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No. 04-129358- -00017-0000

Fecha: 2010-10-08 14:23:56 Dep. 2020 DIR.NUEVASCR
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Act. 444 RESPUESTAREQUE Follos: 6

Re.: Código 011-378-444
Expediente No. 04-129.358
Fase nacional de Solicitud
de Patente PCT
R.P. SCHERER TECHNOLOGIES, INC.

En relación con el expediente de la referencia y dando cumplimiento al oficio No. 8765 de Julio 9 de 2010 adjunto a este escrito una copia del documento YOSHIMURA K., TERASHIMA. M., HOZAN D., NOMURA Y., ISHII Y. AND SHIRAI K.: "Physical properties of shark gelatin compared with pig gelatin" J. AGRIC. FOOD CHEM. Vol. 48, June 2000 (2000-06), pages 2023-2027

De Ustedes Atentamente,


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T.P. No. 68.995 del C.S.J.

Physical Properties of Shark Gelatin Compared with Pig Gelatin

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Physical properties of shark gelatin were examined during gel formation and postgelation in comparison with pig gelatin. Samples with various concentrations and pH values were evaluated by breaking strength, dynamic viscoelasticity, and dynamic light scattering. Sol-gel and gel-sol transition temperatures for shark gelatin were remarkably lower than those for pig gelatin. Shark gelatin gel shows a narrower pH range to form a stable gel compared with pig gelatin. Melting enthalpy of shark gelatin gel was greater than that of pig gelatin gel, and G' of shark gelatin gel changed more extensively with rising temperature in comparison with pig gelatin gel. It is concluded that shark gelatin has different characteristics from pig gelatin not only for gel characteristics but also for the solution property.

Keywords: Gelatin; rheology; viscoelasticity; shark; gel

INTRODUCTION

Gelatin is a denaturation product of collagen and has been widely utilized for foods, photographic uses, medical materials, and microorganism culture materials. In addition, recently, its use is expanding to new applications such as health foods. Gelatin of land animal origin such as bovine and porcine has been mainly used. However, gelatin with new properties is desired to develop expanded applications. We have reported that type I collagen from great blue shark (*Prionace glauca*), which is caught in abundant quantity in connection with tuna fishery, could provide a unique collagen material different from that of land animal origin (Nomura et al., 1995, 1997; Yoshimura et al., 1996, 1997, 1999). Shark skin collagen has such features as less imino acid residue content, lower denaturation temperature, stronger swelling ability, higher solubility in acid medium, and different fibril assembly from that of pig skin collagen. Moreover, the rheological properties of shark collagen solution are also different from those of land animal origin. As the feature of collagen must reflect its derivative gelatin, it is presumed that shark gelatin has also a different characteristic from that of land animal. For gelatin from aquatic animals, however, there are only limited utilizations and studies (Hamada, 1990; Leuenberger, 1991; Gudmundsson and Hafsteinsson, 1997). Especially, there are very few reports on the properties of shark gelatin solution and gel as approached with the rheological technique. The reversible sol-gel transition is one of the most important features of gelatin as a macromolecule and plays an important role in the application. Gelatin gel is formed by the entanglement of molecular chain due to the interaction including hydrogen bonds, ion bonds, and hydrophobic

bonds between the chains or the segments that constitute the chain, and substantially shows a reversible sol-gel transition by the change in temperature, solvent composition, pH, etc. If a point at which there is strong interaction between segments is regarded as a cross-linking point in the wide sense, the number of cross-linking points increases with gelatin concentration and decreases with rising temperature and electrostatic repulsion; hence, it should be reflected in the dynamic parameter. In this paper, the rheological properties of shark gelatin were examined in the process of gel formation and postgelation in comparison with those of pig gelatin.

MATERIALS AND METHODS

Preparation of Gelatin Solution and Gelation. Acid soluble shark collagen (ASSC) was prepared from fresh great blue shark (*Prionace glauca*) skin corium, according to the method of Kimura et al. (1981). The skin was freshly collected from sharks unloaded at Kesennuma port in Japan and transported to the laboratory in the frozen state. The cubes were extracted with acetone, *n*-hexane, and 0.5 M sodium acetate in series. The cubes were thoroughly washed with distilled water and then extracted with 0.5 M acetic acid. Collagen preparation was recovered by salting out and dialyzed against 0.01 M acetic acid. Acid soluble pig collagen (ASPC) was also prepared according to the previously described method (Shirai et al., 1979). All operations were conducted in a room temperature of 4 °C.

