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2 Water/glycerol mixed solvent transportation behavior of mechanically
3 constrained agarose gels

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18 **Abstract**
19   The effect of glycerol on the solvent transportation behavior of mechanically
20 constrained agarose gels was studied. The sample hydrogels were prepared using a
21 water/glycerol mixed solvent containing various concentrations of glycerol. The
22 compression load relaxed under the compression, and the volume of the agarose gels
23 decreased synchronously with mechanical relaxation. This implied that the solvent is
24 squeezed out due to mechanical constraints; the time constants for both the mechanical
25 relaxation and the volume change are directly related to the friction between the solvent
26 and the gel network structure. Both time constants increased with an increase in glycerol
27 concentration. These results indicate that glycerol changes the gel network structure, and
28 this is confirmed by other experimental evidence. The compression fracture stress and
29 strain improved with an increase in glycerol content. Moreover, scanning electron
30 microscope images showed that the mesh size of the gel network decreased with an
31 increase in glycerol content.

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36 Keywords: agarose gel, compression, solvent transportation, glycerol, scanning electron microscope

1 **Introduction**

2   How is the solvent squeezed out from foods when they are compressed in mouth? It is

3 quite interesting research topics. Generally, the taste or flavor of food is due to the

4 solvent (usually water), and therefore, we cannot enjoy the food unless the solvent is

5 squeezed out. Solvent transportation from hydrogels has attracted attention in the field

6 of food science and technology because this process is strongly related to flavor release

7 and water-holding ability. Although solvent transportation from edible gels has been

8 extensively studied (Stieger & van de Velde, 2013), the main focus has been on the

9 passive diffusion of flavor from edible gels (Wang et al., 2014).   Solid like foods

10 obviously must be bit or chewed during mastication. Therefore we should consider not

11 only passive diffusion of solvent, which contains flavor, but also squeeze behavior of

12 the solvent under the compression pressure. The solvent transportation behavior can be

13 explained in terms of the friction between the gel networks and the solvents (Tanaka &

14 Fillmore, 1979). A few studies have focused on clarifying the friction within the gel

15 network for chemically cross-linked gels (Tokita& Tanaka, 1991; Suzuki & Hara, 2001;

16 Fatin-Rougue et al., 2003). The solvent transportation behavior of chemically

17 cross-linked hydrogels under mechanical constraints has also been studied (Kneable &

18 Lequeux, 1998). The solvent is squeezed out from chemically cross-linked

19 polyelectrolyte gels under compression. However, no such clarification has been

20 provided for polysaccharide gels, which are physically cross-linked gels.

21   In this study, we focused on agarose gels as a simple food model. The idealized

22 structure of agarose constitutes a disaccharide repeating unit comprising $(1\rightarrow3)$ linked

23 $\beta$-D-galactose and $(1\rightarrow4)$ linked 3,6-anhydro-$\alpha$-galactose (Morris 1998). Agarose

24 solutions of sufficiently high concentrations give rise to thermally reversible gels that

are slightly turbid and brittle below the gelling temperature. Although the polysaccharides dissolve in water as coils at higher temperatures, they adopt a helical conformation at lower temperatures (Arnott et al., 1974). The mechanism of formation of the network structure of an agarose gel is proposed to be as follows: if the concentration of the polysaccharide is sufficiently high, the helices form bundles, which then associate to generate a network structure. Small angle neutron scattering study of the "swollen gels" has revealed the cross-sectional radius of the agarose cylindrical fibers to be a few nanometers (Boral & Bohidar, 2009). The reported mesh sizes of agarose gel network are various values ranging from the order of a few 10 nm to a few 100 nm depending on the experimental method. For example, the size estimated by using the wave length exponent is on the order of a few 10 nm (Normand et al 2003). The value estimated from the diffusion coefficient of a probe with NMR is also on the order of a few 10nm (Dai and Matsukawa). On the other hand, the size estimated by using electrophoresis of a probe is on the order of a few 100 nm (Xiong et al). These sizes are obviously quite larger than the size of water molecular. Although we observe syneresis that is spontaneous solvent excluding phenomenon, it is clear that the solvent never easily flow out from the hydrogels. Therefore, it is one of the most interesting research topics to investigate the solvent transportation from such hydrogels in a various conditions. Recently, we studied solvent transportation from constrained agarose gels. We observed that the compression load and the volume of the agarose gels decreased with time during compressive restraint, as the time constants for the relaxation of the compression load and the volume change of the constrained agarose gel were changed. It means that the decrease in volume was induced by squeezing of the solvent from the gel under compressive constraint (Kaneda & Iwasaki, 2015).

