

Grafting of Polypeptide from Carbon Black by the Ring-Opening Polymerization of γ -Methyl L-Glutamate *N*-Carboxyanhydride Initiated by Amino Groups on Carbon Black Surface

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ABSTRACT: The introduction of amino, methylamino, or dimethylamino groups onto a carbon black surface was achieved by the reaction of carbon black having acyl azide groups with corresponding ethylenediamines. It was found that in the presence of carbon black having these amino groups, the anionic ring-opening polymerization of γ -methyl L-glutamate *N*-carboxyanhydride (NCA) is initiated to give polypeptide-grafted carbon black. The percentage of grafting of the polypeptide onto carbon black having primary amino groups (0.29 mmol g^{-1}) in dioxane at 40°C was up to 23.7%. The percentage of grafting of polypeptide onto carbon black having tertiary amino groups was lower than that onto carbon black having primary or secondary amino groups. This may be due to the propagation of polypeptide from carbon black surface in the polymerization initiated by carbon black having primary amino groups. On the contrary, in the polymerization initiated by tertiary amino groups, ungrafted polymer was preferentially formed because the polymerization is initiated by γ -methyl L-glutamate NCA anion formed by the abstraction of hydrogen from the NCA by tertiary amino groups.

KEY WORDS Carbon Black / Ring-Opening Polymerization / Grafting of Polypeptide / Polypeptide-Grafted Carbon Black / γ -Methyl L-Glutamate *N*-Carboxyanhydride / *N*-Carboxyanhydride / Amino Group on Carbon Black / Ethylenediamine /

In a series of our papers, we have reported the introduction of reactive functional groups onto carbon black surface, such as isocyanate,^{1,2} acyl azide,¹ and acyl chloride groups.³ By the reaction of these reactive groups with various kinds of polymers having hydroxyl or amino groups, these polymers were able to be grafted onto carbon black surface.¹⁻³

Furthermore, it has been demonstrated that radical, cationic, and anionic graft polymerizations of various monomers are initiated by azo⁴ or peroxyester groups,⁵ acylium perchlorate groups,⁶ and alkali metal carboxylate groups^{7,8} on carbon black, respectively. In the polymerizations, polymers are effectively grafted from a carbon black surface based on the propagation of the polymers from the

functional groups on the surface.

On the other hand, it is well known that the anionic ring-opening polymerizations of α -amino acid *N*-carboxyanhydrides (NCA) are initiated by nucleophilic reagents, bases, and organic metal compounds to give polypeptides.⁹

Dietz and his coworkers have reported that silica having aminophenyl groups initiates the polymerization of α -amino acid NCA to give polypeptide-grafted silica.¹⁰ In addition, the grafting of polypeptide onto water-soluble chitin by the polymerization of α -amino acid NCA initiated by amino groups of the chitin was reported by Kurita *et al.*¹¹ Furthermore, Asami and his coworkers described the graft polymerization of α -amino acid NCA from vinylbenzylamine copolymers.¹² Therefore, it

is expected that carbon black having amino groups is able to initiate the polymerization of α -amino acid NCA to give polypeptide-grafted carbon black.

In this paper, the introduction of primary, secondary, and tertiary amino groups onto a carbon black surface and the grafting of polypeptide from carbon black by the ring-opening polymerization of γ -methyl L-glutamate NCA initiated by amino groups introduced onto carbon black were investigated.

EXPERIMENTAL

Carbon Black

The carbon black used was color channel black Neospectra II (Columbian Carbon Co.; BET specific surface area, $906 \text{ m}^2 \text{ g}^{-1}$; particle size, 13 nm) and dried *in vacuo* at 110°C before use. The contents of carboxyl, phenolic hydroxyl, and quinonic oxygen groups on the surface were estimated to be 0.40, 0.24, and 0.92 meq g^{-1} , respectively.

γ -Methyl L-Glutamate NCA

γ -Methyl L-glutamate NCA obtained from Ajinomoto Co., Inc. Japan was recrystallized twice from chloroform-hexane before use.

