α-AMINO ACID N-CARBOXYANHYDRIDE POLYMERIZATIONS--A MECHANISTIC ANALYSIS
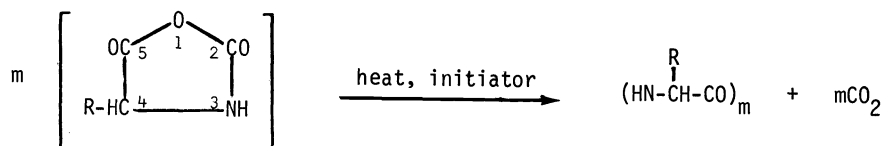
Murray Goodman* and Evaristo Peggion**

*Department of Chemistry, University of California, San Diego, La Jolla,
California 92093 **Institute of Organic Chemistry, University of Padua,
Via Marzolo 1, 35100 Padua, Italy

Abstract - An overview of the mechanisms of polymerization of α-amino acid N-carboxy-
anhydrides is presented. Particular attention is paid to the seminal work of
Bamford and his associates who carefully defined the scope of and the mechanistic
factors involved in the polymerization of N-carboxyanhydrides. Special note is
taken of the "active monomer" mechanism of polymerization originally proposed by
Bamford. The basis for the elucidation of these mechanisms of polymerization is
discussed.

INTRODUCTION

High molecular weight homo- and copolypeptides are useful models for studying the conforma-
tional properties of naturally-occurring peptides and proteins. This important class of
model compounds is usually prepared by polymerization or copolymerization of α-amino acid
N-carboxyanhydrides. Many such polymerizations have been published since 1958 (1-4), but
important aspects of the mechanisms remain to be elucidated. We desire to present our views
of the present state of understanding of the mechanisms of polymerization with special
emphasis on problems relating to the nature of the monomers and the conditions of polymeriza-
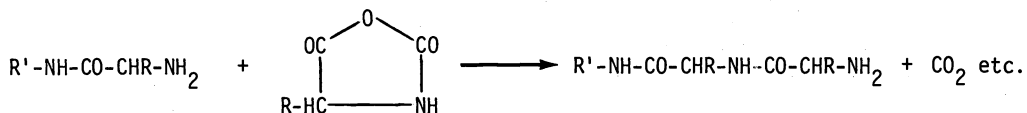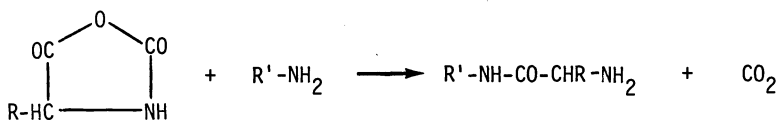tion.

Polymerization of N-carboxyanhydrides may be induced by a number of initiators, including
bases such as amines, alkoxides, hydroxyl anions, various salts, as well as by heat. The
reaction proceeds with evolution of carbon dioxide and under certain conditions may yield
high molecular weight polypeptides, according to the general reaction:

$$m \begin{bmatrix} \overset{O}{\underset{R-HC\underset{4}{\quad}\underset{3}{\quad}NH}{OC_5 \, 1 \, 2 CO}} \end{bmatrix} \xrightarrow{\text{heat, initiator}} \underset{(HN-\overset{R}{\underset{|}{C}H}-CO)_m}{} + \quad mCO_2$$

Using ^{14}C-labeled glycine N-carboxyanhydride, it has been demonstrated that carbon dioxide
arises only from carbonyl-2 of the N-carboxyanhydride ring (5). Despite the apparent simpli-
city of this reaction, a general polymerization mechanism which applies to all amino acid
N-carboxyanhydrides, or more appropriately to all types of initiators, has not been described.
The polymerization of N-carboxyanhydrides is sensitive to a number of chemical and physical
factors including the solubility of the polymer and the conformation assumed by the growing
chain. The tendency of polypeptides to undergo intermolecular aggregation further complicates
the elucidation of the polymerization process. Thus, most conclusions reached to date on the
polymerization mechanism of a given monomer-solvent-initiator system should be extrapolated
to other N-carboxyanhydride systems with great caution.

PRIMARY AMINE-INITIATED POLYMERIZATION

In the presence of primary amines, the polymerization of N-carboxyanhydrides proceeds accord-
ing to the so-called "normal" mechanism. The reaction is a multi-step nucleophilic attack by
the terminal amino group on the 5-carbonyl of the N-carboxyanhydride, followed by ring
opening and evolution of carbon dioxide. There is well-documented evidence that in the
"normal" primary amine-initiated polymerization each amine initiates one polymer chain, so
that the number-average degree of polymerization is given by the molar ratio of monomer (or
anhydride) to initiator (A/I). With the exception of a few cases reported in the literature
(6-7), the concentration of amino groups remains constant during the polymerization, and is

identical to the initial amine concentration (4). When the reactivity of the initiating amine is comparable to the reactivity of the terminal group of the growing chain, the polymerization rate follows simple first-order kinetics:

$$- \frac{d[M]}{dt} = k[M][I]_0$$

in which the rate constants for the individual reaction steps are identical. Under these circumstances, both theoretical and experimental results indicate that a very narrow molecular weight distribution (Poisson) is obtained (8-11). Systems conforming to such a reaction scheme include γ-benzyl-L-glutamate N-carboxyanhydride and N^ε-benzyloxycarbonyl-L-lysine N-carboxyanhydride (12) initiated by n-hexylamine in dimethylformamide, and DL-leucine N-carboxyanhydride initiated by primary amines or preformed polymers (13) in nitrobenzene. Examples of first-order kinetics are shown in Fig. 1 and refer to the polymerization of DL-leucine N-carboxyanhydride. Using ^{14}C-labeled initiators, 100% of the amine was shown to be
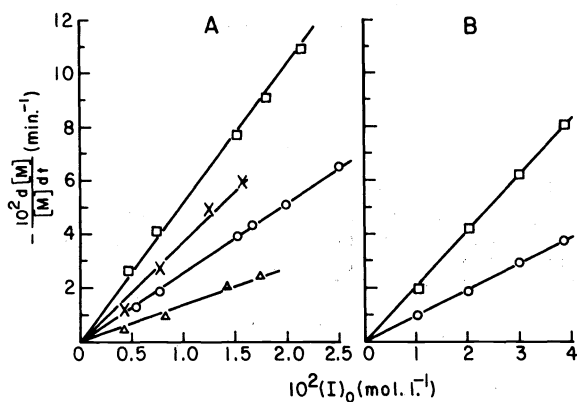

Fig. 1A. Polymerization of DL-leucine N-carboxyanhydride, initiated by preformed polymer $[I]_0$. Key: □ in nitrobenzene at 45°; X in o-nitroanisole at 45°; 0 in nitrobenzene at 25.2°; △ in o-nitroanisole at 25°. B. Polymerization of DL-phenylalanine N-carboxyanhydride in nitrobenzene, initiated by preformed polymer. Key: □ at 45°; 0 at 25° (taken from reference 13).

incorporated in the polymer (Table 1).