Phosphate buffer (0.1 M, pH 6.0) was added to ASSC and ASPC to give a concentration of 30 mg/mL, and the mixture was heated at 100 °C for 5 min and cooled immediately to room temperature. Gelatin concentration was adjusted to 30 mg/mL. The gelatin solution was cooled to 4 °C for 48 h to set. This condition was adopted as standard condition of gelatin gel formation, and the conditions in the following experiment were changed with respect to time, temperature, gelatin concentration, and pH.

Measurement of Breaking Strength on a Creep Meter. The mechanical property of the gels was evaluated as breaking strength on a creep meter (RE-3305, Yamaden Co., Tokyo). A portion of gelatin solution (0.5 mL) was placed in a well of the

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48 hole plate (diameter = 11 mm) and incubated at 4 °C for 1–96 h to set. The plunger (diameter = 5 mm) of the creep meter was pressed against the center of the gel in well with a speed of 5 mm/s. The force versus strain curve was measured, and the first force maximum or the inflection point was regarded as breaking strength. The experiments were carried out at various concentrations (20–60 mg/mL) and pH values (4–12) in this series.

Measurement of Dynamic Viscoelasticity by Rheometer. Dynamic viscoelasticity was measured by rheometer (ARES viscoelasticity measurement system, Rheometric Scientific). A portion of gelatin solution (0.5 mL) was placed between the parallel plates of 25 mm in diameter, and the time course measurement of dynamic viscoelasticity was started after transfer to 4 °C at a constant frequency (1 Hz) and a constant shear strain (5%). The melting process of the gel by heating (transition from gel to sol) was also studied by dynamic viscoelasticity measurement. The gelatin concentration (15, 30, 45, and 60 mg/mL) and pH (4.0, 6.0, and 8.0) were changed. Rheological properties were measured by dynamic time sweep mode (DTS) at constant temperature (4 and 25 °C) or by dynamic temperature ramp mode (DTR) at a range of 4–40 °C. In the latter case temperature was cooled from 40 to 4 °C at a constant rate of 0.3 °C/min and heated from 4 to 40 °C at a constant rate of 1.0 °C/min. Storage modulus (G'), loss modulus (G''), and $\tan \delta$ (G''/G') were determined.

Measurement of Dynamic Light Scattering (DLS). The measurement by DLS (DLS-7000, Otuka Electronic, Tokyo) was carried out to evaluate the internal structure of gelatin gel. Gelatin solution at different pH values (4–12) was filtered through a 0.45 μ m pore size membrane, and a portion of the gelatin solution was cooled at 4 °C for 1–96 h to gel. The autocorrelation function of the scattered incident light from a 10 mW He–Ne laser of wavelength 632.8 nm was acquired as a time function of the scattering intensity at an angle of 90° using a photomultiplier tube operated in a photon counting mode.

Measurement of Differential Scanning Calorimetry (DSC). The melting behavior of gelatin gel was measured by a DSC apparatus (DSC120, data processor SSC5300, Seiko Electronics, Tokyo) according to the method of Takahashi et al. (1986). A 50 μ L portion of 60 mg/mL gelatin solution was sealed in a 70 μ L silver cell and incubated at 4 °C for 48 h to gel. From the DSC curve, the denaturation temperature [onset temperature (T_d), peak temperature (T_p), and conclusion temperature (T_c)] and denaturation enthalpy were recorded.

RESULTS AND DISCUSSION

Change in Mechanical Property during Gelation Process. The breaking strength of gelatin solution placed at 4 °C showed the rapid increase for the first 2 h, and after that the slope slowed (results not shown). The rapid increase in breaking strength and the large variation of data before 24 h reflect the transient process of gelation. Therefore, it is considered that at least 24 h might be required to attain a stable gelatin gel structure.

