

Conformational Studies on Copolypeptides Composed of γ -Benzyl-L-Glutamate and γ -Methyl-L-Glutamate

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Polymer characterization and helix-coil transition of copolypeptides composed of γ -benzyl-L-glutamate and γ -methyl-L-glutamate were investigated by viscometric, osmometric, and spectropolarimetric measurements. The characteristic ratio of the equimolar copolypeptide in random coil conformation was estimated and the data concerning the helix-coil transition were analyzed according to an approximate expression for the helical content proposed for homopolypeptides. It was shown that the helical content was almost proportional to the reciprocal of the degree of polymerization. The results were compared with ones from Allegra's approximate theory on the helix-coil transition of copolypeptides.

INTRODUCTION

Many papers on solution properties of polypeptides have been reported since the discovery of conformational changes of polypeptides in solution, the helix-coil transition, by Doty and Blout.¹⁾ Moreover a number of statistical mechanical theories were proposed by Zimm-Bragg,²⁾ Lifson-Roig³⁾ and many others. These theories related to copolypeptides were developed to give light onto the theoretical treatment of the melting of deoxyribonucleic acids as a heterogeneous chain.⁴⁻⁹⁾ Recently Scheraga and Coworkers¹⁰⁾ have applied both these theories and their experimental results in order to obtain the Zimm-Bragg parameters σ and s for naturally occurring amino acids in water. However the comparison of various theories with experiments is little to date, although the validity of the theories was examined only by means of Monte Carlo calculation.¹¹⁻¹³⁾ Therefore, it is very useful to investigate the helix-coil transition of copolypeptides and to compare the data obtained with ones calculated from Allegra's approximate theory for copolypeptides.^{14,15)}

Let's consider copolypeptides composed of two kinds of amino acids; γ -benzyl-L-glutamate and γ -methyl-L-glutamate. The copolypeptides of both amino acids were regarded as almost random copolymers.¹⁶⁾ It is one more advantage to the copolypeptides of this combination that each homopolypeptide brings about the thermally induced transition in the same mixed solvent, so that the phenomenon with the copolypeptides of all over the composition range seems to be observed only by the temperature change without changing the solvent composition.

EXPERIMENTAL

Materials

Poly- γ -benzyl-L-glutamate (PBLG), poly- γ -methyl-L-glutamate (PMLG), and

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copolypeptides (PMBLG's) composed of BLG and MLG were synthesized by the NCA method according to the procedure reported in the preceding paper.¹⁷⁾ The polymerization was carried out in a 1:1 (*v/v*) mixture of dioxane and methylene chloride at room temperature with triethylamine as an initiator. Polypeptides synthesized thus were roughly fractionated in the system of methylene chloride and methanol. All solvents used for synthesis and as the initiator were purified three times by the usual methods described in the literature. The composition of copolypeptides were determined by means of elementary analysis of C and H. The homopolypeptides and the equimolar PMBLG, having different degrees of polymerization, are listed in Tables I and II, respectively. In addition to the equimolar PMBLG,

Table I. Characterization of Homopolypeptides: PBLG and PMLG

Polymer	$[M]/[I]$	$[\eta]$ (dl/g)	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$
PBLG-1	250	3.55		74.0
PBLG-2	250	3.44		71.4
PBLG-3	150	2.50	24.7	49.4
PBLG-4	100	2.02	20.9	38.7
PBLG-5	25	0.87	14.7	14.7
PMLG-1	300	3.65	33.4	
PMLG-2	250	3.18	27.5	
PMLG-3	150	2.50	19.7	
PMLG-4	100	1.36	8.42	
PMLG-5	45	0.88 ₅	4.69	

Table II. Characterization of Equimolar PMBLG

Polymer	$[M]/[I]$	$[\eta]$ (dl/g)	$M_n \times 10^{-4}$	$A_2 \times 10^4$ mol. ml/g ²
PMBLG-1	230	3.13	33.3	2.2
PMBLG-2	140	2.70	26.6	2.3
PMBLG-3	100	2.32	21.4	2.1
PMBLG-4	25	2.12	18.9	3.0
PMBLG-5	50	1.33	11.0	2.4
PMBLG-6	50	0.66	6.06	3.2

three kinds of copolypeptides were synthesized: The contents of BLG in these copolypeptides were 81.1, 71.2, and 29.0 mole%, and the limiting viscosity numbers were 2.21, 1.58, and 1.72 dl/g, respectively, in dichloroacetic acid (DCA) at 25°C.

