Formation of Stable Gel Colors of Calcium Alginate

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By a newly developed gel-preparation method using binary aqueous mixed solvents, such as a methanol–water or an ethanol–water mixture, before gelation as a sol solvent and after gelation as a gel environmental solvent, stable gel colors and xerogel colors of calcium alginate (Ca alg.) including photosensitive chlorophyll a (chl.a) were obtained. Ca alg. gel containing leucomalachite green (leuco-M) was made by using the same mixed solvents; after gelation by immersion in mixed solvents or in an acidic solution of various pH values, color development was investigated under ultraviolet irradiation (365 nm). From gel rupture stress measurements, absorption spectral measurements, and an X-ray diffraction study, etc., it was found that such gel-color stability mainly depends on the conformational stability of the host gel.

Sodium alginate (Na alg.) is a naturally occurring polysaccharide, isolated from brown seaweeds. It is a heterogeneous binary copolymer of D-mannuronate (M) and L-guluronate (G) residues arranged in a blockwise pattern along a linear chain.[1—3] Each molecule normally contains three types of blocks, i.e., homopolymeric sequences of the constituent sugars of MM blocks, GG blocks, and MG blocks, in which these two uronic acids occur in some sort of alternating pattern. The high uptake behavior of G-rich Na alg. in reducing the absorption of heavy metals without reducing the calcium ion in man has been reported.[4,5] A gel is an infinite crosslinked polymer network immersed in a fluid medium. Crosslinking occurs most strongly in homopolymeric GG block regions in the presence of calcium ions by depending mainly upon autocooperatively formed junctions, for which an "egg-box" type structure has been proposed.[6]

During the course of our investigations on the binding properties of Na alg., many new phenomena have been observed. Among others, the affinity of Na alg. to metal ions markedly increased upon introducing a dialysis gel preparation method and, in some cases, by using a mixture of two metal ions, such as Ca^{2+} and Fe^{3+} nitrate solutions.[7] Some of these gels became very hard and insoluble in 1 mol dm^{-3} HCl. By these methods, the maximum total number of metal ions bound to one uronate was about 2. Especially for both pure G–Na alg. and pure M–Na alg. in the case of the Ca ion, it was just 2.0. If gelation takes place in the presence of some divalent cations as the result of ionic crosslinking between carboxy groups on adjacent polymer chains, it should be less than 0.5, taking into account the sample G/M ratio (ca. 0.91). This indicates that the binding mechanism cannot be explained simply by the "egg box" model.

In one preliminary experiment,[8] in which an aqueous solution of calcium nitrate was added to Na alg. powder, which had been mixed with dye powder in advance, and after mixing when ethanol and 1 mol dm^{-3} HCl were added, stable color development of the dye included in the Ca alg. gels was observed. The stability has not been explained at all, because the dye is usually soluble in ethanol, or changes color in an acidic solution. The present work was initiated in order to examine such inclusion phenomema of a gel phase using chl.a as a color indicator. Chl.a is the main component of natural green food colors having various important functional properties, but is easily decomposed by light, heat or acid. It is also of particular interest since it is possible to control such properties by including it in a polymer gel.

An alginate polymer chain conformation depends on the solvent, temperature, pH, and salt concentration.[9] Based on electron-microscopic observations of metal alginate gel samples, it was shown that their initial fibrous networks changed immediatly to rigid and fine porous ones, even with a treatment using acid or ethanol during the sample-preparation procedure. By adding metal alginate gels, cholesterol dissolved in acetic acid was removed, but that in the ethanol solution was not reduced at all.[8] This indicated that the water content or pH of the gel outer solution plays and important role in the interaction between hydrophobic cholesterol and hydrophilic metal alginates. Cholesterol is an amphiphilic molecule. Although chlorophyll a is also amphiphilic, it is rather hydrophobic. Therefore, upon changing the water content of a binary aqueous mixed solvent, such as a methanol–water mixture, it was expected that some special interaction between the host alg. and the guest chl.a. may be present at a particular methanol concentration of the mixed solvents. On covalently crosslinked polyacrylamide in an acetone–water gel-fluid mixture, it is reported that the existence of a critical point in the phase equilibria and the phase separation have been observed at a certain acetone concentration and at a certain temperature.[10] The phase equilibrium and phase separation were also observed in our system as mentioned below.