1    In this study, we focused on glycerol as a possible additive for gel-type foods

2    containing agarose. The effect of glycerol on the physical properties of agarose gels has

3    already been reported (Nishinari & Watase, 1987). However, to the best of our

4    knowledge, the solvent transportation behavior of mechanically constrained agarose

5    gels has not been reported. Therefore, we studied the effect of glycerol on the solvent

6    transportation from constrained agarose gel using the method developed in the previous

7    study. We observed the time development of the mechanical load and the volume

8    change under compressive strain for agarose gels containing glycerol as a solvent. The

9    relationship between the mechanical response and the volume change, i.e., the mode of

10   solvent transportation from the gel, was also analyzed.

11

12   **Materials and Methods**

13   **Materials**

14   Agarose type IV (Sigma-Aldrich) was purchased and used without further purification.

15   The sample contained less than 0.25% sulfate. Aqueous solutions of glycerol were used

16   as the solvent. The concentrations of glycerol ranged from 0 to 80 wt % in distilled

17   water. Sample gels were prepared as follows: agarose powder was dispersed in the

18   solvent and stirred at room temperature for 18 h to allow sufficient swelling. The

19   swollen dispersion was heated at 95 °C for 1 h to ensure complete dissolution. The hot

20   solution was poured into a polycarbonate tube ($\phi = 20$ mm), and both ends of the tube

21   were sealed with polyvinylidene chloride film. Then, the tube was immediately placed

22   in a water bath for temperature control. After 24 h of quenching in the water bath at 10

23   °C, the sample gels were squeezed out of the tube and cut to a length of about 20 mm

24   using a razor blade. The cylindrical gels were immersed in the solvent and incubated at

1  5 °C for at least 5 days so that they reached the equilibrated state before the experiments.

2  The dimensions of the gels (diameter and length) were measured with a caliper

3  immediately prior to the experiments. The sample codes used herein are "G-XX", where

4  "XX" indicates the molar ratio of glycerol in the solvent.

5  **Monitoring of compression load and volume change**

6  For experiments evaluating the mechanical properties and volume change of the sample,

7  the polymer concentration was fixed at 1.5 wt%. The temporal evolution of the

8  compression load and changes in the shape of the agarose gel were simultaneously

9  monitored. The details of the measurement system are described in elsewhere (Kaneda

10 & Iwasaki, 2015). Compression and monitoring of the compression load were

11 performed with an INSTRON MINI 55 (Instron, USA) instrument. The sample gel was

12 compressed at a rate of 1 mm/s, and when the compression strain reached 0.05, the

13 compression was held constant. The time development of the compression load was

14 monitored for 18 h. The side view of the sample was monitored with a CCD camera,

15 and still images were acquired at regular time intervals. The diagram of the

16 measurement setup is shown in Fig.1. In this study, the top and bottom surfaces of the

17 cylindrical gel were sealed with cyanoacrylate to prevent slipping. The digital images of

18 the samples and the sample width were analyzed using image analysis software

19 (Image-J). Since the gel deformed to a barrel-like shape, the width was measured at five

20 different points and the average width ($w_m$) was obtained. The height of the gel could be

21 determined during the INSTRON MINI 55 measurements; therefore, the volume ($v$) of

22 the gel was calculated using Eq. 1, and each measurement was performed at least three

23 times.

1 $$v = \pi \left( \frac{w_m}{2} \right)^2 \cdot h \qquad (1)$$

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3 **Fracture behavior**

4   To study the general mechanical properties of the agarose gels containing glycerol,

5 compression fracture tests were performed. The cylindrical gels were compressed by the

6 INSTRON MINI 55 system. The crosshead speed was 1 mm/s. The values of fracture

7 stress and strain were estimated from the peak position in the stress-strain curves. In this

8 estimation, we assumed that the compression area was constant during compression

9 fracture.

10 **Viscosity measurement**

11 The apparent viscosities of water/glycerol mixed solvents were measured ARES (TA

12 instruments) equipped a bob (16mm diameter) and cup (16.5mm diameter) fixture. The

13 steady state viscosity at 50 s$^{-1}$ was measured at 30°C.

