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THERMOMECHANICAL ANALYSIS OF CALCIUM ALGINATE HYDROGELS IN WATER

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Abstract

Spherical hydrogels of Ca alginate (CaAlg) were prepared by conversion of Na alginate (NaAlg) to CaAlg in $CaCl_2$ aqueous solution. The degree of conversion was varied by changing the conversion times. NaAlg samples with mannuronic and guluronic component ratios (M/G ratio) 0.21 and 0.88 were used. The degrees of conversion and water contents (W_c=mass of water in CaAlg/mass of dry CaAlg) were measured by weighing. The degrees of conversion from NaAlg to CaAlg increased with increasing immersion time and the content of G. W_c of CaAlg hydrogels decreased with increasing immersion time and levelled off at 75 g g^{-1} when immersion time exceeded about 10 min. Viscoelastic properties of CaAlg hydrogels in water were measured using a thermomechanical analyzer (TMA) equipped with a quartz compression probe. Dynamic modulus (E') of CaAlg with M/G=0.88 increased with increasing immersion time when immersion time is less than 5 min. When immersion time exceeded 5 min, E' reached a constant value at $4.5\cdot10^4$ Pa. In contrast, E' of CaAlg with M/G=0.21 increased with increasing immersion time. This suggests that densely crosslinked hydrogels are formed when the guluronate sequence of the samples is rich and Na ions are fully converted to Ca ions.

Keywords: alginic acid, dynamic modulus, hydrogel, sphere gels, TMA

Introduction

Alginic acid is a copolysaccharide consisting of mannuronic (M-component) and guluronic acid (G-component) which is extracted from seaweed and some kinds of bacteria [1, 2]. The chemical structure is shown in Fig. 1. Sodium salt of alginate (NaAlg) is water soluble. It is well known that Na ions of aqueous solution of NaAlg are replaced by Ca ions in an aqueous media, such as $CaCl_2$ solution and hydrogels are formed [3–5]. It is thought that Ca ions are enclosed by the guluronic component and a junction zone is formed. The interaction between Ca ions and carboxyl groups of the guluronate is described by the egg box model involving a two-stage process of initial dimerization and subsequent aggregation [6, 7]. On this account, the size of the junction zone depends on the mannuronic/guluronic (M/G) ratio of the sample [8].

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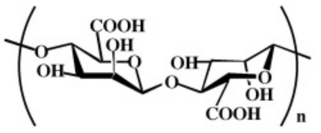
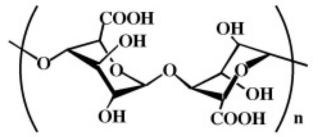
When NaAlg aqueous solution is extruded using a syringe into $CaCl_2$ aqueous solution as fibres, CaAlg gel fibres are formed [9]. In this case, molecular chains align along the b axis and CaAlg gel fibres containing a large amount of water show a liquid crystalline structure [9, 10]. When aqueous NaAlg solution is dropped into $CaCl_2$ aqueous solution, spherical CaAlg gels are formed. This characteristic property is utilized to produce man-made fish eggs which are widely used in food industries [11].



Mannuronic acid



Guluronic acid

Fig. 1 Chemical structure of alginic acid

It is thought that physical properties of CaAlg hydrogels depend not only on *M*/*G* ratio and molecular mass but also gel preparation conditions, such as, concentration of NaAlg solution, Ca concentration, substitution time and homogenisation of aqueous solution. When sphere type gels are formed, Na ion is instantaneously substituted with Ca ions. After the junction zone is completed on the surface, Ca ions diffuse into the core, and this process is time dependent.

Viscoelastic properties of hydrogels have been investigated by rheometry in order to determine the sol–gel transition [12–16]. In the above studies, cone–plate or plate–plate type rheometers have extensively been utilised. In our previous study, we introduced the application of a TMA to measure dynamic modulus of hydrogels in water using a special sample cell attached to the TMA [17, 18]. The swelling behaviour of gel films has also been measured by static measurements by TMA [17]. TMA enables us to carry out the static and dynamic mechanical properties of hydrogels in water [18]. In this study, alginate spherical hydrogels were prepared using samples with various *M*/*G* ratios and different degrees of conversion. Dynamic modulus was measured by TMA.

