EFFECT OF ANNEALING ON CALCIUM PECTIN GEL FORMATION BY THERMOMECHANICAL ANALYSIS

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Abstract

Pectin solutions were annealed at various temperatures and time intervals. Calcium pectin gels were prepared by mixing aqueous solutions of pectin and calcium chloride ($CaCl_2$). The degree of Na to Ca substitution (*DS*) was calculated based on the data obtained by atomic absorption spectrometry. *DS* increased with increasing Ca content and levelled off at the characteristic Ca content which corresponds to the saturated value assuming all –COOH was converted into –COO_2Ca. The characteristic saturated values depended annealing temperature and time at sol state. *DS* was calculated and it was found that *DS* decreased with increasing annealing temperature. Viscoelastic properties of gels in water were investigated using a thermomechanical analyzer (TMA). Dynamic modulus increased and tanδ decreased with increasing *DS*. Swelling ratio of gels decreased with increasing annealing temperature and time at sol state showing a similar tendency to the results of *DS*. It can be concluded that a large network structure was formed when the samples were annealed at high temperature for a long time at sol state.

Keywords: *dynamic modulus, hydrogels, pectin, TMA*

Introduction

Pectin is a polysaccharide found in many plant tissues. It consists of galacturonic acid having some carboxyl groups which are esterified by methyl groups [1–3]. Chemical structure of pectin is shown in Fig. 1. When the degree of methyl esterification (*DE*) is smaller than 43%, gels are formed in the presence of divalent cations [4, 5]. The gelation of pectin occurs by ionic linkages via calcium bridges between two carboxyl groups belonging to two different chains in close contact [6, 7]. The interaction between Ca ions and carboxyl groups of the pectin is described by the egg box model involving a two-stage process of initial dimerization and subsequent aggregation of formed egg boxes [7, 8]. The gel formation of an 'egg box' structure is also reported for Ca alginate hydrogel [8, 9]. Alginic acid is a copolysaccharide consisting of poly-*L*-guluronic and poly-*D*-mannuronic acids. Pectic acid has the same uronic acid structure. Poly-*L*-guluronate and poly-*D*-galacturonate are stereochemically analogous, in that they are exact mirror images other than at C-3 [10].

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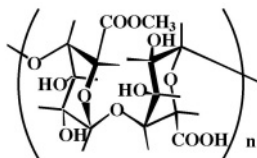
Fig. 1 Chemical structure of pectin

Many research works on Ca pectin gels have been carried out [11–23], such as conformation of gels by X-ray analysis [16], thermodynamics on association mechanism [17] and viscoelasticity [18–20]. Since the gelling mechanism of pectin differs according to DE, conflicting results have been reported on the thermorevisibility of pectin gels. It is reported that Ca pectin gels are thermo-irreversible [21]. On the other hand, gel–sol transition temperature is reported to be 130°C [22] or 46–74°C [23], suggesting that Ca pectin gels are thermo-reversible. In contrast, Ca alginate are thermo-irreversible gels which are categorised as the same as ionic bonding gel [24].

We have studied the gel conformation of many polysaccharide hydrogels, such as alginate [25–27], gellan gum [28–30], xanthan gum [30–33], hyaluronan [34, 35] and locust bean gum [36]. In our previous studies, we reported that gelation of many thermoreversible polysaccharide gels such as xanthan gum, gellan gum and hyaluronan was affected by annealing at sol state [28–34]. It is reported that gellan gum hydrogels which were prepared by cooling after annealing at sol state at a temperature higher than gel-sol transition formed more rigid gels than non-annealed gels [28–30]. Though xanthan gum and hyaluronan have been considered as non-gelling polysaccharides, it was found that xanthan gum aqueous solutions form the gel state when they are fully annealed in the sol state [30–33]. It is thought that polysaccharide solutions are in a thermodynamically non-equilibrium state. However, affect of annealing at sol state on gelation via ionic bonding has scantly been investigated.

