

Dielectric Properties of Poly(γ -methyl D-glutamate) and Copoly(γ -methyl D,L-glutamate) Films

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ABSTRACT: Dielectric measurements of poly(γ -methyl D-glutamate) (PMDG) and copoly(γ -methyl D,L-glutamate) (PMDLG) films were performed over the frequency range from 30 Hz to 1 MHz and over the temperature range from -50°C to 70°C . A dispersion peak arising from the molecular motion of side chains was found and discussed. For the PMDG films cast from chloroform, 1,2-dichloroethane (EDC), and dichloromethane (DCM) solutions, differences in the side chain mobility were interpreted in relation to the molecular packing of α -helices. The Cole–Cole plots showed that the relaxation strength of the PMDG film cast from chloroform solution is smaller than that of the other films. This fact was interpreted in terms of the side-chain structure, which presumably restricts the side-chain motion. The PMDG and PMDLG films cast from trifluoroacetic acid (TFA)—EDC solutions showed two dispersions, which were attributed to the side-chain motions in two different phases.

KEY WORDS Dielectric Properties / Poly(γ -methyl D-glutamate) / Side-Chain Motion / Dispersion / Cole–Cole Plots /

The motion of side chains for many polypeptides has been studied by mechanical,^{1–3} dielectric,^{4–8} and NMR methods.^{9–12} Recently, we have investigated the effect of casting solvent on the structure and mechanical properties of PMDG films. X-ray measurements indicated that films cast from chloroform and DCM solutions had a well-ordered structure consisting of α -helices packed on a regular hexagonal lattice, while films cast from EDC solution showed less crystallinity and maintained the same twisted structure as the cholesteric liquid crystal structure in concentrated solutions.¹³ The mechanical properties for these two types of films were also different from each other. Furthermore, X-ray diffraction patterns of PMDG films cast from chloroform showed the appearance of some reflections on “extra” layer lines which cannot be explained by the α -helix.¹³ We suggested that these reflections are caused by the side-chain structure where the three residues make up an asymmetric unit, as in the case of poly(L-alanine).¹⁴ These reflections disappeared

by annealing the film at a temperature above 150°C . From the differential scanning calorimeter (DSC) measurements, such a breakdown of side-chain structure was associated with the irreversible endothermic peak observed at about 140°C .

In this paper, the dielectric behavior due to the side-chain motion was studied with respect to the difference of the packing of α -helical molecules in PMDG cast films and in view of the phase transition observed for PMDG films cast from chloroform solutions. The dielectric investigations were also carried out for PMDG and PMDLG films cast from TFA—EDC solutions and the side-chain motion was discussed in relation to the secondary structure of the main chains.

EXPERIMENTAL

Materials

The PMDG sample (Ajicoat A-200, $M_n=100,000$) was kindly supplied by Ajinomoto

Table I. Samples of copoly (γ -methyl D,L-glutamate)

Sample	$M_v \times 10^{-4}$
D-0	30.2
D-15	18.4
D-30	8.9
D-50	5.8

Co., Ltd. Four kinds of PMDG films were examined in this work. Three kinds of films were cast from chloroform, DCM, and EDC solutions. The other was prepared from a two-component solvent which contained 15-ml TFA and 10-ml EDC.

The PMDLG samples were synthesized by the NCA method. D- and L-N-carboxyamino acid anhydride, which were obtained by the Fuchs-Farthing method,¹⁵ were mixed at various ratios (D/L=0-1) and were polymerized in EDC-N, N-dimethylformamide (95 : 5) mixed solvent with trimethylamine initiator at 25°C. The molecular weight of PMDLG is shown in Table I, where D-X indicates a PMDLG film containing X-mol% of D-isomer. PMDLG films were prepared from TFA-EDC mixed solvent as mentioned above.

Films 0.2-0.3 mm thick were prepared on a glass plate at room temperature. After being dried, the films were introduced into methyl alcohol in order to remove the residual solvent and again dried under vacuum for 1 week at room temperature.