Experimental

Preparation of alginate spherical hydrogels

Sodium alginate (NaAlg) in powder form was obtained from Kibun Food Chemifer Co. There were two samples, one having *M*/*G* ratio 0.21 (viscosity=0.53 Pa s, measured at shear rate=8.55 s^{-1}) and the other 0.88 (viscosity=0.55 Pa s, measured at shear rate=

8.55 s^{-1}) according to the manufacture's report. Degree of substitution of NaAlg was 1.0. Powder samples were solved in deionized water to obtain 1% solutions and heated at 105°C for 3 h. The solution was equilibrated at 25°C for 2 h. The solution was dropped into an excess amount of 0.5% aqueous solution of calcium chloride ($CaCl_2$) using a 1 mL syringe from 0.10–0.15 m high. The mass of one drop of NaAlg solution was weighed and the average value (0.060±0.0005 g) was obtained by measuring ten drops. Transparent, flexible hydrogels in spherical shape were formed immediately after NaAlg solution came into contact with $CaCl_2$ solution. The hydrogels were kept in deionized water at 25°C for 20 min. The gels were wiped and the diameter and mass were quickly measured. A syringe needle was used to separate NaAlg aqueous solution that was not converted. The mass was measured again. The gels were then dried at 120°C for 3 h in an oven and the dry mass of the CaAlg was recorded. A Sartorius micro-balance (MC210S, $\pm 0.1\cdot 10^{-4}$ g) was used for sample mass measurements.

In order to avoid confusion in the subsequent description, the superscripts will be used, for Na alginate sample and Ca alginate designate ($^{Na}Alg_{dry}$), ($^{Ca}Alg_{dry}$), and subscript gel or sol, ($^{Na}Alg_{sol}$), ($^{Ca}Alg_{gel}$) means the above samples are in sol or in gel state. ($^{Ca+Na}Alg_{gel}$) designates that the gel contains non-substituted Na alginate aqueous solution. The times (t_{CaCl_2}) shows immersing time in $CaCl_2$ aqueous solution. Water content (W_c) was calculated according to the following Eq. (1).

$$W_c\ (\mathrm{g\ g^{-1}}) = (\text{mass of water in } {}^{Ca}Alg_{gel})/(\text{mass of } {}^{Ca}Alg_{dry}) \qquad (1)$$

TMA measurements

Viscoelastic properties of gels were studied using a TMA (Seiko Instruments TMA/SS 150). A quartz rod with uniform cross-sectional area ($9.616\cdot 10^{-6}$ m^2) was used as a probe. The gel sample was placed in an aluminium sample pan with a diameter of 7 mm and height of 3 mm. The gel was immersed in water at 25°C to prevent evaporation during the measurement. Operating frequency was 0.05 Hz. Measurements were carried out for 5 min. The above conditions were employed for all measurements. From output data, a dynamic stress-strain hysteresis loop (Lissajous diagram) was obtained [14].

Results and discussion

When 1 mass% $^{Na}Alg_{sol}$ is dropped in $CaCl_2$ aqueous solution, transparent spherical $^{Ca+Na}Alg_{gel}$ was immediately formed. $^{Ca}Alg_{gel}$ membranes were too thin to form spheres, when immersion time in $CaCl_2$ aqueous solution was less than 30 s. These $^{Ca+Na}Alg_{gel}$ spheres were easily broken during sample handling. Figure 2 shows the relationship between degree of conversion from $^{Na}Alg_{sol}$ to $^{Ca}Alg_{gel}$ of one sphere and immersion time in $CaCl_2$ aqueous solution. The degree of conversion was calculated according to the following Eq. (2).

$$\text{degree of conversion}\ (\mathrm{g\ g^{-1}}) = (\text{mass of } {}^{Ca}Alg_{dry})/(\text{mass of } {}^{Na}Alg_{dry}) \qquad (2)$$

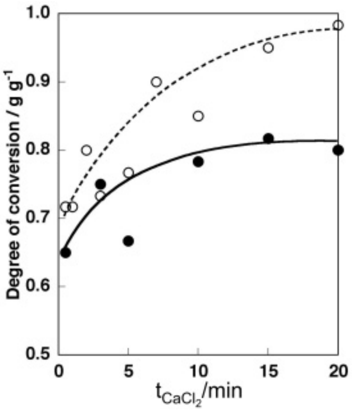


Fig. 2 Relationships between degree of conversion (mass of $^{\mathrm{Ca}}\mathrm{Alg}_{\mathrm{dry}}$/mass of $^{\mathrm{Na}}\mathrm{Alg}_{\mathrm{dry}}$) and immersion time in $\mathrm{CaCl_2}$ aqueous solution ($t_{\mathrm{CaCl_2}}$) of the sample with various M/G ratios; ● – M/G ratio=0.88, o – 0.21

