Macromolecular Research, Vol. 17, No. 10, pp 785-790 (2009)

A Comparative Study on the Dielectric and Dynamic Mechanical Relaxation Behavior of the Regenerated Silk Fibroin Films

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Received February 2, 2009; Revised April 21, 2009; Accepted April 21, 2009

Abstract: In this paper, the relaxation behavior of the regenerated silk fibroin (SF) films was investigated using dielectric thermal analysis (DETA), and compared with the dynamic mechanical behavior obtained from dynamic mechanical thermal analysis (DMTA), in order to gain a better understanding of the characteristics of dielectric behavior of SF film and identify the differences between the two analyses. Compared to DMTA, DETA exhibited a higher sensitivity on the molecular relaxation behaviors at low temperature ranges that showed a high γ-relaxation peak intensity without noise. However, it was not effective to examine the relaxation behaviors at high temperatures such as α- and α_c-relaxations that showed a shoulder peak shape. On the contrary, DMTA provided more information regarding the relaxation behaviors at high temperatures, by exhibiting the changes in width, intensity and temperature shift of the α-relaxation peak according to various crystallinities. Conclusively, DETA and DMTA can be utilized in a complementary manner to study the relaxation behavior of SF over a wide temperature range, due to the different sensitivity of each technique at different temperatures.

Keywords: regenerated silk fibroin, dielectric thermal analysis, dynamic mechanical thermal analysis.

Introduction

Silk fibroin (SF), the typical biological macromolecules spun by *Bombyx mori* silkworm, has been mainly used as textile fibers with excellent performances. Recently, many researchers have investigated SF as one of the promising resources of biotechnology and biomedical materials with advantages of useful properties, such as good cell attachment and growth,[1] good blood compatibility,[2] and enzyme entrapment.[3] For the application of SF to those fields, SF needs to be regenerated in the forms of powder, film or fiber. Researchers became interested in the structural characteristics of the regenerated SF due to the importance of the strong relationship between its structure and properties.[4-6]

Dynamic mechanical thermal analysis (DMTA) has been used as an effective tool in order to understand the structural characteristics of a polymer. An examination of the temperature dependence of the dynamic modulus gives the structural information regarding polymer by providing the knowledge on glass transition, secondary relaxations, etc. In addition, it is well known that DMTA could be used as a sensitive method in order to evaluate the miscibility of polymer blends through its detection at the molecular level.[7] Based on these benefits, DMTA has been utilized in order to study the structural characteristics of the regenerated SF films[8-11] as well as the miscibility of SF blends.[12,13]

In spite of these advantages of DMTA, the sample preparation for the DMTA measurement is neither easy nor practical, due to the brittleness of regenerated SF film, which can result in cracking when the rectangular film is prepared with a knife or scissors. Therefore, careful handling of samples is required. Furthermore, the powder sample can not be applied to the DMTA technique.

Dielectric thermal analysis (DETA) provides similar informa-

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tion with DMTA displaying peaks at temperatures where the molecular relaxations are present. Moreover, DETA has several advantages over DMTA when considering the following facts: First, the sample preparation of DETA is much easier in comparison to DMTA for measurement purposes requiring an appropriate film size or powder amount, which can cover the upper and lower plates of the dielectric thermal analyzer. Second, it is possible to perform DETA measurement with the powder sample. Finally, it is possible to conduct a frequency sweep test at even at high frequencies, including 10,000 Hz. With those advantages, many studies on the structural characteristics of polymer[14] and miscibility of polymer blends[15] have been performed, using DETA.

It is expected that the structural characteristics of various SF samples can be also investigated, using DETA with easy measurement and simple sample preparation. However, the detailed study on the regenerated SF using DETA, has not been performed yet, though Magoshi *et al.* reported on the dielectric properties of SF.[8] In particular, the comparative study between DETA and DMTA in regards to the regenerated SF films has not been conducted even though those techniques are similar.