Methods

Dielectric measurements were carried out with a mutual inductance bridge (TR-1C), a detector (BDA-1B), and an oscillator (WBG-3G) manufactured by Ando Electric Co., over a frequency range from 30 Hz to 1MHz. Samples were painted with conductive silver paste in order to assure complete contact with the electrodes. X-ray measurements were carried out with a Rigaku Denki X-ray generator (Ni-filtered $\text{CuK}\alpha$ Radiation).

RESULTS AND DISCUSSION

The dielectric measurements were performed for the films cast from chloroform, DCM, and EDC solutions. Figure 1 represents the frequency

dependence of dielectric constant ϵ' and dielectric loss ϵ'' at various temperatures. For these films a dispersion which is referred to as the β -dispersion is observed over the temperature range from -50° to 50°C . This β -dispersion is associated with the onset of side-chain motion, as presumed from NMR measurements.^{10,11} The slope of the Arrhenius plots which show the relation between the frequency at maximum loss f_m and the reciprocal absolute temperature T^{-1} yields the activation energy ΔH associated with the side-chain motion. As estimated from Figure 2, ΔH for all these films are similar, about 23 kcal/mol. The dispersion temperatures

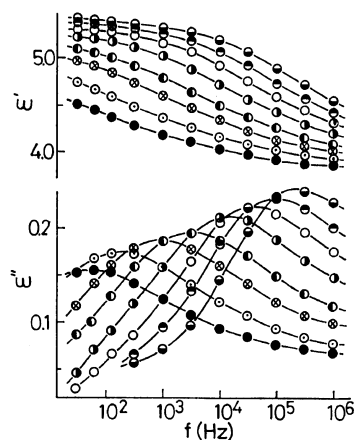


Figure 1. The dielectric constant ϵ' and ϵ'' as a function of frequency at various temperatures for the PMDG film cast from DCM solution: \ominus , 55.4°C; \bullet , 46.8°C; \circ , 38.8°C; \oplus , 29.9°C; \odot , 20.0°C; \otimes , 10.5°C; \odot , -1.1°C ; \bullet , -10.6°C .

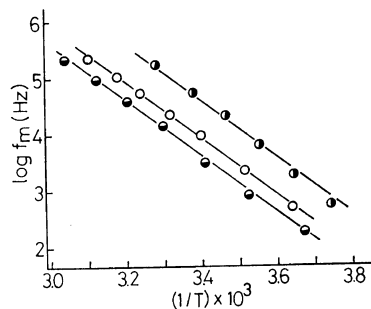


Figure 2. Relation between the frequency at maximum loss (f_m) and reciprocal absolute temperature (T^{-1}) for the PMDG films cast from chloroform (\circ), DCM (\bullet), and EDC (\odot).

Table II. The β -dispersion temperature at 1 kHz

Film	The β -dispersion temperature at 1 kHz, °C
Film cast from chloroform solution	7
Film cast from DCM solution	11
Film cast from EDC solution	-3

at 1 kHz are summarized in Table II. The dispersion temperature for the film cast from chloroform is a little lower than that for the film cast from DCM, while that for the film cast from EDC is much lower. Such differences in the dispersion temperature should be attributed to the difference in the molecular packing of α -helices, since these films consist of α -helices.¹³ As already observed from X-ray measurements,¹³ the films cast from chloroform and DCM solutions were highly crystalline and the α -helical molecules were packed into a hexagonal lattice with $a=11.95$ Å and $c=27$ Å. In contrast to these films, the film cast from EDC maintained the cholesteric twisted structure seen in the concentrated solutions, with a rather large angle of twist per molecular layer (1–2 degrees).¹⁶ Although the nature of the twisted structure in the solid state is still not clear, α -helical molecules in twisted array are considered to be more loosely packed in comparison with those in the hexagonal array. Thus, the density of the film cast from EDC was 1.287–8 g/ml and smaller than that of the films cast from DCM and chloroform (1.296–8 g/ml). Thus, it is reasonable that the film cast from EDC shows the β -dispersion at a lower temperature.