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15 **Scanning electron microscopy (SEM) observation**

16   The microstructure of the agarose gels was observed with a scanning electron

17 microscope (HITACHI S-2460N). The sample gels were immersed in D.W. for at least

18 10 days to remove the glycerol present in them. The washed gels were rapidly cooled

19 under liquid nitrogen before being placed in a freeze dryer. The completely freeze-dried

20 samples were cleaved to expose the torn surfaces, and were coated with Pt-C on the

21 SEM sample table.

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**Results and discussion**

**Fracture behavior**

Fig. 2 shows the glycerol concentration dependence of the fracture stress (Fig. 2a) and the fracture strain (Fig. 2b). Both parameters clearly increase with glycerol concentration. Increasing fracture stress indicates that the gel network structure in agarose gels became sturdier with the addition of glycerol Additionally, the fracture strain increased with increasing glycerol concentration. From these results, it is clear that glycerol changes the network structure when added at the aforementioned concentrations.   It is reported that sugars or polyols make agarose gels less brittle and the gels show a more strain hardening behavior due to be changing the mechanical property and the number density of the junction zones (Nishinari, Fang 2016). Therefore it is expected that the samples containing glycerol showed a larger failure stress and strain.


**Relaxation of compression load and volume change**

 Careful experiments have revealed that the solvent was squeezed out from mechanically constrained agarose gels and that the solvent transportation speed synchronized with the relaxation of the compression loads. The time constant of volume change, namely, the solvent transportation speed, must therefore be strongly affected by the gel network structure. Figs 3 and 4 show the time development of the compression load and the volume change, respectively. For ease of comparison across samples, the volume change was converted to a relative value using Eq. 2.

$$V(t) = \frac{v(t)}{v_0} \qquad (2)$$

where $v(t)$ and $v_0$ are the volume of the gel at time "t" and the volume at time zero,

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1 respectively.

2 The four panels in Figs 2 and 3 are as follows: G-20 (a), G-40 (b), G-60 (c), and G-80,

3 as typical examples.

4 It was clear that L(t), the time development of the compression load, and $V$(t), decayed

5 exponentially. Doi (1990) proposed the stress-diffusion coupling theory (SDCT), which

6 is a continuum theory describing the deformation and solvent transport during the

7 swelling of gels. The solvent transportation behavior of a cylindrical gel was studied by

8 using SDCT (Yamane and Doi 2005). The result of the theoretical study shows that the

9 transportation of the solvent follows a single exponential type function. Therefore, the

10 data were subjected to analysis using an empirical stretched exponential function (Eqs.

11 3 and 4). The experimental data were fitted to Eqs.3 and 4 using a non-linear

12 least-squares method.

13 $$L(t) = L_0 \exp\left(-\left(\frac{t}{\tau_M}\right)^{\alpha}\right) + L_r \qquad (3)$$

14 $$V(t) = V_0 \exp\left(-\left(\frac{t}{\tau_V}\right)^{\beta}\right) + V_r \qquad (4)$$

15 where $L_0$ and $V_0$ are the relaxation intensities for the compression load and volume

16 change, respectively; $\tau_M$ and $\tau_V$ are the time constants of the decay for the compression

17 load and volume change; $L_r$ and $V_r$ are the residual compression load and volume,

18 respectively; $\alpha$ and $\beta$ are the respective power indices for each function.  The solid

19 lines in Figs. 3 and 4 are the best fit results of the analysis using Eqs. 3 and 4; the results

20 of the analyses for all samples are listed in Tables 1 and 2. Although we needed a

21 stretched exponential function to obtain a good agreement fitting results, its reason is

22 unknown in this time. Recently, the compression load is un-uniform in the compression

23 cross section when agar cylindrical gel is compressed (Matsukawa et al 2014). It means

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that there is distribution of the compression load in its cross section. It is expected that a stretched exponential function is needed to be analyzed the data containing distribution.

First we focused on the effect of glycerol on the time constants. As mentioned above, the solvent transportation speed from the mechanically constrained agarose gels synchronized with the mechanical relaxation speed (Kaneda & Iwasaki, 2015). From Figs. 3 and 4 (and Tables 1 and 2), we can see that the time constants increased with glycerol concentration in the mixed solvent. To demonstrate this in a simpler manner, the glycerol concentration dependence of the time constants is plotted in Fig. 5. The glycerol concentration dependence for both time constants shows a similar trend, although the shapes of the time decay curves are slightly different as a result of the differences in the power indices $\alpha$ and $\beta$ (Eqs 3 and 4).

As shown above, the fracture stress and strain of the agarose gels were affected by the addition of glycerol. This confirms that glycerol changed the network structure. This response was also observed in the compression load relaxation measurements. Fig. 5 shows the glycerol concentration dependence on $L_0$ and $L_r$, which are defined in Eq. 3. The physical meanings of $L_0$ and $L_r$ are the part of the relaxed compression load and the remaining compression load, respectively. It can be seen that $L_0$ increased with glycerol concentration, whereas $L_r$ decreased. This result also provides evidence for the change in the network structure of the agarose gels by the addition of glycerol.