Therefore, in this study, we investigated the relaxation behavior of the regenerated SF films using DETA, and performed a comparative study based on results from DMTA measurement in order to achieve a better understanding regarding the dielectric thermal behavior of regenerated SF films and to identify differences between the two measurements.

Experimental

Preparation of SF Films. The regenerated SF solutions were obtained by the same method as reported in previous literature.[9] Bombyx mori silk cocoons were degummed twice with marseillus soap 0.5% (o.w.f.) and a sodium carbonate 0.3% (o.w.f.) solution at 100 °C for an hour, and rinsed thoroughly in warm distilled water. The degummed silk cocoon was first dissolved in a ternary solvent system, consisting of a $CaCl_2/H_2O/EtOH$ solution (1/8/2 mole ratio), for 30 min at 85 °C and dialyzed to remove salts in a cellulose tube (molecular weight cut off = 12,000~14,000) against distilled water for 4 days at room temperature. Then, the aqueous SF solution obtained was cast onto a polystyrene plate and dried at room temperature in order to prepare the regenerated SF film. The crystallinity of the regenerated SF films was controlled by heat treatment (annealing). The films were stored in a drying oven at different temperature (150, 170, and 200 °C) for an hour.

Characterization. The dynamic mechanical response of the samples was monitored using Rheometric Scientific DMTA Mark IV (USA) in the tensile mode. Rectangular samples, 20×5×0.05 mm, were scanned isochronally at a heating rate of 4 °C/min (between -30 °C and 280 °C). The strain was controlled to 0.05%, due to the brittle film state. The dynamic tests were performed at 0.1, 1 and 10 Hz in order to examine the frequency effect. A dielectric thermal analyzer (DETA 500, Rheometric Scientific, USA) was used to examine the dielectric responses of sample. Circular film shapes with a diameter of 33 mm and thickness of 0.05 mm, were prepared for the measurement. The dielectric measurements were performed at temperatures ranging from -30 to 250 °C, with a heating rate of 4 °C/min. The frequencies used were 100, 1,000, 10,000, and 100,000 Hz. The X-ray diffraction (XRD) analysis was performed on small-angle X-ray scattering, with the General Area Detector Diffraction System (GADDS, Bruker-Axs, Germany) using Cu K_α radiation. Irradiation conditions were 40 kV and 30 mA.

Results and Discussion

Many researchers have utilized DMTA to study the molecular motion and relaxation of polymers by measuring storage (E') and loss modulus (E''). That is, in regards to DMTA measurement, a steady state, alternating strain is applied to a polymer film. As a result, the storage modulus, related to the mechanical energy stored, as well as, the loss modulus, which is the energy converted to heat through viscous dissipation, can be obtained. Furthermore, the loss tangent (tan δ), the ratio of loss modulus to storage modulus, has also been used primarily to investigate the relaxation of polymer.

DETA measures polymer property changes when it is subjected to a periodic electric field. Dielectric constant and dielectric loss can be obtained by DETA measurement, and have been utilized in the study on the molecular relaxation of polymer. The dielectric constant and dielectric loss are defined as the measure of the degree of alignment of dipoles in the applied electric field, and the energy required to align the dipoles and to move the free ionic impurities, respectively.[16]

Therefore, those quantities of DMTA and DETA in regards to the regenerated SF film were compared in order to understand differences between dynamic mechanical and dielec-

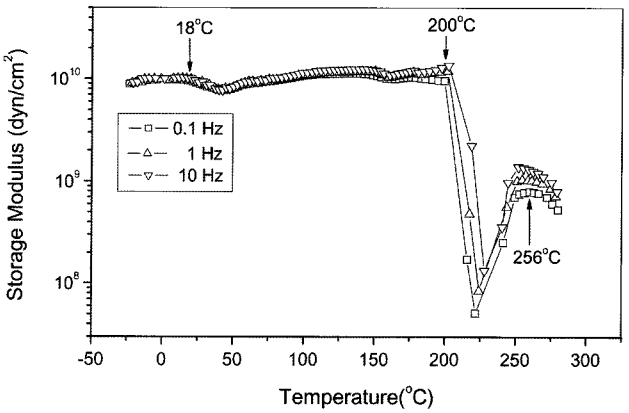

Figure 1. The frequency dependence of storage modulus for the regenerated SF film measured by DMTA.