TABLE 1. Polymerization of γ-benzyl-L-glutamate N-carboxyanhydride in dimethylform-
amide and dioxane initiated by ^{14}C-labeled amines (14-15)

Initiator	A/I	% Radioactivity in the Polymer DMF	A/I	% Radioactivity in the Polymer DIOXANE
Isopropylamine	20	100.0	16	100.0
Diisopropylamine	17	4.3	24	10.9
Diisopropylamine	20	2.8	15	11.4
Methyldiisopropylamine	26	0.3	12	0.0
Methyldiisopropylamine			100	0.5

On the whole, the "normal" reaction scheme applies to N-carboxyanhydride polymerizations carried out in <u>polar</u> solvents such as dimethylformamide, o-nitroanisole, or nitrobenzene, using aliphatic primary amine-initiators, provided that the resulting polymer is soluble in the reaction medium. However, deviations from the above reaction pattern prove to be the rule rather than the exception. For example, the polymerization of γ-benzyl-L-glutamate N-carboxyanhydride initiated by n-hexylamine shows an entirely different kinetic behavior when an apolar solvent such as dioxane or tetrahydrofuran is used in place of dimethylformamide. Under these conditions the reaction becomes autocatalytic and the molecular weight distribution broadens (16). In fact, two first-order stages (Fig. 2) were observed for this polymerization in dioxane (17) with the second stage 5 times faster than the first. Since the
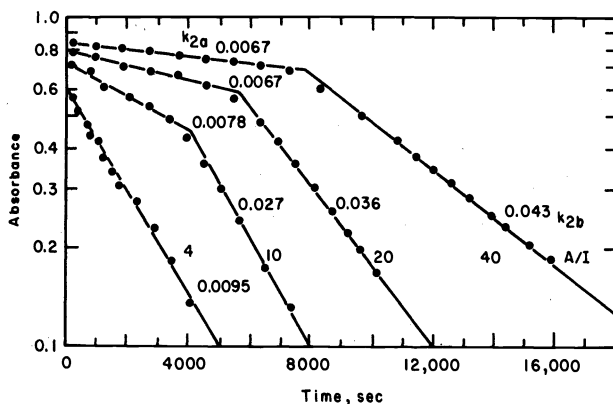

Fig. 2. Absorbance at 1860 cm^{-1} (corresponding to the C=O band of the N-carboxyanhydride) measured as a function of time during n-hexylamine-initiated polymerizations of the N-carboxyanhydride of γ-benzyl-L-glutamate at M/I = 4, 10, 20, and 40. The initial rate constants k_{2a}, are shown below the symbol. The final rates, k_{2b}, are shown in a horizontal line with the symbol (taken from reference 17).

break in the kinetic curve occurs at 20-40% conversion, when the degree of polymerization of the growing chain is of the order of 6-12, Idelson and Blout (17) suggested that the higher reaction rate may be due to the onset of helical structure for the growing chain. This suggests an effect of the polymer conformation on the rate of polymerization.

It has been shown that the critical chain length for L-glutamate oligomers to form an α-helix can be of the order of <u>7</u> amino acid residues (18). Accordingly, a model (shown in Fig. 3) was proposed (19) which accounts for the acceleration of the reaction rate when the chain folds into the helical structure. Fractionation experiments on polymers obtained in dioxane with primary amine-initiation show a bimodal distribution of molecular weights (20). One maximum is centered at a chain length of about 5 and the other at a chain length of about 135. A very broad molecular weight distribution is found ($\overline{M}_w/\overline{M}_n$ = 7) consistent with previous literature data (16-17) and with a theoretical analysis of the two-stage propagation process (21). These findings give further support to the theory of the conformational effect on polymerization.
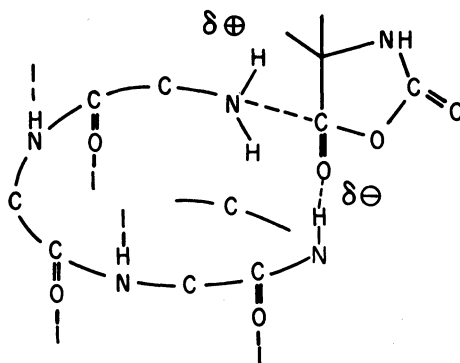
Fig. 3. Schematic diagram depicting the N-carboxyanhydride molecule interacting with a helical amine terminal end of the growing polymer chain (taken from reference 14).

The problem is actually more complex than suggested. Although poly(γ-benzyl-L-glutamate) assumes the α-helical form in dimethylformamide, no effect of the polymer conformation is observed on the kinetics of polymerization. In addition, at the monomer concentrations normally used in the kinetic experiments (2-4%) polypeptide chains tend to form intermolecular aggregates in apolar solvents such as dioxane and tetrahydrofuran. At the beginning of the reaction, growing oligomers have been shown to aggregate into β-structures (17). It has been recently suggested by Williams and Brown (22-23) that the coil-helix transition cannot be entirely responsible for the kinetic behavior. These authors interpreted their results in terms of formation of polymer aggregates at a critical concentration and chain length. The local concentration of monomer increases because of absorption on the aggregates which leads to the observed acceleration.

At present, the autocatalysis observed for N-carboxyanhydride polymerizations in apolar solvents remains incompletely understood. Apparently, contributions from both peptide aggregations and conformation affect the polymerization. However, in apolar solvents radioactive primary amines are totally incorporated into the polymer chain (Table 1). Thus, the initiation process occurs by quantitative nucleophilic attack at C-5 of the N-carboxyanhydride ring in a manner analogous to initiation in polar media.

Some of the complex kinetic behavior observed in solvents such as dioxane, has also been traced to monomer purity. Results from different laboratories are often conflicting. For example, the two-stage polymerization in dioxane can only be observed with very pure monomer. Finally, catalysis by the carbon dioxide evolved during chain growth has been observed (24).

In view of the above complications for primary amine-initiated polymerizations in apolar solvents, it is clear that the synthesis of polypeptides is better controlled if conducted in polar solvents such as dimethylformamide. To obtain peptide chains of well-defined degrees of polymerization, unhindered primary amines should be used as initiators, the polypeptide should be soluble in the solvent medium, and N-carboxyanhydride/initiator ratios should never exceed 100. At higher ratios polymerization is very slow and adventitious impurities may act as initiators or lead to side reactions. The above criteria are especially important when block sequences of defined length from various amino acid residues are needed for conformational analysis.

