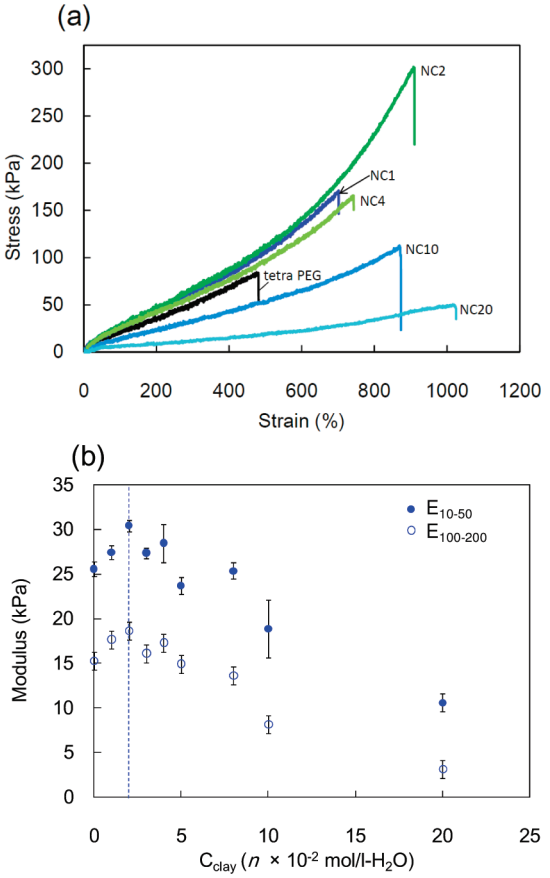


Figure 5. Effects of clay content on the tensile properties of tP-NC gels synthesized using pyrophosphate-Na buffer. (a) Stress−strain curves for tP-NC gels with different C_{clay} values (n = 1−20) and tetra-PEG gel. (b) Changes in E_{10-50} and $E_{100-200}$, the tensile moduli for tP-NC gels, as a function of C_{clay} (n = 0−20). E_{10-50} and $E_{100-200}$ are calculated from the slopes in the ranges of 10−50% and 100−200% elongation, respectively. Initial cross-sectional area was used for calculating the modulus and strength.

To reveal the effect of the size of tetra-PEG network (i.e., network density) on the mechanical properties of tP-NC gels, we further prepared two types of tP-NC2-A gel consisting of macromonomers with different molecular weights (20K and 5K). For the two tP-NCn gels in Figure 6, altering C_{clay} produced changes in the initial modulus (E_{10-50}). E_{10-50} of tP-NCn-A (5K) gels increased with increasing C_{clay} and reached a maximum at NC8, whereas the maximum occurred at NC2 in tP-NCn-A (20K) gels. That is, the value of C_{clay} which produces the maximum modulus in tP-NCn-A (5K) gels (n=8) is 4 times that in tP-NCn-A (20K) gels (n=2). This result indicates that the number of clay platelets which can be effectively incorporated in tP-NC gels without disturbing the formation of tetra-PEG network depends on the length (molecular weight) of macromonomer. That is, low-molecular-weight macromonomers can form regular networks even with short interclay distances, although long macromonomers appear to have a problem under the same conditions since clay platelets in close proximity may act as obstacles for the alternate arrangement and reaction of TN-PEG and TA-PEG. This may be another reason why tP-NCn-A (20K) gels with high C_{clay} show inferior tensile properties (Figure 5).

In order to reveal the effect of C_p on the tensile properties, we prepared tP-NC2-A gels as well as tetra-PEG gels with different C_p values in the range of 80−240 mg/mL in pyrophosphate-Na buffer. The tensile stress−strain curves