From the Cole–Cole plots, the relaxation strength $\Delta\epsilon$ and the distribution of relaxation times were obtained at various temperatures; these are shown in Figure 3. The $\Delta\epsilon$ and the distribution of relaxation times for the films cast from EDC and DCM are estimated to be 1.2–1.3 and about 0.45, respectively. As these values are similar to those for poly(γ -benzyl glutamate) films in which no benzyl groups of the side chains stack,^{6,8} it is considered that all of the side chains are freely mobile and contribute to this relaxation. For the film cast

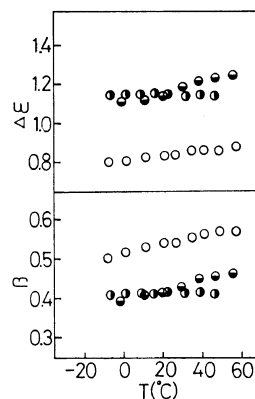


Figure 3. Relaxation strength $\Delta\epsilon$ and distribution of the relaxation times β as a function of temperature for the PMDG films cast from chloroform (○), DCM (◐), and EDC (◑).

from chloroform solution, however, the relaxation strength is smaller than that for the film cast from DCM or EDC solution, about 0.9. This suggests that the side-chain motion may be partially restricted because of a special interaction between the side chains. Indeed, the X-ray diffraction patterns of the film cast from chloroform have shown some reflections (for example, a 4.5-Å meridional reflection) which cannot be interpreted in terms of an α -helix according to the diffraction theory of helical molecules;¹⁷ such results have suggested the existence of a side-chain structure where the three residues make up an asymmetric unit. Thus, it is sure that there exists a side-chain structure in the film cast from chloroform. In its DSC thermogram, this film clearly showed an irreversible endothermic peak at about 140°C.¹³ This endothermic peak was attributed to the breakdown of a side-chain structure, such as was mentioned above. In order to examine the relationship of this endothermic peak and the β -dispersion, the dielectric measurements were carried out for the film annealed at 150°C for 10 min. As estimated from the Cole–Cole plots (Figure 4), the relaxation strength $\Delta\epsilon$ for the 150°C annealed sample was about 1.3, which is the value expected if all of the side chains can freely rotate and contribute to β -dispersion, while $\Delta\epsilon$ did not change by heat treatment at 110°C. The result also shows that the restriction of side-chain rotation disappeared as the result of

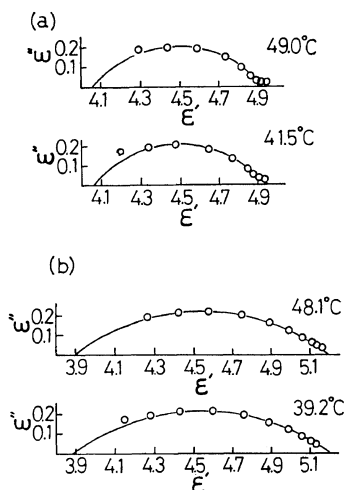


Figure 4. Cole—Cole plots for the original PMDG film cast from chloroform solution (a) and the PMDG film annealed at 150°C for 10 min (b).

the phase transition at 140°C. According to the Debye equation, $\Delta\epsilon$ may be expressed as

$$\Delta\epsilon \approx 4\pi N\mu^2/3kT \quad (1)$$

where N , μ , k , and T are, respectively, the number of dipoles per unit volume, the dipole moment of the carbonyl group of the side chain, Boltzman's constant, and the absolute temperature. We here make an assumption that the dipole moment of the carbonyl group in the side chains is the same for the original and the 150°C annealed films. This assumption may be reasonable since the activation energies for both films were the same, 23 kcal/mol. The number of side chains which do not contribute to the β -dispersion because of the restricted rotation can be estimated from the following equation, which expresses the relative ratio to the number of all the side chains:

$$(N_{\text{ann}} - N_{\text{ori}})/N_{\text{ann}} = (\Delta\epsilon_{\text{ann}} - \Delta\epsilon_{\text{ori}})/\Delta\epsilon_{\text{ann}} \quad (2)$$

where $\Delta\epsilon_{\text{ann}}$ and $\Delta\epsilon_{\text{ori}}$ indicate $\Delta\epsilon$ at the same temperature for the 150°C annealed and the original films, respectively. Using $\Delta\epsilon_{\text{ann}} = 1.3$ and $\Delta\epsilon_{\text{ori}} = 0.9$ at about 50°C (Figure 4),

$$(N_{\text{ann}} - N_{\text{ori}})/N_{\text{ann}} = (1.3 - 0.9)/1.3 \doteq 1/3$$