Solvents

Dioxane and tetrahydrofuran (THF) were refluxed over sodium and distilled twice. Acetonitrile was refluxed over CaH_2 and distilled. Subsequently, it was refluxed over KHSO_3 to remove a trace of amine and fractionally distilled. *N*-Methyl-2-pyrrolidone (NMP) was distilled twice under reduced pressure. Dimethyl sulfoxide (DMSO) was dried over CaH_2 with stirring for three days and fractionally distilled twice under reduced pressure. Dichloroacetic acid obtained from Kanto Chemical Co., Inc. was used without further purification.

Others

Guaranteed-reagent-grade ethylenediamine,

N,N'-dimethyl-ethylenediamine, and *N,N*-dimethylethylenediamine obtained from Tokyo Kasei Kogyo Co., Ltd. were dried over potassium hydroxide and distilled.

Introduction of Primary Amino Groups onto Carbon Black

The introduction of primary amino groups onto carbon black was achieved by the treatment of carbon black having acyl azide groups or acyl chloride groups with ethylenediamine. The procedures for the introduction of acyl azide groups or acyl chloride groups onto carbon black are described in our previous paper.^{3,4}

A typical example for the introduction of amino groups was as follows: Into a tear-drop type flask equipped with a refluxed condenser, 5.0 g of carbon black having acyl azide groups (or acyl chloride groups), 30.0 ml of ethylenediamine, and 30.0 ml of DMSO as solvent were charged. The mixture was stirred with a magnetic stirrer at 130°C for 20 h. After the reaction, the reaction mixture was filtered, and the solid was washed with water and then methanol. The resulting carbon black was extracted with methanol by use of a Soxhlet extractor and dried *in vacuo* at 110°C for 48 h.

The introduction of primary amino groups onto carbon black by the hydrolysis of acyl azide group with water was carried out by refluxing the carbon black having acyl azide group with distilled water for 20 h. After the reaction, the carbon black was filtered, washed with water, and dried *in vacuo*.

Introduction of Secondary or Tertiary Amino Groups onto Carbon Black

The introduction of secondary or tertiary amino groups onto carbon black was carried out by the treatment of carbon black having acyl azide groups with *N,N'*-dimethylethylenediamine and *N,N*-dimethylethylenediamine, respectively. The reaction procedures are similar to that of the introduction of primary amino groups described above.

Determination of the Content of Amino Groups on Carbon Black

The content of amino groups introduced onto the surface of carbon black was determined by titration. The carbon black having amino groups was treated with excess of 0.01 N HCl aqueous solution and unreacted HCl was titrated with 0.01 N NaOH aqueous solution using phenolphthalein as an indicator.

Polymerization of γ -Methyl L-Glutamate NCA by Carbon Black Having Amino Groups

Into a tear-drop type flask equipped with a CaCl₂ tube, 0.30 g of carbon black having amino groups, 1.0 g of γ -methyl L-glutamate NCA, and 20.0 ml of a solvent were charged in a drybox to avoid contamination by moisture into the polymerization system. The mixture was stirred with a magnetic stirrer at 20–40°C under dry nitrogen. After the reaction, the reaction product was filtered, washed with dioxane, and dried in vacuo at 50°C for 48 h. The conversion was calculated by the following equation:

$$\text{Conversion (\%)} = \frac{\text{Product (g)} - \text{Carbon black (g)}}{\gamma\text{-methyl L-glutamate NCA used (g)}} \times 100$$

Determination of the Percentage of Grafting

To dissolve the ungrafted polypeptide, the

product containing carbon black was dispersed in dichloroacetic acid and stirred with a magnetic stirrer at 50°C. After 2 h, the dispersion was centrifuged at 1.2×10^4 rpm. The carbon black precipitated was dispersed again in dichloroacetic acid at 50°C and centrifuged. The procedure was repeated until no polypeptide was detected in the supernatant solution. The percentage of grafting of polypeptide onto carbon black was calculated by the following equation:

$$\text{Percentage of grafting (\%)} = \frac{\text{Polymer grafted (g)}}{\text{Carbon black used (g)}} \times 100$$