Measurements

The number-average molecular weight, M_n , of PBLG and PMBLG was determined in dimethylformamide (DMF) at 30°C with an Electronic High-Speed Membrane Osmometer (Knauer Co.), in which regenerated cellulose membrane SD (Sartorius Membrane-Filter GmbH) was used as the semipermeable membrane after successive conditionings.

The limiting viscosity number, $[\eta]$, was determined in DCA at $25 \pm 0.01^\circ\text{C}$ with Ubbelohde type viscometers.

The optical rotatory dispersion (ORD) was measured with a Yanagimoto OR-

100 Type Spectropolarimeter using a tungsten lamp as light source. The temperature fluctuation was kept within $\pm 0.02^\circ\text{C}$. Polypeptide solutions were prepared at a concentration of 1 g/dl throughout these measurements. The results of ORD measurements were analyzed in terms of the Moffitt plot¹⁸⁾ with $\lambda_0=212$ nm to determine the parameter b_0 .

RESULTS AND DISCUSSION

Molecular Weight of Samples

The results from viscosity and osmotic pressure measurements of PBLG, PMLG, and equimolar PMBLG are summarized in Tables I and II, where $[M]/[I]$ is the mole ratio of NCA monomer to the initiator and A_2 the second virial coefficient. The weight-average molecular weight, M_w , of PBLG was obtained from the relation between $[\eta]$ and the molecular weight proposed by Doty, Bradbury, and Holtzer.¹⁹⁾ The M_n of PMLG was obtained from the relation between $[\eta]$ and M_n proposed by Tanaka;²⁰⁾ $[\eta]=2.9 \times 10^{-4} M_n^{0.74}$. As shown in Tables I and II, the molecular weight of these three polypeptides increases with increasing $[M]/[I]$.

Characteristic Ratio of Equimolar PMBLG

The limiting viscosity number of the equimolar PMBLG is plotted against the number-average molecular weight on a logarithmic scale in Fig. 1. From the straight

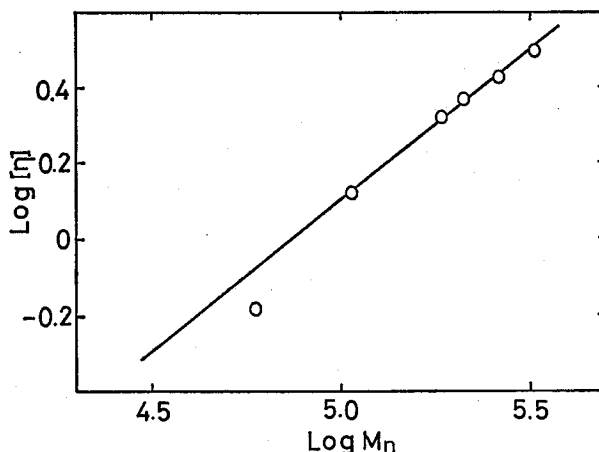


Fig. 1. Double-logarithmic plots of $[\eta]$ vs. M_n for equimolar PMBLG in DCA at 25°C .

line in Fig. 1, Mark-Houwink-Sakurada equation is written as:

$$[\eta]=1.3 \times 10^{-4} M_n^{0.80} \quad (1)$$

The value of 0.80 for the exponent in this equation suggests that the equimolar PMBLG exists in random coil conformation in DCA at 25°C , and nearly equals to the value of 0.74 for PMLG obtained by Tanaka²⁰⁾ and to the value of 0.76 for PBLG obtained by Nakagawa *et al.*²¹⁾

Figure 2 shows the Kurata-Stockmayer-Fixman plot²¹⁾ of the equimolar PMBLG,

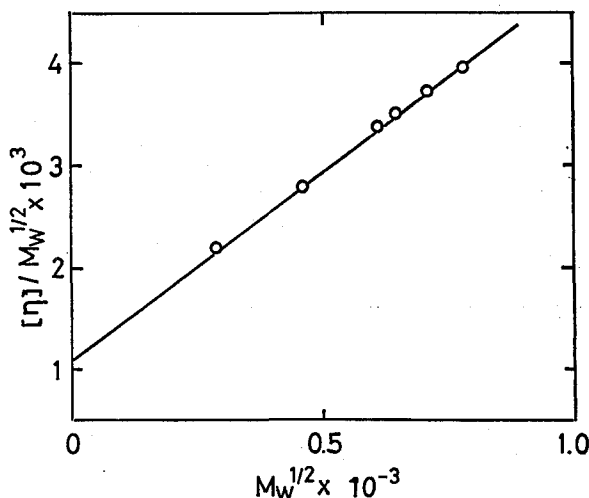


Fig. 2. Kurata-Stockmayer-Fixman plot for equimolar PMBLG.