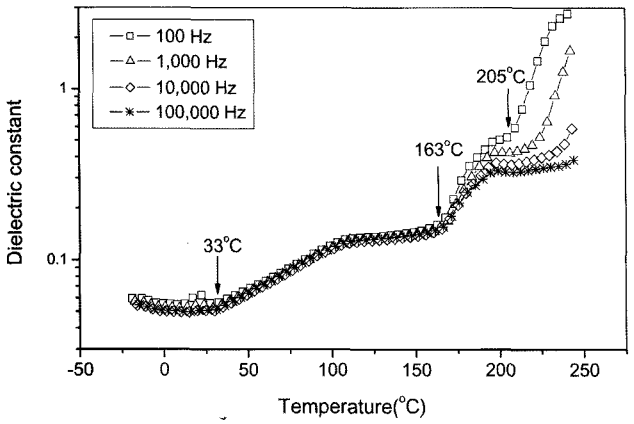

Figure 2. The frequency dependence of dielectric constant for the regenerated SF film measured by DETA.

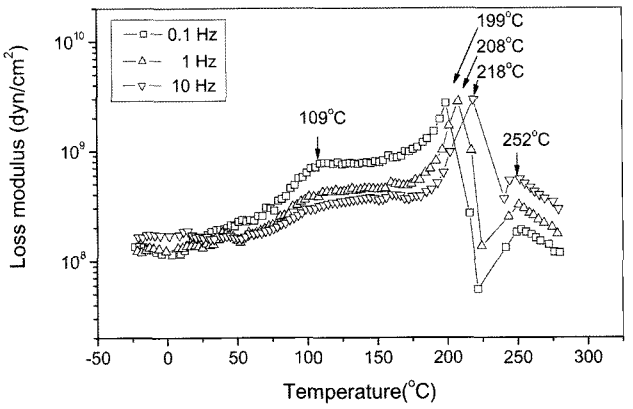

Figure 3. The frequency dependence of loss modulus for the regenerated SF film measured by DMTA.

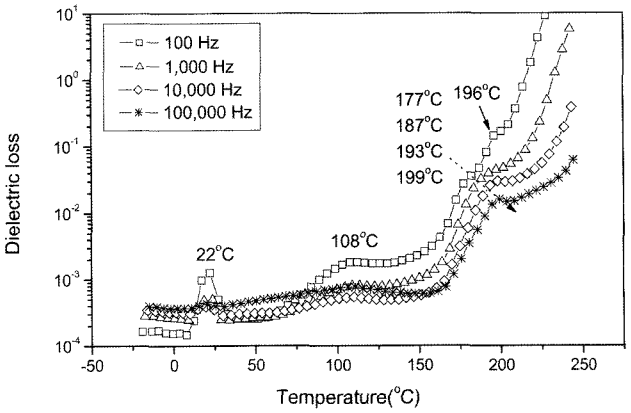

Figure 4. The frequency dependence of dielectric loss for the regenerated SF film measured by DETA.

tric behaviors of the regenerated SF film. First, the storage modulus and dielectric constant of untreated regenerated SF film with various measuring frequencies as a function of temperature were examined. As shown in the Figure 1, the storage modulus of the regenerated SF at 0.1 Hz decreased slightly at 18 °C, declined abruptly at 200 °C and then showed a peak of storage modulus at 256 °C. The storage modulus drop at 200 °C is attributed to the glass transition of SF molecules, and the peak at 256 °C is associated with the cold crystallization of amorphous SF molecules.[8] The slight modulus decrease at 18 °C might be due to the molecular motion of the SF side chain.