From these experiments, we propose a scenario where the decline in solvent transportation from the mechanically constrained gels is due to a change in their network structure by the addition of glycerol. However, a problem still remains: glycerol is a thick liquid, and aqueous solutions of glycerol also have high viscosity.

The influence of compression velocity on gel structure using gellan gel, which is a

1    polysaccharide gel similar to agarose gel, is reported (Nakanura et al 2001). When

2    gellan gel is compressed in very slow speed, the solvent is squeezed out. Moreover,

3    quite narrow cylindrical pores are observed using a confocal lasar scanning microgram

4    (Nakamura 1999). It is expected that there are the similar solvent transport channels

5    in agarose gels. If the bold simplification is allowed, the solvent transportation behavior

6    can be described by the Hagen-Poiseuille law. This theoretical law declares that the flow

7    rate (Q) of laminar flow in a cylindrical tube is inversely proportional to the viscosity of

8    the fluid (η) if the pressure gradient and the radius of the tube are constant.

9    $$Q \propto \frac{1}{\eta} \qquad\qquad (5)$$

10   Therefore, if the viscosity of the solvent containing glycerol increases, the solvent

11   transportation rate would decrease. To estimate the viscosity thickening effect of the

12   solvent, the apparent viscosity of the solvent was measured. Fig. 7 shows the apparent

13   viscosity of the solvents measured at 50 s$^{-1}$. The apparent viscosity of the solvent is

14   increased by adding glycerol; particularly, it is 20-fold at 80 wt% aqueous glycerol

15   solution. This glycerol concentration dependence is similar to the behavior of the time

16   constants shown in Fig.5. This result indicates that the observed delay in the solvent

17   transportation is partially due to the viscosity thickening of the solvent.

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19   **Observation of the microscopic structure of agarose gels**

20   Because specimens for electron microscopy are completely dried, it is impossible to

21   observe the "wet state" of hydrogels. However, a relative comparison between the

22   different samples would be of interest. Although the samples for the experiments

23   mentioned above contained 1.5 wt% agarose, the samples for SEM contained 3 wt%

24   agarose to permit easier observation. Representative SEM images are shown in Figs. 8 a

(G-0) and b (G-80). A network structure is observed in both images. Electro-microscopic observation of agarose gel demonstrates that the cylindrical fibers of agarose comprise a network structure with a diameter of several tens of nanometers and the mesh size is of the sub-micrometer order (Amici et al 2002; Nordqvist and Vilgis 2011). The mesh size seen in Figs. 8 is similar to these previous reports. On the other hand, the image of G-80 (80 wt% glycerol in the solvent) was different from that of G-0. We observed the torn surfaces of the freeze-dried gels. It is clear that the surface texture is different for each gel; in particular, the torn surface of G-80 seemed smoother than that of G-0. Agarose gel networks are formed by the junction zones consisting of aggregates of helices. The effect of polyols on the mechanical and thermal properties of agarose gels is already discussed using a theoretical model for thermo-reversible physical gels (Nishinari and Watase 1987). It is reported that glycerol affects the numbers or spatial density of the junction zones. Therefore, the difference in these SEM images is considered to be due to change of the junction zones by adding glycerol.

Of cause, the thickened viscosity after the addition of glycerol is one of the causes of the delayed solvent transportation from the mechanically constrained agarose gels. However, glycerol caused molecular-level structural changes as shown fig.8. The mechanical properties of the gels also changed by adding glycerol as mentioned above.



**Conclusion**

It has been revealed that solvent transportation from mechanically constrained agarose gels containing glycerol can be analyzed simultaneously for mechanical properties and volume change measurements using a previously reported system. The solvent

transportation speed decreased with glycerol concentration. Moreover, the relaxation speed of the compression load decreased in the same manner, and these behaviors were synchronized. It was considered that glycerol changed the gel network structure in agarose gels, and resultantly, the solvent transportation speed decreased. We obtained two different evidence sources for this: the results of the compression fracture test and the real-space SEM observations. Although it was expected that the viscosity thickening effect of glycerol would alter the gel network structure, the effect of glycerol on the solvent transportation due to the changing of the gel network structures was confirmed.

The solvent transportation behavior may relate to flavor release from gel-type foods during mastication. Therefore, such a study would be worthwhile for the food industry. We are currently conducting further investigation on the effect of adding sugar, and plan to report a series of studies in the near future.

**References**
Amici E, Clark AH, Normand V, Johnson NB (2002), Interpenetrating network formation in agarose - κ-carrageenan gel composites. Biomacromolecules, 3:466-474.