represented by

$$[\eta]/M_w^{1/2} = K + (3/2\pi)^{3/2} C \Phi_0 B M_w^{1/2} \quad (2)$$

where Φ_0 is the Flory constant at the theta state, B and C are constants. The K is related to the characteristic ratio $\langle R_0^2 \rangle / nl^2$, which is expressed by the following equation,

$$\langle R_0^2 \rangle / nl^2 = (K/\Phi_0)^{2/3} (M_0/l^2) \quad (3)$$

where $\langle R_0^2 \rangle$ is the mean square unperturbed end-to-end distance, n the number of amino acid residues in a chain, l the length of a virtual bond, 3.8 Å, M_0 the molecular weight of the amino acid residue.

The M_w of our equimolar PMBLG may be estimated from M_n of them. Because it is known that the molecular weight distribution of the polypeptide is influenced by initiator for polymerization. For example, M_w/M_n of PBLG is reported to be 1.16 using *n*-butylamine as an initiator,²²⁾ and the values range from 1.38 to 2.27 with triethylamine.²³⁾ In addition, as is seen in Table I, M_w/M_n of PBLG-4 and PBLG-3 are 1.85 and 2.0, respectively. The polymerization condition of our PMBLG was resemble to that of PBLG. Therefore it was assumed that the molecular weight distribution of PMBLG obtained by the polymerization initiated by triethylamine is of the most probable distribution type.

Assuming Φ_0 to be $(2.3 \pm 0.3) \times 10^{21}$ and substitution $K = 1.1 \times 10^{-3}$ the value obtained from Fig. 2, into Eq. (3), we obtain 7.7 ± 0.7 for the characteristic ratio of the equimolar PMBLG. This value is in good agreement with experimental and theoretical values for other α -L-polypeptides whose side chains are of $-\text{CH}_2\text{R}$ type, e.g., 7.7 ± 0.8 for PMLG determined experimentally by Tanaka,²⁰⁾ 7.5 ± 1.2 for PBLG by Fujita *et al.*,²⁴⁾ and the calculated value of 8.38 for poly-L-alanine.²⁵⁾ Our

experimental result also leads to a conclusion that the characteristic ratio, *i.e.*, the unperturbed chain dimension, of polypeptide is dependent mostly on the main chain structure and little on the side chain structure, and supports the validity of the theoretical treatments^{25,26)} on the conformational analysis for polypeptides in random coil conformation.

Helix-Coil Transition

The helical content, θ_N , of PBLG, PMLG, and various PMBLG's in a DCA-DCE (7:3 by volume) mixture is plotted against temperature in Fig. 3, where θ_N is related¹³⁾ to the parameter b_0 by