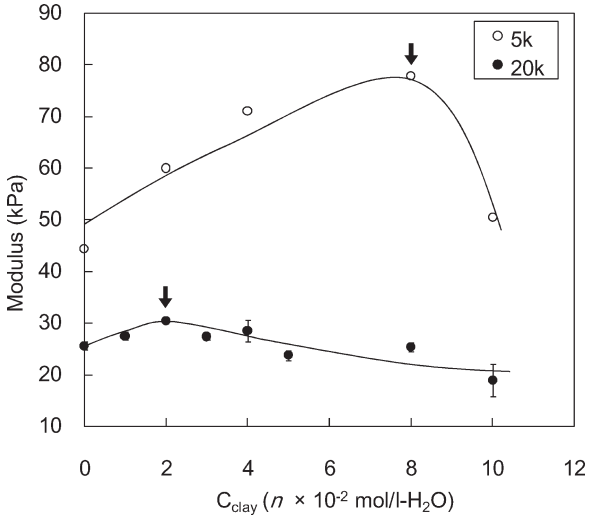


Figure 6. Change in tensile modulus of two tP-NCn-A gels with different C_{clay} values (n = 1−10). The molecular weight of the macromonomer was 5300 (5K: open circles) or 20 000 (20K: closed circles). Gels synthesized using pyrophosphate-Na buffer. The modulus is calculated from the slopes in the range of 10−50% elongation.

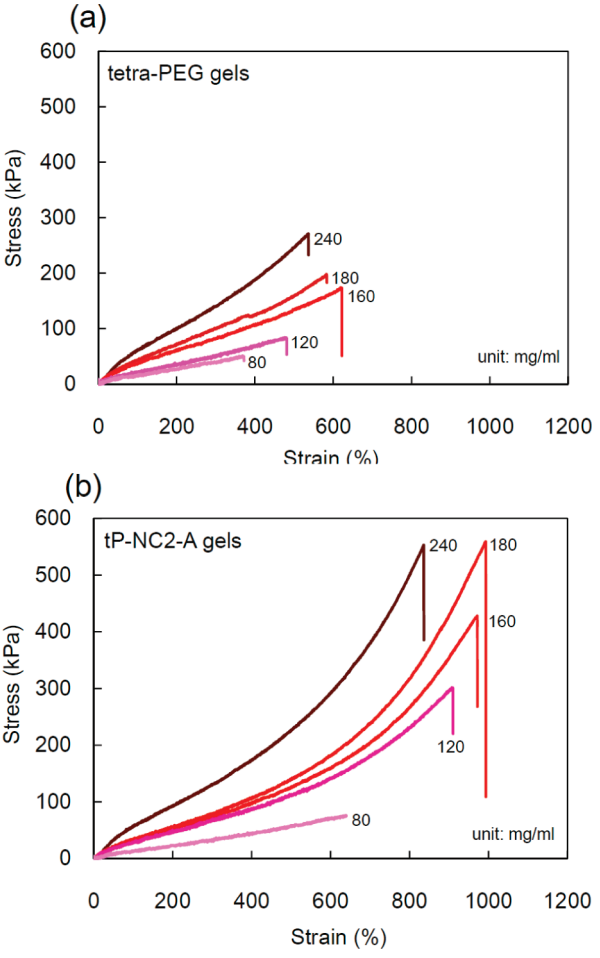


Figure 7. Changes in tensile stress−strain curves for (a) tetra-PEG gels and (b) tP-NC2-A gels by altering polymer content from 80 to 240 mg/mL. Synthesized using pyrophosphate-Na buffer.

are shown in Figure 7a (tetra-PEG gels) and Figure 7b (tP-NC2-A gels). As shown in Figure 7a, TS and E increased with increasing C_p. Consistent with normal NC gels with different C_p,[7] this trend could occur because of an increase in polymer