Arnott S, Fulmer A, Scott WE, Dea ICM, Moorhouse R, Rees DA (1974), The agarose double helix and its function in agarose gel structure. J. Mol. Biol., 90:273-284.

Boral S, Bohidar HB (2009), Hierarchical structure in agarose hydrogels. Polymer, 50: 5585-5588.

Dai B, Matsukawa S (2013) Elucidation of gelation mechanism and molecular interactions of agarose in solution by 1H NMR. Carbohydr. Res., 365: 38-45

Doi M (1990) Dynamics and Patterns in Complex Fluids. Onuki A and Kawasaki K (eds), Springer, Berlin pp100.

1

2    Fatin-Rouge N, Milon A, Buffle J, Goulet RR, Tessier A (2003), Diffusion and partitioning of solutes in

3    agarose hydrogels: The relative influence of electrostatic and specific interactions. J. Phys. Chem. B, 107:

4    12126-12137.

5

6    Kaneda I, Iwasaki S (2015), Solvent transportation behavior of mechanically constrained agarose gels.

7    Rheol Acta, 54: 437-443.

8

9    Kneabel A, Lequeux F (1998) Force relaxation of single spherical gel beads under compression. Polymer

10    Gels and Networks, 5: 577-584.

11

12    Matsukawa S, Ding Y., Zhao Q, Mogi A, Tashiro Y, Ogawa H., (2014) Effect of solvent transfer in agar

13    gels on stress relaxation under large deformation. Carbohydrate Polymers. 109: 166-170

14

15    Morris VJ (1998), Gelation of polysaccharide in Functional properties of food macromolecules (Ed. Hill

16    SE, Ledward A, Mitchell JR). Aspen Publication, pp148

17

18    Nakamura K (1999) The influence of deformation rate on the strength in compression for food gels. J.

19    Japanese Soc. Biorheology. 13 (3); 112-118 (in Japanese)

20

21    Nakamura K, Shinoda E, Tokita M (2001) The influence of compression velocity on strength and

22    structure for gellan gels. Food Hydrocolloids. 15; 247-252

23

24    Nishinari K, Watase M (1987), Effects of polyhydric alcohols on thermal and rheological properties of

25    polysaccharide gels. Agric Biol Chem. 51: 3231-3238.

26

27    Nishinari K, Fang Y. (2016) Sucrose release from polysaccharide gels. Foof & Function, View article

28    online (DOI: 10.1039/ c5fo01400j)

29

30    Nordqvist D, Vilgis TA (2011), Rheological study of gelation process of agarose-based solution. Food

31    Biophysics, 6: 450-460.

32

33    Normand V., Aymard P., Lootens DL, Amici E, Plucknett KP, Frith WJ. (2003) Effect of sucrose on

34    agarose gels mechanical behavior. Carbohydr. Polym. 54: 83-95

35

36    Stieger M, van de Velde F (2013), Microstructure, texture and oral processing: New ways to reduce sugar

1 and salt in foods. Cur. Opin. Colloid & Intr. Sci., 18:334-348.

2

3 Suzuki A, Hara T (2001,) Kinetics of one-dimensional swelling and shrinking of polymer gels under

4 mechanical constraint. J. Chem. Phys., 114: 5012-5015.

5

6 Tanaka T, Fillmore D (1979), Kinetics of swelling gels. J. Chem. Phys., 70:1214-1218.

7

8 Tokita M, Tanaka T (1991), Friction coefficient of polymer networks of gels. J. Chem. Phys., 95:

9 4613-4619.

10

11 Wang Z, Yang Y, Brenner T, Kikuzaki H, Nishinari K (2014), The influence of agar gel texture on sucrose

12 release. Food Hydrocolloids, 36: 196-203.

13

14 Xiong J-X, Narayanan J, Liu X-J, Chong TK, Chen SB, Chung T S (2005) Topology evolution and

15 gelation mechanism of agarose gel.   J. Phys. Chem B., 109: 5638-5643

16

17 Yamane T, Doi M (2005) The stress diffusion coupling in the swelling dynamics of cylindeical gels. J.

18 Chem. Phys. 122, 084703.

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Table 1 Characteristic parameters of the compression load relaxation, analyzed using Eq. 3

| Sample | $L_0$ [N] | $\tau_M$ [s] | $\alpha$ | $L_r$ [N] |
|---|---|---|---|---|
| G-0 | $1.06 \pm 0.180$ | $132 \pm 18.8$ | $0.387 \pm 0.0144$ | $0.733 \pm 0.0359$ |
| G-20 | $1.13 \pm 0.105$ | $167 \pm 22.4$ | $0.346 \pm 0.0578$ | $0.868 \pm 0.0492$ |
| G-40 | $1.20 \pm 0.115$ | $304 \pm 30.0$ | $0.349 \pm 0.0114$ | $0.842 \pm 0.0484$ |
| G-60 | $1.55 \pm 0.119$ | $786 \pm 116$ | $0.310 \pm 0.0162$ | $0.853 \pm 0.0512$ |
| G-80 | $1.46 \pm 0.203$ | $3973 \pm 1948$ | $0.239 \pm 0.0691$ | $0.538 \pm 0.0691$ |