Several attempts have been made to control the conformation of the polymer chains with light. Photostimulated color changes of some leuco-M derivatives in-

cluded in poly-*N*,*N*-dimethylacrylamide, which is one type of photosensitive polymer, were reported.[11] In the present study, some properties of alginate gels containing leuco-M upon photoirradiation were also examined.

Here, we report on a new two-step gel preparation method as well as the formation mechanism of gel colors of Ca alg., which was proposed in relation to the stability of the host gel at room temperature.

Experimental

Materials. Sodium alginate was obtained from Wako Pure Chemical Industries, Ltd. The G/M of this sample was ca. 0.91 and the $\overline{DP}$ was ca. 470. Chlorophyll a and leuco-malachite green were also obtained from the same company. All other commercially available chemicals used were of the highest available purity without further purification.

Apparatus. The gel rupture stress was measured for the film gel using an IIO Rheolometer (MAX RX 1600). Data concerning thermal analysis were obtained using a Rigaku (TAS 2000) system attached to DSC, TG, and DTA. An X-ray diffraction analysis was carried out with copper $K\alpha$ radiation, and using a 0.15 mm slit under 15 mA, 30 kV and a small-angle diffraction meter. Ultraviolet irradiation was produced by 12 W UV lamp; a sample immersed in various solvents was placed at a distance of 10 cm from the lamp.

Gel Preparation. i) Two-Step Method: The new method, called a "two-step method", consists of the 1st step and the 2nd step. It was applied to each type of gel form, i.e., the film form or the bead form, prepared by either a dialysis or dropwise method. In the typical two-step method, binary aqueous mixed solvents were used for both the 1st and 2nd steps. The gel was prepared as follows:

1st step—— 10 ml of a 0.4% or 1.0% Na alg. aqueous solution and 5 ml of a 0% (water)–100% methanol or ethanol aqueous solution containing chl.a (0—5×10^{-6} mol) or leuco-M (0—2.5×10^{-6} mol) were mixed with stirring, resulting in the methanol or ethanol concentration of the mixture being 0—33.3%. The mixture filled in a bottom petri dish or a 20 ml beaker was covered and sealed with a cellulose membrane (visking dialysis 36/32), turned upside down and dialyzed in 20 ml of 0.1 or 1 mol dm^{-3} $Ca(NO_3)_2$ aq soln filled in the upper petri dish or a 50 ml beaker for 3 d in the dark. The gel was a thick film formed in a petri dish or in a 20 ml beaker; here, we call it the film gel. By the dropwise method, a bead-form gel was prepared under the same conditions, except for the gelation time (about 30 min) in the calcium nitrate aqueous solution. The film gel was removed from the membrane, and the bead gel was separated by using a nylon cloth; both types of gels were slightly dried with filter paper.

2nd step—— After the 1st step, each gel was immersed in 20—100 ml of a methanol–water (0—100%) or ethanol–water (0—100%) mixture or in 10—20 ml of various pH solutions adjusted with HCl or NaOH. Bead gels immersed in 40—60% methanol or ethanol became stacked upon each other and formed a lump-form gel. For gels containing leuco-M or blank gels immersed in various concentrations of methanol, ethanol or in various pH solutions, ultraviolet irradiation (λ=365 nm) was carried out, usually for 2h. All of the gels were stored in the dark, except during UV irradiation or gel weight measurements.

ii) One Step Method: A mixture of 10 ml of 0.4% or 1.0% Na alg. aq solution and 5 ml of 0% (water)—90% methanol–water or ethanol–water mixed solutions containing chl.a (0—5×10^{-6} mol dm^{-3}) was dialyzed in a mixture of 20 ml of 0.1 or 1.0 mol dm^{-3} $Ca(NO_3)_2$ aq solution and 10 ml of 0% (water)—90% mathanol or ethanol with the same methanol or ethanol concentration as the initial solution (the final concentration was 0—30%), in the dark for 3 d. The film gels were used for DSC measurements after gradient drying with 50—100% ethanol.