In the case of DETA (Figure 2), the dielectric constant increased with several steps as a function of temperature. Three inflection points appeared at 33, 163, and 205 °C in the measurement with 100 Hz of frequency.

It is well known that the storage modulus decreases at the temperature where a molecular motion exists, whereas the dielectric constant increases at this temperature. Since the storage modulus is related to the mechanical energy stored, it is reduced when the molecular relaxation is present. On the contrary, the dielectric constant increases at temperatures where the molecular motion exists as the degree of dipole alignment for polymer molecules is increased in the applied electric field.

In the meanwhile, the frequency effect on the storage modulus and dielectric constant of the regenerated SF film showed somewhat different aspects. The storage modulus and dielectric constant remained constant up to 200 and 163 °C, respectively. However, after that temperature, the storage modulus increased slightly and the peak shifted to a slightly higher temperature as the frequency increased. On the other hand, the dielectric constant was reduced with increasing frequency.

Unlike the storage modulus and dielectric constant, the relaxation behavior is shown as a peak type in the loss modulus or dielectric loss curve. Therefore, those quantities are known as a more useful tool, and have been utilized extensively in order to investigate the relaxation behavior of polymers.

Figures 3 and 4 displayed the loss modulus and dielectric loss curves of regenerated SF film, respectively. Three relaxation peaks were observed at 109 °C (β), 199 °C (α), and 252 °C (α_c), respectively in loss modulus curves of SF film when the frequency of measurement was 0.1 Hz. The α-relaxation is related to the glass transition of the SF amorphous phase due to the segmental motion of the SF backbone. The α_c-relaxation is associated to cold crystallization of SF molecules as mentioned earlier. Though the β-relaxation process could not be precisely assigned, it might be related to the molecular motion of SF when the water evaporated.

As shown in Figure 4, four peaks appeared at 22, 108, 177, and 196 °C in dielectric loss curves with 100 Hz of frequency. Those peaks correspond to γ, β, α, and α_c-relaxations, respectively, considering the result of loss modulus by DMTA. Though γ-relaxation could not be detected by loss modulus curves in DMTA, it was observed by means of the DETA measurement. The γ-relaxation might occur due

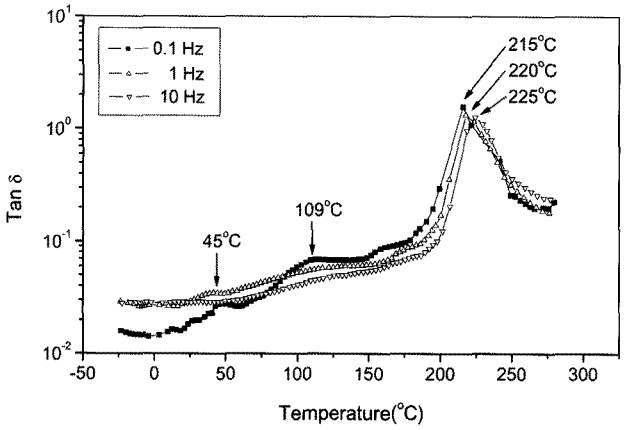

Figure 5. The frequency dependence of tan δ for the regenerated SF film measured by DMTA.

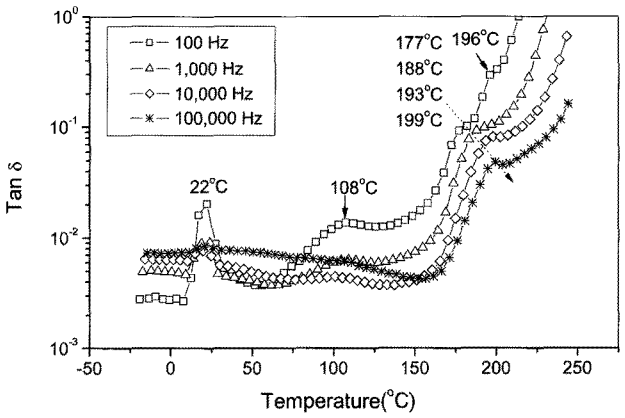

Figure 6. The frequency dependence of tan δ for the regenerated SF film measured by DETA.

to the molecular motion of the SF side chain.

