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Thermochimica Acta 431 (2005) 68–72

thermochimica acta

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Swelling behaviour of calcium pectin hydrogels by thermomechanical analysis in water

Mika Iijima[a, *], Tatsuko Hatakeyama[b], Hyoe Hatakeyama[c]

[a] *Department of Environmental and Information Sciences, Faculty of Environmental and Information Sciences, Yokkaichi University, 1200 Kayo-cho, Yokkaichi 512-8512, Japan*
[b] *Department of Textile Science, Faculty of Home Economics, Otsuma Women's University, 12 Sanban-cho, Chiyoda-ku, Tokyo 102-8357, Japan*
[c] *Department of Environmental and Biotechnology, Faculty of Engineering, Fukui University of Technology, Gakuen, Fukui 910-8505, Japan*

Received 21 October 2004; received in revised form 6 January 2005; accepted 21 January 2005
Available online 22 February 2005

Abstract

Aqueous solutions of pectin annealed at various temperatures at sol state were mixed with $CaCl_2$ solution and calcium pectin gels with various cross-linking ratios were prepared. A thermomechanical analyser (TMA) equipped with a sample holder, which was newly designed at our laboratory was used. The sample holder was immersed in water whose temperature was controlled from 30 to 80 °C. Swelling behaviour of hydrogels during equilibration was measured as a function of immersing time. Creep coefficient was calculated as functions of time and temperature using a compression type probe. The creep coefficient of hydrogels increased with increasing swelling temperature and decreased with increasing cross-linking ratio. Dynamic modulus (E') was measured in water in a temperature range from 30 to 60 °C. E' of the gel with cross-linking ratio 0.36 decreased from 5.5×10^4 to 3.5×10^4 Pa with increasing swelling temperature from 30 to 60 °C.

Keywords: Pectin; Thermomechanical analysis; Hydrogels; Swelling behaviour; Dynamic modulus

1. Introduction

A large number of water-soluble polysaccharides are known to form physical gels in aqueous media. The gelation ability of each polysaccharide depends on various factors, such as chemical structure, higher order structure and gelation conditions. We have reported that gellan gum forms rigid gels when it is annealed at a temperature higher than gel–sol transition [1,2]. Although xanthan gum and hyaluronan have been considered as non-gelling polysaccharides, it was found that gels are formed when they are fully annealed in the sol state [3–5].

Pectin is a polysaccharide found in many plant tissues. It consists of galacturonic acid having some carboxyl groups, which are esterified by methyl groups [6–9]. When the degree of methyl esterification (DE) of pectins is low, pectin molecules react with divalent cations and hydrogels are easily formed [10,11]. The gelation mechanism of polyguluronic acid of alginate has mainly been explained by the "egg-box" model. The gelation of low methoxyl pectin is also explained in the same mechanism [12,13]. We have reported that calcium pectin hydrogels, which were prepared after annealing at sol state forms a greater number of soft gels than non-annealed gels [14]. When pectin sol was annealed at a temperature higher than 50 °C, Ca pectin hydrogels formed more soft gels than those annealed at low temperature. The swelling ratio measured by tea-bag method decreased with increasing annealing temperature at sol state. The above fact indicates that the effect of annealing temperature at sol state affects the higher order structure of pectin molecular assembly, which can be fixed by Ca ion.

Mechanical properties of hydrogels have mainly been investigated by viscoelastic measurement using a rheometer

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* Corresponding author. Tel.: +81 776 22 8111; fax: +81 776 29 7891.
E-mail address: h-hatake@ccmails.fskui-ut.ac.jp (H. Hatakeyama).

0040-6031/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2005.01.041

[15–17]. Even when the gel is rigid, mechanical testing of hydrogels using a custom made machine is limited, since the gel frame is frequently destroyed by cramping. Furthermore, vaporization of water during measurements is neglected in the above method. In our previous study, we introduced a thermomechanical analyser (TMA) to measure dynamic modulus of hydrogels in water [18,19]. By TMA, both static and dynamic mechanical properties of hydrogels in water can be measured [18]. Dynamic viscoelasticity measured by TMA covers dynamic modulus range from 10^4 to 10^5 Pa. Although dynamic viscoelastic properties can be measured using a conventional dynamic mechanical analyser (solid state) and a rheometer (liquid state), it is difficult to measure the values shown in the above dynamic modulus range. In our previous study [18,19], it was suggested that TMA is appropriate in measuring viscoelastic properties of ionically linked hydrogels, such as pectin or alginate. By introduction of a new sample probe, it is possible to measure the hydrogels in water and other solvents.