iii) Photostimulated Dilation of Some Lump Gels Containing Leuco-M Immersed in Water: A mixture of 1 ml of ethanol or 1.6×10^{-3} mol dm^{-3} leuco-M ethanol soln (without sufficient mixing) and 4 ml of 0.4% Na alg. aq soln was added to 10 ml of a 0.1 mol dm^{-3} calcium nitrate aq soln without mixing. In this case a lump-form gel was formed. Five gels containing leuco-M and a leuco-M blank gel were prepared. After immersing in 20 ml of water in the dark for 24 h, the weight of each gel (W_1) was measured and immersed in 10 ml of water. One of them was stored in the dark and others were irradiated under UV with 365 nm for 1, 2, 3, and 4 h, respectively. After storing for 0, 48, and 96 h in the dark, each gel weight (W_2) was measured again.

iv) Effect of pH of the Gel Outer Solution of Some Bead Gels Containing Leuco-M in an Aqueous System: 26.4 mg leuco-M and 400 mg Na alg. were dissolved in water and made up to 100 ml.

5 ml of the solution was poured into 10 ml of a 0.02 or 0.1 mol dm^{-3} $Ca(NO_3)_2$ aq solution. These 11 bead gels were immersed in 20 ml of an aq solution adjusted to pH 0.95—12 with hydrochloric acid and sodium hydroxide.

Swelling or Shrinkage Ratio. V/V_0, of the gel volume or W/W_0 of the gel weight was determined by measuring the gel volume (V) or the gel weight (W) to the gel volume (V_0) or the gel weight (W_0) of a methanol- or an ethanol-free gel or a gel immersed in water. For measuring the gel volume in the 1st step, each gel was immersed in water filled in a measuring cylinder for an instant.

UV-vis Spectral Measurements. The gel sample was cut so as to fit a 1×4 cm cubic cell, and attached to the cell wall. A dye blank gel was also attached to the reference side cell wall.

Gel Rupture Stress. A film gel was measured with dyn (=10^{-5} N) per cm^{-2} (D). The mean value (D) of several data was calculated. The gel stability ratio (D/D_0) was obtained from the ratio of the gel rupture stress (D) to the gel rupture stress (D_0) of a gel having 0% methanol or 0% ethanol, or a gel immersed in water.

The methanol or ethanol concentration (v/v) of the methanol–water or the ethanol–water mixture is expressed under the assumption that a volume change does not occur after mixing methanol or ethanol with water.

Results

Gel Colors of Chl.a. Methanol or ethanol solution of chl.a was green and showed typical absorptions at around 420 and 660 nm, respectively. These intensities decreased if it was not shielded to light. Figure 1a shows the changes in the absorption spectra of chl.a in methanol (dotted lines) and that in a film gel prepared

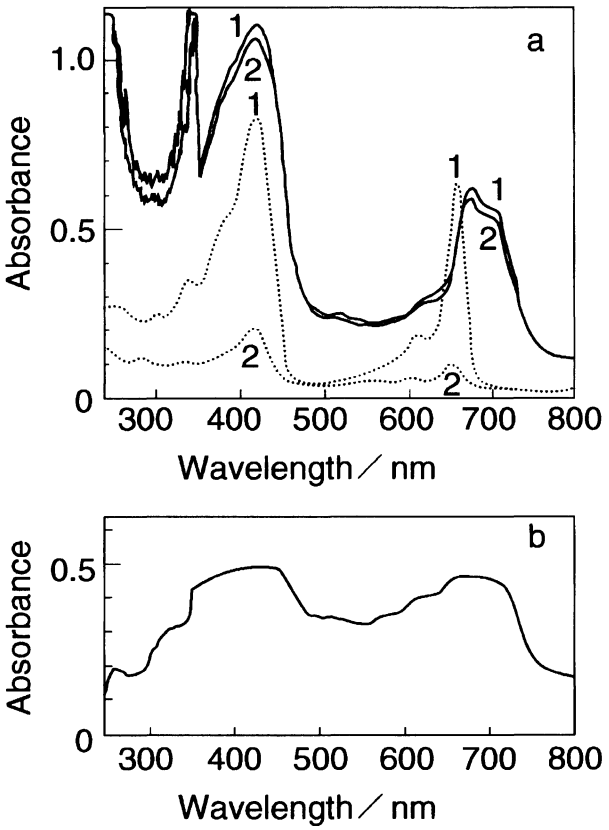

Fig. 1. Absorption spectra of chl.a. a) A stable film form gel (——) and a methanol (---) solution before (1) and after (2) exposure to light for 7 d. b) A stable film form xerogel. The gel film was prepared under the condition: 1st step at 33.3% methanol and 2nd step at 60% methanol.

by the two-step method (solid lines) before and after being exposed to sunlight for one week. The difference between dotted lines 1 and 2 shows that exposure to light yielded significant decolorization of the methanol solution of chl.a. However, for chl.a in gels prepared by the two-step method such a color degradation was scarcely observed, as indicated by the solid lines.

