212. *The Heterogeneous Polymerization of α-*N*-Carboxyamino-acid Anhydrides.*

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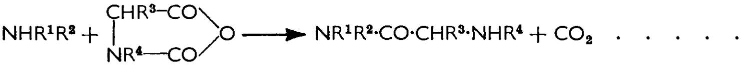
The polymerizations of the *N*-carboxy-anhydrides of sarcosine, L-alanine, L-leucine, and L-proline initiated by primary bases have been studied in conditions such that polymers are precipitated at an early stage of reaction. In each case separation of polymer is accompanied by an increase in rate. It is concluded that the increase arises primarily from the heterogeneity, and possible mechanisms are discussed. The bearing of these results on the postulated existence of two successive propagation coefficients in the polymerization of the *N*-carboxy-anhydrides of optically active α-amino-acids is considered.

THE mechanism of polymerization of the *N*-carboxy-anhydrides of DL-α-amino-acids in solution has been the subject of numerous investigations. When primary or secondary bases are used as initiators the base adds to $C_{(5)}$, and subsequent ring opening and elimination of carbon dioxide lead to formation of a substituted amide of the amino-acid: [1,2]



(1)

This product, being a base, can react similarly with further molecules of anhydride to form a linear polypeptide. The general propagation reaction is shown in (2):

(2)

Kinetic investigations have indicated that if $R^4 = H$ this reaction is of first order in anhydride and base concentrations. In the general case the velocity coefficients of initiation and of propagation are different, but all the propagation steps, with the exception of the first two, probably have similar coefficients.[1] A particularly simple situation arises when initiation is carried out by a preformed short polymer; the overall reaction is then of first order throughout its whole course. With *N*-carboxysarcosine anhydride ($R^3 = H$, $R^4 = Me$) the propagation reaction is complicated by catalysis by carbon dioxide,[1] probably through the participation of carbamate ions.

Some *N*-carboxy-anhydrides, particularly those derived from optically active amino-acids, cannot be polymerized in homogeneous systems on account of the insolubility of the resulting polypeptides in suitable reaction media. When studying one such polymerization—that of α-*N*-carboxy-L-alanine anhydride in nitrobenzene—we found that a marked acceleration occurs when the system becomes heterogeneous. About this time Doty and Lundberg [3] and Lundberg and Doty [4] published results on the polymerization of the γ-benzyl ester of α-*N*-carboxy-L-glutamic anhydride in dioxan solution which were interpreted in terms of two successive propagation coefficients; the first corresponded to the growth of the chains up to a certain critical size, and the second, about five times as large, was considered to apply to subsequent growth. Similar observations were later reported by Idelson and Blount [5] and Weingarten.[6] Lundberg and Doty, on the basis of optical-rotation studies by Mitchell, Woodward, and Doty,[7] suggested that chains shorter than the critical size were predominantly randomly coiled, while those larger were mainly

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[1] Ballard and Bamford, *Proc. Roy. Soc.*, 1954, *A*, **223**, 495.

[2] Breintenbach and Allinger, *Monatsh.*, 1953, **84**, 1103.

[3] Doty and Lundberg, *J. Amer. Chem. Soc.*, 1956, **78**, 4810.

[4] Lundberg and Doty, *ibid.*, 1957, **79**, 3961.

[5] Idelson and Blout, *ibid.*, p. 3948.

[6] Weingarten, *ibid.*, 1958, **80**, 352.

[7] Mitchell, Woodward, and Doty, *ibid.*, 1957, **79**, 3955.

α-helical; the latter configuration was therefore supposed to give rise to an increased rate of propagation. This idea has been elaborated by Weingarten,[6] who has considered in detail the nature of the transition state in the mechanism of propagation involving α-helical polypeptides. We have not been able to confirm these findings with the γ-benzyl ester of α-*N*-carboxy-L-glutamic anhydride.[8] The anhydride is not ideal for kinetic work because it cannot be purified by sublimation and some of the recorded differences may be due to the presence of traces of unidentified impurities.

Lundberg and Doty[4] recently extended their work to include the polymerization of α-*N*-carboxy-L-leucine anhydride in nitrobenzene and reported essentially similar results to those they obtained with the γ-benzyl ester of α-*N*-carboxy-L-glutamic anhydride. They mentioned that the system is heterogeneous but did not consider that this feature affected their interpretation in terms of configurational changes of the growing chains. It appeared to us that this polymerization is similar to that of α-*N*-carboxy-L-alanine anhydride in nitrobenzene already mentioned and we have undertaken a kinetic investigation of these and other heterogeneous polymerizations. This work is reported in the present paper. All four systems studied show similar kinetic features which we attribute to the onset of heterogeneity.