In this study, TMA equipped with a sample holder, which was newly designed at our laboratory was used. Creep coefficient is calculated as functions of time and temperature using a compression type probe. Dynamic modulus is measured at various temperatures. The Ca pectin gel formation mechanism was investigated.

2. Experimental

2.1. Sample preparation

Pectin with the degree of methylesterification (DE) = 25.6% was obtained from Taiyo Kagaku Co., Japan. Molecular weight calculated from viscosity measurement was 8.5×10^4 [20]. Pectin was solved in deionised water at 25 °C to obtain 2% solutions. The solutions were stirred at 25, 50, 80 and 98 °C for 180 min. An aqueous solution of calcium chloride ($CaCl_2$) 2.28% was poured into an aqueous solution of pectin in a 20 ml glass container and mixed at 25 °C. Transparent and flexible gels were formed immediately. The pectin concentration of samples at this stage was 1.7%. After stirring, samples were maintained at room temperature for 24 h in order to complete the substitution.

Quantitative analysis of Ca in Ca pectin gel was carried out by atomic absorption spectrometer (Shimadzu AA-630-01). The degree of substitution (DS) of the Ca pectin gel was obtained using the measured concentration of Ca ion in the sample and calculated value assuming 100% substitution. In this case, all COOH is assumed to be substituted by Ca ions.

$$\mathrm{DS} = \frac{\text{measured value of Ca}}{\text{calculated value of Ca}} \times (1 - \mathrm{DE}) \qquad (1)$$

Equilibrium DS values decreased with increasing annealing temperature at sol state. DS was 0.423, 0.381, 0.355 and 0.311 at annealing temperature at sol state 25, 50, 80 and 98 °C, respectively [14].

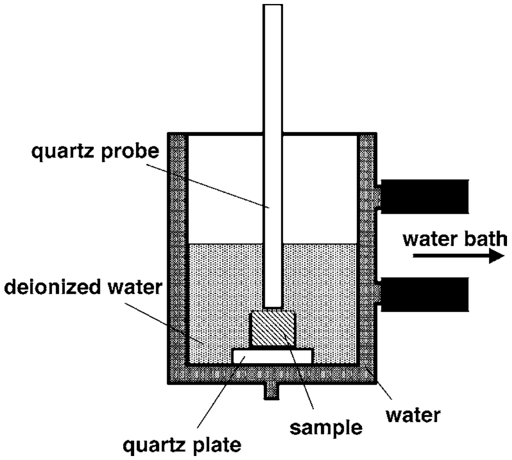

Fig. 1. Schematic conformation of TMA sample probe.

2.2. Measurements

A Seiko thermomechanical analyser (TMA, Seiko Instruments TMA/SS 150) equipped with a newly designed sample holder was used. Quartz rod with uniform cross-sectional area (9.616×10^{-6} m^2) was used as a probe. Sample setting in the probe is illustrated in Fig. 1. The sample holder was immersed in water whose temperature was controlled from 30 to 80 °C by circulation of water from a constant temperature bath (HAAKE F8). In this stage, errors of water temperature were within ±1 °C. The gel sample was placed in a quartz sample pan with diameter 7 mm (inner diameter) and height 5 mm and immersed in 70 ml water. And the sample was compressed by quartz probe. Swelling behaviour by creep measurement and dynamic modulus was calculated.

2.2.1. Swelling behaviour by creep measurement

Creep measurements were carried out as follows:

(1) the sample was compressed in water (at 30–60 °C),
(2) applied maximum stress was varied from 1.04×10^3 to 5.2×10^3 Pa,
(3) initial sample length decreased until applied load reached predetermined stress and then the load was maintained at constant,
(4) compressed height at a constant load was measured as a function of time and
(5) measurement was completed in 60 min.