STRONG BASE OR TERTIARY AMINE INITIATION

One of the most important methods developed for the preparation of high molecular weight polypeptides from N-carboxyanhydrides involve the use of strong base or aprotic initiators. In the presence of initiators such as carbanions, alkoxides, alkaline hydroxides, and aprotic bases (such as tertiary amines), the polymerization of N-carboxyanhydrides exhibits entirely different features from those observed for primary amine-initiated polymerizations. The reaction rate is much faster and the degree of polymerization is much higher than the monomer-to-initiator molar ratio. In non-polar solvents such as dioxane, tetrahydrofuran or benzene,

the relevant kinetic characteristic is the presence of autocatalysis. Two reaction mechanisms
have been proposed to account for the experimental results, i.e., the carbamate mechanism and
the "active monomer" mechanism.

The carbamate mechanism

According to this mechanism of Idelson and Blout (25), a strong base initiator such as sodium
methoxide reacts with the monomer via nucleophilic attack at the 5-carbonyl of the N-carboxy-
anhydride ring:
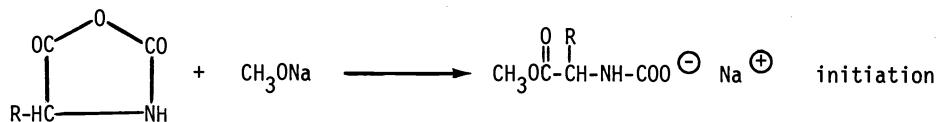
The nucleophilic attack of the base results in opening of the ring and formation of a carba-
mate species which is responsible for chain propagation. The chain grows by addition of
carbamate to a new N-carboxyanhydride molecule, with formation of the intermediate carbamic-
carboxyl mixed anyhydride, followed by carbon dioxide evolution and generation of a new re-
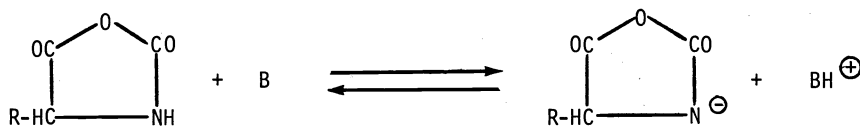active carbamate ion, according to the following reactions:
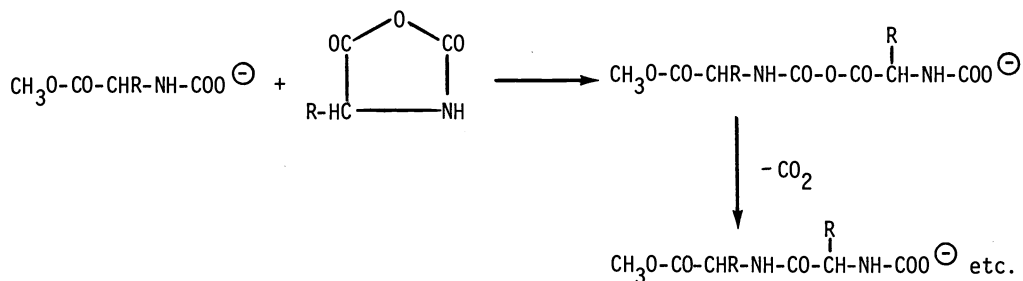
This reaction mechanism was originally proposed on the basis of the fact that at equimolar
concentrations the reaction of N-carboxyanhydrides with sodium methoxide leads to the forma-
tion of two products: the sodium salt of the carbamate ester and the sodium salt of the N-
carboxyamino acid ester.
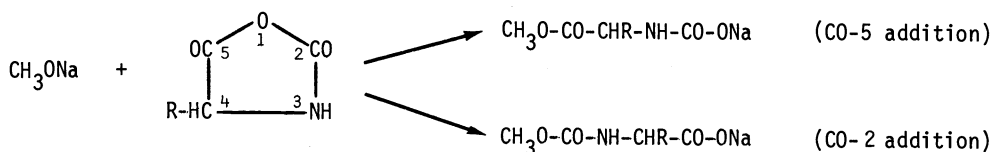
According to the above mechanism, each polymer chain must contain a fragment of the initiator.
Furthermore, in order to account for autocatalysis, the propagation step, namely carbamate
attack on an N-carboxyanhydride species, must be assumed to be much faster than methoxide
attack on an N-carboxyanhydride. Actually, since intermediate carbamate ions are formed even
with primary amine initiation (24), the carbamate mechanism does not appear to account for
the difference between strong base-initiated polymerization and primary amine-initiated
polymerization.

The active monomer mechanism

To account for polymerizations not conforming to the carbamate mechanism, Bamford and Block(24)
proposed a polymerization mechanism which was subsequently modified by Szwarc (3). According
to this mechanism, abstraction of a proton from the monomer leads to an "active monomer:"

The "active monomer", i.e. the N-carboxyanhydride anion, is responsible for very fast propagation according to the following series of reactions:
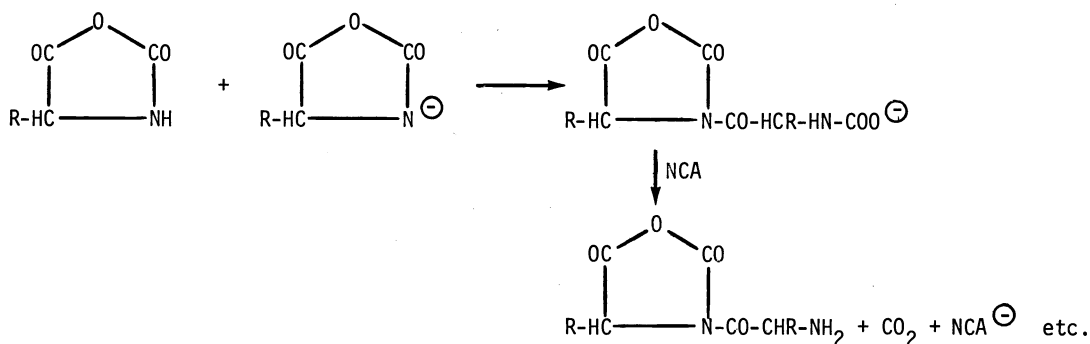
The chain grows by nucleophilic attack by the "active monomer" on the cyclic end of the bi-functional intermediate to form a carbamate which abstracts a proton from another N-carboxy-anhydride molecule. This regenerates the "active monomer" which can then attack the cyclic end of the growing chain. The driving force for the polymerization is the evolution of carbon dioxide. The polymerization is anionic, with the negative charge not on the growing chain (as in vinyl anionic polymerization) but on the "active monomer." This is completely analogous to the well-established "active monomer" mechanism for the anionic polymerization of cyclic lactams in the presence of strong base (26). In this case, abstraction of the amide proton by the base yields lactamate anions, the active species responsible for the rapid chain growth.

EXPERIMENTAL APPROACHES CONCERNING THE STRONG BASE OR TERTIARY AMINE-INITIATED POLY-MERIZATION

Most of the controversies on the polymerization of N-carboxyanhydrides concern the mechanism in the presence of strong bases such as alkoxides, or tertiary amines as initiators. In this section, we present a critical evaluation of the work carried out in the field, stressing the ambiguities in the literature.