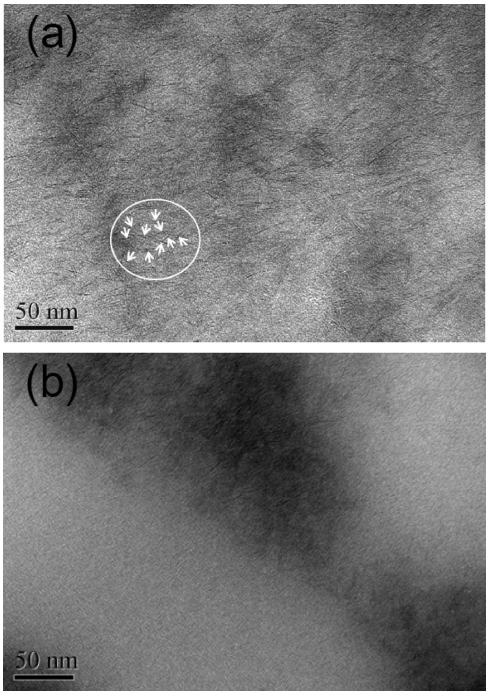


Figure 8. TEM micrographs of dried tP-NC2 gel synthesized in (a) pyrophosphate-Na and (b) phosphate-Na buffer. Magnification = 500 000. In (a), the arrows indicate exfoliated platelets.

concentration. By comparing parts a and b of Figure 7, we found that the tensile properties of tetra-PEG gels were significantly increased by incorporating clay ($n = 2$) regardless of C_p. TS and ε_b reached 560 kPa and 1000%, respectively, in tP-NC2-A (180 mg/mL) gel. Thus, by altering C_{clay} and C_p, the mechanical properties of tP-NC gels can be controlled over a wide range.

Network Structure of tP-NC2 Gel. The outstanding improvement in tensile properties of tP-NC2-A gels described above could arise from the effective incorporation of clay nanoparticles into the tetra-PEG network. The resulting tetra-PEG/clay network was characterized by XRF, TGA, and TEM measurements for dried tP-NC2-A and dried tetra-PEG gels, which were obtained by drying in vacuum at 25 °C after washing with water to remove the pyrophosphate-Na buffer. From XRF measurements (Figure S1), we found that phosphorus (from pyrophosphate-Na) was removed from both tetra-PEG and tP-NC2-A gels by washing, while silicon (from clay) was detected at the same level as in that of the as-prepared gel. These results indicate that clay is not eliminated by washing but remains incorporated in the tetra-PEG/clay network, even though pyrophosphate-Na can readily be eliminated from the gel by washing. From TGA analyses (Figure S2), we confirmed that the weight ratios of inorganic clay/organic PEG in tP-NC2-A gel as well as the amount of pyrophosphate-Na were in fairly good agreement with those calculated from the solution composition.

The exfoliation of clay and the dispersion of platelets in tP-NC2-A gels were directly evaluated by TEM measurements. Figure 8 shows TEM micrographs of ultrathin films of two types of dried tP-NC2-A gel, i.e., dried tP-NC2$_{py}$-A gel (Figure 8a) and dried tP-NC2$_{ph}$-A gel (Figure 8b). In both cases, the buffer was removed by washing with water before drying. Clearly, the exfoliated clay platelets are uniformly dispersed in tP-NC2$_{py}$-A gel but are aggregated in tP-NC2$_{ph}$-A gel. The different distributions of clay platelets are consistent with the differences in transparency and tensile properties between tP-NC2$_{ph}$-A and tP-NC2$_{py}$-A gels (Figures 1 and 3).

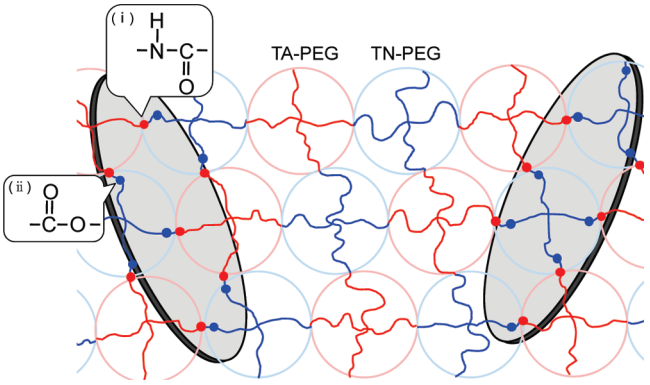


Figure 9. Schematic representation of the structural model for the tetra-PEG/clay network in tP-NC2-A gels synthesized using pyrophosphate-Na buffer. In the model, two probable interactions between clay and tetra-PEG segment/network are shown.