The standard deviations are reported as the errors

Table 2 Characteristic parameters of the solvent transportation behavior, analyzed using Eq. 4

| Sample | $V_0 \times 10^2$ [-] | $\tau_V$ [s] | $\beta$ | $V_r \times 10^2$ [-] |
|---|---|---|---|---|
| G-0 | $2.27 \pm 0.872$ | $98.1 \pm 54.1$ | $0.416 \pm 0.159$ | $98.9 \pm 0.976$ |
| G-20 | $1.70 \pm 0.321$ | $239 \pm 28.0$ | $0.832 \pm 0.200$ | $99.2 \pm 0.889$ |
| G-40 | $1.60 \pm 0.736$ | $350 \pm 124$ | $0.761 \pm 0.445$ | $98.0 \pm 1.10$ |
| G-60 | $1.79 \pm 0.221$ | $715 \pm 239$ | $0.640 \pm 0.192$ | $98.0 \pm 1.27$ |
| G-80 | $1.96 \pm 0.516$ | $7629 \pm 4452$ | $0.564 \pm 0.213$ | $99.2 \pm 1.86$ |

The standard deviations are reported as the errors
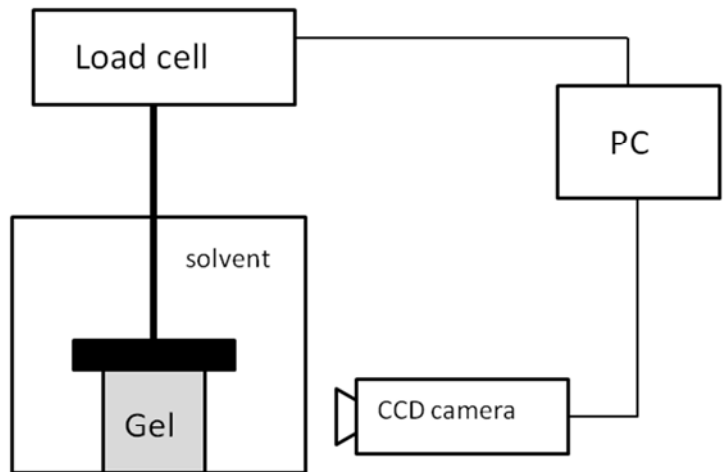
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3 Fig. 1 Block diagram of the experimental apparatus for mechanical property and
4 volume change analysis
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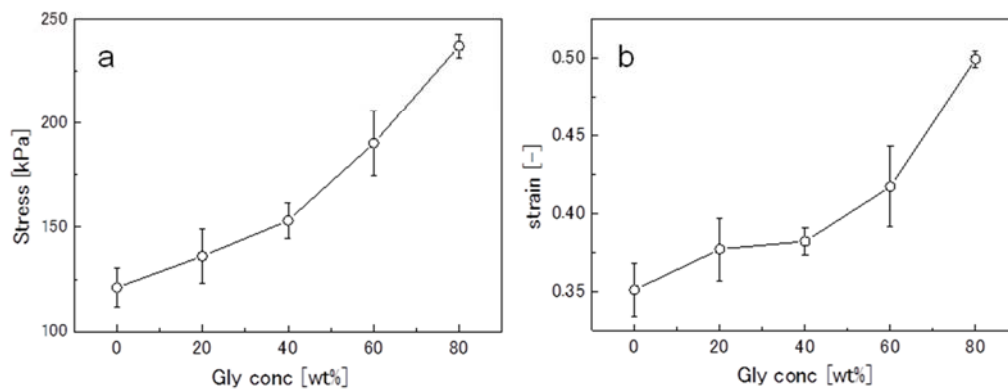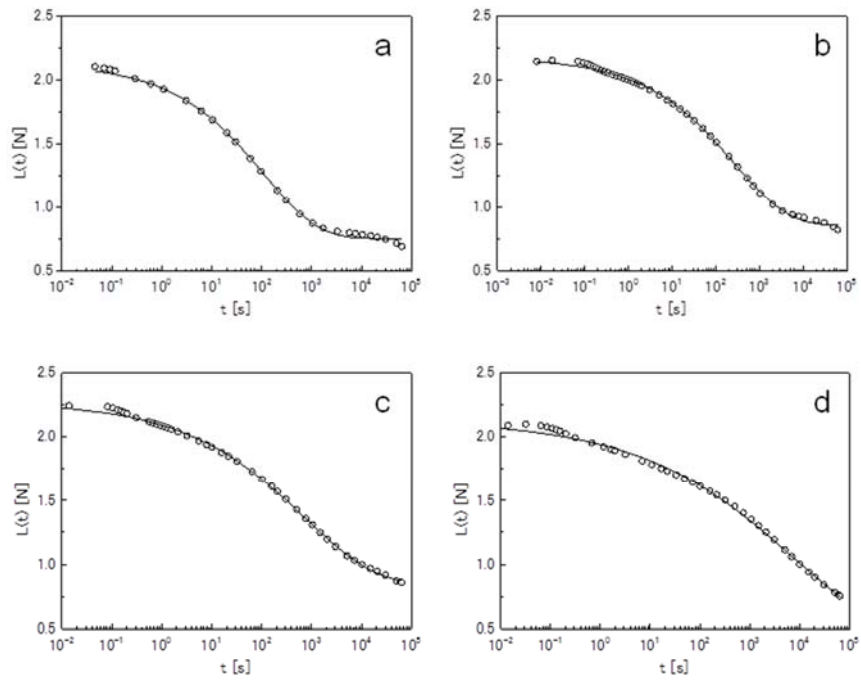