Initiation by sodium methoxide and related compounds
The presence or absence of initiator fragments in the polymer provides the clearest distinc-tion between "active monomer" mechanism and "carbamate" mechanism. A number of experiments have been designed to detect initiator fragments in the polypeptide chains formed in strong base-initiated polymerizations. Goodman and coworkers (27-28) used ^{14}C-labeled sodium methoxide and 9-fluorenyl potassium to initiate the polymerization of γ-benzyl-L-glutamate N-carboxyanhydride in apolar solvents such as dioxane or tetrahydrofuran. The alkoxide is insoluble in the reaction medium and was used as a powder. The potassium salt of 9-fluorene is soluble both in dioxane or tetrahydrofuran, and the reaction therefore proceeds under homogeneous conditions. After the characteristic induction period of the polymerization, a high molecular weight polymer is formed. The sodium methoxide-initiated polymer contained only 6% radioactivity instead of 22% if each polymer chain were to possess an initiator fragment. The potassium fluorenyl-initiated polymer did not contain any fluroenyl fragments. In fact, using radioactive sodium benzyl carbamate as initiator in the same monomer-solvent systems, Goodman and Hutchison (28) found only 2.5% of the total radioactivity in the poly-mer. This figure is only 15% of the radioactivity expected if each polymer chain would contain an initiator fragment. The polymerization was very rapid, (90% conversion after 2 hours) yielding polymer with degree of polymerization ($\overline{DP}$)≈300 for a ratio of anhydride to initiator A/I = 52. Most important, the induction period in the kinetic curve was not eliminated as would be expected for propagation proceeding via carbamate. The results of this work appear to rule out the carbamate mechanism and give strong support to the active monomer mechanism.

On the other hand, recent results have been published which favor the carbamate mechanism for strong base-initiated polymerization. Seeney and Harwood (29) carried out polymerizations

with radioactive initiators. They used ^{14}C-sodium methoxide in <u>radioactive</u> methanol as the
initiator for the polymerization of γ-benzyl-L-glutamate N-carboxyanhydride, O-benzyloxy-
carbonyl-L-tyrosine N-carboxyanhydride, sarcosine N-carboxyanhydride,and L-leucine N-carboxy-
anhydride in dioxane. The authors showed the presence of methoxide groups in the polymers
either by radioactivity measurements or by nuclear magnetic resonance. Earlier Zilkha and co-
workers (30-31)were able to graft peptide chains to polysaccharide molecules using the sodium
derivative of the polysaccharide as an initiator. Such a result obviously implies nucleo-
philic addition of alkoxide units to N-carboxyanhydride molecules:
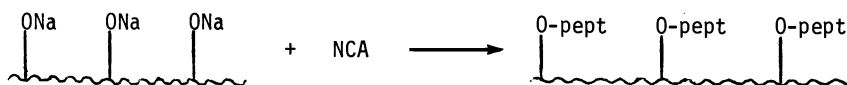
Giannakidis and Harwood (32) carried out experiments using radioactive sodium benzyl carba-
mate as the initiator for the polymerization of DL-valine N-carboxyanhydride and DL-leucine
N-carboxyanhydride in dioxane. In contrast to experiments of Goodman and Hutchison (28), the
polymerization rates appear unusually slow, reaching ∿70% conversion after 12 hours.

Sekiguchi and Doussin (33) reported that the sodium methoxide-initiated polymerization of
α-amino isobutyric acid N-carboxyanhydride can be explained by the co-existence of carbamate
and "active monomer" mechanisms. The equilibrium

$$NCA \quad + \quad CH_3ONa^{\oplus} \quad \rightleftharpoons \quad NCA^{\ominus} \quad Na^{\oplus} \quad + \quad CH_3OH$$
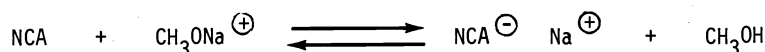
is shifted to the left in the presence of large amounts of methanol with consequent predomi-
nance of the carbamate mechanism over the "active monomer" mechanism.

The above results can all be rationalized by recognizing that the "active monomer" mechanism
obtains under certain conditions while the carbamate route may predominate under others.
Thus the use of ^{14}C-labeled methoxide in labeled methanol would be expected to involve some
polymerization via carbamates. In addition,with an heterogenous macromolecular initiator, it
is difficult to establish the "local" ratio of monomer-to-initiator, which depends upon the
concentration of the monomer within the macromolecular domain of the polysaccharide chain.
As noted above, sodium methoxide adds to the N-carboxyanhydride ring when the ratio of
alkoxide to anhydride is near to or greater than unity.

With regard to initiation by radioactive sodium benzylcarbamate,it is known that 50% of the
initiator decomposes into benzylamine and carbon dioxide in 24 hours. Therefore, it is not
clear whether the radioactivity is introduced into the polymer from the original initiator
or from the decomposition product, benzylamine.

Initiation by secondary and tertiary amines
Experiments carried out by independent research groups using ^{14}C-labeled amines (14-15,28)
provide a rather complete picture about the polymerization mechanisms. For the system
γ-benzyl-L-glutamate N-carboxyanhydride in dioxane with radioactive tertiary amine-initiators
are not incorporated into the polymer chain (Table 1). This is supporting evidence for
initiation via proton abstraction from the monomer by the tertiary amine (i.e., the "active
monomer" route). When ^{14}C-labeled secondary amines are used as initiators, the extent of
radioactivity in the polymer depends upon basicity and steric character of the initiating
amines. Highly hindered secondary amines behave the same as tertiary amines. Using [14]C-
labeled diisopropyl amine for the polymerization of γ-benzyl-L-glutamate N-carboxyanhydride
in dioxane between 10% and 20% of the initiator is incorporated in the polymer, depending
upon the monomer-to-initiator molar ratio (Table 1). These results are consistent with the
co-existence of nucleophilic addition of the initiator to the monomer, and "active monomer"
formation. The first mechanism leads to a radioactivelow molecular weight polymer, while the
second leads to a high molecular weight polypeptide which is not radioactive. It was in fact
demonstrated that practically all the radioactivity is extracted by hot methanol, which
dissolves species with chain length of about 30 or below.