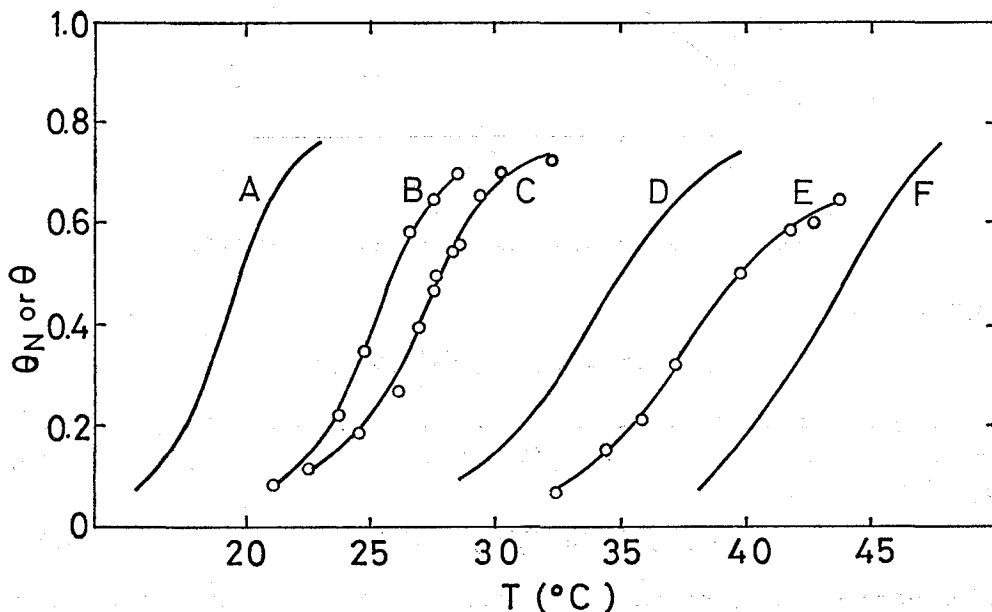


Fig. 3. Temperature dependence of helical content for PMBLG's, PBLG, and PMLG in DCA-DCE (70 vol% DCA) mixture; A, PBLG; B, PMBLG (81.1 mole% BLG); C, PMBLG (71.2 mole% BLG); D, equimolar PMBLG; E, PBMLG (29.0 mole% BLG); F, PMLG.

$$\theta_N = (b_0 - b_0^C) / (b_0^H - b_0^C) \quad (4)$$

where b_0^C and b_0^H denote the b_0 values for random coil and helix, respectively. For PBLG, PMLG, and PMBLG's, b_0^C was assigned to be +30 from ORD measurements in DCA at 20°C, while b_0^H was assigned to be -630 from ones in DCE at 20°C. In the figure the helical content of PBLG, PMLG, and the equimolar PMBLG is extrapolated to the infinite chain length. It is obvious from Fig. 3 that the existence of comonomers, BLG and MLG, in PMBLG's affords a retardation effect for helix formation in comparison with that of homopolypeptides, PBLG and PMLG, *i.e.*, this means that the transition of the copolypeptide takes place not independently but cooperatively with respect to two comonomers.

In order to evaluate the transition parameters, the nucleation parameter σ and the enthalpy of transition ΔH , from the ORD data, we adopted the procedure pro-

posed by Okita *et al.*²⁷⁾ Provided that the degree of polymerization $N \gg 1$, $\sigma^{1/2} \ll 1$, and $N\sigma^{1/2} > 2$, the helical content is written by

$$\theta_N = \theta - 2\theta^{3/2}(1-\theta)^{1/2}/N\sigma^{1/2} \quad (5)$$

where N is the degree of polymerization and θ the helical content of a polypeptide having infinite degree of polymerization. Eq. (4) predicts that a plot of θ against N^{-1} at a fixed solvent condition gives a straight line, where the ordinate intercept $\beta(=\theta)$ is related to the solpe α by Eq. (6).

$$\sigma^{1/2} = -(2\beta/\alpha)[\beta(1-\beta)]^{1/2} \quad (6)$$

The value of ΔH is evaluated from the value of $\sigma^{1/2}$ derived from Eq. (6) by the following equation.²⁸⁾

$$\lim_{N \rightarrow \infty} (d\theta/dT)_{T=T_m} = \Delta H/4RT_m\sigma^{1/2} \quad (7)$$

where T_m is the transition temperature, at which $\theta_N = 0.5$, and $d\theta/dT$ the transition sharpness at T_m .

Figures 4, 5, and 6 show the plots of θ_N against N^{-1} at various temperature in DCA-DCE mixture, for PBLG, PMLG, and the equimolar PMBLG, respectively. As interpreted from Eq. (5), the data points fall on a straight line for the equimolar PMBLG as well as the homopolypeptides, PBLG and PMLG, at each temperature. Thus the theories for the helix-coil transition of homopolypeptides may be available for that of copolypeptides, which have the same type of helical structure, *i.e.*, α -helix, as homopolypeptides and are regarded as almost random copolypeptides. Roig and Cortijo²⁹⁾ also reported a similar conclusion from a different treatment as to the