When comparing the results of DMTA and DETA, it seems that DMTA and DETA have a different sensitivity on the relaxation behaviors of SF molecules, depending on the temperature range. DMTA exhibited a lower sensitivity with regard to the molecular motion of SF at a low temperature ranges without any γ-relaxation detected, while it exhibited a higher peak sensitivity at high temperature ranges displaying clear peaks for α- and α_c-relaxations. On the other hand, in the case of DETA, only a shoulder peak type was present at 196 °C in dielectric loss curves with 100 Hz of frequency implying a low sensitivity. However, a clear peak of γ-relaxation could be observed at 22 °C, indicating a high peak sensitivity in low temperature ranges compared to DMTA.

The intensity of loss modulus decreased and the α-relaxation temperature shifted from 199 to 218 °C as the frequency increased. Also, the β-relaxation peak at 109 °C was reduced with increasing frequency, indicating the peak sensitivity was deteriorated. In the case of dielectric loss, the peak sensitivity decreased with a higher frequency, showing that the peak heights of γ- and β-relaxation at 22 and 108 °C, respectively, became lower and the shoulder peak type at 196 °C disappeared.

Many researchers have often utilized the loss tangent (tan δ) because this provides better information regarding relaxation behaviors of polymer. Figures 5 and 6 showed tan δ curves of the regenerated SF film measured by DMTA and DETA, respectively. Tan δ curves obtained from DMTA and DETA exhibited similar feature in regards to the loss modulus and dielectric loss, respectively. However, the peaks were more prominent in the tan δ curves and the peak sensitivity had also improved, except that the peak of α_c-relaxation had disappeared in the DMTA measurement.

In the case of damping curves by DMTA measurement, γ-relaxation behavior was exhibited as a small peak at around 45 °C, with a better peak resolution. However, though the peak of α-relaxation became more pronounced than the loss modulus curve, α_c-relaxation could not be observed in the damping curves. On the contrary, DETA produced similar feature regarding tan δ curves with the result of the dielectric loss, displaying that the relaxation peaks became more pronounced by the increased peak height.

The comparison between dynamic mechanical and dielectric thermal behavior of the regenerated SF film can be summarized as follows: First, DETA exhibited a higher detection sensitivity regarding the SF molecular motion at low temperatures, including γ-relaxation (especially, below 50 °C). Second, DETA provides less information for molecular relaxations at high temperatures, such as α- and α_c- relaxations (especially, above 150 °C), while DMTA displayed two separated peaks attributed to the glass transition and the crystallization of SF. Third, as the frequency decreased, the peak sensitivity regarding both measurements had improved. Finally, DETA yielded a smoother curve in comparison to DMTA.

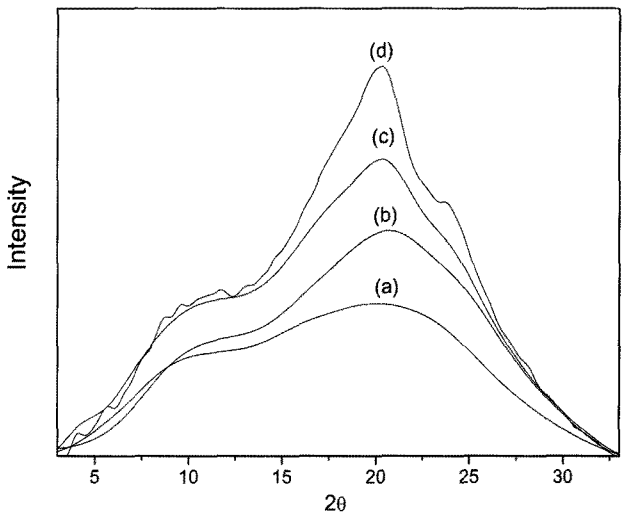

Figure 7. X-ray diffractograms of the regenerated SF films with various annealing temperatures; (a) untreated, (b) 150 °C, (c) 170 °C, and (d) 200 °C.