RESULTS AND DISCUSSION

Introduction of Amino Groups onto Carbon Black

In the preceding papers, it was reported that acyl azide groups or acyl chloride groups introduced onto a carbon black surface react with various kinds of polymers having amino groups to give polymer-grafted carbon black.¹⁻³ Therefore, the introduction of primary amino groups onto carbon black by the reaction of ethylenediamine with acyl chloride (eq 1) or acyl azide groups (eq 2) was examined. Furthermore, it is expected that by the reaction of acyl azide groups with water, primary amino groups can be introduced onto the surface of carbon black (eq 3).¹³

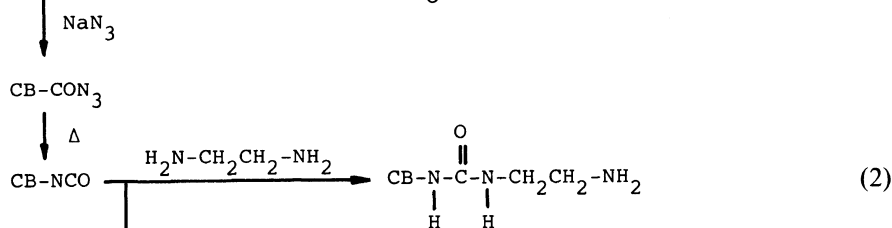
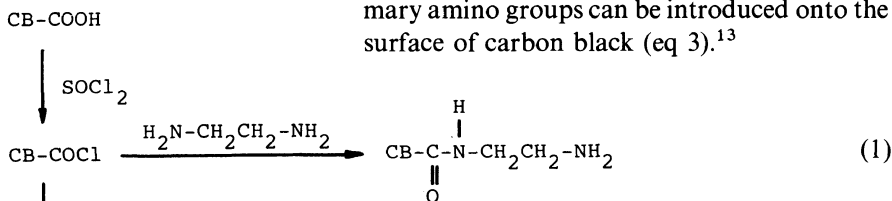


Table I. The content of primary, secondary, and tertiary amino groups introduced onto the carbon black surface

Carbon black	Introduction reaction	Amino group
		mmol g ⁻¹
CB-NH ₂	Eq. 1	0.23
CB-NH ₂	Eq. 2	0.29
CB-NH ₂	Eq. 3	0.17
CB-NH(CH ₃)	Eq. 4	0.20
CB-N(CH ₃) ₂	Eq. 5	0.29

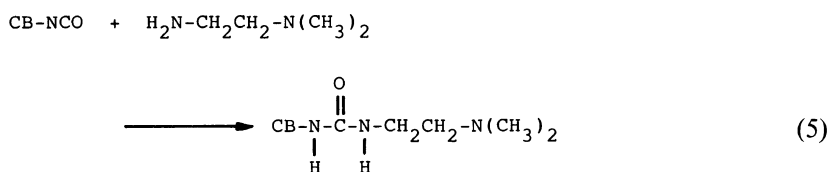
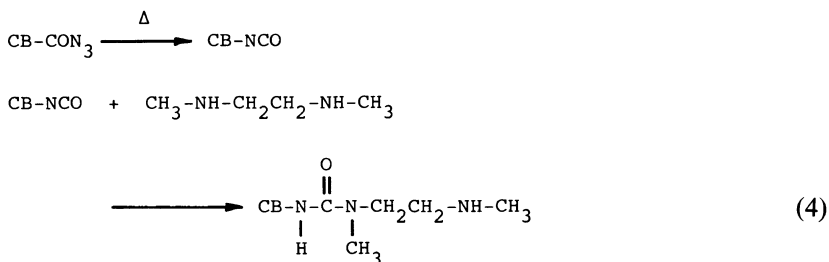
The content of amino groups onto carbon black introduced by the reaction shown in eq 1, 2, and 3 was determined by titration. The results are summarized in Table I.