Pectin is representative gels formed by ionic linkages via divalent cations. In this study, Ca pectin gels were prepared with pectin with various annealing conditions at sol state, and the dynamic viscoelastic properties of the gels were investigated by TMA.

Experimental

Preparation of Ca pectin gels

Pectin (Pec_{dry}) in powder form was obtained from Taiyo Kagaku Co. Pec_{dry} with various degrees of methyl esterification (*DE*), 25.6% ($Mw=8.5 \cdot 10^4$), 29.6% ($8.5 \cdot 10^4$), 60.7% ($1.0 \cdot 10^5$) and 71.9% ($1.5 \cdot 10^5$) was used [37]. Pec_{dry} was solved in deionized water at 25°C to obtain 2% solutions. The solutions were stirred at 25, 50, 80 and 98°C for 30, 90 and 180 min. An aqueous solution of calcium chloride ($CaCl_2$) (0.43–3.42%) was poured into an aqueous solution of pectin in a 20 mL glass container and mixed at 25°C. Transparent, flexible gels were formed immediately. The pectin concentration of samples at this stage was 1.0–1.8%. After stirring, samples were maintained at room temperature for 24 h in order to complete the substitution.

The mass of $CaCl_2$ was divided by mass of Pec_{dry} and $CaCl_2$/pectin ratio was calculated according to the following equation.

$$CaCl_2/\text{pectin (g g}^{-1}) = (\text{mass of } CaCl_2)/(\text{mass of } Pec_{dry}) \qquad (1)$$

Gels were formed and the separated sol portion was observed. The gels were removed from the glass vessel and weighed quickly. The gels were desalted by dialysis in flowing water for 24 h. The mass of the gels ($^{Ca}Pec_{gel}$) was calculated before and after dialysis.

The $^{Ca}Pec_{gel}$ was then dried at 120°C for 3 h in an oven and the dry mass of the gel ($^{Ca}Pec_{dry}$) was recorded. Water contents (W_c) of the samples were calculated according to following equation [38].

$$W_c \text{ (g g}^{-1}) = (\text{mass of water})/(\text{mass of } ^{Ca}Pec_{dry}) \qquad (2)$$

The $^{Ca}Pec_{dry}$ was again immersed in water at 25°C. The W_c of once dried samples was also calculated. A Sartorius micro-balance (MC210S) was used for sample mass measurements. Precision was $\pm 1.0 \cdot 10^{-5}$ g.

In order to avoid confusion in the subsequent description, the superscripts will be used, for pectin sample and Ca pectin designate (Pec_{dry}), ($^{Ca}Pec_{dry}$), and subscript gel or sol, (Pec_{sol}), ($^{Ca}Pec_{gel}$) means the above samples are in sol or in gel state. The temperature (T_a) shows annealing temperature at sol state. The times (t_a, t_{water}) show annealing time at sol state or immersing time in water.

Quantitative analysis of Ca

Quantitative analysis of Ca in $^{Ca}Pec_{gel}$ was measured by atomic absorption spectrometer (Shimadzu AA-630-01). A certain amount of each $^{Ca}Pec_{dry}$ was weighed and dissolved in concentrated hydrochloric acid and sulfuric acid. A certain amount of deionized water and lanthanum chloride solution were added to the acid solution with the sample in order to obtain 2–12 ppm solution. The dilute solution was measured. Flame gas was a mixture of acetylene and air, and the absorbance at wavelength 422.7 nm was measured. The commercial standard solutions of Ca (Wako Chemical Co.) were used to obtain calibration curves. The glassware and tools used in this experiment were treated with nitric acid.