Thus, a third of the side chains in the film cast from chloroform solution cannot contribute to the β -dispersion. It is easy to envisage that

such a side chain exists in one residue per every three residues. This model for the side-chain structure corresponds to that presumed from the X-ray diffraction pattern, although it still remains a question why a third of the side chains cannot rotate.

The dielectric measurements were carried out for the films cast from TFA—EDC (3 : 2) solutions. These films showed two dispersions in the temperature range from -50°C to 70°C . They were named β_1 - and β_2 -dispersion in order of increasing temperature. When prepared by quick evaporation of solvent (for 2 or 3 days), the film showed predominantly the β_2 -dispersion (Figure 5). For the film obtained by slow evaporation, two dispersions were observed (Figure 6). The Arrhenius plots for β_1 - and β_2 -dispersions are shown in Figure 7. The activation energies of β_1 - and β_2 -dispersions are 18 kcal/mol and 27 kcal/mol, respectively. The β_2 -dispersion temperature is a little higher than that for the films cast from DCM or chloroform, while the β_1 -dispersion temperature is much lower. From X-ray measurements, it was indicated that the crystalline form of these films is hexagonal, with the unit cell of $a = 11.8 \text{ \AA}$ and $c = 27 \text{ \AA}$. Since the molecular diameter is appreciably smaller than that of the films cast from chloroform or DCM solution, the β_2 -dispersion with the higher transition temperature

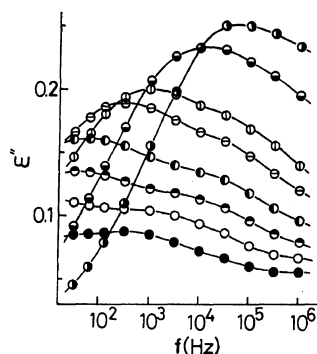


Figure 5. The dielectric loss ϵ'' as a function of frequency at various temperatures for the PMDG film cast from TFA—EDC solution: \bullet , 43.6°C; \ominus , 33.0°C; \oplus , 19.0°C; $\omin�$, 9.6°C; \bullet , -0.2°C ; $\omin�$, -12.0°C ; \circ , -25.0°C ; \bullet , -36.2°C . The film was prepared by quick evaporation of solvent (for 2—3 day).

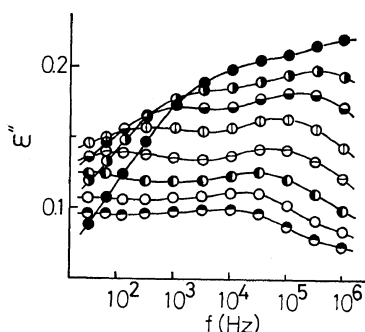


Figure 6. The dielectric loss ϵ'' as a function of frequency at various temperatures for the PMDG film cast from TFA—EDC solution: ●, 49.7°C; ○, 39.7°C; ◐, 31.8°C; ◑, 20.6°C; ◒, 11.9°C; ◓, -0.2°C; ◔, -10.7°C; ◕, -20.5°C. The film was prepared by slow evaporation of solvent (for 1 week).