To clarify the early stage of the gelation process in detail, Figure 1 shows the change in rheological properties of gelatin solution during cooling from 40 to 4 °C. The value of G' increased sharply at ~30 °C for pig gelatin and at ~21 °C for shark gelatin. The value of $\tan \delta$ decreased at the described temperature, indicating the decrease in the viscous contribution to viscoelastic property. It is therefore clear that the described parameter change during cooling reflects the transition from sol to gel. Consequently, the sol–gel transition temperature for shark gelatin is remarkably lower than that of pig gelatin. From the above results, dynamic viscoelasticity was measured at 25 °C, at which pig gelatin was presumed to be in the gel, whereas shark gelatin

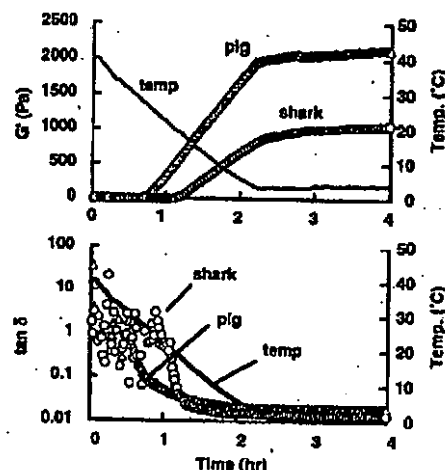


Figure 1. Change in rheological properties of 30 mg/mL solutions of shark and pig gelatins during a cooling from 40 to 4 °C. Conditions: frequency, 1 Hz; strain, 5.0%.

was presumed to be in the sol (Figure 2a). The rapid increase in G' and the decrease in $\tan \delta$ in the early stage of measurement for pig gelatin demonstrate that gelation occurred. However, shark gelatin indicated no sign of the gelation even after 90 min and remained in the sol.

In the next experiment, dynamic viscoelasticity was measured at 4 °C at which both gelatins were presumed to form the gel (Figure 2b). The sol state can be represented by a rheological parameter function $G'' > G'$, whereas the gel state is represented by $G' > G''$ due to an increased elasticity. Therefore, the gelling point can be defined as a point of $\tan \delta = 1$ ($G' = G''$) (Winter and Chambon, 1986). According to this definition, the gelling point was reached within 30 s at 4 °C, very rapidly for both gelatins. The level of G' for shark gelatin is lower than that of pig gelatin. This may suggest in turn that the intermolecular interaction of shark gelatin is weaker than that of pig gelatin.

As shown in Figure 1, the value of G' and G'' rapidly increased and $\tan \delta$ decreased in the earliest stage of measurement, and the values reached a plateau after 2 h and afterward changed very little. The described time course indicates that the reaction between gelatin chains during the gelation process contains at least two terms, one with a rapid velocity within 2 h and the other with a slow velocity after 2 h. It is therefore possible to distinguish the two steps; the first is referred to as the gel-forming stage and the second as the gel-aging stage.

From the above results, dynamic viscoelasticity measurement can assess effectively the features in the early stage of gelation. However, the change in rheological parameters diminished strongly as the incubation time was >10 h (results not shown). This suggests that dynamic viscoelastic measurement is not so valid in the assessment of the late stage of gelation. Therefore, DLS was examined for applicability to the late stage of the gelation of shark gelatin (Figure 3); taking in account the theory of Tanaka (1985) on the applicability of DLS to the polymer gel study. The diffusion coefficient (D) rose immediately after incubation at 4 °C, corresponding with the rapid increase in breaking strength or the rapid