The compression was expressed by negative value. Creep coefficient was calculated as functions of time and temperature using a compression type probe. Sample compression during equilibration was measured as a function of immersing time. Creep coefficient function of time ($(l_e - l_c)/l_0 t$) calculated.

2.2.2. Dynamic measurement

Dynamic measurements were carried in water at 30–60 °C by using a quartz rod probe. The probe was the same as that

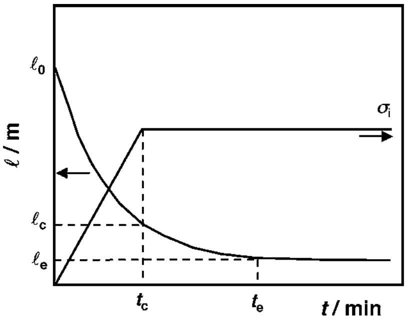

Fig. 2. Schematic TMA curves of creep of hydrogel in water: t, time (min); t_c, time reached predetermined stress (min); t_e, time when thickness reached constant (min); l, thickness as a function of time (m); l_0, initial thickness (m); l_c, thickness at t_c; l_e, thickness at t_e (m); σ, applied stress as a function of time (Pa)

used in creep measurements. Each sample was measured after equilibration swelling was attained at various temperatures. After the gels were saturated by swelling, measurements were started at various temperatures. Operating frequency was 0.05 Hz. Measurements were carried out for 5 min. The above conditions were employed for all measurements to facilitate comparison of the variation in E' and $\tan\delta$ as a function of DS and DE. From Lissajous diagram, E' and $\tan\delta$ were obtained [18].

3. Results and discussion

3.1. Swelling behaviour of calcium pectin hydrogels

Fig. 2 shows schematic TMA curves of creep of hydrogel in water, where t, time (min); t_c, time reached predetermined stress (min); t_e, time when sample thickness reached a constant value (min); l_0, initial thickness (m); l, thickness as a function of time (m); l_c, thickness at t_c (m); l_e, thickness at t_e (m); σ, applied stress (Pa) as a function of time and σ_i, constant stress at i (Pa), respectively [18].

Effect of stress on creep of Ca pectin hydrogels was investigated. Fig. 3 shows relationship between $((l_e - l_c)/l_0) \times 100$ and stress. Values of $((l_e - l_c)/l_0) \times 100$ increased linearly with increasing stress. A similar relationship is also observed in other hydrogels [21]. In the following measurements, samples were measured at the constant stress of 2.08×10^3 Pa (20 mN). It was confirmed that the size of gels was completely recovered when applied stress was 2.08×10^3 Pa.

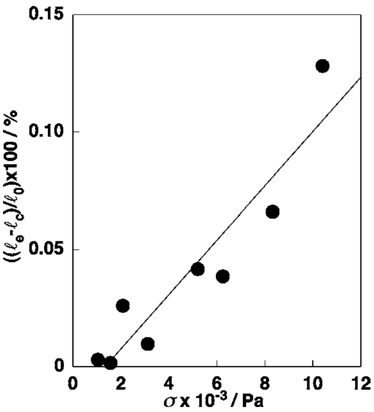

Fig. 3. Relationship between $((l_e - l_c)/l_0) \times 100$ and stress (σ).

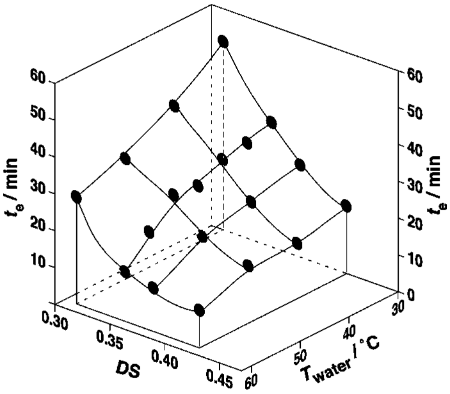

Fig. 4. Three-dimensional relationship between t_e, DS and temperature (T_{water}).

Fig. 4 shows three-dimensional relationship between t_e, DS and water temperature (T_{water}). The t_e decreased with increasing T_{water} and DS. It is thought that pectin molecular chains are extended at high temperatures and water easily penetrates cross-linking networks, hence the time for equilibration decreases. In contrast, as shown in Fig. 4, t_e decreases with increasing DS. This suggests that time molecular rearrangement in water under applied stress becomes short when the number of pectin molecules between cross-linking points decreases, i.e. time for equilibration under applied stress decreases.