Regarding the two-step method, for example, when each green chl.a–Ca alg. bead gel formed under the condition of 33.3% methanol during the 1st step was immersed in a methanol–water mixture with methanol concentrations of 20, 40, 60, 80, and 100%, respectively, a clear green color developed in each gel. The clear green-colored gel remained stable in the mixed solvents, except for the gel immersed in 100% methanol.

It was found that at a methanol concentration of 60% of the methanol–water mixture a remarkably clear gel was formed for both the bead-form and the film-form gel. At 60% methanol, not only the best color transparency of chl.a, but also a special interaction between binary aqueous-mixed solvents and chl.a or sodium alg., or both chl.a and sodium alg., were observed.[12] In the case of chl.a or a mixture of chl.a and sodium alg., precipitation began to occur, and then the solution became colorless at this particular concentration. For the ethanol–water mixture, the best color of the gel was obtained for an ethanol content of 50%.

Usually, not only decolorization, but also spectral shifts of the gel colors of chl.a were not clearly observed. The spectral shifts shown by the absorption spectra with full lines 1 and 2 in Fig. 1a indicate that the shifts occurred before exposure. It is the most extreme case of a spectral shift to longer wavelengths among film-form gels containing chl.a. Usually, the absorption spectrum of chl.a in the film gel was almost the same as that of chl.a in methanol, as shown by dotted line 1. Under the fixed condition of 60% methanol in the 2nd step for film gels, upon increasing the methanol concentration during the 1st step to 30%, two typical absorptions moved to around 440 and 680 nm; when it was increased up to 33.3% these spectra changed to 420 nm (doublet) and 700 nm (doublet), as shown in Fig. 1a. It is therefore obvious that the porphyrin ring of the chl.a moiety which hydrated under the former condition was protected from protonation, and then returned to the stable state as a result of the formation of $[(\text{chl.a } 2H_2O)_n]$. The doublet signals suggest that there are two different types of chemical species upon increasing the methanol content of the methanol–water mixed solvents by about 33% during the 1st step and by 60% during the 2nd step in the mixture.

Most gel colors remained stable in the mixed solvents for more than a few years in a room without any protection against light. Especially, film-form xerogels prepared at 6.7% MeOH during the 1st step and 60% MeOH during the 2nd step were stable upon exposure to sunlight, in an oven at 60 °C for more than 3 d, in either 1 mol dm^{-3} HCl or 100% MeOH, though, in 100% EtOH chl.a in the gels they were slightly soluble. The clearest color was observed at 20% MeOH for film gels and 33.3% MeOH for bead gels during the 1st step, when immersed in 60% methanol during the 2nd step. Figure 1b shows the absorption spectrum of a xerogel containing chl.a. The two broadened peaks indicate the formation of irrevesible phases by the above-mentioned steps.

Change of the Chl.a Gel Color. Under various conditions, the chl.a gel color stability was examined, and several systematic facts concerning color change of chl.a in Ca alg. were obtained. While immersing the gels in mixed solvents, the typical absorption spectra of chl.a in some green-bead gels not stored in the dark shifted to longer wavelengths of 745 and 445 nm, indicating the formation of $[(\text{chl.a } 2H_2O)_n]$ and a conformational change of the porphyrin ring of chl.a, respectively. Figure 2 shows several changes in the absorption spectra of chl.a in gels. Such spectral shifts were not observed for chl.a in the film-form gels; however, for bead gels having an ethanol–water gel fluid, the shifts appeared relatively soon and the green-colored