The amount of Ca ion in the gel sample was calculated by normalising the mass of gel as follows.

$$\text{Ca/gel (g g}^{-1}) = (\text{mass of measured Ca})/(\text{mass of } ^{Ca}Pec_{dry}) \qquad (3)$$

The degree of substitution (*DS*) of the $^{Ca}Pec_{gel}$ 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.

$$DS = (\text{measured value of Ca})/(\text{calculated value of Ca})(1-DE) \qquad (4)$$

Viscoelastic properties

Viscoelastic properties of gels were studied using a thermomechanical analyzer (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 $^{Ca}Pec_{gel}$ was placed in an aluminium

sample pan with a diameter 7 mm and height of 3 mm. The gel was immersed in water at 25°C to prevent evaporation during the measurements. 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 tanδ as a function of DS and DE. From output data, a dynamic stress-strain hysteresis loop (Lissajous diagram) was obtained [39].

Swelling behaviour of gels

The swelling behaviour of gels was measured by the tea bag method. Tea bags were polyester nonwoven fabric (thickness 0.2 mm) at 40×80 mm and the bag was prepared by sealing at 250°C. Weighed $^{Ca}Pec_{gel}$ in a tea bag was swollen in water at 40 and 60°C for 10, 30, 60 and 180 min. The swollen gels were weighed and swelling ratio was calculated according to following equation.

$$\text{Swelling ratio} = (\text{mass of } ^{Ca}Pec_{gel} \text{ at 40 or 60°C})/(\text{mass of } ^{Ca}Pec_{gel} \text{ at 25°C}) \quad (5)$$

Results and discussion

Among the samples used in this study, gels were formed for the samples with DE 25.6 and 29.6%. In contrast, no gels were formed when DE was 60.7 and 71.9%. In order to calculate the degree of crosslinking of $^{Ca}Pec_{gel}$, the amount of Ca consumed for gel formation was calculated based on the data obtained by atomic absorption spectrometry. The mass of substituted Ca ions was measured for the samples prepared by various conditions. Figure 2a shows relationships between mass ratio of Ca/gel ($R_{Ca/gel}$) and CaCl$_2$/pectin ($R_{Ca/p}$) of the sample with DE=29.6%. Pec$_{sol}$ was annealed at various temperatures for 180 min before mixing with CaCl$_2$ solution.

As shown in Fig. 2a, Ca concentration of gels reaches a constant value at CaCl$_2$/pectin=0.2 g g^{-1}. The broken line shows calculated values assuming that all of COOH groups in the Pec$_{dry}$ were replaced with Ca ions in the gels. It is assumed that gelation completes by pairing two –COOH groups and one Ca. It is clear that the experimental values are found at ca 45–55% of calculated values at around 0.2 g g^{-1}. This means that 45–55% Ca
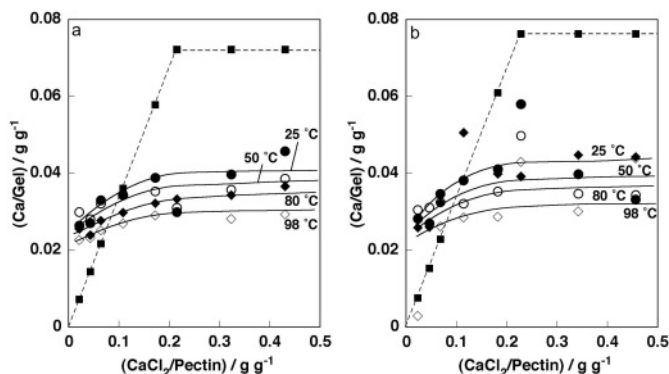

Fig. 2 Relationships between Ca/gel and CaCl$_2$/pectin annealed at various temperatures at sol state (annealing time=180 min); Annealing temperature at sol state= ● – 25°C, o – 50°C, ◆ – 80°C, ◇ – 98°C; a – DE=29.6%, b – 25.6%

in the mixture contributes the gelation. At the same time, it was found that equilibrium values of Ca in gels depend on a temperature at which Pec_{sol} was annealed. Equilibrium value of Ca content decreases with increasing annealing temperature. When $CaCl_2$/pectin is less than 0.1 g g^{-1}, Ca/gel is larger than calculated values. It is thought that non-crosslinked pectin molecules were eluted during desalting process. Similar relationships were also obtained as shown in Fig. 2b for the sample with DE=25.6%, although equilibrium DS values are slightly higher than those of DE=29.6%.