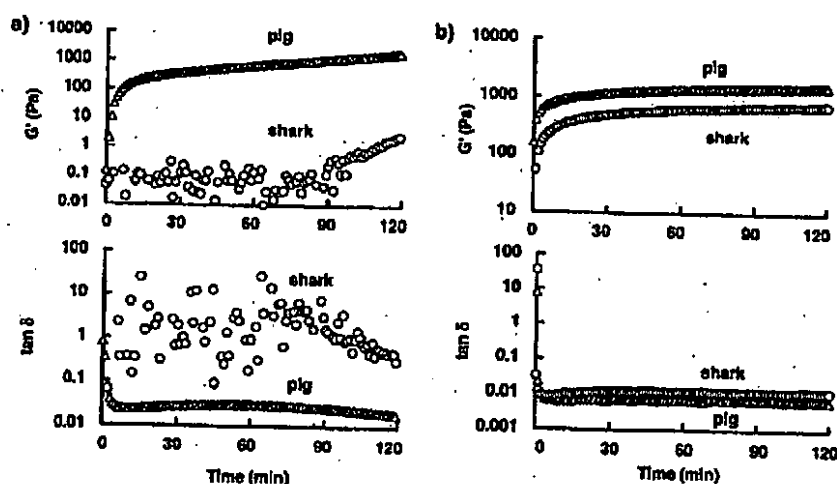


Figure 2. Change in rheological properties of shark and pig gelatins (30 mg/mL in 0.1 M phosphate buffer, pH 6.0) incubated at (a) 25 °C and (b) 4 °C. Conditions: frequency, 1 Hz; strain, 5.0%.

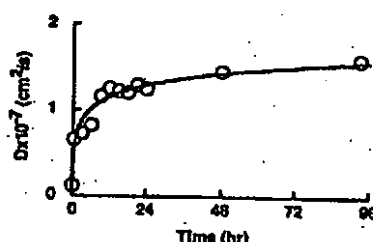


Figure 3. Change of diffusion coefficient (D) in the gel formation of shark gelatin (25 mg/mL in 0.1 M phosphate buffer, pH 6.0) with DLS at 4 °C.

decrease in $\tan \delta$ (Figure 2). D is a quantity in proportion to the reciprocal of the correlation distance in Brownian motion of the scatter. In the case of the gel, the increase in D means the decrease in the distance between entangled points of the molecular chain segment in the gel and, hence, the increase in the number of entangled points in the network. Therefore, the progress of the gelation of gelatin must be possible to evaluate by analyzing the change in D . The value of D still increased gradually even after 96 h of incubation as shown in Figure 3. This implies that such a change in the internal structure of the gel, which cannot be detected by dynamic viscoelasticity measurement, progresses still for a considerably long time. As a similar reaction, it had been reported that the specific viscosity of the dilute solution of gelatin continues to increase after 48 h at 10 °C (Nomura et al., 1995).

Concentration Dependency Gel Property. The concentration dependency gel property was examined by change in the breaking strength and dynamic viscoelasticity at 4 °C. The breaking strength of the gel increased almost linearly with the increase in the concentration for both gelatins (Figure 4a). However, shark gelatin gel at a concentration <30 mg/mL was so weak that breaking strength could not be measured with this method. This may suggest that the critical concentration of gel formation of shark gelatin is higher than that of pig gelatin. It is interesting that the 60 mg/

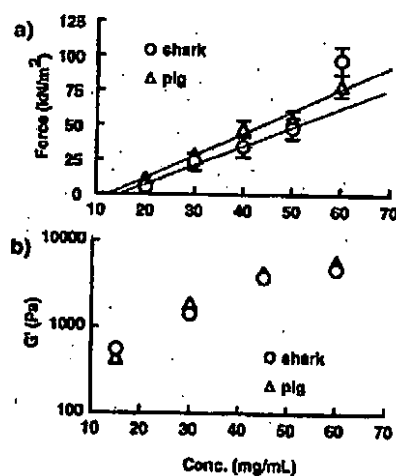


Figure 4. Effect of gelatin concentration on (a) breaking strength and (b) G' of shark and pig gelatin gels (in 0.1 M phosphate buffer, pH 6.0) incubated at 4 °C. Breaking strength was measured by creep meter after 48 h of incubation. Value of G' was measured by rheometer after 2 h of incubation. Conditions of breaking strength: plunger diameter, 5 mm; plunger speed, 5 mm/s. Conditions of rheological study: frequency, 1 Hz; strain, 5%. The bars represent standard deviation of five measurements.

mL concentration of shark gelatin gel is higher than that of pig gelatin gel.