EXPERIMENTAL

Materials.—The *N*-carboxy-anhydrides of sarcosine, L-alanine, L-leucine, and L-proline, prepared by standard methods,[9,10] were recrystallized from the solvents mentioned in the literature until completely free from chlorine ($<0{\cdot}01$ mole %) and were sublimed in a vacuum before use.

The purification of nitrobenzene has already been described.[1] " AnalaR " benzene was stored over sodium wire and distilled.

" AnalaR " *n*-hexylamine was dried (KOH pellets), then distilled in a vacuum into ampoules which were subsequently sealed off. The preparation and purification of sarcosine dimethylamide were carried out as described earlier.[1]

Apparatus and Technique.—The rates of polymerization of the *N*-carboxy-anhydrides were generally measured by the rates of evolution of carbon dioxide in a constant-volume apparatus,[1] but a few experiments were carried out in a constant-pressure apparatus [1] or under conditions of virtually zero carbon dioxide pressure.[11] The last experiments were necessary to establish that catalysis of the reactions by carbon dioxide [1] did not play a significant part in the constant-volume experiments.

Access of atmospheric moisture to the reactants was excluded by conducting all manipulations involved in preparing the reaction vessel in a dry box.[1]

In all the experiments described below, the *N*-carboxy-anhydrides were initially completely dissolved in the reaction media.

RESULTS AND DISCUSSION

(i) *The* α-N-*Carboxysarcosine Anhydride–Benzene System.*—The results of initiation by *n*-hexylamine are shown in Figs. 1, 2(*a*), and 2(*b*) for three different base concentrations, $[M]/[M]_0$ being plotted logarithmically as a function of time. Here [M] is the anhydride concentration at time *t*. Each curve consists of four portions. The initial steep portion *AB* corresponds to the reaction of *n*-hexylamine with the anhydride; this base is stronger than the basic derivative subsequently produced and hence reacts faster. This type of behaviour has already been referred to and has no special interest here. From *B* to *C*, $\log [M]/[M]_0$ is approximately linear in *t*; at *C* the slope of the plot begins to increase but eventually a second nearly linear portion is observed, extending to *D*. After *D* the slope begins to decrease progressively. A comparison of Figs. 1, 2(*a*), and 2(*b*) shows that the relative importance of the various steps depends on the initiator concentration.

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[8] Ballard and Bamford, *J. Amer. Chem. Soc* 1957 **79**, 2336.
[9] Hanby, Waley, and Watson, *J.*, 1950, 3009.
[10] Katchalsky, Kurtz, Fasman, and Berger, *Bull. Res. Council Israel*, 1956, **5**, *A*, No. 4.
[11] Ballard, Bamford, and Weymouth, *Proc. Roy. Soc.*, 1955, *A*, **227**, 155.

As far as can be ascertained visually, the system becomes heterogeneous just before the point *C* is reached in each case. Between *C* and *D* the polymer is dispersed, while after *D* coagulation to a more compact precipitate occurs.

The calculated number average degrees of polymerization corresponding to the points *C* and *D* are given in the Table, together with the approximate velocity coefficients, $-(\mathrm{d[M]/d}t)/[\mathrm{M}][\mathrm{X}]$, for the steps *BC*, *CD*. Here [X] represents the (constant) base concentration. A run was carried out with sarcosine dimethylamide as initiator in place of *n*-hexylamine, having [M]/[X] = 10 initially. The results were indistinguishable from those with *n*-hexylamine.

FIG. 1. *Reaction of* N-*carboxysarcosine anhydride with* n-*hexylamine in benzene at* 25°: $[\mathrm{M}]_0 = 0{\cdot}100$ mole l.$^{-1}$; $[\mathrm{X}]_0 = 10^{-2}$ mole l.$^{-1}$.