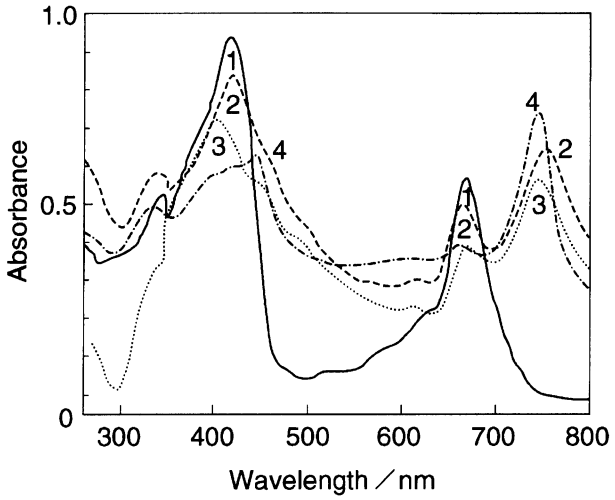

Fig. 2. Absorption spectral changes of chl.a in the bead gel immersed in 60% methanol or 60% ethanol. Line number 2 and 3 show the cases of without sufficient mixting at 1st step. 1st step: 20% methanol for 1, 2, and 3 and 20% ethanol for 4. 2nd step: 1. (——) indicates for gels before immersion. 2. (---) indicates for gels immersed in 60% methanol for 17 d, 3. (····) indicates for gels immersed in 60% methanol for 19 d, and then immersed in 0.1 mol dm^{-3} hydrochloric acid for 1 h. 4. (–·–) indicates for gels immersed in 60% ethanol for 4 d.

gels changed to light green, but were stable. When a mixture of an aqueous sol solution and a methanol solution was prepared with a sodium alginate content of more than 0.4% or a MeOH content of more than 60%, homogeneously mixed sol solutions were not obtained. However, when bead gel colors were prepared without mixing before gelation, a broadened absorption peak at around 420—440 nm (Fig. 1a) shifted to shorter wavelengths at around 400 nm accompanied by a degradation of the gel color.

The clear and stable green color of the bead gels prepared by this experiment remained almost fadeless when the gels were stored in a room without any protection from light for more than one year. When these gels were exposed to ultraviolet irradiation (wavelength at 365 nm) for 1 h, spectral changes were not observed; but, when the gels were exposed to sunlight directly after UV irradiation, the green color of all the bead gels changed immediatly to dull green. From this experiment concerning the effect of sunlight and ultraviolet irradiation on the gel color, it was found that under such irradiation the formation of hydrophobic chl.a dihydrates was accelerated; then, the dihydrates changed to the hydrophilic type accompanied by color degradation. Using the one-step method, clear green gel films were also obtained, although their color stability against acid, organic solvents, and light (even in a room) was poor, and the color faded within a few days.

X-Ray Diffraction Patterns of Wet Film Gels Containing Chl.a. The data indicated that the structure was almost the same as those of gels containing iron chlorophyllin, 5-iodo eosine or the host gel (dye blank) prepared using binary aqueous mixed solvents, such as methanol–water or ethanol–water by the two-step method used in the preparation experiment. During consecutive and repeated measurements, two large peaks appeared at around $2\theta=28.3°$ and 13.2°, corresponding to 3.15 and 6.70 Å, respectively. The former distance is almost the same as the typical distance of I–I for iodine included in metal alginates immersed in an iodine –KI solution adjusted to pH 1.[8] The latter, also observed for alginic acid powder, was shorter than a distance of 8.7 Å (fiber axis), which was observed for alginic acid or metal alginates.[13] After 5 or 6 consecutively repeated runs, these peaks changed to one broad peak at around $2\theta=17.0°$, which corresponds to 5.2 Å. This change indicates that the wet crystal-like gel color collapsed during the X-ray measurements. Based on a small angle X-ray diffraction analysis, it was found that chl.a in the film gel is arranged at a distance of about 110 Å, two dimensionally in the dialyzed layers of the film gel.

Infrared Absorption Spectral Data (in KBr) or Chl.a Gel Samples. The data of the original Na alg. powder and both the film-form and bead-form types of clear green gels and a chl.a blank film gel prepared under the condition of 33.3% methanol during the 1st step and 60% methanol during the 2nd step from a 0.4% Na alg. aq solution showed excellent agreement concerning the hydrogen bond, which was indicated by absorption at 3350 cm^{-1}. In some bead-form chl.a gels and chl.a-free and methanol- or ethanol-free gels the typical absorption peak at 1620 cm^{-1} ascribed to crystal water in Ca alg. gels was observed.