Figure 4b shows changes in the rheological properties of the gel with varying concentration of gelatin. The value of G' increased with the increase in gelatin concentration and showed a parallelism to the breaking strength at <50 mg/mL concentration. It is therefore considered that the elastic term G' can represent appropriately the physical property of gelatin gel. G' values of shark gelatin were at all times similar to those of pig gelatin. The present experiment indicating that the dynamic viscoelasticity measurement was possible

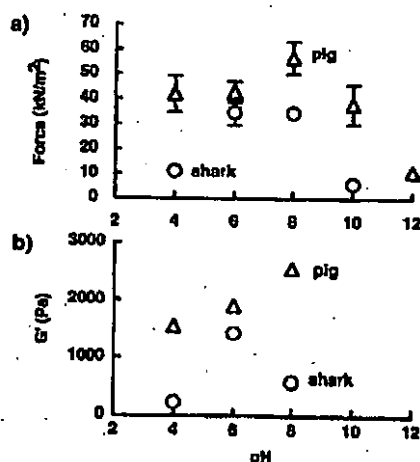


Figure 5. Effect of pH on (a) breaking strength and (b) G' of shark and pig gelatin gels (30 mg/mL in 0.1 M phosphate buffer) incubated at 4 °C. For conditions, see Figure 4. The bars represent standard deviation of five measurements.

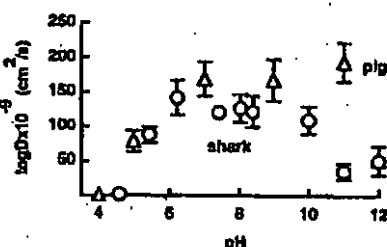


Figure 6. Effect of pH on diffusion coefficient (D) of shark and pig gelatin gels (25 mg/mL in 0.1 M phosphate buffer, pH 5.0) measured by DLS at 4 °C. The bars represent standard deviation of five measurements.

for shark gelatin gel at 15 mg/mL concentration suggests that it provides also an effective technique for characterizing the low-concentration or weak gel.

pH Dependency Gel Property. The breaking strength of the gel was also influenced by pH (Figure 5a). The breaking strength of shark gelatin gel at pH 4 and 10 was remarkably lower than that at pH 6–8. The value of pig gelatin gel was almost constant in a pH region from 4–10. The results indicate that the breaking strength of shark gelatin gel is more susceptible to high or low pH than that of pig gelatin.

G' of gelatin gel at different pH values was measured (Figure 5b). G' of shark gelatin gel indicated a significant decrease at pH 4 and 8, whereas pig gelatin gel indicated no decrease. The result agrees substantially with the result on breaking strength as obtained with the creep meter (Figure 5a).

Furthermore, gelatin gel was examined by DLS at different pH values (Figure 6). In the case of shark gelatin, the value of D was almost constant in a range 80–120 × 10⁻⁹ cm²/s at pH 5–10. However, at an alkaline pH of 11, the D value of shark gelatin gel was considerably lower than that of pig gelatin gel. It is considered that such a low D value at a pH outside the neutral region for shark gelatin is correspondent with the low value of breaking strength and G' (Figure 5).

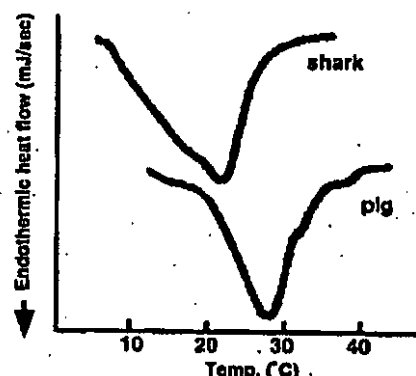


Figure 7. DSC curves of shark and pig gelatin gels (60 mg/mL in 0.1 M phosphate buffer, pH 6.0).

These results on the pH dependency gel property suggest that shark gelatin gel is more affected by the pH-induced electrostatic repulsion between charges and hence shows a narrower pH range to form a stable gel compared with pig gelatin. The greater mobility of shark gelatin chains enhances the effect of the electrostatic repulsion between charges. Nomura et al. (1997) reported that when the electrophoretic mobility of shark and pig gelatin was measured by electrophoretic light scattering, the mobility of both gelatins was zero in a wide range from pH 6 to 10.5, and there was no difference in the mobilities between shark and pig gelatins. They also indicated that there were quite similar pI and ζ -potential values for both gelatins. These results indicate that there is no difference in the net charge between shark and pig gelatins. However, the present study demonstrated a difference in the pH effect between shark gelatin and pig gelatin. This suggests that the intramolecular distribution of charge is more important than total or averaged charge of the whole molecule in determining the network structure of gelatin gel at the level of dynamic characteristics and optical behavior.