Figure 3 shows the relationships between equilibrium DS values which were calculated from Figs 2a and 2b and annealing temperature of Pec_{sol}. DE of the samples was 25.6 and 29.6% and annealing time was 180 min. DS decreased with increasing annealing temperature at sol state for both samples and increasing DE.
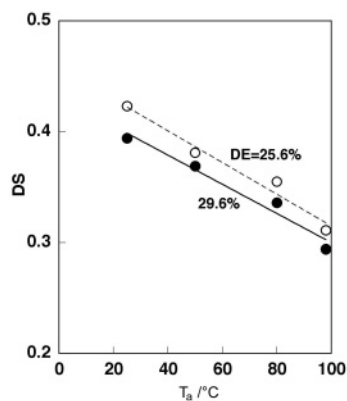

Fig. 3 Relationships between DS and annealing temperature at sol state (annealing time=180 min); DE= ● – 29.6%, o – 25.6%

Figure 4 shows relationships between mass ratio of Ca/gel ($R_{Ca/gel}$) and $CaCl_2$/pectin ($R_{Ca/p}$) using Pec_{sol} annealed for various times at 98°C. DE of the samples was 29.6%. It is clearly seen that the amount of Ca ions attaining equilibrium values decreases with increasing annealing time. A similar tendency was obtained for the samples with DE=25.6%.

When Pec_{sol} was annealed at high temperature for a long time, molecular motion of pectin molecules is enhanced and molecular chains are fully equilibrated. The cross-linking frequency of Ca ions established between thoroughly relaxed molecular chains is smaller than that of entangled molecular chains. It was found that a Ca linkage is formed at every 1.2 repeating units of pectin when Pec_{sol} was treated at 25°C. Once the Pec_{sol} is annealed at 98°C the values increase by 1.7. Furthermore, equilibrium DS values of the samples with DE=25.6% were always higher that those of DE=29.6%. It was found that cross-linking density increased with decreasing DE of pectin. It is quite reasonable that carboxyl groups in pectin serve as cross-linking points.

Water content of the above gels at various stages of Ca substitution was calculated using Eq. (2). Figure 5a shows the relationships between DS and concentration of $^{Ca}Pec_{gel}$ various annealing temperatures. W_c of gel is shown in x-axis together with concentration

in this figure. *DE* of pectin was 29.6%. Figure 5b shows similar relationships for the samples with *DE*=25.6%. Figure 6a shows relationships between *DS* and concentration of $^{Ca}Pec_{gel}$ for various annealing times at 98°C. *DE* of pectin was 29.6%. Figure 6b shows
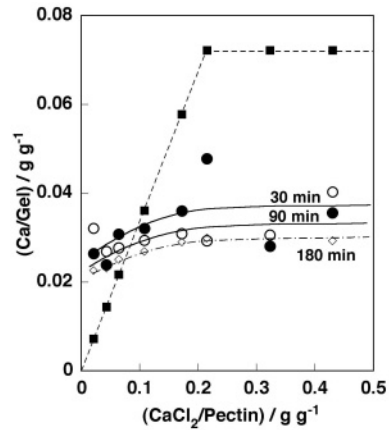