Swelling and Shrinkage. Upon increasing the methanol or ethanol concentration of sol-mixed solvents, gels shrink at low alcohol concentrations compared to an alcohol-free gel in the case of the Na alg. sample used here, and then swell. Especially, gels swell significantly from around 20% to 33.3% methanol or ethanol, respectively. At 33.3% methanol or ethanol the swelling ratio to an alcohol-free gel is about 2. During the 2nd step, the gels shrink or do not change in volume, but do not swell at all. The final swelling ratio (V/V_0) for the bead-type gel as a function of the methanol concentration during the 2nd step (V) to the bead gel formed at 0% MeOH (water, V_0) is shown in Fig. 3. This indicates that the gel volume was most stable when the gel was formed at 33.3% methanol and immersed in 60% methanol. The maximum volume was 1.7-times that of the methanol-free host bead gel immersed in water. The condition for the gel volume stability for both gels containing chl.a and chl.a-free is in good agreement with the condition for the chl.a gel color transparency described in the above section.

Gel Rheology. In Fig. 4, D/D_0 shows the ratio of

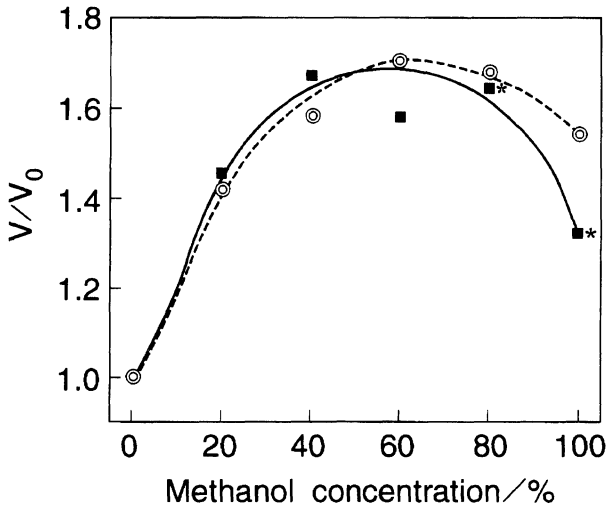

Fig. 3. Effect of the addition of methanol at immersion step on the gel volume stability. The sol mixed solutions at the second step for the chl.a blank gel (◎) and the gel containing chl.a (■) prepared at 33.3% methanol at 1st step using 0.4% Na-alg. ⋆ indicates gel collapse of a part of the sample.

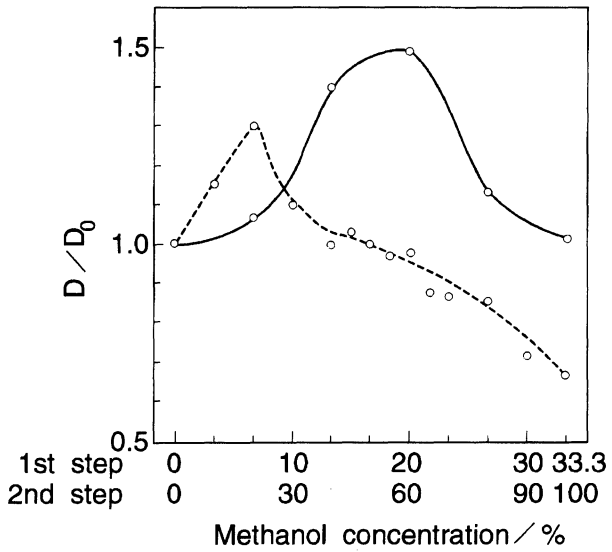

Fig. 4. Effect of the addition of methanol at the 1st step (---) and the 2nd step (——) on the gel rupture stress per 1 cm^2 of a gel film. 1st step: 5 ml of 0—100% methanol. for 10 ml of 0.4% Na-alg. (0—33.3% methanol) 2nd step: 20 ml of 0—100% methanol, for the film gel prepared for 10 ml of 0.4% Na-alg. at 1st step.

the gel rupture stress measured by dyn cm^{-2} of a host film gel to that of a methanol-free gel as a function of the methanol concentration of the sol solvent (1st step) or the gel outer solution of a methanol–water mixture (2nd step). For the 1st step, D/D_0 is 1.3 at 6.7% methanol and 0.7 at 33.3% methanol. For the 2nd step it is 1.5 at 60% methanol. This suggests that the gel structure is remarkably strengthened under a combination of concentrations of 6.7% methanol during the 1st step and 60% methanol during the 2nd step.