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3  Fig. 2 The compression fracture stress (a) and strain (b) for the samples containing
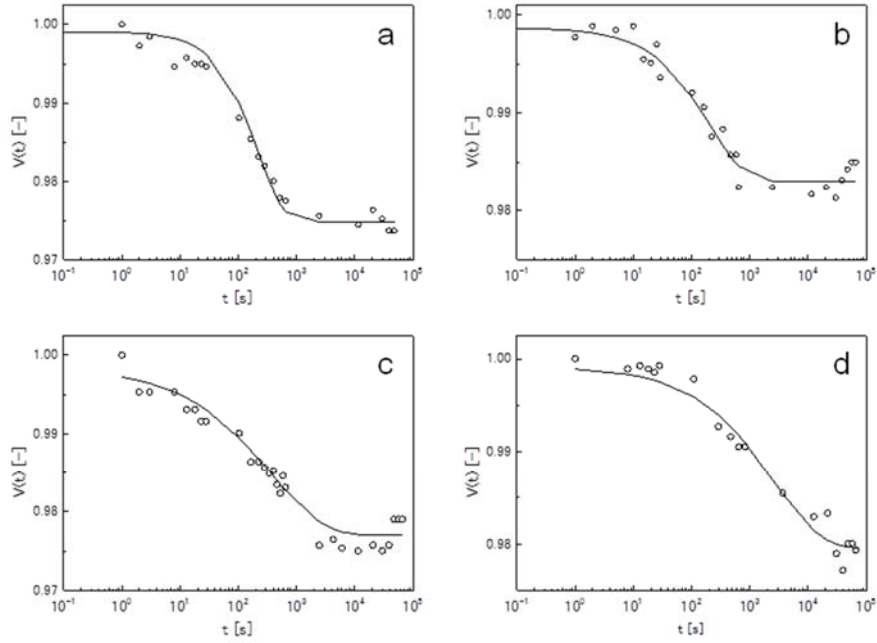4  various concentrations of glycerol.
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4 Fig. 3 Plots of the temporal variation of the compression load (open circles) for the

5 samples G-20 (a), G-40 (b), G-60 (c), and G-80 (d). The lines show the best fit results of
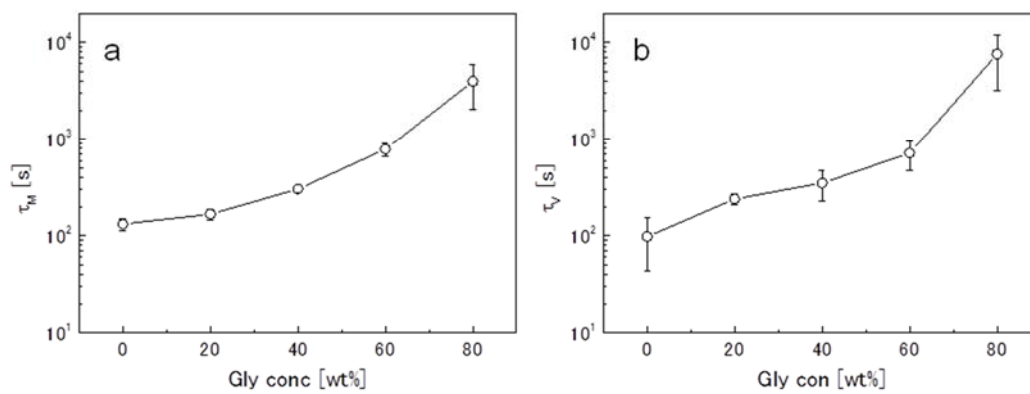
6 the analysis with Eq. 3.