Fig. 4 Relationships between Ca/gel and CaCl$_2$/pectin annealed at various times at sol state (annealing temperature=98°C, *DE*=29.6%); Annealing time at sol state = ● – 30 min; o – 90 min; ◇ – 180 min
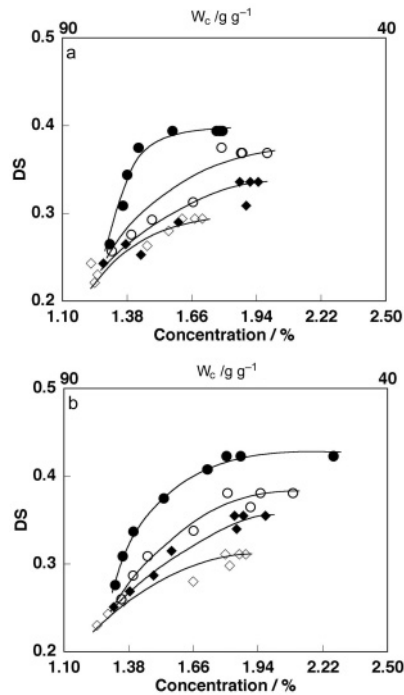

Fig. 5 Relationships between *DS* and concentration annealed at various temperatures at sol state (annealing time=180 min); Annealing temperature at sol state= ● – 25°C, o – 50°C, ◆ – 80°C, ◇ – 98°C; a – *DE*=29.6%, b – 25.6%

the similar relationships for the samples with DE=25.6%. W_c and concentration of gel are varied only by the amount of Ca. On this account, concentration varied only from 1.37 to 2.22 mass%. Both figures indicate that the largest W_c of gel is, ca 82 g g^{-1} at DS=0.22. When DS is smaller than ca 0.2, sol portion increases and it was difficult to maintain gel shape by the experimental procedure stated in section 2. As already shown in Fig. 3, each sample has own equilibrium DS values. As shown in Figs 5a and 5b, W_c of almost all samples is found at around 50 to 55 g g^{-1}. In contrast, with increasing annealing temperature in sol state, equilibrium DS values decrease, i.e. amount of water retained by one network structure increases. The relationship between equilibrium DS values at W_c=55 g g^{-1} and annealing temperature is exactly the same as Fig. 3. As already been shown in Figs 2–3, a large network of gels can be obtained by high temperature annealing. On this account, it is appropriate to consider that a gel having a large network structure retains a large amount of water.

All of the gels were dried and again immersed in water. W_c of once dried gels immersed in water at 25°C was 5 to 9 g g^{-1}. This fact indicates that the structures of the junction zones were highly established during the drying process.

$^{Ca}Pec_{gel}$ was immersed in water at 25°C for 24 h as stated in the experimental section. Swelling behaviour at a temperature higher than 25°C was examined in order to investi-
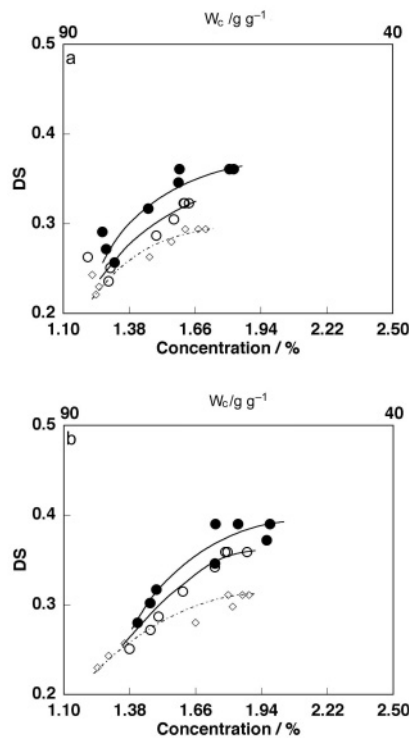

Fig. 6 Relationships between DS and concentration annealed at various times at sol state (annealing temperature=98°C); Annealing time at sol state= ● – 30 min; o – 90 min; ◇ – 180 min; a – DE=29.6%, b – 25.6%
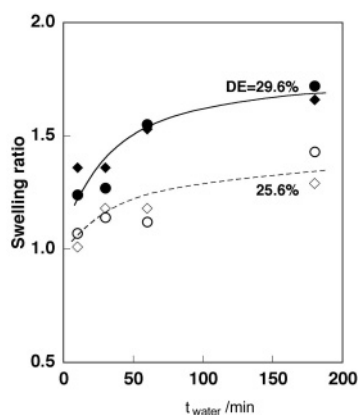
Fig. 7 Relationships between swelling ratio and swelling time; ● – *DE*=29.6%, swelling temperature=40°C; ◆ – *DE*=29.6%, swelling temperature=60°C; o – *DE*=25.6%, swelling temperature=40°C; ◇ – *DE*=25.6%, swelling temperature=60°C