Table I shows that primary amino groups can be introduced onto the carbon black surface by the reaction of reactive carbon black having acyl azide or acyl chloride groups with ethylenediamine. The content of amino groups introduced by the reaction of ethylenediamine

with acyl azide groups is larger than that with acyl chloride groups. This may be due to the higher reactivity of isocyanate groups formed by the rearrangement of acyl azide groups.

In addition, primary amino groups were found to be introduced onto carbon black by the hydrolysis of acyl azide groups. However, the amount of amino groups introduced onto carbon black by the reaction was less than that of acyl azide groups with ethylenediamine.

Furthermore, the introduction of *N*-methylamino and *N,N*-dimethylamino groups onto carbon black surface by the reaction of acyl azide groups with *N,N'*-dimethylethylenediamine and *N,N*-dimethylethylenediamine was attempted. The results are also shown in Table I. As shown in Table I, secondary amino and tertiary amino groups can be introduced onto carbon black surface by the reaction of carbon black having acyl azide groups with the corresponding ethylenediamines as shown in eq 4 and 5.



Proof of Initiation by Primary Amino Groups on Carbon Black Surface

The polymerization of 1.0 g of γ -methyl L-glutamate NCA and 0.30 g of carbon black having primary amino groups was carried out at 30°C. The results are shown in Figure 1.

Both in the absence of carbon black and in

the presence of untreated carbon black, no polymerization of γ -methyl L-glutamate NCA could be detected. In contrast, the polymerization of the NCA was initiated by carbon black having primary amino groups to give poly(γ -methyl L-glutamate), *i.e.*, polypeptide. Furthermore, Figure 1 shows that the rate of

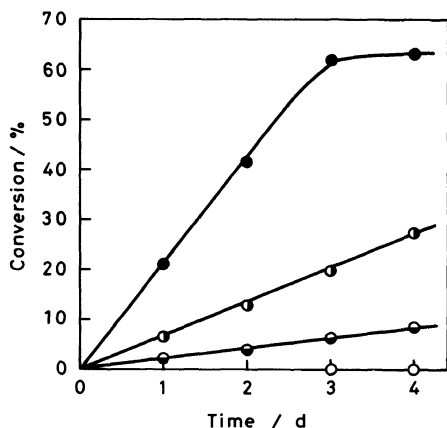


Figure 1. Polymerization of γ -methyl L-glutamate NCA initiated by primary amino groups on carbon black. Carbon black, 0.30 g; γ -methyl L-glutamate NCA, 1.0 g; dioxane, 20.0 ml; 30°C. (○), in the absence of carbon black and in the presence of untreated carbon black; (●), in the presence of carbon black having amino groups (0.29 mmol g^{-1}); (◐), 0.27 mmol g^{-1} ; (◑), 0.17 mmol g^{-1} .

the polymerization of γ -methyl L-glutamate NCA increases with an increase in the content of primary amino groups on carbon black.

Based on the above results, it is concluded that the polymerization of γ -methyl L-glutamate NCA is initiated by primary amino groups on carbon black.

Proof of Grafting onto Carbon Black

The carbon black obtained by the polymerization gave a stable colloidal dispersion in dichloroacetic acid. For instance, when the carbon black was dispersed in dichloroacetic acid and the dispersion was allowed to stand at room temperature, the carbon black particles precipitated could scarcely be observed even after one month. The phenomenon suggests that poly(γ -methyl L-glutamate) was grafted onto the carbon black surface.

Figure 2 shows the relationship between conversion and the percentage of grafting of the polypeptide onto carbon black obtained from the polymerization of γ -methyl L-glutamate NCA at 30°C by carbon black having amino groups (0.29 mmol g^{-1}).