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5    Fig. 4 Plots of the temporal variation of the volume change (open circles) for samples
6    G-20 (a), G-40 (b), G-60 (c), and G-80 (d). The lines show the best fit results of the
7    analysis with Eq. 4.
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3 Fig. 5 Dependence of time constants $\tau_M$ (a) and $\tau_V$ (b) on glycerol concentration. The
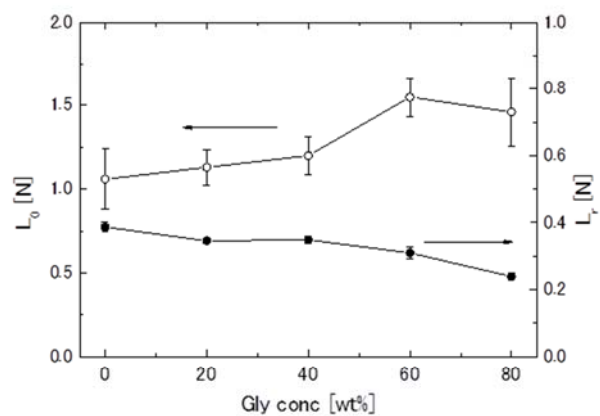4 error bars show the standard deviation.
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3　Fig. 6 The mechanical properties under a small compression strain for the samples

4　containing various concentrations of glycerol. The open circles and closed circles denote

5　$L_0$ and $L_r$, respectively.
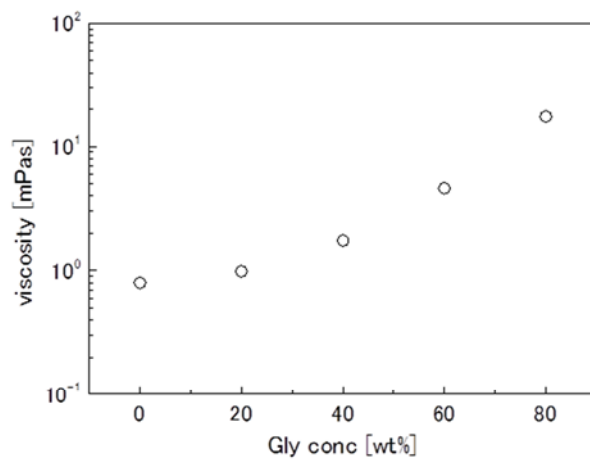
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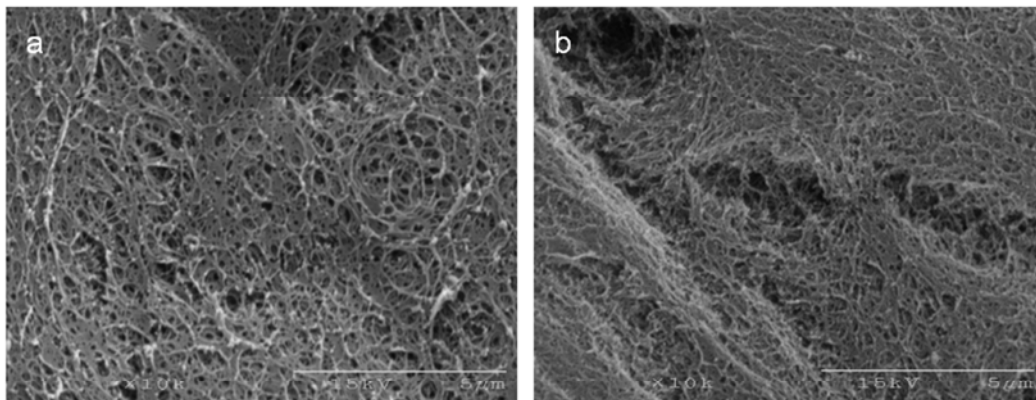4     Fig. 7 The apparent viscosity at 50 s$^{-1}$ of the water/glycerol mixed solvent.

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4    Fig. 8 SEM images of G-0 (a) and G-80 (b)
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