Fig. 5 shows three-dimensional relationship between $((l_e - l_c)/l_0) \times 100$, DS and T_{water}. The values of $((l_e - l_c)/l_0) \times 100$ increased with increasing T_{water}, and decreasing with DS. It is thought that pectin molecular chains are extended with increasing immersion temperature. Soft gel is obtained when the number of cross-linking points

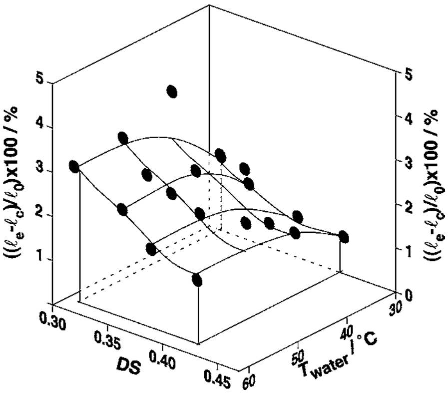

Fig. 5. Three-dimensional relationship between $((l_e - l_c)/l_0) \times 100$, DS and temperature ($T_{water}$).

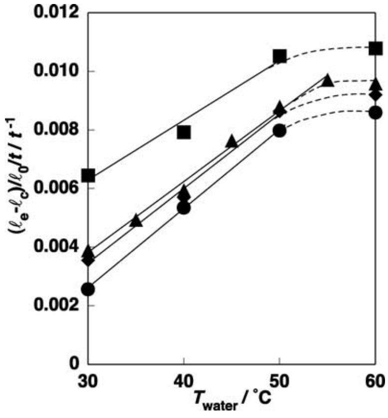

Fig. 6. Relationships between $(l_e - l_c)/l_0t$ and temperature (T_{water}): (■) DS = 0.311; (▲) 0.355; (◆) 0.381; (●) 0.423.

is small and molecular chain length between cross-linking points is large [22] and soft hydrogels deform easily. It is clearly seen in Fig. 5, $((l_e - l_c)/l_0) \times 100$ shows the highest values at DS = 0.311.

Fig. 6 shows relationship between $(l_e - l_c)/l_0t$ and T_{water}. The values of $(l_e - l_c)/l_0t$ decrease with increasing DS and gradients of straight lines are varied according to the change of DS. As shown in the figure, values of $(l_e - l_c)/l_0t$ decreases with increasing DS at 50 °C, however, they are saturated when T_{water} exceeds 50 °C. It is thought that entangled intermolecular chains expand with increasing T_{water}. The fact that $(l_e - l_c)/l_0t$ values are maintained constant when T_{water} exceeds 50 °C suggests that molecular chains fully expand at a temperature higher than 50 °C under the applied stress employed in this study.

Creep coefficients were calculated as a function of temperature from the gradient of each straight line in a temperature from 30 to 50 °C. The values of $(l_e - l_c)/l_0T$ increased with increasing DS. In our previous study, it was found that static swelling ratio measured by tea-bag method increased with increasing DS [14]. This suggests that swelling ratio of Ca pectin hydrogels is influenced by the higher order structure at sol state, although effect of DS is not ignored. Generally, the swelling ratio increases when the molecular chains are allowed to expand. Accordingly, molecular chain expansion is restricted during swelling when gels have high cross-linking density, which are formed by annealing at low temperature in the sol state. The results shown in Fig. 7 support the creep coefficient obtained by TMA, showed a similar tendency to that of swelling ratio measured by tea-bag method.

Fig. 7 shows the relationship between $(l_e - l_c)/l_0T$ and swelling ratio at 60 °C by tea-bag method. It is clear that $(l_e - l_c)/l_0T$ closely correlates with swelling ratio at 60 °C by tea-bag method. Large value of $(l_e - l_c)/l_0T$ indicate that the shape of hydrogels markedly depends on temperature. From this result, it is considered that $(l_e - l_c)/l_0T$ values can be used as an index of swelling behaviour under constant pressure of hydrogels.