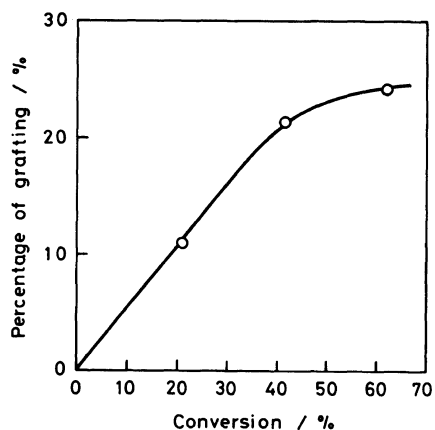


Figure 2. Relationship between conversion and percentage of grafting of poly(γ -methyl L-glutamate) onto carbon black having amino groups (0.29 mmol g^{-1}). Polymerization conditions are given in Figure 1.

From the results shown in Figure 2, it is apparent that in the polymerization, poly (γ -methyl L-glutamate) is grafted onto the carbon black surface and that the percentage of grafting increase gradually with an increase in conversion. This suggests that polypeptide is propagated from the carbon black surface.

Initiating Ability of Primary, Secondary, and Tertiary Amino Groups on Carbon Black

The effects of primary, secondary, and tertiary amino groups on the initiating ability of the polymerization of γ -methyl L-glutamate NCA and the grafting of polypeptide were examined. The results are shown in Table II.

As shown in Table II, the initiating ability of tertiary amino groups tended to be larger than that of primary amino groups. However, the percentage of grafting of polypeptide obtained from the polymerization by use of tertiary amino groups was lower than that of primary amino groups.

It is well known that when a primary amine is used as an initiator in the polymerization of α -amino acid NCA, the polymerization is initiated by nucleophilic attack of the amino group to the carbonyl carbon of α -amino acid NCA and further propagation is performed by

Table II. Polymerization of γ -methyl L-glutamate NCA initiated by carbon black having amino groups^a

Carbon black	Introduction reaction	Amino group	Conversion	Percentage of grafting/%
		mmol g ⁻¹	%	
CB-NH ₂	Eq. 1	0.23	35.6	17.4
CB-NH ₂	Eq. 2	0.29	62.0	23.7
CB-NH(CH ₃)	Eq. 4	0.20	52.4	19.6
CB-N(CH ₃) ₂	Eq. 5	0.29	75.1	5.3

^a Neospectra II, 0.30 g; γ -methyl L-glutamate NCA, 1.0 g; dioxane, 20.0 ml; 30°C; 4d.

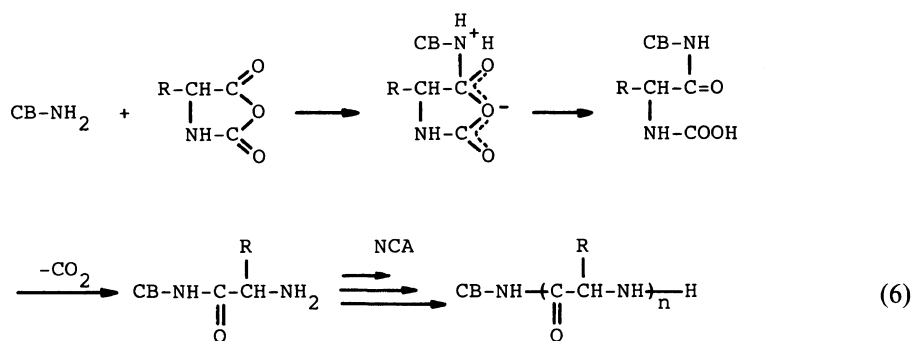
the attack of the amino group of the polymer to the carbonyl carbon of α -amino acid NCA.^{14,15}

On the other hand, when the polymerization is carried out by use of a tertiary amine as catalyst, the polymerization is initiated by nucleophilic attack of the α -amino acid NCA anion, which is formed by proton abstraction by the tertiary amine from the imino nitrogen of α -amino acid NCA, to the carbonyl carbon of α -amino acid NCA.^{16,17} Further propagation is considered to occur by nucleophilic attack of the NCA anion on the polymer.