The results obtained with tertiary amines are of particular importance since they lead to the
conclusion that if proton abstraction from an N-carboxyanhydride molecule is the initiating
step, N-substituted N-carboxyanhydrides cannot polymerize. Extremely pure sarcosine or
proline N-carboxyanhydrides cannot be polymerized by tertiary amines. Sarcosine N-carboxy-
anhydride has been reported to polymerize very slowly with tertiary amines that are not
rigorously purified. However, if 3-methyl hydantoin is added to the reaction mixture in

dimethylformamide, rapid polymerization takes place even with the most pure tertiary amine. This observation strongly favors the initiation via proton abstraction. The 3-methyl hydanto-in reacts with the tertiary amine as follows:
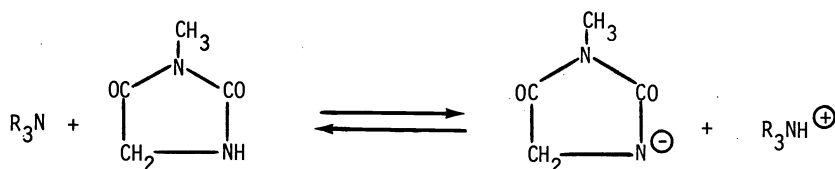
The resulting anion adds to an N-carboxyanhydride molecule, according to the reaction:
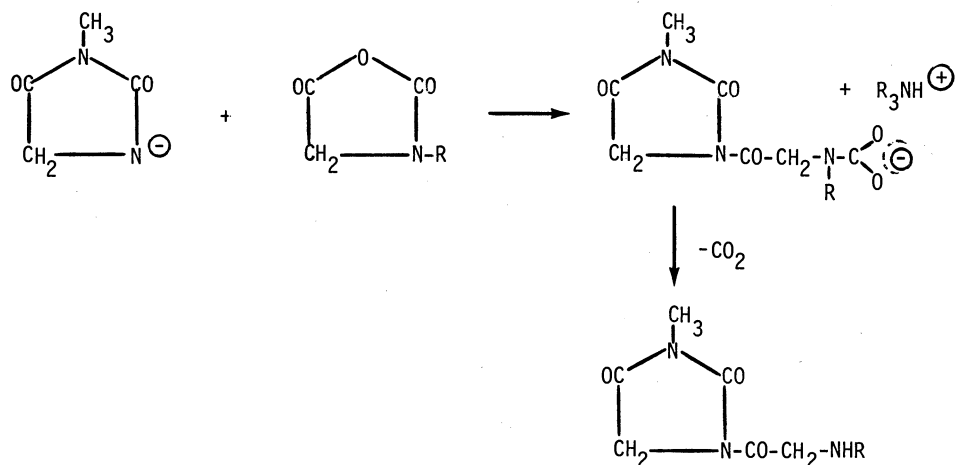
Polymerization proceeds then via nucleophilic attack by the amine terminal groups on monomer molecules. Bamford et al., (34) allowed N-phenylglycine N-carboxyanhydride to react with 3-methyl hydantoin in the presence of tertiary amines. They obtained the expected N-phenylglycyl derivative of the hydantoin, according to the reaction:
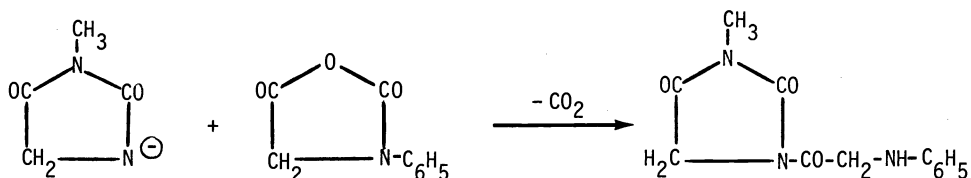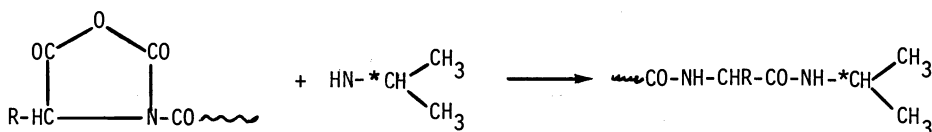

The above reaction mechanism requires incorporation of 3-methyl hydantoin at the end of the polymer chain. This was shown by Bamford and his associates for the polymerization of sarcosine N-carboxyanhydride (34).

One of the most important consequences of the Bamford mechanism is the formation of a bifunctional intermediate containing an amine and a cyclic end.
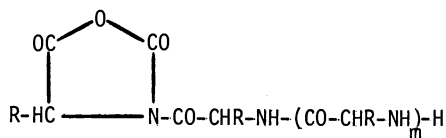
For tertiary amine-initiated polymerization of γ-benzyl-L-glutamate N-carboxyanhydride in dimethylformamide, direct evidence has been obtained for the existence of the bifunctional intermediate (35). Addition of a large excess of ^{14}C-labeled primary amine leads to the destruction of cyclic ends, according to the reaction:

$$OC \overset{O}{\underset{R-HC———N-CO \sim\sim\sim}{\diagup\diagdown}} CO \quad + \quad HN-*CH \overset{CH_3}{\underset{CH_3}{\diagup\diagdown}} \quad \longrightarrow \quad \sim\sim CO-NH-CHR-CO-NH-*CH \overset{CH_3}{\underset{CH_3}{\diagup\diagdown}}$$

with resulting incorporation of radioactivity in the polymer. It has also been shown that there is a marked increase of the molecular weight when the dimethylformamide solvent is reduced in volume. This arises from extensive coupling between bifunctional growing chains.

Recent work carried out by Kricheldorf (35-36) provides additional evidence that nucleophilic addition and "active monomer" formation can co-exist as mechanistic processes in the case of secondary amine initiation, and that the relative importance of the two mechanisms depends upon the balance between nucleophilicity and basicity of the initiator. The authors investigated the polymerization of glycine N-carboxyanhydride, L-alanine N-carboxyanhydride, and L-phenylalanine N-carboxyanhydride initiated by a variety of secondary amines. The reaction products were isolated and carefully analyzed, using both chemical and physical methods. The most important results are collected in Table 2.
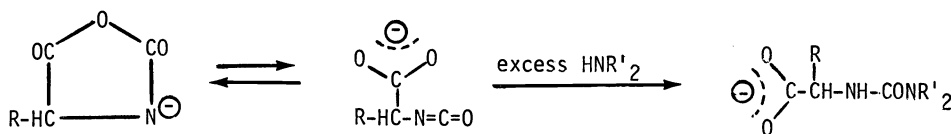
Table 2. Conversion of α-amino acid N-carboxyanhydrides (NCAs) with stoichiometric amounts of secondary amines in dioxane (36)

| No. of experiment	NCA of	Secondary amine	Mole ratio NCA/amine	Yield of polypeptide in %	Yield of subst. hydantoic acid in %	Reaction[a] temp. in °C
1		Diethylamine	1:1	0	32.0	25-40
2		Diethylamine	1:3	0	75.0	25-40
3	DL-Phenyl-		1:6	0	86.0	25-40
4	alanine	Diisopropyl-amine	1:1	97.0	0	25-30
5		Dicyclohexyl-amine	1:1	98.0	0	25-30
6		Morpholine	1:2	0	38.5[b]	25-45
7		Diethylamine	1:2	1.0	75.5[b]	25-45
8	Glycine	Diisopropyl-amine	1:2	95.5	0	25-45
9		Dicyclohexyl-amine	1:2	97.0	0	25-45

a) The temperature rises during the exothermic reaction.
b) Products soluble in alkaline water.