DSC Measurements. For two xerogel film samples prepared by a one-step gel preparation procedure having 0% (water) and 20% ethanol gel fluids, DSC measurements were carried out. As shown in Table 1, the gel having 20% ethanol gel fluid had a slightly stronger structure than the gel having an ethanol-free gel fluid. The water contents in these xerogel samples were found to be 14.43 and 14.54%, respectively. If the binding ratio of calcium ions to one uronate in the case of film gels was 1.0 for the Na alg.[7] used in the present study, where the one-step method was applied, the water contents correspond to two water molecules bound to one uronate.

Photostimulated Dilation. The initial color of leuco-M in gels is colorless. Usually, upon sunlight or ultraviolet irradiation the gel color turns blue. Figure 5 shows the photostimulated dilation of Ca alg. lump-form gels containing or not containing leuco-M prepared without mixing, as a function of the storage time, in the dark after ultraviolet irradiation (λ=365 nm). The dilation curves indicate that the effect of irradiation on the photoresponsed dilation clearly appeared 2 d later and disappeared within 4 d. However, photostimulated dilation for the film gels prepared by the two-step method was not observed, the developed blue color of the film gel did not turn colorless.

Effect of pH. Figure 6 shows the pH effect of the gel outer solution on W_2/W_1, i.e., the gel swelling and shrinkage of calcium alginate gels containing leuco-M. For 0.1 mol dm^{-3} calcium nitrate, data for more

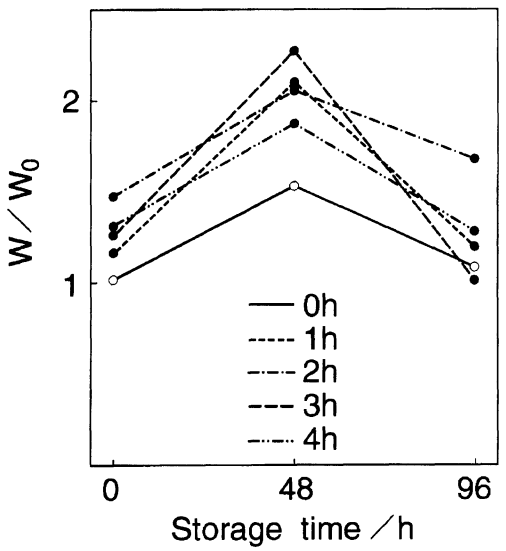

Fig. 5. Effect of ultraviolet irradiation on the photostimulated dilation for the lump form gel containing leucomalachite green. 1st step: 20% ethanol, 2nd step: water. W_0 and W indicate the gel weight before the irradiation (W_0) and after storing in the dark for 0, 48, and 96 h after the irradiation (W).

Table 1. DSC Data of Dry Film Gels[a] of Calcium Alginate

Sample gel fluid	Dehydration temp °C	Peak temp °C	Water content %
Ca-alg. water	47.0—131.4	85.9	14.43
Ca-alg. 20% ethanol	52.9—136.3	89.4	14.54

a) Both film gels were prepared as follows: A mixture of 10 ml of 0.4% Na-alg. aqueous solution and 5 ml of 0 and 60% ethanol–water mixtures (20% ethanol) was dialysed in the mixture of 20 ml of ethanol concentration of 20% containing calcium nitrate.

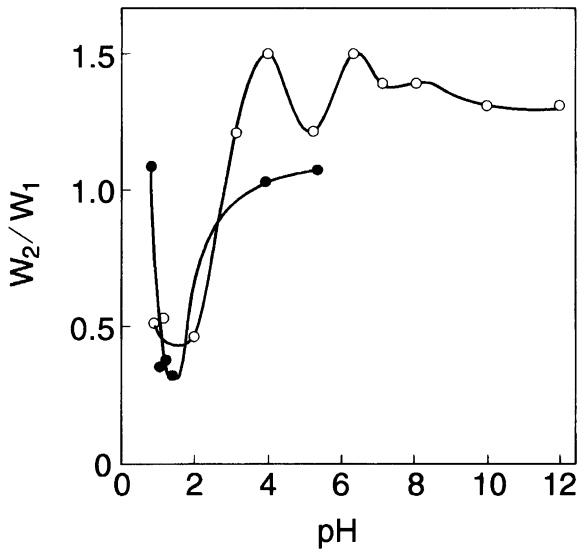

Fig. 6. Effect of pH on the gel swelling and the gel shrinkage for the gel containing leuco-M formed in 0.02 mol dm^{-3} $Ca(NO_3)_2$ (○) and 0.1 mol dm^{-3} $Ca(NO_3)_2$ (●). W_1: each gel weight 3 h after gelation. Bead form gels are formed from 5 ml of aqueous solution of 0.4% Na-alg. containing 4 mol% leuco-M (to Na-alg.). W_2: each gel weight after immersion in 20 ml aqueous solution of adjusted pH, for 24 h in the dark.