We propose the following mechanism for the formation of the tetra-PEG/clay networks in tP-NC2$_{py}$-A gels. First, clay is exfoliated in aqueous pyrophosphate-Na buffer. Next, TA-PEG macromonomer is added to the clay suspension and coexists with clay with only a mild interaction which prevents any increase in the viscosity of the suspension. Then, the highly transparent, low-viscosity TA-PEG/clay suspension is mixed with the aqueous TN-PEG solution. The reaction between TA-PEG and TN-PEG then proceeds with interactions forming between clay platelets and the resulting amide linkages as well as the ester-groups in TN-PEG, while the uniform dispersion of clay platelets is simultaneously retained. Figure 9 depicts a schematic representation of the structural model for a tetra-PEG/clay network.

As possible sites for interactions between clay and the tetra-PEG network, the following three possibilities were considered: (i) −NHCO− in the main chain, (ii) −COO− in TN-PEG, and (iii) $-NH_2$ in TA-PEG. Here, (i) is formed by reacting TA-PEG and TN-PEG. The evidence for each interaction is shown and discussed in the following section based on FTIR spectra.

Interactions between Clay and Tetra-PEG Segment/Network. Interactions between clay and the tetra-PEG segment/network were investigated by classifying the following three groups: (1) TN-PEG and clay, (2) TA-PEG and clay, and (3) clay and amide linkage in the tetra-PEG network.

TN-PEG and Clay. FTIR spectra of TN-PEG and freeze-dried samples of clay/TN-PEG solutions with different C_{clay} ($n = 0-5$) are shown in Figure 10a. TN-PEG possesses two ester groups in each arm: esters 1 and 2 (see the formula in Figure 10a). We observed absorption by the C=O stretching vibration ($\nu_{C=O}$) from both ester groups at 1739 cm^{-1} (Figure 10a(1)). By adding clay, the $\nu_{C=O}$ partly shifted from 1739 to 1716 cm^{-1}, presumably due to the hydrogen bonding between C=O of TN-PEG and Si−OH of clay. The peak height at 1716 cm^{-1} increased with increasing C_{clay} up to $n = 2$ and was nearly saturated at $n > 2$ (Figure 10a(1−4)). These results suggest that either or both ester groups in TN-PEG interact with clay so that $\nu_{C=O}$ shifts to a lower frequency.

In order to decide which ester group in TN-PEG is responsible for the interaction with clay, a similar experiment was carried out using TC-PEG, the arms of which possesses only ester 2 (Figure 10b). The $\nu_{C=O}$ from ester 2 observed at 1739 cm^{-1} was not shifted to 1716 cm^{-1} by adding clay (Figure 10b(1−3)). This indicates that the shift of $\nu_{C=O}$ in TN-PEG (Figure 10a) is attributable to the interaction