The reactions of α-amino acid N-carboxyanhydrides with stoichiometric amounts of secondary amines in dioxane show that amines with small substituents react preferentially as nucleophiles. However, the yield of hydantoic acid increases in the presence of a large excess of secondary amine. The large excess of amine increases the basicity of the reaction medium thus favoring the formation of N-carboxyanhydride anion which leads to the hydantoic acid, according to the following reactions:

$$OC \overset{O}{\underset{R-HC———N^{\ominus}}{\diagup\diagdown}} CO \quad \rightleftharpoons \quad O \overset{\ominus}{\underset{R-HC-N=C=O}{\diagdown C \diagup}} O \quad \xrightarrow{\text{excess } HNR'_2} \quad O^{\ominus} \overset{O}{\underset{O}{\diagup\diagdown}} C-CH \overset{R}{\underset{}{}} -NH-CONR'_2$$

Kricheldorf showed that under identical conditions, the yield of glycine N-carboxyanhydride anion and subsequently of hydantoic acid derivatives increases with the bulkiness of the substituents. Diethylamine gives more hydantoic acid than does morpholine. Bulky groups on the amine, such as diisopropyl or dicyclohexyl completely prevent nucleophilic attack on the N-carboxyanhydride ring or the isomeric α-isocyanatocarboxylates. Such secondary amines react as tertiary amines, producing high molecular weight polypeptides in quantitative yield. Kricheldorf (35) also showed that the identification of the reaction product of DL-phenylalanine N-carboxyanhydride with diethylamine by Seeney and Harwood (29) was not carbamate as they claimed, but rather hydantoic acid:

$$(C_2H_5)_2N\text{-}CO\text{-}NH\text{-}\underset{\underset{CH_2C_6H_5}{|}}{CH}\text{-}CO\text{-}OH$$
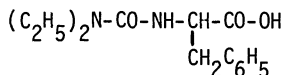
In a most recent approach to tertiary amine-initiated polymerization, Kricheldorf employed a series of co-catalysts for the polymerization of glycine N-carboxyanhydride, alanine N-carboxyanhydride, and sarcosine N-carboxyanhydride in dioxane (37). The co-catalyst effect was evaluated by reaction rate measurements and end group analysis. It turns out that N-acyl co-catalysts such as:


where X = O, S
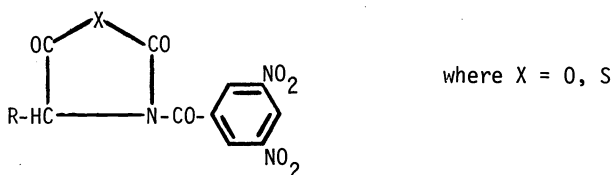
enhance the reaction rate and are incorporated into the polymer chain. Both these effects are entirely consistent with the "active monomer" mechanism. In the following reactions:

(1)

(2)

(3)
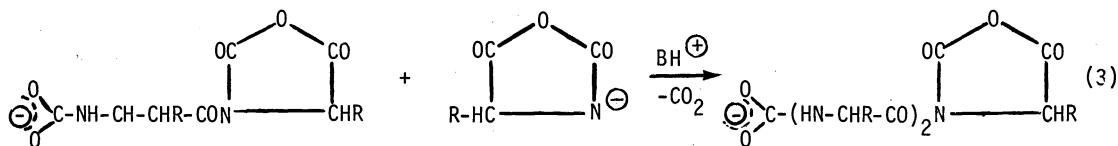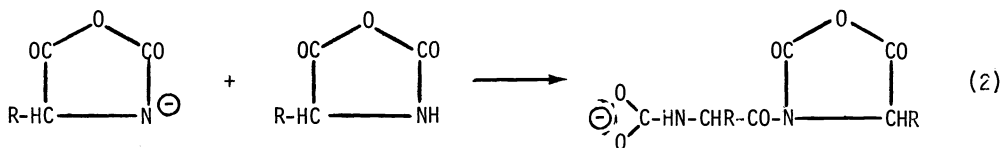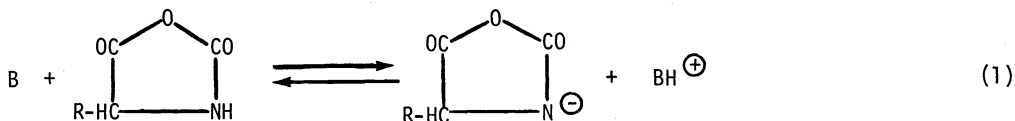
if reaction (2) is slower than reaction (3), it becomes the rate-determining step. With a co-catalyst, the slow reaction (2) is replaced by the faster reaction (4):

$$\text{(4)}$$

(co-catalyst)

where X = 0,S
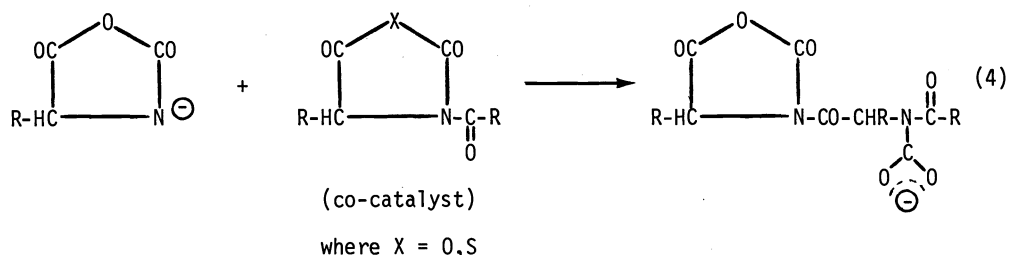
As a consequence, a fragment of the <u>co-catalyst</u> becomes the end residue of the polymer chain. The co-catalyst must be more reactive than the monomer toward N-carboxyanhydride anions in order to accelerate the polymerization. Thus, anhydrides such as:

or
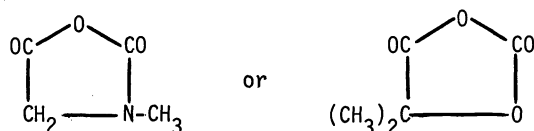
"cannot act as accelerants." However, since they compete with the monomer in the reaction with N-carboxyanhydride anions, they do form end groups in the polypeptide chain.

In the same work (37), it was also shown that sarcosine N-carboxyanhydride in the presence of triethylamine polymerizes very slowly by an entirely different mechanism. The opening of the ring must be catalyzed by adventitious impurities. The polymerization then proceeds via nucleophilic addition of monomer molecules. Substantial evidence has been accumulated to show that polymerization of L-phenylalanine N-carboxyanhydride, glycine N-carboxyanhydride, and L-alanine N-carboxyanhydride in dioxane initiated by tertiary amines or hindered secondary amines proceeds by the "active monomer" mechanism.