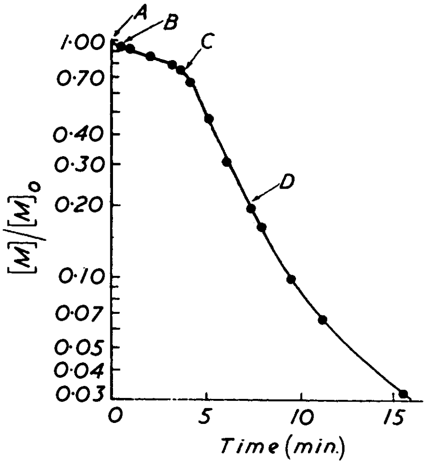
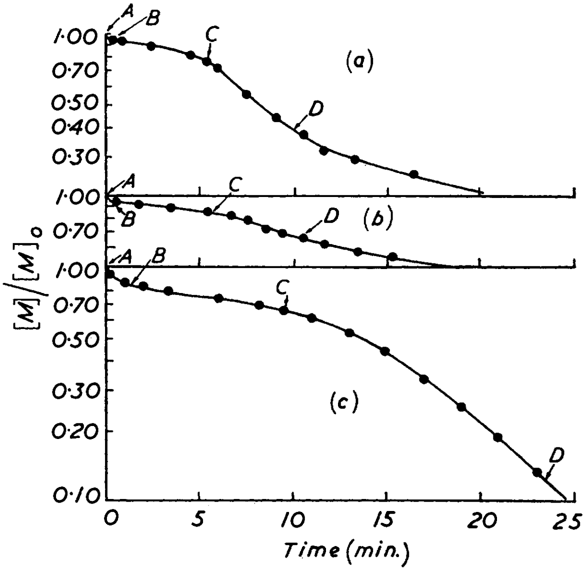


FIG. 2. (*a*), (*b*) *Reaction of* N-*carboxysarcosine anhydride with* n-*hexylamine in benzene at* 25°: $[\mathrm{M}]_0 = 0{\cdot}100$ mole l.$^{-1}$; $[\mathrm{X}]_0 =$ (*a*) $5{\cdot}3 \times 10^{-3}$, (*b*) $2{\cdot}6 \times 10^{-3}$ mole l.$^{-1}$. (*c*) *Reaction of* N-*carboxy*-L-*alanine anhydride with* n-*hexylamine in nitrobenzene at* 25°: $[\mathrm{M}]_0 = 0{\cdot}102$ mole l.$^{-1}$; $[\mathrm{X}]_0 = 10^{-2}$ mole l.$^{-1}$.



[M]/[X] (initial)	Approx. velocity coeff. (mole^{-1} l. min.$^{-1}$) *B–C*	*C–D*	Number average degree of polymerization *C*	*D*
10·0	6·6	38	3	8
18·9	7·8	34	4	12
38·4	7·1	39	6	12

The increase in rate at the point *C* corresponds to a factor of 5 or 6, while the number average degree of polymerization is in the range 3—6. It is most unlikely that polysarcosine undergoes any definite configurational change in this range. In particular, since polysarcosine is an *N*-substituted polypeptide it cannot adopt an α-helical configuration, hence explanations of the rate data based on special steric features of this configuration [6] do not apply.

The rate of the polymerization reaction (2) in nitrobenzene solution at zero carbon dioxide pressure is of strictly first order in base and anhydride concentrations throughout,[12] so it is unlikely that any chemical factor is responsible for the effects observed in the present case.

(ii) *Systems containing* α-N-*Carboxy*-L-*alanine Anhydride.*—A typical run with this anhydride in nitrobenzene is shown in Fig. 2(*c*). The curve is essentially similar to that

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[12] Ballard and Bamford, to be published.

in Fig. 1 for α-*N*-carboxysarcosine anhydride in benzene. The rate of polymerization increases markedly—by a factor of about 5·5—when the number average degree of polymerization reaches 4 (in the neighbourhood of *C*) and the reaction eventually follows a course in which log [M] is approximately linear in *t*. In this case gelation occurs just before the increase in rate sets in.

Similar polymerizations carried out in benzene are represented in Figs. 3(*a*) and (4*a*). Fig. 3(*a*) is similar to Fig. 2(*c*); the rate increases by a factor of 8·5 when the number average reaches 3 (near *C*). Fig. 4(*a*), which refers to a higher initial value of [M]/[X], shows four portions similar to those observed in the experiments with α-*N*-carboxysarcosine anhydride (Fig. 1). Here the rate increases by a factor of 14·5 when the number average degree of polymerization attains a value of 5 (near *C*). As is the case with the sarcosine derivative, polymer is precipitated and remains suspended during the reaction represented by *CD*; after *D* has been passed the precipitate consolidates.