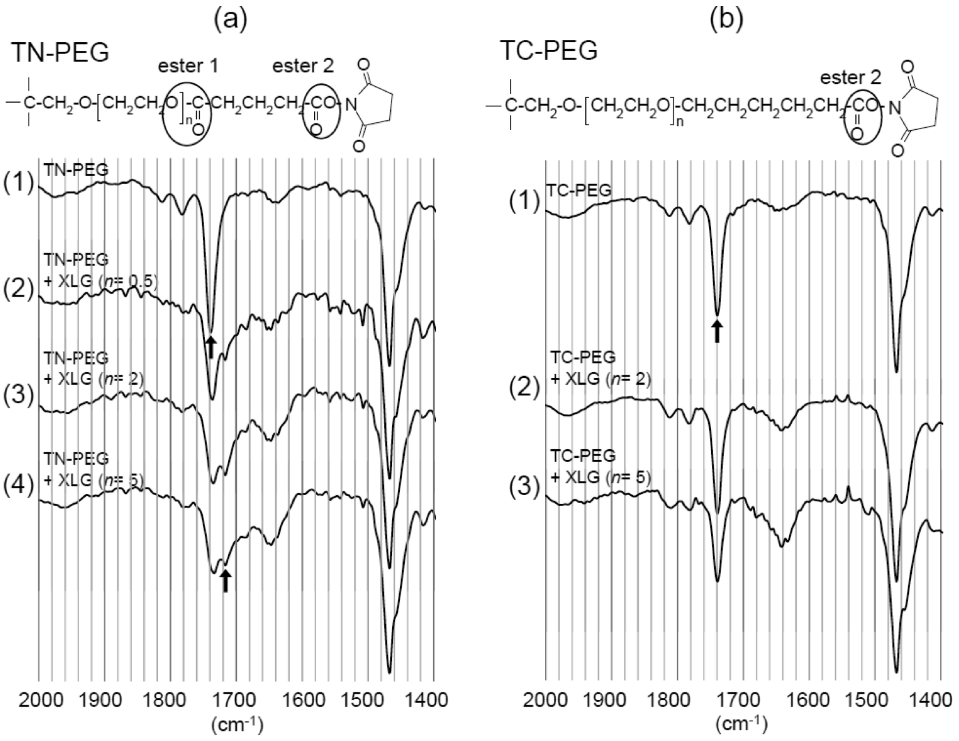


Figure 10. Changes in FTIR spectra by adding clay (n = 0−5) to (a) TN-PEG and (b) TC-PEG. The formulas of TN-PEG and TC-PEG are shown above.

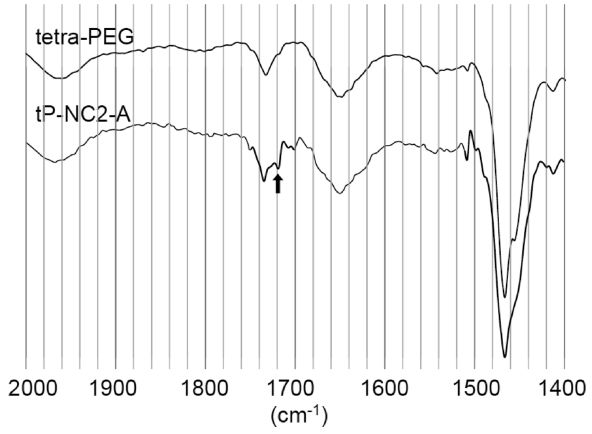


Figure 11. FTIR spectra of dried tP-NC2-A and dried tetra-PEG gels. Synthesized using pyrophosphate-Na buffer.

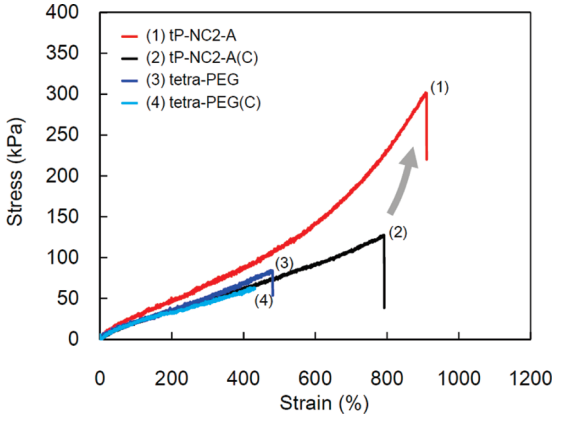


Figure 12. Tensile stress−strain curves for (1) tP-NC2-A gel, (2) tP-NC2-A (C) gel, (3) tetra-PEG gel, and (4) tetra-PEG (C) gel. In the case of (C), TC-PEG was used instead of TN-PEG. Synthesized using pyrophosphate-Na buffer.

between clay and ester 1. The same peak shift of $\nu_{C=O}$ was also observed in the FTIR spectra of dried tP-NC2-A gel when compared with that of dried tP-NC0 gel (≡ tetra-PEG gel) (Figure 11).