The difference of molecular mass between $^{\mathrm{Ca}}\mathrm{Alg}_{\mathrm{gel}}$ and $^{\mathrm{Na}}\mathrm{Alg}_{\mathrm{sol}}$ was negligible since two $\mathrm{Na^+}$ ions are converted into one $\mathrm{Ca^{2+}}$ ion by gelation. Degree of conversion increases with increasing immersion time in $\mathrm{CaCl_2}$ aqueous solution ($t_{\mathrm{CaCl_2}}$ /min). It is found that the degree of conversion in the initial stage is high 0.6–0.7 g g^{-1}. In the case of $^{\mathrm{Na}}\mathrm{Alg}_{\mathrm{sol}}$ with M/G=0.21, the degree of conversion reaches a constant of 0.95 g g^{-1} when immersion time exceeds 15 min. In the case of $^{\mathrm{Na}}\mathrm{Alg}_{\mathrm{sol}}$ with M/G=0.88, the degree of conversion is constant (=0.8 g g^{-1}) when immersion time exceeds 10 min. It is thought that G segments of $^{\mathrm{Na}}\mathrm{Alg}$ molecules are incorporated with Ca ions and form a crosslinking zone.

After immersion in $\mathrm{CaCl_2}$ aqueous solution, each sphere gel was transferred into water and maintained for 20 min. After removal of the water soluble portion from the $^{\mathrm{Ca+Na}}\mathrm{Alg}_{\mathrm{gel}}$, the remaining amount of ($^{\mathrm{Ca}}\mathrm{Alg}_{\mathrm{gel}}$) was weighed. It was assumed that the water soluble portion was $^{\mathrm{Na}}\mathrm{Alg}_{\mathrm{sol}}$ and although a small amount of $^{\mathrm{Na}}\mathrm{Alg}_{\mathrm{sol}}$ might re-

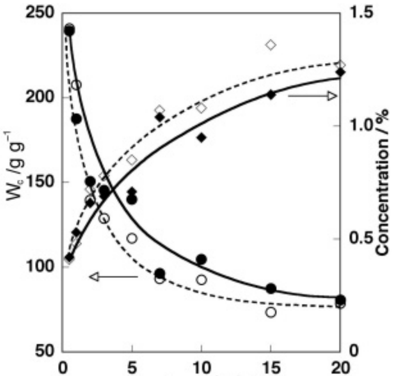


Fig. 3 Relationships between water content (W_c) and immersion time in $\mathrm{CaCl_2}$ aqueous solution; ●◆ – M/G ratio=0.88, o◇ – 0.21; W_c= (mass of water)/(mass of $^{\mathrm{Ca}}\mathrm{Alg}_{\mathrm{gel}}$)

main in the sphere gel. Accordingly it was considered that the mass value of ${}^{Ca}Alg_{gel}$ contained an experimental error. Water content in ${}^{Ca}Alg_{gel}$ was obtained by drying using Eq. (1). Figure 3 shows relationships between water content (W_c), concentration of ${}^{Ca}Alg_{gel}$ (%) and immersion time in $CaCl_2$ aqueous solution (t_{CaCl_2}). Concentration of the ${}^{Na}Alg_{sol}$ was 1%. As shown in Fig. 3, apparent concentration of the ${}^{Ca}Alg_{gel}$ increases when immersing time (t_{CaCl_2}) was less than 10 min. W_c decreased with increasing immersion time (t_{CaCl_2}). In initial stage of conversion, the water permeates into the ${}^{Ca}Alg_{gel}$ membrane and W_c markedly increases. When immersion time exceeds about 10 min, W_c reaches a constant at 75 g g^{-1}. It is clear that densely cross-linked junction zones are formed with increasing immersion time. At the same time, the samples with rich mannuronic component sorb a slightly larger amount of water than guluronic component rich samples.

A skin-core structure was formed from the surface to the core in the ${}^{Ca}Alg_{gel}$ sphere gels. From visual observation, the sphere surface was smooth and the inner surface showed irregular structure. This suggests that gradation of Ca content may occur in the gel. At the same time, the thickness of ${}^{Ca}Alg_{gel}$ membrane varies with increasing immersion time. Thickness of ${}^{Ca}Alg_{gel}$ (m) membrane was calculated according to the following Eq. (3).

$$\text{Thickness of } {}^{Ca}Alg_{gel}(m) = (\text{radius of } {}^{Ca+Na}Alg_{gel}) - (\text{radius of } {}^{Na}Alg_{sol} \text{ in } {}^{Ca+Na}Alg_{gel}) \quad (3)$$

Radius of ${}^{Na}Alg_{sol}$ included in ${}^{Ca+Na}Alg_{gel}$ was calculated using mass of ${}^{Na}Alg_{sol}$ ($={}^{Ca+Na}Alg_{gel} - {}^{Ca}Alg_{gel}$), assuming the density of ${}^{Na}Alg_{sol}$ is the same as water. The radius was also calculated using the mass of ${}^{Ca+Na}Alg_{gel}$ assuming the density of the gel is the same as that of water. The calculated radius agreed well with measurement values.