than pH 6 were not obtained because of a gel collapse. For 0.02 mol dm^{-3} calcium nitrate at around pH 6—3, a periodical change of the value of W_2/W_1 between 1.25—1.5 appeared. Drastic gel shrinkage (W_2/W_1 is about 0.5) was observed for gels at around pH 1, and the initial color of the gels was not changed upon UV or sunlight irradiation.

Discussion

The color of a film-form gel is more stable than that of a bead form gel as mentioned above. From a microscopic observation for a film-form gel of calcium alginate, it was confirmed that it comprised regulary arranged multiple layers of a glassy fine network.[8] The network layers were formed one after another by dialysis. In an earlier experiment, it was found that the calcium content in a film-form gel is about two-times that in a bead-form gel. This suggests that film gels have a more rigid and regularly arranged structure than that of bead gels.

However, it is obvious that the gel color transparency, and even the gel color stability, basically do not depend on the calcium content. The gel color properties seem to be induced by two different conformational stabilities during the 1st and 2nd steps.

During the 1st step the maximum gel stability was observed to be around 6.7% methanol or ethanol, accompanied by gel shrinkage. This may be explained by the high viscosity of the sol solvent at a low content of alcohol. It is known that the viscosity of sodium alginate in an acetone–water mixture of ca. 2.5% acetone is larger than that of water. The curve of the viscosity ratio to the viscosity of Na alg. aqueous solution as a function of the acetone concentration is very similar to the curve of the gel rupture stress as a function of the methanol concentration during the 1st step, as shown by the dotted line in Fig. 4.

For the 2nd step, the gel colors were developed at 60% methanol and 50% ethanol for chl.a and leuco-M, respectively. For a gel containing leuco-M immersed in a solution adjusted to around pH 1 the color of the gel containing leuco-M was not developed upon ultraviolet irradiation. The X-ray diffraction data, the gel volume change and the IR data mentioned above indicate the formation of cavities. The hydrophilic site (V part) of a porphyrin ring of chl.a is supposed to be plunged into the cavity and chl.a would thus be stably settled in the network of Ca alg. The absorption spectrum of chl.a was usually the same as that in a methanol solution, though in some cases it was slightly shifted, as shown by the full line in Fig. 1a. In such a case, the porphyrin ring would be deformed by exposure to light in the room during sample preparation.

Tanaka reported on a critical behavior associated with a phase separation of the binary mixture of the network and fluid medium, namely, a shrinkage of the network in a fluid medium on a polyacrylamide gel.[10] In a methanol–water solution containing more than 60% methanol, Ca alg. gel also shows a phase separation, because chl.a dissolved into the solution. This was espe-

cially obvious for the 100% methanol solution.

Without using the 2nd step of the two-step gel preparation method, or without sufficient mixing of Na alg. in the methanol–water mixtures, color degradation of the gel colors was easily observed.

As shown in Fig. 5, in the case of a lump-form gel containing leuco-M, photostimulated dilation was observed. This may be explained as follows: during the 1st step, polymer dihydrates of Ca alg. were formed as a solid state on the surface of the lump gels, and leuco-M was protected from protonation; then, by diffusion and/or irradiation, the dihydrates turned to the gel state with swelling, and a blue color developed 48 h later; however 96 h later, gel shrinkage occured.

Conclusions

1. Very clear and stable green gel colors or xerogel colors of calcium alginate containing chl.a were prepared by a two-step method which uses binary aqueous-mixed solvents, such as methanol–water mixtures, before and after gelation.

2. The conditions for the gel color stability agreed with those for the maximum gel stability of the host gel of Ca alg. The conditions for the gel color transparency agreed with those for the final maximum gel volume of the host gel.

3. The color development of leuco-M in the gel by UV irradiation was observed, except for around pH 1.

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