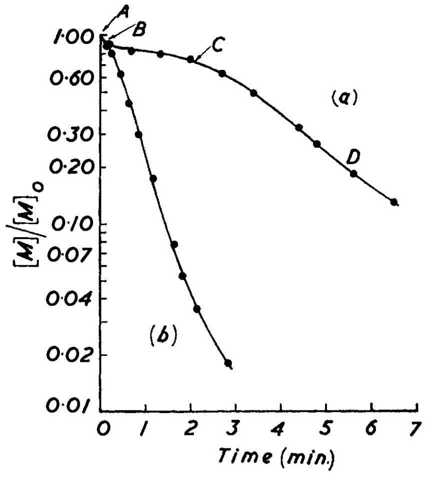

FIG. 3. (*a*) *Reaction of* N-*carboxy*-D-*alanine anhydride with* n-*hexylamine in benzene at* 25°: [M]$_0$ = 0·113 mole l.$^{-1}$; [X]$_0$ = 10^{-2} mole l.$^{-1}$. (*b*) *Reaction of* N-*carboxy*-L-*proline anhydride with* n-*hexylamine in nitrobenzene at* 25°: [M]$_0$ = 9·8 × 10^{-2} mole l.$^{-1}$; [X]$_0$ = 10^{-2} mole l.$^{-1}$.

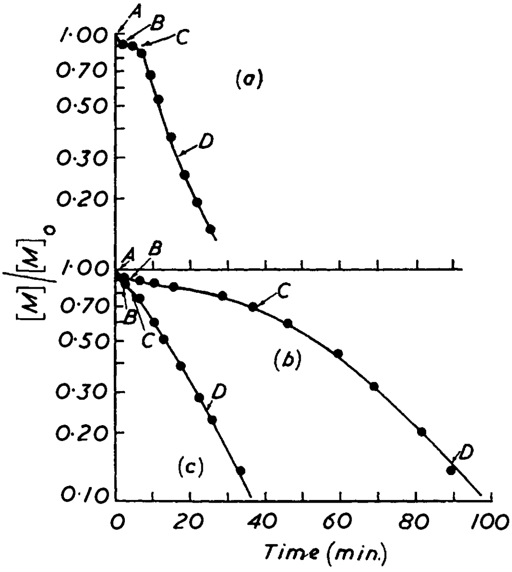

FIG. 4. (*a*) *Reaction of* N-*carboxy*-D-*alanine anhydride with* n-*hexylamine in benzene at* 25°: [M]$_0$ = 0·107 mole l.$^{-1}$; [X]$_0$ = 2·08 × 10^{-3} mole l.$^{-1}$. (*b*), (*c*) *Reaction of* N-*carboxy*-L-*leucine anhydride with* n-*hexylamine in nitrobenzene at* 25°: (*b*) [M]$_0$ = 0·134 mole l.$^{-1}$; [X]$_0$ = 6·6 × 10^{-3} mole l.$^{-1}$. (*c*) [M]$_0$ = 0·32 mole l.$^{-1}$; [X]$_0$ = 2·15 × 10^{-2} mole l.$^{-1}$.

The rate coefficient corresponding to the portion of the curve between *B* and *C* in Fig. 2(*c*) is 2·9 mole^{-1} l. min.$^{-1}$. This is a reasonable value for the normal propagation reaction (1) and may be compared to that for α-*N*-carboxy-DL-phenylalanine anhydride,[1] *viz.*, 0·94 mole^{-1} l. min.$^{-1}$. The corresponding values deduced from Figs. 3(*a*) and 4(*a*) referring to polymerizations in benzene are 3·8 and 3·0 mole^{-1} l. min.$^{-1}$, respectively.

(iii) α-N-*Carboxy*-L-*leucine Anhydride in Nitrobenzene*.—Using concentrations of this anhydride in nitrobenzene similar to those reported by Lundberg and Doty,[4] we found no difficulty in reproducing their results. A typical experiment is shown in Fig. 4(*b*). In this case also the relative sizes of the various steps were found to depend on the concentrations of anhydride and base used; Fig. 4(*c*) shows that with rather high initial concentrations the *BC* stage is very small.

(iv) α-N-*Carboxy*-L-*proline Anhydride in Nitrobenzene*.—The polymerization of α-*N*-carboxy-L-proline anhydride initiated by *n*-hexylamine in nitrobenzene solution [Fig. 3(*b*)] shows the characteristic features associated with the polymerization of other anhydrides which form sparingly soluble polymers. Poly-L-proline separates at an early stage, and the rate of reaction subsequently increases by a factor of approximately 1·9 in the experiment illustrated. Towards the end of the reaction a decrease in the velocity coefficient occurs.