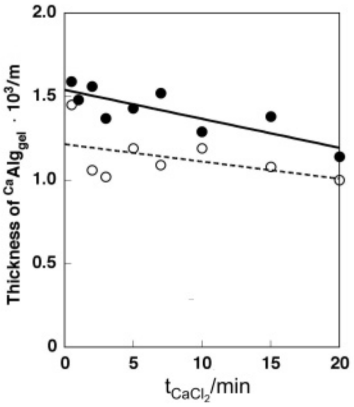


Fig. 4 Relationships between thickness of ${}^{Ca}Alg$ and immersion time in $CaCl_2$ aqueous solution; ● – M/G ratio=0.88, o – 0.21

Figure 4 shows relationships between thickness of ${}^{Ca}Alg_{gel}$ with different M/G ratios and immersing time in $CaCl_2$ aqueous solution. Thickness of ${}^{Ca}Alg_{gel}$ membrane decreases with increasing immersing time, and the thickness of $^{Ca}Alg_{gel}$ membrane with *M*/*G*=0.88 is greater than that of *M*/*G*=0.21. High crosslink density gels are formed at the initial stage of conversion for G-component rich samples. A large amount of water is retained in the M-component rich samples. The mass of $^{Ca+Na}Alg_{gel}$ is 2.5 to 3 times higher than that of orignal $^{Na}Alg_{sol}$.

The fact that the size of sphere hydrogels decreases with increasing immersion time in $CaCl_2$ aqueous solution indicates water is excluded from hydrogels during both Na–Ca conversion process and water sorbing process. On this account, the amount of water which increases or decreases during gel formation ($W_{c\,s}$) was calculated according to the following Eq. (4). The original amount of water is calculated from the concentration of $^{Na}Alg_{sol}$.

$$W_{c\,s}\,(\mathrm{g\,g^{-1}}) = -[(\text{mass of }{}^{Na}Alg_{sol}) - (\text{mass of }{}^{Ca+Na}Alg_{gel})]/(\text{mass of }{}^{Ca}Alg_{dry}) \quad (4)$$

The change of water content during gel formation can be estimated from the above equation. The difference between $W_{c\,s}$ and W_c indicates the increase or decrease in the amount of water immersion. In the above equation, when water content in the gels is larger than original aqueous solution, $W_{c\,s}$ is shown as positive. On this account, the amount of water obtained by syneresis is shown in negative values. As shown in Fig. 5, $W_{c\,s}$ decreases with increasing immersion time. When immersion time exceeded about 10 min, W_c maintained constant value at 75 g g^{-1}. When immersion time is less than 5 min, W_c of $^{Ca}Alg_{gel}$ is higher than $^{Na}Alg_{sol}$. This comes from the fact that $^{Ca}Alg_{gel}$ sorbs water in the soaking process which was carried out for 20 min as stated in the experimental section. When immersion time is longer than 5 min, W_c of $^{Ca}Alg_{gel}$ is lower than $^{Na}Alg_{sol}$ because syneresis occurs.

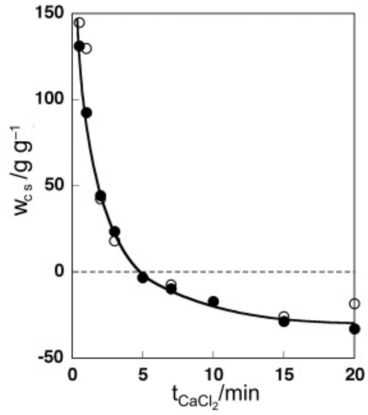


Fig. 5 Relationships between amount of increased or decreased by synerisis ($W_{c\,s}$) and immersion time in $CaCl_2$ aqueous solution; ● – *M*/*G* ratio=0.88, o – 0.21

Figure 6 shows the variation of dynamic modulus (*E*') of $^{Ca}Alg_{gel}$ measured in water as a function of immersion time in $CaCl_2$ aqueous solution (t_{CaCl_2}). As clearly seen, *E*' of $^{Ca}Alg_{gel}$ *M*/*G*=0.88 alginate increased with increasing immersion time when immersion time in $CaCl_2$ aqueous solution is shorter than 5 min. When immer-