Therefore, the mechanisms of the polymerization of α -amino acid NCA initiated by the

primary and tertiary amino groups on carbon black were considered as follows.

When primary amino groups on carbon black are used as initiators, the polymerization of γ -methyl L-glutamate NCA is initiated by nucleophilic attack of amino groups on carbon black to the carbonyl carbon of the NCA and further propagation is performed by the attack of the amino group of the polymer on the carbonyl carbon of the NCA. Therefore, the polypeptide was propagated from the primary amino group on the carbon black surface (eq 6). As a result, polypeptide was effectively grafted from the carbon black surface.

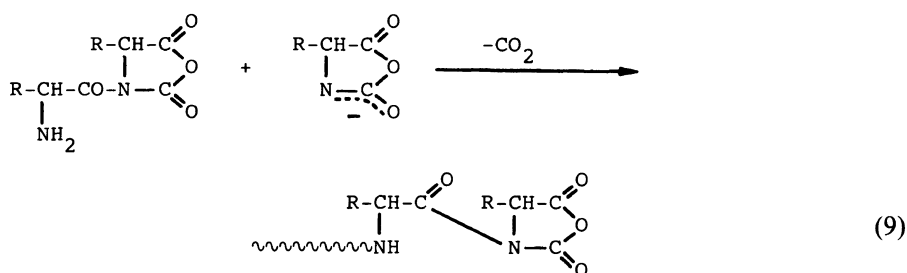
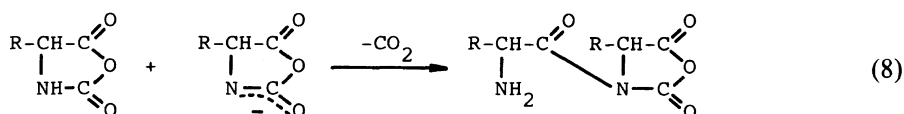
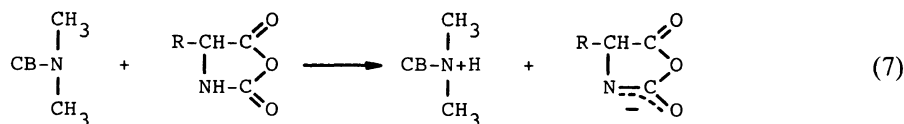


However, in the case of initiation by tertiary amino groups on carbon black, the tertiary amino groups abstract the proton from the imino nitrogen of γ -methyl L-glutamate NCA to generate the γ -methyl L-glutamate NCA anion (eq 7). The polymerization of γ -methyl

L-glutamate NCA is then initiated by the NCA anion (eq 8) and further propagation is performed by nucleophilic attack of the NCA anion to the polymer (eq 9). Therefore, the formation of ungrafted polymer preferentially proceeds and the grafting of polypeptide onto carbon

black scarcely occurs. Although a small amount of polypeptide remains on the carbon black surface obtained by the polymerization,

this may be due to the adsorption of the polymer on to the surface.



On the other hand, when secondary amino groups on carbon black were used as catalysts, the polymerization of γ -methyl L-glutamate NCA was considered to be initiated by the nucleophilic attack of both the amino groups on the carbon black (eq 6) and the NCA anion on the carbonyl carbon of NCA (eq 7).^{9,7}

Effects of Solvent on the Polymerization of γ -Methyl L-Glutamate NCA by Amino Groups on Carbon Black

By use of carbon black having primary or tertiary amino groups, the effects of solvent on the polymerization of γ -methyl L-glutamate NCA were investigated. The solvents used were dioxane, tetrahydrofuran (THF), acetonitrile, and *N*-methyl-2-pyrrolidone (NMP). The results with primary amino and tertiary amino groups are shown in Figures 3 and 4, respectively.