(v) *General Conclusions.*—The experiments described lead to the following conclusions. (*a*) Increases in the velocity coefficient during polymerization are commonly encountered when the polypeptide product is precipitated; the increase coincides approximately with the appearance of visible heterogeneity. (*b*) Similar results are obtained with *N*-substituted and unsubstituted anhydrides. (*c*) With unsubstituted anhydrides the main increase in rate can occur when a mean degree of polymerization as low as 3 or 4 has been reached [point *C*, Figs. 1, 2(*c*), and 3(*a*)]. This value is too low to permit the growing chains to adopt the α-helical configuration since the α-helix contains close to 3·6 residues per turn. It may be argued that a small fraction of the chains at the points *C* [Figs. 1, 2(*c*), and 3(*a*)] will be α-helical on account of the spread in molecular weights; if this were the cause of the acceleration the latter should continue to a very marked extent considerably beyond *C*. However, the acceleration is substantially complete by the time the chains have reached a degree of polymerization of five; it is unlikely that they are completely α-helical at this stage. Idelson and Blout [5] suggested that the terminal base groups of low polymers of γ-benzyl esters of L-glutamic acid may be less accessible, and therefore react more slowly, than those of the α-polymer. In principle this could arise if the low polymers were associated by inter-chain hydrogen bonds. A mechanism of this kind cannot apply to *N*-substituted polymers, and, in view of (*b*) above, is unlikely to be important in general. Further, it is difficult to believe that the terminal groups are less accessible in solution than in the precipitated polymer. The acceleration is not a function merely of the nature of the solvent alone, since, for example, α-*N*-carboxysarcosine anhydride at constant carbon dioxide pressure polymerizes normally in nitrobenzene whereas the *N*-carboxy-anhydrides of L-leucine and L-proline give accelerating reactions in this medium.

It appears to us that the increase in rate observed at the points *C* in Figs. 1—4 must have its origin in the development of heterogeneity, which seems to be the only common feature in the systems studied. In view of these observations it is debatable whether there is any evidence for a dependence of the propagation rate on a specific chain configuration. Such evidence could only be obtained from strictly homogeneous polymerizations, and to reach a decision further work is required; however, in our experience polymerizatons of this kind can be described by a single propagation coefficient. [For example, we have confirmed the findings of Lundberg and Doty [4] that in highly purified *NN*-dimethylformamide the (homogeneous) polymerization of α-*N*-carboxy-L-glutamic anhydride γ-benzyl ester is of strictly first order throughout its whole course.] Most of the evidence cited in favour of such a dependence is derived from work on the γ-benzyl ester anhydride in dioxan. It is possible that the polymerization of this anhydride is markedly affected by the presence of trace impurities which can affect the precipitation of the polymer. Indeed, Lundberg and Doty [4] have recorded that impurities can influence the physical state of solution of this polymer.

The *N*-carboxy-anhydrides, like the polypeptides, are highly polar substances, and it is reasonable to suppose that they are preferentially soluble in a phase consisting of swollen polypeptide in contact with a poor solvent for the polymer. Since the reactive base residues are attached to the polymer chains, precipitation followed by absorption of the anhydride will increase the effective concentration of both reactants and so lead to an increase in the rate of reaction. Although the chains may be immobilised in such an aggregate, the small monomer molecules are comparatively mobile. In order that this mechanism alone should result in a large increase in the velocity it is necessary that the volume of the anhydride-rich precipitated phase should be small compared with the total liquid volume. This appears to be the case in the systems described above. However, the high dielectric constant of the swollen phase may be an additional factor tending to increase the rate of reaction.

An alternative, though somewhat similar, mechanism may be visualised in which the anhydride molecules are reversibly adsorbed on the precipitated polymer molecules, and then react particularly readily on account of the proximity of the base at the end of the chains and possible activation arising from the adsorption. It is not clear whether any specific conformation of the end portions of the chains would be required to produce this enhancement in rate, but the observation that anhydrides of different structure behave similarly make this seem rather unlikely. This mechanism has analogies with the chain-effect encountered in homogeneous polymerizations initiated by polysarcosine,[13] the adsorption of anhydride by the precipitated polymer taking the place of the attachment of anhydride molecules to polysarcosine by hydrogen bonds.

Phenomena of the type considered above will generally affect the nature of the molecular-weight distribution, and will lead to departures from the Poisson distribution expected for homogeneous polymerization. The result will be a broadening of the distribution; this has been observed by Mitchell, Woodward, and Doty[7] in the case of the γ-benzyl ester of *N*-carboxy-L-glutamic anhydride, and was previously interpreted[4] in terms of the two-stage propagation suggested by Doty and Lundberg.[3]

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[13] Ballard and Bamford, *Proc. Roy. Soc.*, 1956, *A*, **236**, 384.