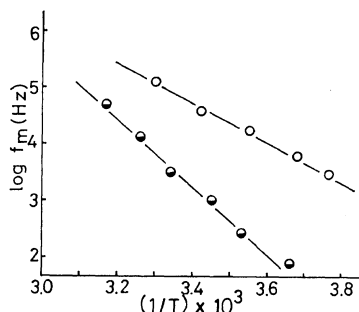


Figure 7. Relation between the frequency at maximum loss (f_m) and reciprocal absolute temperature (T^{-1}) for the PMDG films cast from TFA—EDC solution: ○, β_1 -dispersion; ◐, β_2 -dispersion.

is attributed to the side-chain motion in the hexagonal crystalline phase. It was found that the peak height of the β_1 -dispersion increased with the decrease of the magnitude of the β_2 -dispersion (Figures 5 and 6). This phenomenon indicates that the β_1 -dispersion is also associated with the side-chain motion in the other phase, the non-hexagonal one. Such a phase, however, was not clearly observed from IR and X-ray measurements. We found that the films cast from TFA—EDC solutions cannot be dissolved in helicogenic solvents such as EDC, DCM, and chloroform, but only swollen to some extent. Therefore, these films seem to contain some chains with interchain hydrogen bonds as well as α -helical chains. It may be

considered that the structure of the side chains is disturbed if hydrogen bonds occur partially between α -helical molecules in the hexagonal array. The motion of such disoriented side chains should be associated with the β_1 -dispersion with the lowest transition temperature. If this is the case, the behavior of the β_1 -dispersion observed in Figures 5 and 6 indicated that the formation of interchain hydrogen bonds is promoted by the slow evaporation of the solvent. This result is interesting to compare with that for the formation of a β -chain structure of PMLG films reported by Nakajima, *et al.*¹⁸

The dielectric losses, ϵ'' , for PMDLG films cast from TFA—EDC solutions are shown in Figure 8a—c. All of the films were prepared by quick evaporation of solvent (for 2—3 days). The dielectric properties and structure of D-0 (PMLG) were the same as those of PMDG. The side-chain motion for all of the PMDLG films is described by β_1 - and β_2 -dispersions observed for PMDG films, although the β_1 -dispersion shifts to a lower temperature with the increase of D-isomer content. As shown in Figure 8a, b, the β_2 -dispersion decreased gradually for D-15 and D-30. Finally, for D-50, only the β_1 -dispersion was observed (Figure 8c). From X-ray measurements, it was shown that a hexagonal crystalline phase decreased abruptly for D-30 and disappeared for D-50. Such a disappearance of

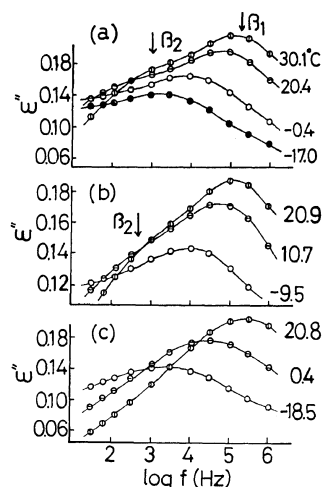


Figure 8. The dielectric loss ϵ'' as a function of frequency at various temperatures for D-15 (a), D-30 (b), and D-50 (c).

the crystalline phase is due to the breakdown of helical conformation of main chains, as is well known from IR measurements.^{19,20} The results support the association of the β_2 -dispersion with the side-chain motion in the hexagonal crystalline phase. Furthermore, the β_1 -dispersion can be attributed to the side-chain motion in the disordered part of the main chains.

CONCLUSION

The film cast from EDC solution showed a lower β -dispersion temperature compared to the films cast from DCM and chloroform solutions. This shows that the α -helical molecules in the cholesteric twisted array are more loosely packed than those in the hexagonal array.

From the examination of the relaxation strength for the β -dispersion, it was concluded that in the film cast from chloroform solution a third of the side chains cannot rotate. This dielectric result is consistent with the suggestion from X-ray measurements that each side chain is not equivalent and that three residues make up an asymmetric unit.

The PMDG and PMDLG films cast from TFA—EDC solutions showed two dispersions, β_1 - and β_2 -dispersions. The β_2 -dispersion was attributed to the motion of side chains in the hexagonal crystalline phase, and the β_1 -dispersion to that of the disoriented side chains in the main chains with the interchain hydrogen bonds and/or in the disordered part of the main chains.

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