Melting Behavior of Gels. As shown in Figure 7, the DSC curve of shark gelatin gel as well as that of pig gelatin gel showed a single endothermic peak. However, the former shifted to a lower temperature region and had a broad shoulder in the lower side of the peak. Such DSC curves indicate that the melting of shark gelatin gel takes place in a wide range of temperature, and the structural heterogeneity of shark gelatin gel is greater than that of pig gelatin gel. The lower melting temperature (T_p) of shark gelatin gel (21.8 °C compared with 28.5 °C for pig gelatin gel) indicates that the structural stability of shark gelatin is weaker than that of pig gelatin.

Figure 8 shows the results of dynamic viscoelasticity measurement of gelatin gel from 4 to 40 °C. G' and G'' of shark gelatin gradually decreased with rising temperature and suddenly decreased at a characteristic temperature. Pig gelatin indicated also a similar but gentler change. $\tan \delta$ of shark gelatin gel also increased rapidly at 23 °C. This temperature is close to the T_p value measured by DSC. In the case of pig gelatin, $\tan \delta$ rapidly increased at 32 °C. These results show that the dynamic viscoelasticity measurement reflects clearly the melting behavior of gelatin gel. In this meaning, it

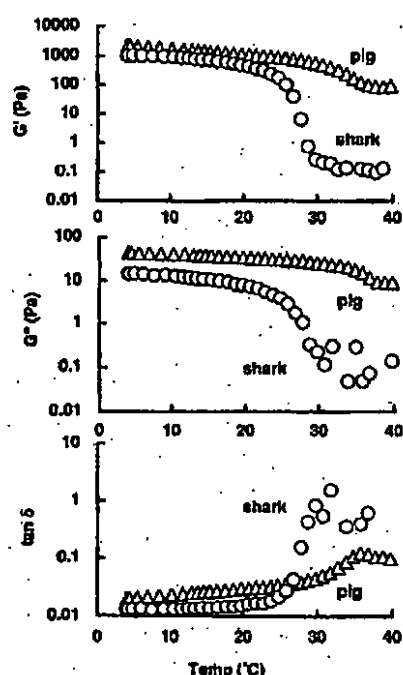


Figure 8. Change in rheological properties of shark and pig gelatins (30 mg/mL in 0.1 M phosphate buffer) during a rise of temperature from 4 to 40 °C. Conditions: frequency, 1 Hz; strain, 5%.

Is possible to consider that substantial correspondence exists on the measurement of the melting temperature between DSC and dynamic viscoelasticity. However, in the case of pig gelatin, the decrease in G' and the increase in $\tan \delta$ above 35 °C were much smaller. This suggests that pig gelatin sol retains a certain extent of the interchain interaction after the melting of the gel network. In other words, shark gelatin seems to show a more ideal gel-sol transition than pig gelatin.

Conclusion. It has been reported that percolation theory was applicable to the sol-gel transition of gelatin (Djabourov, 1988; Okawa et al., 1997). As a model of the heterogeneous gel structure, the fractal heterogeneous gel structure model has been proposed (Vilgis and Heinrich, 1992), in which some percolation clusters with a fractal nature combine with each other to form a network. In such a model, cross-linkage exists inside and between percolation clusters. In the case of the deformation of such a gel, the cross-linkage between clusters is regarded to play a major role in the mechanical behavior of system. The described idea is supported by the effect of gelatin concentration, temperature, and pH on breaking strength and dynamic viscoelasticity in the present study, especially as the dynamic viscoelasticity measurement revealed the significant effect of these variable factors. From this view, the elastic modulus G' of shark gelatin is somewhat lower than that of pig gelatin at the same concentration (Figure 4). This means that shark gelatin gel contains fewer cross-linkages between clusters compared to pig gelatin or exhibits weaker interaction between clusters.

It is concluded, consequently, that shark gelatin has different characteristics from pig gelatin not only for the gel characteristics but also for the solution property. This is important knowledge because gelatin is used generally at high concentration in practical applications.

ABBREVIATIONS USED

ASSC, acid soluble shark skin collagen; ASPC, acid soluble pig skin collagen; DLS, dynamic light scattering; DSC, differential scanning calorimetry.

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Received for review August 6, 1999. Revised manuscript received March 16, 2000. Accepted March 23, 2000.

JF990887M