To confirm the effect of the clay−ester 1 interaction on the mechanical properties of tP-NC gels, the tensile properties of two types of tP-NC2-A gels, i.e., tP-NC2-A gel prepared with normal TN-PEG and tP-NC2-A(C) gel prepared with TC-PEG instead of TN-PEG, were compared with the corresponding tetra-PEG gel and tetra-PEG(C) gel. As shown in Figure 12, two tetra-PEG gels (tetra-PEG gel and tetra-PEG(C) gel) displayed nearly identical stress−strain curves. By incorporating clay, both tP-NC2-A and tP-NC2-A(C) gels showed significant increases in tensile properties although the magnitudes of the increase differed. TS and ε_b increased substantially in tP-NC2-A gel relative to the corresponding tetra-PEG (≡ tP-NC0) gel (Figure 12). On the other hand, the increase in TS was much smaller for tP-NC2-A(C) gel. This difference can be attributed to the existence of ester 1; that is, tP-NC2-A(C) gel showed a smaller increase in TS because there is no clay−ester 1 interaction. In other words, the interaction between clay and ester 1 plays the most important role in increasing the tensile properties of tP-NC gel.

The interaction between clay and ester 1 was also observed in the changes in viscosity for clay/macromonomer suspensions. As shown in Figure 4, a clay/TN-PEG suspension showed increased viscosity compared with clay/TC-PEG suspension. This increase in viscosity is attributed to the clay−ester 1 interaction from the difference of structures.

TA-PEG and Clay. We examined the interaction between TA-PEG and clay in a similar manner. Although absorption by the NH stretching vibration (ν_{NH}) of NH_2 in TA-PEG was observed at 1645 cm^{-1}, it did not shift in the presence of clay (n = 2) as shown in Figure S3. This indicates that the

interaction between clay and TA-PEG with NH_2 is so weak that any shift in the IR peak is not detectable. This is consistent with the results on changes in viscosity by mixing TA-PEG with clay suspension.

Amide Linkages and Clay. It has been reported that NC gels consisting of clay and hydrophilic polymers with amide (−NHCO−) groups such as poly(*N*-isopropylacrylamide) and poly(*N,N*-dimethylacrylamide) form well-defined polymer/clay networks and thereby provide NC gels with excellent mechanical properties.[16,17] Here, clay acts as a multifunctional cross-linking agent through an interaction (hydrogen bonding) between clay (Si−OH or Si−O−Si) and an amide group of the polymer.[18] Thus, the amide group is able to interact with clay in aqueous media, even though in the dried state of NC gels, there were hardly any changes in the FTIR spectra due to the formation of hydrogen bonds between the amide group of PNIPA and clay.[18] In the present study, FTIR spectra were also compared between dried tP-NC2-A gel and dried tetra-PEG gel. Similar to dried NC gels, there were no distinctive differences in the peak for the amide I band (1648 cm^{-1}) (Figure 11).

Conclusion

We have established synthetic procedures for tetra-PEG-based nanocomposite gels (tP-NC gels) with outstanding optical and mechanical properties. In-situ polymerization of TA-PEG and TN-PEG in the presence of clay, under the same conditions as those of previously reported for the synthesis of tetra-PEG gels, did not result in transparent tP-NC gels with superb mechanical properties. We found that the buffer solution plays important roles in retaining the uniform dispersion of exfoliated clay and in limiting the increase of viscosity in aqueous clay suspensions. The use of pyrophosphate-Na as a buffer, and a preparative sequence in which the clay suspension is first mixed with TA-PEG, were the most suitable conditions for synthesizing tP-NC gel with excellent properties. Consequently, a transparent tP-NC2 gel with high tensile properties (TS = 300 kPa and ε_b = 900%) was obtained at C_p = 120 mg/mL. The effects of clay and polymer content on the mechanical properties of tP-NC gel were investigated by varying them across a wide range [$n(C_{clay})$ = 1−20 and C_p = 80−240 mg/mL]. We found that the tensile properties of tP-NC*n* gel exhibit a maximum at n = 2 and that they increased with increasing C_p, attaining TS and ε_b values of 560 kPa and 1000%, respectively, for tP-NC2 (180 mg/mL) gel. From instrumental analyses and mechanical tests of the tetra-PEG/clay network structure, we found that exfoliated clay platelets are uniformly dispersed and stably incorporated into the tetra-PEG network via an interaction (i.e., hydrogen bonding) between clay and the tetra-PEG network. In addition, concerning the interaction between clay and the tetra-PEG segment/network, we revealed that a specific ester group (ester 1) present in the arm of a tetrahedral macromonomer (TN-PEG), as well as amide linkages resulting from the polymerization reaction, is responsible for the interactions between clay and tetra-PEG. The facts that tP-NC*n* gels with high C_{clay} (n > 2) exhibit poor mechanical properties distinct from those of conventional NC gels consisting of a poly(*N*-alkylacrylamide)/clay network may be attributed to the limitation in the number of clay platelets which can be incorporated without disturbing the formation of tetra-PEG network. Thus, PEG-based NC gels with excellent properties were prepared for the first time by incorporating clay nanoparticles into the tetra-PEG networks under selected conditions. The resulting tP-NC gels may extend the potential applications of both NC gels and tetra-PEG gels in various biomedical fields including tissue engineering, drug delivery systems, and artificial organs.

