SYNTHETIC OLIGOPEPTIDES AS POLYMER PRECURSORS

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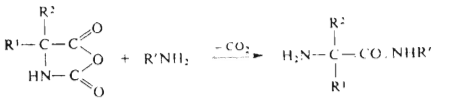
Dedicated to Academician O. Wichterle on the occasion of his 70th birthday.

By reacting N-carboxyanhydrides of L-phenylalanine, DL-phenylalanine, L-leucine or of a mixture of DL-phenylalanine and L-leucine with hexamethylenediamine (15–20 mol.% related to monomer) in absolute benzene at 25°C, which was followed by an additional treatment with hexamethylenediamine (60°C, 4 h), oligopeptides were prepared with the average functionality ($\bar{F}_n$) 1·72–1·96 with respect to primary amino groups. The reaction between these oligopeptides and poly(oxyethylene) terminated with isocyanate groups ($\bar{F}_n$ 1·69–1·94) in anhydrous chloroform at 25°C leads to copolymers of the type A-B and A-B-A with a minority content of copolymers $(A\text{-}B)_m$ for $m \geqq 2$. The low efficiency of the coupling reaction is attributed to the formation of an important amount of monofunctional oligopeptide in the preparation reaction.

Synthetic peptides are studied with increasing attention, because they are analogs of proteins. One of the types of synthetic polypeptides usually investigated are their block copolymers. The copolymers of peptides described so far were prepared by the polymerization of N-carboxyanhydrides of α-amino acids in the presence of macroinitiators bearing one[1–3] or two[4–6] amino endgroups which are the growth centers of condensation polymerization. Accordingly, the copolymers are either of the A-B or A-B-A type, where the segment A is a regular block composed of amino acid and the segment B is a block prepared, *e.g.*, from a vinyl or diene monomer, or of the types $A^1\text{-}A^2$, $A^2\text{-}A^1\text{-}A^2$, $A^1\text{-}A^2\text{-}A^3$-..., where each block A consists of a different amino acid. Copolymers containing only amino acid constitutional structural units have been lately examined with respect to their secondary structure[7] and interaction with detergents[8]. Block copolymers of natural polysaccharides with synthetic polypeptides were also prepared[9]. Generally, a domain structure formed as a result of microphase separation is characteristic of block copolymers. In some cases this structure has also been studied for copolymers of peptides[1,2,5], and its importance in the preparation of bioanalogous membranes has been pointed out[6]. The secondary structure of high-molecular weight polypeptides in solution is almost exclusively studied by using samples prepared from N-carboxyanhydrides of α-amino acids. The patent literature contains an almost endless series of procedures suggested for the preparation of copolymers of polypeptides based on N-carboxyanhydrides, but their structure is usually not adequately defined.

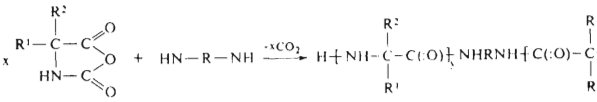
In the polymerization of N-carboxyanhydrides of α-amino acids unsubstituted on the nitrogen atom with amines as initiators, two different mechanisms of propagation are usually operative. In the case of initiation with primary amines, a nucleophilic attack takes place in position 5 of the 2,5-dioxo-1,3-oxazolidine ring, followed by

ring-opening with the release of CO_2 from the carbamic carboxyl group and by the regeneration of the amino group[10]:



(A)

This mechanism is usually called the „mechanism of primary amines". Propagation reaction occurs due to a reaction between another molecule of N-carboxyanhydride with the amino group. If diamine with primary amino groups is used as initiator, a large amount of the particles formed consists of telechelic molecules:



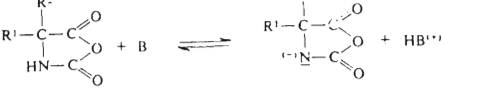
(B)

Assuming that the carbamic carboxyl group can be preserved at least transitionally[11], the end groupings of carbamic acid were also regarded as the growth centre

(C)

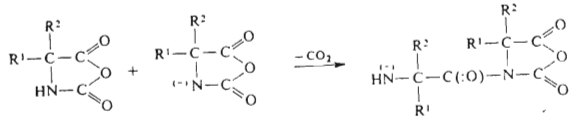
and the decarboxylateion takes place on the internal amide group.This mode of propagation[12] was considered as a little probable or minority variant of the reaction mechanism of the primary amine.

With bases, and consequently also amines as initiators, a mechanism called „the mechanism of activated monomer" is always operative[10,13-19]. This is an ionic mechanism: acidobasic equilibrium



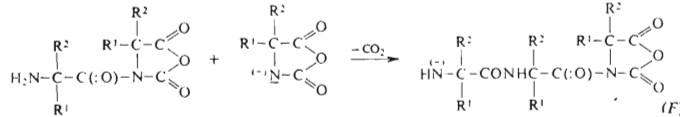
(D)

gives rise to an N-anion which is acylated with another molecule of N-carboxyanhydride



(E)

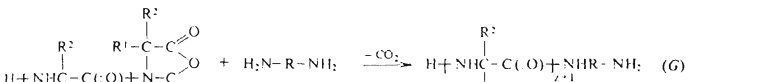
In the propagation reaction proceeding *via* this mechanism the growth centre is represented by the oxazolidine end ring:



(F)

Thus, the chain is terminated with the N-acyl-2,5-dioxo-1,3-oxazolidine grouping possessing electrophilic character at one end, and with the amino group possessing nucleophilic reactivity, which at the same time represents the growth centre of the mechanism of the primary amine, at the other. Moreover, the aminolytic combination of both chain terminations should also be borne in mind. Quite frequently, the "quasi-living" character of polymerization of N-carboxyanhydrides of α-amino acids is mentioned. Up to now, no end rings have been proved in the products, either analytically or spectroscopically; moreover, nothing has been reported in the literature about the transformation of end groupings which may take place in the isolation of the polymer. Even though the dependence of the content of individual endgroups on the type of N-carboxyanhydride, initiator and reaction conditions has not yet been studied in detail, it is known from the radiometric analysis of poly(γ-benzyl-L-glutamate) after initiation of the polymerization with ^{14}C-benzylamine[18] that 50–70% of the particles are formed by the mechanism of primary amine; the same study showed that in the initiation with diethylamine the mechanism of primary amine was operative to a small extent only (10%).