In general, the relaxation behavior of polymer is affected by many structural parameters and crystalline/amorphous nature of supramolecular structure is the most important parameter. In this study, the effect of crystallinity on the dynamic mechanical and dielectric behavior of SF film was investigated in more detail by comparing the differences between the two measurements.

For preparing the SF films of different crystallinities, the samples were annealed at different temperature (150, 170, and 200 °C) for one hour. Figure 7 showed that the diffraction peak at 20.4°, which corresponds to the β-sheet crystalline spacing of 4.5 Å,[17] appeared for the annealed films. The degree of crystallinity of SF films was determined by the ratio of integrated crystalline scattering to the total scattering, in both the crystalline and amorphous regions.[18] In this study, we used the X-ray diffraction curve of untreated SF film as an amorphous scattering pattern in order to determine the degree of crystallinity, since the phase of untreated SF film is known to be an amorphous as reported in the previous study.[9] Using this method, the degree of crystallinity of the regenerated SF films treated at 150, 170, and 200 °C was calculated as 24%, 41%, and 48%, respectively.

The effect of crystallinity on tan δ curves obtained from

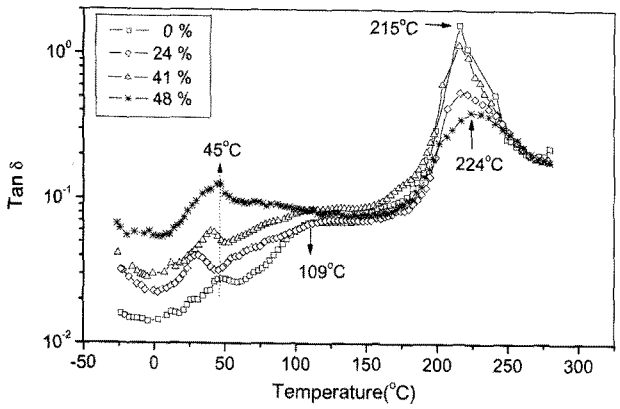

Figure 8. Tan δ curves of DMTA for the regenerated SF film with various crystallinities.

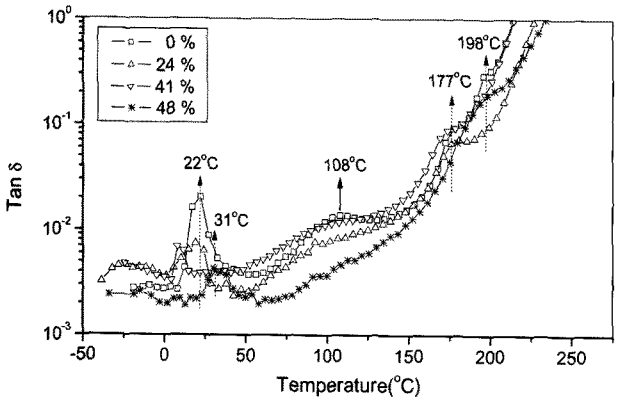

Figure 9. Tan δ curves of DETA for the regenerated SF film with various crystallinities.

DMTA and DETA was presented in Figures 8 and 9, respectively. In the case of DMTA measurement, γ-, β-, and α-relaxation behaviors were shown in three peaks at 45, 109, and 215 °C, respectively in the damping curves with 0.1 Hz. As the degree of crystallinity increased, several changes were observed in the damping peaks. First, the peak height of α-relaxation had been reduced and the relaxation temperature shifted to a higher temperature (224 °C) accompanying the peak broadening. This phenomenon is one of the representative changes in the relaxation behavior of semicrystalline polymer at the glass transition stage when the degree of polymer crystallinity has increased.[16] This is due to the fact that the segmental motion of SF molecules in amorphous regions is restricted by the growing crystalline region.