Bamford and his associates have long ago established that tertiary amines initiate the polymerization of N-unsubstituted N-carboxyanhydrides in polar solvents. They have also ruled out the need to consider adventitious impurities as catalysts. Furthermore, sodium hydride has once again been shown to be a powerful initiator for the polymerization of γ-benzyl-L-glutamate N-carboxyanhydride and Nε-benzyloxycarbonyl-L-lysine N-carboxyanhydride (38). Katchalski and Sela (1), Bamford and Block (24), and Bailey (39) demonstrated that carbamates are formed under appropriate conditions. Kopple and his associates (40-42) showed that the rate of decarboxylation of the mixed carbamic-carboxylic anhydride is too slow to account for the high reaction rates observed with tertiary amines. Other strong bases such as methoxide (28) hydride (38), and the fluorenyl anion (27) similarly promote rapid polymerizations which proceed via the "active monomer" mechanism.

KINETIC STUDIES OF STRONG BASE OR TERTIARY AMINE-INITIATED POLYMERIZATION OF N-CARBOXYANHYDRIDES

The problem of strong-base or tertiary amine-initiated polymerization has been also approached using kinetic methods, and the determination of molecular weight distribution. For the sake of clarity, we will discuss results obtained in apolar solvents such as dioxane, tetrahydrofuran, or benzene separately from the results obtained in polar solvents (such as dimethylformamide).

The characteristic feature of the sodium methoxide or tertiary amine-initiated polymerization of γ-benzyl or γ-ethyl-L-glutamate N-carboxyanhydride in <u>apolar</u> solvents is the presence of autocatalysis occurring at 2-30% conversion (Fig. 4). Kinetic analysis showed that a very
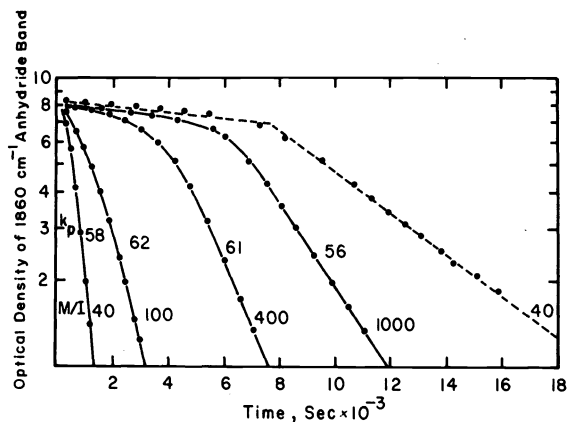
Fig. 4. Plots of optical density of 1860 cm^{-1} anhydride C=O band measured as a function of time during sodium methoxide-initiated polymerization of γ-benzyl-L-glutamate N-carboxyanhydride in dioxane solution (4 g/100 ml) at M/I = 40, 100, 400, and 1000. The dashed line is a rate curve for an M/I = 40 n-hexyl-amine-initiated polymerization. The polymerization rate constants (k_p) are shown in a horizontal line with the symbol (taken from reference 17).

simple reaction scheme accounts reasonably well for the experimental data. Initiator, I, reacts with a monomer molecule, forming the active species P_1. The active species P_1 then adds monomer very rapidly according to the following reactions:

$$I + M \xrightarrow{k_1} P_1$$

$$P_1 + M \xrightarrow{k_2} P_2$$

$$P_2 + M \xrightarrow{k_2} P_3$$

$$P_n + M \xrightarrow{k_2} P_{n+1}$$
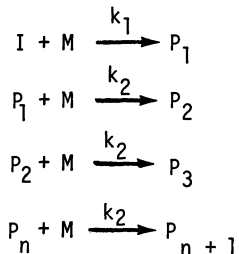
A complete kinetic treatment of this reaction scheme has been formulated by Shalitin (4). If [P] is the concentration of all growing chains, and assuming $k_2 \gg k_1$, for the rate of monomer consumption we can write:

$$- \frac{d[M]}{dt} = k_2[P][M] \tag{1}$$

The rate of formation of growing chains is given by:

$$\frac{d[P]}{dt} = k_1[I]_0[M] \tag{2}$$

Dividing (2) by (1) we obtain:

$$\frac{d[P]}{d[M]} = \frac{k_1}{k_2} \frac{[I]_0}{[P]} \tag{3}$$

or, in the integrated form:

$$[P] = \left\{ \frac{2k_1}{k_2} [I]_0 ([M]_0 - [M]) \right\}^{1/2} \tag{4}$$

which gives the concentration of growing chains as a function of the kinetic parameters. By combining (4) and (1) we obtain:

$$- \frac{d[M]}{dt} = [M] \sqrt{2k_1 k_2 [I]_0 ([M]_0 - [M])} \tag{5}$$

Integration of this equation gives:

$$\ln \frac{[M]_0^{1/2} - ([M]_0 - [M])^{1/2}}{[M]_0^{1/2} + ([M]_0 - [M])^{1/2}} = -t(2k_1 k_2 [I]_0 [M]_0)^{1/2} \tag{6}$$

The number-average degree of polymerization is given by:

$$\overline{DP}_n = \frac{[M]_0 - [M]}{[P]} \tag{7}$$

Combining (7) with (4), after complete conversion we obtain:

$$\overline{DP}_n = \frac{k_2}{2k_1} \frac{[M]_0}{[I]_0} \tag{8}$$

As shown in Fig. 5, equation (6) fits with the experimental data in the case of the sodium methoxide-initiated polymerization of γ-benzyl-L-glutamate N-carboxyanhydride in dioxane giving k_2/k_1 1.5 x 10^4. Similarly, data obtained for the polymerization of N^ε-benzyloxycarbonyl-L-lysine N-carboxyanhydride gave $k_2/k_1 \simeq$ 2 x 10^5.

A detailed theoretical analysis of the molecular weight distribution function, equation (8), predicts a heterogeneity ratio $\overline{M}_w/\overline{M}_n$ of 1.33 when $k_2 >> k_1$. Experimental determinations of the distribution function have shown that for sodium methoxide or tertiary-amine-initiated poly(γ-benzyl-L-glutamate), $\overline{M}_w/\overline{M}_n$ is of the order of 1.35 - 1.40 (43-44).

The carbamate mechanism cannot account for such a large value for the ratio k_2/k_1. Initiation and the subsequent steps are chemically identical, and even a change of the polymer conformation such as that suggested for the primary amine-initiation could at most account for a change of the rate constant by a factor of 10-100. On the other hand, the initiation and propagation steps are intrinsically different in the "active monomer" mechanism. Initiation, in fact, involves nucleophilic attack by an N-carboxyanhydride anion molecule on a monomer molecule (see above).