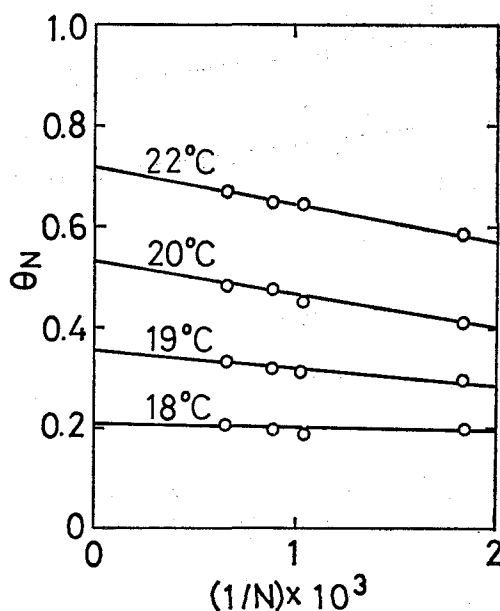


Fig. 4. Plots of θ_N vs. N^{-1} for PBLG in DCA-DCE (70 vol% DCA) mixture at various temperatures.

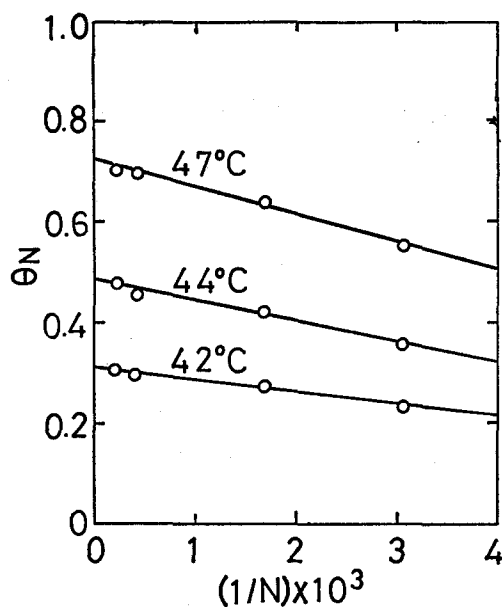


Fig. 5. Plots of θ_N vs. N^{-1} for PMLG in DCA-DCE (70 vol% DCA) mixture at various temperatures.

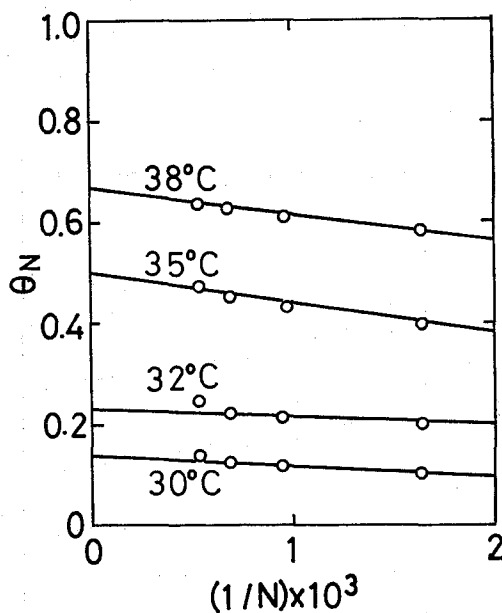


Fig. 6. Plots of θ_N vs. N^{-1} for equimolar PMBLG in DCA-DCE (70 vol% DCA) at various temperatures.

helix-coil transition of copolyptide.

Thus, the parameters characterizing the helix-coil transition of the copolyptide were deduced by the same analysis as for homopolyptides, and numerical data for

$\sigma^{1/2}$, ΔH , and T_m are summarized in Table III. The ΔH obtained thus may bring in some molecular meanings for the transition by further comparison with the transition enthalpy change directly obtained from the calorimetric measurement. The values of σ for each polypeptide given in Table III are nearly constant in the temperature range of helix-coil transition. The difference between σ 's of PBLG and those of PMLG may be attributed to the difference in their side chains. On the other hand, σ 's of the PMBLG are in-between ones of both homopolypeptides, which means that the effect of heterogeneity of the side chains on the value of σ is not remarkable.