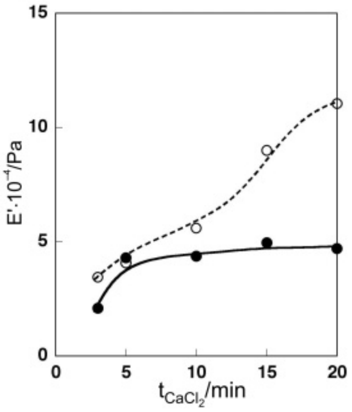


Fig. 6 Relationships between dynamic modulus and immersion time in $CaCl_2$ aqueous solution; ● – *M*/*G* ratio=0.88, o – 0.21

sion time exceeded 5 min, *E*' reaches a constant value at $4.5\cdot10^4$ Pa. In contrast, *E*' of $^{Ca}Alg_{gel}$ with *M*/*G*=0.21 increased with increasing immersion time. It is known that association of polyguluronate sequences contributes to Ca^{2+} binding [19]. It is reasonable to consider that hydrogels formed by guluronate sequence rich alginate have many crosslinking points.

Conclusions

From the experimental results, the following facts are clarified: (1) degree of conversion from $^{Na}Alg_{sol}$ to $^{Ca}Alg_{gel}$ increased with increasing conversion time and *M*/*G* ratio; (2) water content of $^{Ca}Alg_{gel}$ decreased with increasing conversion time and *M*/*G* ratio; (3) thickness of $^{Ca}Alg_{gel}$ membrane decreased with increasing conversion time and *M*/*G* ratio; (4) dynamic modulus increased with increasing conversion time. It is concluded that guluronate sequence forms densely crosslinked structures by Na–Ca conversion. On this account, dynamic modulus of G rich $^{Ca}Alg_{gel}$ is high. The mannuronic sequence retains a larger amount of water than the guluronic sequence and dynamic modulus shows moderately low values. When the membrane is thin, the amount of water maintained in the sphere gels is larger than that of the original $^{Na}Alg_{sol}$. This suggests that network molecules expand several times more than in the initial state.

References

1 E. D. T. Atkins, I. A. Nieduszynski, W. Mackie, K. D. Parker and E. E. Smolko, Biopolymers, 12 (1973) 1865.
2 R. Kohn, I. Furda, A. Haug and O. Smidsrød, Acta Chemica Scandinavica, 22 (1968) 3098.
3 A. Haug and O. Smidsrød, Acta Chemica Scandinavica, 24 (1970) 843.
4 R. Kohn and B. Larsen, Acta Chemica Scandinavica, 26 (1972) 2455.
5 D. Thom, G. T. Grant, E. R. Morris and D. A. Rees, Carbohyd. Res., 100 (1982) 29.

6 G. T. Grant, E. R. Morris, D. A. Carbohyd. Rees., P. J. C. Smith and D. Thom, FEBS Letters, 32 (1973) 195.

7 E. R. Morris, D. A. Rees, D. Thom and J. Boyd, Carbohydrate Research, 66 (1978) 145.

8 O. Smidsrød, Faraday Discussions of the Chemical Society, 57 (1974) 263.

9 F. X. Quinn, T. Hatakeyama and H. Hatakeyama, SEN-I GAKKAISHI 50 (1994) 418.

10 K. Nakamura, T. Hatakeyama and H. Hatakeyama, SEN-I GAKKAISHI 47 (1991) 421.

11 K. Nishinari, ‘Gums and Stabilisers for the Food Industry 4’ G. O. Phillips, P. A. Williams and D. J. Wedlock Eds, IRL Press, Oxford, Washington D. C. 1988, p. 373.

12 J.-T. Fu and M. A. Rao, Food Hydrocolloids, 15 (2001) 93.

13 N. A. Abdulmola, R. K. Richardson and E. R. Morris, Food Hydrocolloids, 14 (2000) 569.

14 K. Nishinari, K. E. Hofmann, H. Moritaka, K. Kohyama and N. Nishinari, Macromol. Chem. Phys., 198 (1997) 1217.

15 M. Rinaudo and J. Desbrieres, ‘Hydrocolloids – Part 1’, K. Nishinari Ed., Elsevier Science B. V., Amsterdam 2000, p. 111.

16 M. Hirashima, T. Takaya and K. Nishinari, Thermochim. Acta, 306 (1997) 109.

17 K. Nakamura, E. Kinoshita, T. Hatakeyama and H. Hatakeyama, Thermochim. Acta, 352–353 (2000) 171.

18 M. Iijima, T. Hatakeyama, M. Takahashi and H. Hatakeyama, J. Therm. Anal. Cal., 64 (2001) 617.

19 O. Smidsrød and A. Haug, Acta Chemica Scandinavica, 26 (1972) 79.