The β-relaxation peak at 109 °C declined as the annealing temperature increased. As mentioned earlier, it is assumed that β-relaxation is related to the molecular motion of SF molecules during the water evaporation stage. Therefore, increased crystallinity levels as the result of higher annealing temperatures might be responsible for the reduction of β-relaxation, considering that water molecules existing in SF molecules can be reduced by heat treatment. It is interesting to note that the β-relaxation peak did not disappear in the SF films crystallized by formic acid casting or methanol treatment processes.[9] This implies that the disappearance of β-relaxation is not just due to the SF crystallization process. In the case of γ-relaxation (around 45 °C), the effect of crystallinity on the peak intensity and shift could not be elucidated clearly because the temperature shift was inconsistent and the change of peak intensity was also ambiguous.

Different features appeared in the results of DETA measurement. DETA measurement at 100 Hz produced four peaks consisting of γ-, β-, α-, and α_c-relaxations at 22, 108, 177, and 198 °C, respectively, in the tan δ curve of amorphous SF film (crystallinity 0%). First, α_c-relaxation disappeared as the degree of crystallinity increased. Considering the relaxation process was attributed to crystallization of the amorphous region in the regenerated SF film, it can be easily understood that the α_c-relaxation behavior of SF became weaker, according to the increase of crystallinity. Second, α-relaxation shoulder peak appeared in the results of DETA measurement not giving enough information for segmental motion of SF while various α-relaxation peak shapes and locations were shown in DMTA measurement. That is, any change in the height and broadness of peak could not be observed though the highly crystallized SF film (48% crystallinity) showed a peak shift to a higher temperature (about 198 °C). Therefore, it can be concluded that DETA does not provide enough information regarding the segmental motion of SF molecules compared to the DMTA measurement.

The β-relaxation peak at around 108 °C showed similar results in comparison to DMTA, in displaying that the peak intensity was reduced by increasing crystallinity. On the

other hand, the γ-relaxation peak at around 25 °C was evident when compared to results based on DMTA. The peak intensity had decreased gradually, and the peak shifted to a higher temperature as the degree of crystallinity increased, though this trend is not so significant.

The above results regarding tan δ curves of the regenerated SF films treated with various annealing temperatures can be summarized as follows: The α_c-relaxation behavior of SF disappeared with increasing crystallinity. The segmental motion of SF had been restricted due to SF crystallization, therefore showing a reduced peak intensity, broadened peak, and peak shift to a higher temperature. The β-relaxation behavior became weaker as the annealing temperature had increased.

Conclusions

In this paper, the molecular relaxation behavior of the regenerated SF films was investigated using DETA and a comparative study with DMTA measurement was also performed to have a better understanding of dielectric thermal behavior for the films. Analytical differences between both measurements were also discussed. DMTA, conventionally used in studying the relaxation behavior, was more useful in obtaining information regarding changes in α-relaxation behavior of SF rather in contrast to DETA. On the contrary, DETA provided more comprehensive information regarding relaxation behaviors at lower temperature ranges with high sensitivity and it produced a smoother curve, thereby increasing its reliability as a measurement tool. Based on these findings, it can be concluded that DMTA and DETA can be utilized complementarily to study the relaxation behavior of SF molecules at various temperatures. Furthermore, since the DETA sample preparation process is simple and easy, this measurement tool can be utilized as a positive alternative to investigate the molecular relaxation behavior of brittle regenerated SF films or powders.

Acknowledgements. The authors of this paper thank the Korea Science and Engineering Foundation (KOSEF) for sponsoring this research through the SRC/ERC Program of MOST/KOSEF (R11-2005-065). The authors are also grateful to The National Instrumentation Center for Environmental Management (NICEM, Seoul National University, Republic of Korea) for their kind assistance in using XRD, DETA and DMTA.

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