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Supporting Information Available: XRF analysis, TGA, and FT-IR analysis of tetra-PEG and tP-NC2 gel. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) De Rossi, D., Kajiwara, K., Osada, Y., Yamauchi, A., Eds.; *Polymer Gels*; Plenum: New York, 1991.
(2) Osada, Y.; Khokhlov, A. R. *Polymer Gels and Networks*; Marcel Dekker: New York, 2002.
(3) Haraguchi, K. *Macromol. Symp.* **2007**, *256*, 120–130.
(4) Okumura, Y.; Ito, K. *Adv. Mater.* **2001**, *13*, 485–487.
(5) Haraguchi, K.; Takehisa, T. *Adv. Mater.* **2002**, *14*, 1120–1124.
(6) Haraguchi, K.; Takehisa, T.; Fan, S. *Macromolecules* **2002**, *35*, 10162–10171.
(7) Haraguchi, K.; Farnworth, R.; Ohbayashi, A.; Takehisa, T. *Macromolecules* **2003**, *36*, 5732–5741.
(8) Gong, J. P.; Katsuyama, Y.; Kurokawa, T.; Osada, Y. *Adv. Mater.* **2003**, *15*, 1155–1158.
(9) Bae, S. J.; Joo, M. K.; Jeong, Y.; Kim, S. W.; Lee, W. K.; Sohn, Y. S.; Jeong, B. *Macromolecules* **2006**, *39*, 4873–4879.
(10) Joo, M. K.; Sohn, Y. S.; Jeong, B. *Macromolecules* **2007**, *40*, 5111–5115.
(11) Schultz, K. M.; Baldwin, A. D.; Kiick, K. L.; Furst, E. M. *Macromolecules* **2009**, *42*, 5310–5316.
(12) Mahou, R.; Wandrey, C. *Macromolecules* **2010**, *43*, 1371–1378.
(13) Baghdadi, H. A.; Sardinha, H.; Bhatia, S. R. *J. Polym. Sci., Part B: Polym. Phys.* **2005**, *43*, 233–240.
(14) Sakai, T.; Matsunaga, T.; Yamamoto, Y.; Ito, C.; Yoshida, R.; Suzuki, S.; Sasaki, N.; Shibayama, M.; Chung, U. I. *Macromolecules* **2008**, *41*, 5379–5384.
(15) Matsunaga, T.; Sakai, T.; Akagi, Y.; Chung, U. I.; Shibayama, M. *Macromolecules* **2009**, *42*, 1344–1351.
(16) Haraguchi, K.; Li, H. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 6500–6504.
(17) Haraguchi, K.; Li, H. J. *Macromolecules* **2006**, *39*, 1898–1905.
(18) Haraguchi, K.; Li, H. J.; Matsuda, K.; Takehisa, T.; Elliott, E. *Macromolecules* **2005**, *38*, 3482–3490.
(19) Cline, G. W.; Hanna, S. B. *J. Org. Chem.* **1988**, *53*, 3583–3586.
(20) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839–1842.
(21) Mongondry, P.; Nicolai, T.; Tassin, J. F. *J. Colloid Interface Sci.* **2004**, *275*, 191–196.
(22) Dijkstra, M.; Hansen, J. P.; Madden, P. A. *Phys. Rev. Lett.* **1995**, *75*, 2236–2239.