As shown in Figures 3 and 4, the decreasing

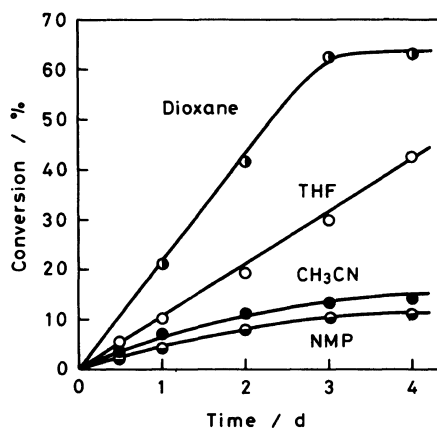


Figure 3. Effects of solvent on the polymerization of γ -methyl L-glutamate NCA initiated by carbon black having primary amino groups. Carbon black-NH₂, 0.30 g; γ -methyl L-glutamate NCA, 1.0 g; solvent, 20.0 ml; 30°C.

dielectric constant of solvent tends to increase the rate of the polymerization of γ -methyl L-glutamate NCA. Therefore, it was found that

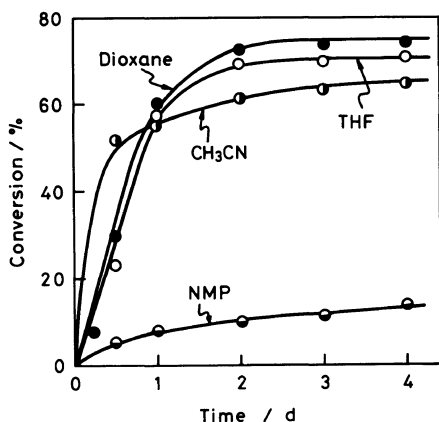


Figure 4. Effects of solvent on the polymerization of γ -methyl L-glutamate NCA initiated by carbon black having tertiary amino groups. Polymerization conditions are given in Figure 3.

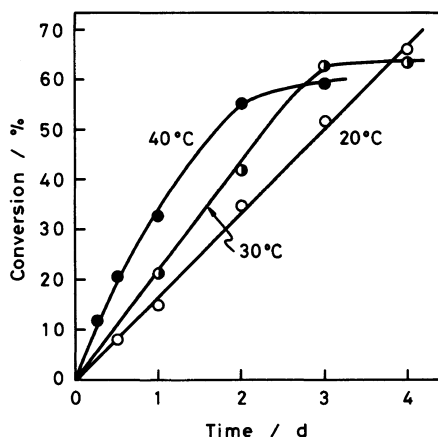


Figure 5. Effects of temperature on the polymerization of γ -methyl L-glutamate NCA initiated by carbon black having primary amino groups. Polymerization conditions are given in Figure 1.

to obtain a higher conversion, the polymerization should be carried out in a solvent with a lower dielectric constant such as dioxane or THF.

Effect of Temperature on the Polymerization of γ -Methyl L-Glutamate NCA

The effect of temperature on the polymerization of γ -methyl L-glutamate NCA by carbon black having primary amino groups was

examined. The results are shown in Figure 5.

The rate of polymerization was found to increase with rise in temperature. An Arrhenius plots for the above results gave a straight line and the apparent activation energy of the polymerization was estimated to be $15.9 \text{ kcal mol.}^{-1}$

CONCLUSIONS

It was found that primary, secondary, and tertiary amino groups can be introduced onto the carbon black surface by reaction of carbon black having acyl azide groups with ethylene diamine, N,N' -dimethylethylenediamine, and N,N -dimethylethylenediamine. The carbon black having these amino groups was capable of initiating the polymerization of γ -methyl L-glutamate NCA. The percentage of grafting of polypeptide obtained by the polymerization of the NCA initiated by primary amino groups on carbon black was larger than that initiated by tertiary or secondary amino groups. This may be due to the differences in the initiation and propagation mechanisms of the polymerization.

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