Table III. Helix-Coil Transition Parameters of PBLG, PMLG, and Equimolar PMBLG

Polymer	Temp. (°C)	$\sigma^{1/2} \times 10^2$	ΔH (cal/mole)	T_m (°C)
PBLG	19	0.88	920	19.8
	20	0.81		
	22	0.87		
PMLG	42	1.20	990	44.2
	44	1.19		
	47	1.12		
PMBLG	32	0.91	560	35.0
	35	0.73		
	37	0.98		

According to Dreele, Poland, and Scheraga,¹²⁾ approximate theories are available for the helix-coil transition of copolypeptides, if the magnitude of the Zimm-Bragg parameters of two homopolypeptides are close to each other. The difference in T_m 's of PBLG and PMLG is 24.4°C (Table III), which may be regarded as small difference. Therefore, we applied the low order approximation. The curve in Fig. 7 is calculated from Allegra's approximate theory for helix-coil transition of copolypeptides by the use of the parameters listed in Table III. As shown in Fig. 7, the calculated values are in good agreement with experimental values for the copolypeptides having BLG as major component, while they deviate gradually with the increase of MLG component. Comparison of Allegra's approximation with the Monte Carlo calculation by Dreele *et al.*¹²⁾ indicates that better agreement with the experimental data could not be expected by the use of the higher order approximation.

It is noted in Fig. 7 that the replacement of MLG residues by a small amount of BLG residues gives such a quick decrease as not expected from the theory. According to Roig and Cortijo,²⁹⁾ this peculiarity was attributed to tighter side chain packing of MLG side chains than that of BLG side chains in the polar solvent, which gave influence on PMBLG molecules containing BLG as minor component. Thus the transition behavior of copolypeptides should not be described only by the properties of the component homopolypeptides. Also it is quite important to take into consideration both the specific interaction among side chains and the interaction between side chains and solvent. The latter is induced by the heterogeneity of side chains.

Figure 8 shows the relation between T_m and MLG mole fraction. The values of T_m for PMBLG's fall on between those of PBLG and PMLG, *i.e.*, the stability of the helices in the copolypeptides is intermediate, although the curves in Fig. 8 does not change linearly. The discrepancy of T_m from the calculated values by Allegra's

approximate theory stressed also the importance of interactions induced by the heterogeneity of side chains.

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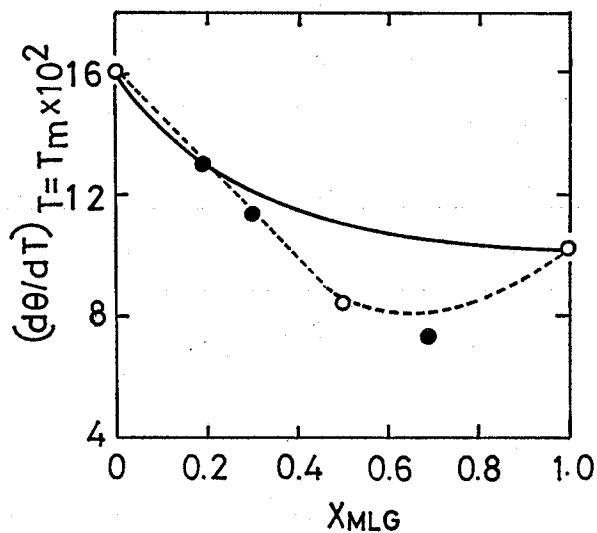


Fig. 7. Dependence of transition sharpness $(d\theta/dT)_{T=T_m}$ on mole fraction of MLG, X_{MLG} , in copolyptide; ○, values extrapolated to infinite degree of polymerization; ●, values for finite degree of polymerization. Solid line represents the curve calculated by Allegra's approximate theory.

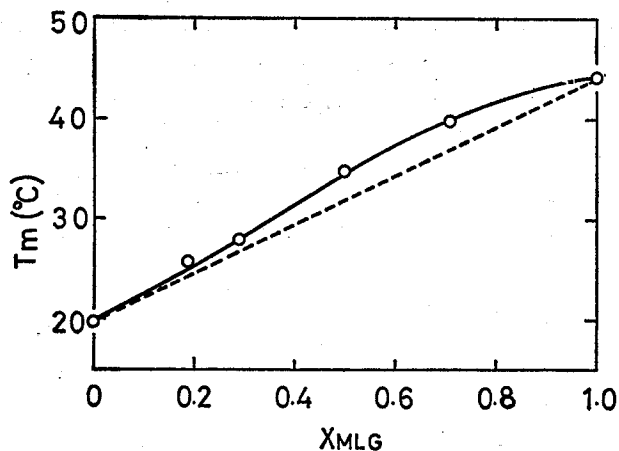


Fig. 8. Dependence of T_m on X_{MLG} . Dashed line represents the curve calculated by Allegra's approximate theory.

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