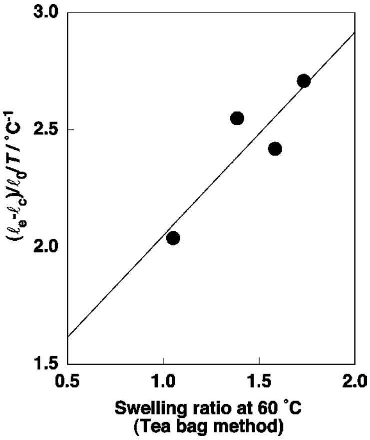

Fig. 7. Relationship between $(l_e - l_c)/l_0T$ and swelling ratio at 60 °C by tea-bag method.

3.2. Dynamic modulus of calcium pectin hydrogels

Dynamic viscoelasticity was measured by the procedure described in Section 2. Fig. 8 shows relationship between E' and T. E' decreases with increasing temperature. Values of $\tan\delta$ were ca. 0.2, although data was not shown in figure. As already shown in Figs. 5 and 6, sample size, in this case $(l_e - l_c)/l_0$, increases with increasing temperature. This suggests that the network structure expands due to swelling, and that the chain length between cross-linking points increases. It is reasonable to expect that dynamic modulus decreases as a function of temperature when DS is constant. The results indicate that gel networks are readily swollen with increasing temperature and that swollen gels were soft.

Fig. 9 shows relationships between E' at 40 and 60 °C, and DS. We have reported that E' values of the same sample at 25 °C ranged from 2.0×10^5 to 2.7×10^5 Pa [14]. As shown in this figure, E' values at 40 °C range from 4 to 5×10^4 Pa, accordingly, it is clear that E' of Ca pectin gels remarkably decreases with increasing temperature. When temperature is constant, E' increases with increasing DS. E' decreased with increasing water temperature (T_{water}). It is thought that Ca

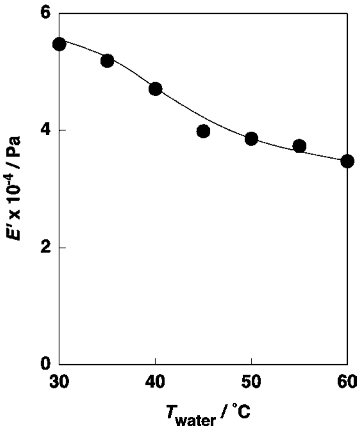

Fig. 8. Relationship between E' and temperature (T_{water}).

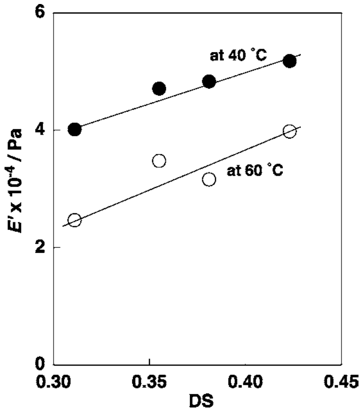

Fig. 9. Relationships between E' at 40 and 60 °C, and DS: (●) at 40 °C; (○) at 60 °C.

pectin gel is swollen at a high temperature than desalting water temperature and E' decreased. In Ca pectin hydrogels, E' decreases with increasing annealing temperature at sol state. In gellan gum and xanthan gum hydrogels, it is reported that E' increases with increasing annealing temperature at sol state. It is considered that high-temperature annealing leads to an increase in the size of the junction zones and the elastic modulus of the gels that are subsequently formed on cooling decreases. Size distribution, the junction zone of most hydrogels has been explained by the Gaussian statistics model. E' of networks decreases with an increase of swelling degree [23]. On the other hand, Ca ion takes a role of junction point in Ca pectin hydrogels, and on this account, the Gaussian statistics model is difficult to apply. When the samples were annealed at high temperature for a long time at sol state, pectin molecules are fully expanded and a large network structure was formed by the introduction of Ca ions.

4. Conclusion

From the experimental results, the following facts are clarified:

(1) creep coefficient was calculated as functions of time and temperature,
(2) swelling behaviour under constant pressure of hydrogels can be measured by $(l_e - l_c)/l_0 T$ and
(3) swelling behaviour of Ca pectin hydrogels can be measured by TMA.

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