gate the thermal stability in water. Figure 7 shows the relationships between swelling ratio and swelling time. Swelling temperature was 40 and 60°C and *DE* of the samples were 29.6 and 25.6%. $^{Ca}Pec_{gel}$ shown in Fig. 7 was annealed at 80°C for 180 min. In this figure, swelling ratio increased with increasing time, however no large difference was found among the swelling ratios measured at 40 and 60°C. The swelling ratio of pectin with *DE*=29.6% was higher than that of *DE*=25.6%, suggesting that the difference in crosslinking density afffects the swelling behaviour.

The effect of annealing conditions $^{Na}Pec_{sol}$ was examined. Figure 8 shows a three dimensional diagram of swelling ratio at 60°C (t_{water}=30 min), annealing temperature and time at sol state. *DE* of the sample was 29.6%. Swelling ratio decreased with increasing annealing temperature and time at sol state. It is thought that high crosslinking density, formed using a low temperature annealing sol, disturbs the molecular chain expansion during swelling. The results of Fig. 8 support the fact that swelling ratio increases when the molecular chains are allowed to spread in restricted networks. Generally, the swelling
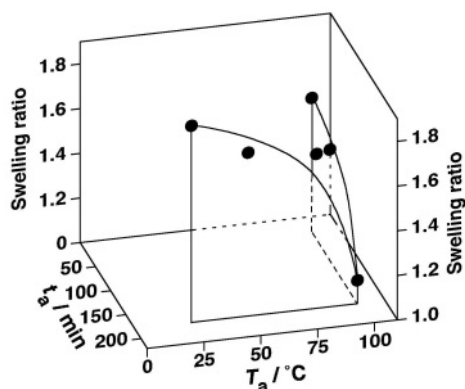

Fig. 8 Relationships between swelling ratio, annealing temperature at sol state and annealing time at sol state
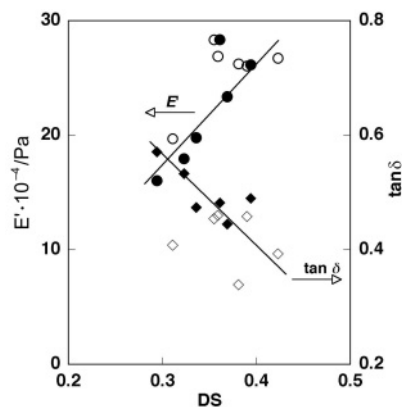
Fig. 9 Relationships between E', tanδ and DS; $DE=$ ●◆ – 29.6%; o◇ – 25.6%

ratio decreased with increasing DS, and depended on annealing conditions at sol state. The swelling ratio also decreased with decreasing DE, however, the effect of annealing temperature at sol state was more pronounced.

Figure 9 shows relationships between E' and tanδ of gels measured in water, and DS. E' of gels increased with increasing DS and tanδ of gels decreased with increasing DS. TMA data accorded well with the results stated in the above sections.

Conclusions

From the experimental results, the following facts are clarified: (1) degree of substitution (DS) which was calculated based on the data obtained by atomic absorption spectrometry increased with increasing Ca content and levelled off at a characteristic value, (2) the characteristic DS values depend on the annealing condition of $^{Na}Pec_{sol}$. DS decreased with increasing annealing temperature and annealing time at sol state; (3) swelling ratio increased when DS was small, and (4) dynamic modulus increased with increasing DS and tanδ decreased with increasing DS. It can be concluded that a large network structure was formed when the samples were annealed at high temperature for a long time at sol state.

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