In the same way there is a difference between initiation and propagation steps in the anionic polymerization of ε-caprolactam where the faster addition of lactamate ions to N-acyl lactam units accounts for the observed autocatalysis in the polymerization process (26). However, even in this case, a rate increase of the order of 10^5 - 10^6 for the addition of N-carboxyanhydride anion to N-acyl N-carboxyanhydride is far greater than expected.
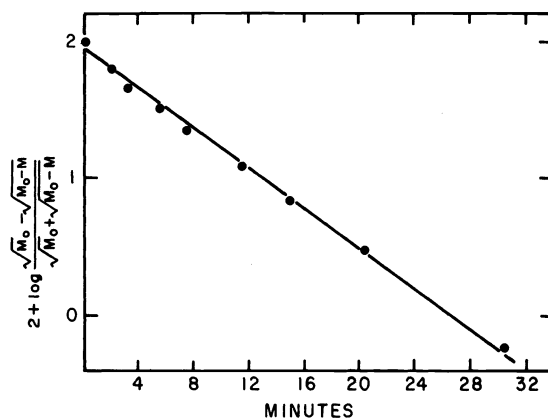
Fig. 5. The polymerization reaction depicted as the function $\log [(\sqrt{M_0} - \sqrt{M_0 - M})/$ $(\sqrt{M} + \sqrt{M_0 - M})]$ versus time. M_0 denotes the initial concentration of the α-amino acid N-carboxyanhydride and M is the concentration of intact α-amino acid N-carboxyanhydride (taken from reference 4).

When discussing the primary amine-initiated polymerization in apolar solvents, physical factors such as changes of conformation of the growing chain and, most importantly, adsorption of monomers on polymer aggregates have been invoked to account for autoacceleration. It is very likely that these physical factors also play a significant role in the strong base or tertiary amine-initiated polymerization in apolar solvents. For instance, an increase of the local concentration from adsorption of monomer on growing chain aggregates could be responsible for part of the autoacceleration. The kinetic behavior is complicated by these factors, and it is difficult to account for them in a quantitative way. It is therefore difficult to draw very definite conclusions from kinetic experiments.

The polymerization kinetics of tertiary amine-initiated polymerization of N-carboxyanhydrides in polar solvents such as dimethylformamide appear simpler. In this solvent, polymerizations do not exhibit autocatalysis, but the degree of polymerization of the final polymer is always much higher than the monomer-to-initiator ratio. Bamford and Block (24) used a series of tertiary amines such as pyridine, α-picoline and 2,6-lutidine for the initiation of the γ-ethyl-L-glutamate N-carboxyanhydride polymerization. The most effective of these is 2,6-lutidine, the most basic (and least nucleophilic) amine of the series (Fig. 6). This is a strong argument in favor of the initiation via proton abstraction from the N-carboxyanhydride rather than nucleophilic attack by the amine on the 5-carbonyl of the monomer.

It has also been shown (45) that initiation by tertiary amines in dimethylformamide leads to poly(γ-benzyl-L-glutamate) having the "most probable" Flory distribution with $\overline{M}_w/\overline{M}_n = 2$. This type of distribution function can be due to random coupling of bifunctional intermediates as postulated by Bamford:

$$x = m + n + 1$$
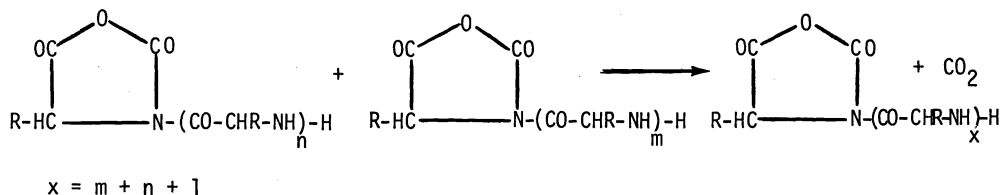
Again, these experimental results represent kinetic evidence for the presence of bifunctional intermediates.
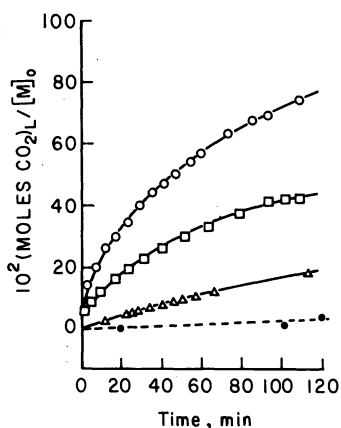
Fig. 6. Conversion-time curves for the polymerization of γ-ethyl-L-glutamate N-carboxyanhydride (0.2 mole 1.$^{-1}$) in N,N-dimethylformamide by pyridine and homologues (2.0 mole 1.$^{-1}$) at 25°C.

  O 2,6-Lutidine.
  □ α-Picoline.
  △ Pyridine.

The broken curve shows a comparative experiment with sarcosine N-carboxyanhydride (0.2 mole 1.$^{-1}$) and 2,6-histidine (2.0 mole 1.$^{-1}$) (taken from reference 25).

Ballard and Bamford (46) derived the following kinetic equation for the initial reaction rate:

$$\left(\frac{d[CO_2]}{dt}\right)_{initial} = K[M]_o^2$$

which is consistent with the experimental results. However, no kinetic treatment has been developed which accounts for the kinetic behavior over the whole range of conversion.

The kinetic approach to N-carboxyanhydride polymerization in apolar solvents leads to a reaction scheme which differentiates between the carbamate mechanism and "active monomer" mechanisms. The latter involves intrinsically different initiation and propagation steps while the former is based on essentially identical initiations and propagations. Thus, qualitatively the observations based on equations (6) and (8) clearly favor an "active monomer" mechanism. The kinetic results appear to be affected by physical factors (monomer adsorption on the growing chains, etc.), which make it difficult to use kinetics for the quantitative explanation of polymerization mechanisms.

CONCLUSION

We have presented an overview of the mechanistic aspects of the polymerizations of N-carboxy-anhydrides. We have stressed an distinguished between the "active monomer" and carbamate routes for polypeptide formation. Both mechanisms are operative under appropriate conditions of initiator, promoter, solvent,and the monomer-to-initiator ratio. Kinetic studies are included to indicate the complexity of these polymerizations. Qualitative support for the "active monomer" route can be obtained while quantitative information cannot be derived from these experiments.

Much remains to be done to explain N-carboxyanhydride polymerizations and copolymerizations. Other lectures in this symposium treat the areas of stereoselectivity (47) and β-amino acid N-carboxyanhydride polymerizations (48). Modern techniques such as high frequency nuclear magnetic resonance and other spectroscopic tools will play central roles in the elucidation of the mechanisms of these polymerizations. These techniques will allow the determination of

conformational effects and perhaps even provide a basis for the quantitative analysis of the kinetics of amino acid N-carboxyanhydride polymerization.

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