Our study aimed at such preparation of telechelic oligopeptides from N-carboxyanhydrides of α-amino acids where each chain would be terminated with two amino groups[20]. Such bifunctional prepolymers (precursors) may be used as peptide segments in alternating block copolymers of the type $(A\text{-}B)_n$ which could not be synthesized by using the procedures so far in existence. We started from the possibility of transformation of cyclic endgroups contained in a synthetic polypeptide into amino endgroups based on the assumption that it is just the end N-acyl-2,5-dioxo-1,3-oxazolidine grouping which possesses electrophilic character (stronger than that of the free monomer) and undergoes aminolysis at elevated temperature. If, on completion of the polymerization, aminolysis with primary diamine is carried out, chains at the two ends bearing the amino groups are formed.



also, chains may join while giving rise to macromolecules with amino endgroups. If the aminolysis is carried out using the same primary diamine as that used in the initiation, the extended macromolecule has the same structure as the chain formed by propagation with the participation of the mechanism of primary amine.

EXPERIMENTAL

Prepolymers

N-Carboxyanhydrides of L-phenylalanine, DL-phenylalanine and L-leucine were prepared from the respective amino acids (Fluka A.G., purissimum) by phosgenation[21]. The polymerization and copolymerization of N-carboxyanhydrides (1—2 g) initiated with hexamethylenediamine (Fluka; freed from carbonate and distilled) were carried out in solution in absolute benzene (total monomer concentration 30—50 mmol/kg) at 25°C. The extent of the reaction (conversion of monomers into polymer) was determined in parallel experiments in which the amount of released carbon dioxide was measured. On completion of the polymerization, the polymerizate was aminolyzed directly in the reaction solution by adding hexamethylenediamine (50% of its initial amount) at 60°C for 4 h. After that, the reaction mixtures were freed from gel fractions by filtration, evaporated to dryness, extracted with water five times at 50°C for 4 h and dried *in vacuo* at 50°C.

The poly(oxyethylene) samples with isocyanate endgroups were prepared by reacting polyoxirane (commercial poly(ethylene glycol), molar masses 600, 3 000, 4 000 and 10 000 $gmol^{-1}$) with hexamethylene diisocyanate at 60°C (ref.[22]); after dissolution in absolute benzene to a 10% solution and filtration in an inert atmosphere, the solutions or evaporated residues were stored at −25°C.

Block Copolymers

The coupling reaction of prepolymers was carried out in solution in $CHCl_3$ at 25°C, similarly to the reaction described for telechelic polyamides and poly(oxyethylene)[22]. At the end of the reaction, isopropylamine was added in a tenfold molar excess (related to the initial content of —NCO groups in the reaction mixture), the reaction solutions were filtered (by a quantitative procedure, rinsed with chloroform), evaporated freely in Petri dishes, extracted with cyclohexane (Reanal, reagent grade) five times for 2 h, and weighed after drying (24 h at 50—55°C/0·15 Pa).

Characterization of Precursors and Block Copolymers

Viscosities of the solutions of samples in dichloroacetic acid (25°C) were measured (Ubbelohde's viscometer) at four concentrations of each sample (0·1—0·5 g/dl); the intrinsic viscosities were determined using Kramer's plot.

The basic and acid functional groups (amino and carboxylic endgroups) were determined by the conductivity titration of polymer solutions (10 ml DMF and 0·5—3·0 ml water). Dimethylformamide was carefully purified[23] and redistilled water was freshly boiled. Basing on preliminary determinations, the weighed amounts of samples were chosen so as to make the consumed amount of 0·1M-NaOH (carbonate-free) or of 0·1M-HCl amount to $1 \cdot 10^{-6} - 3 \cdot 10^{-6}$ mol; the amount consumed for the solvents used (continuous blank control tests) was subtracted. The titration curves were the same as with model titrations of hexamethylenediamire and adipic acid and as in the determination of basic and acid groups of phenylalanine.

The isocyanate groups were determined as reported in ref.[22].

The molar mass of the polymer ($\bar{M}_n$) was determined by the V.P.O. method ($CHCl_3$, Hitachi–Perkin–Elmer, model 115). Prior to determination, benzene solutions of telechelic poly(oxyethylene) were heated at 60°C for 10 h with 1% of methanol, evaporated and dried as block copolymers.

The content of amino acid constitutional units in some products was determined by amino acid analysis according to Spackman, Stein and Moore[24].

The IR spectra of N-carboxyanhydride of DL-phenylalanine and oligopeptides were recorded in KBr discs (5 mg of the sample, 1 g KBr) using a Perkin–Elmer 508 B apparatus.

Solubility in water was determined by the extraction of 0·4—0·5 g of samples in weighing bottles three times for two hours using 50—100 ml water at 50—55°C and stirring with a magnetic stirrer; liquid fractions were removed each time after cooling by filtration through glass filters S2—S4 (according to the consistence of the solid fraction). This extraction procedure was checked by parallel extraction in a Soxhlet apparatus (three times, 8 h), and by extraction with a mixture of polyoxirane (commercial poly(ethylene glycol) M 4000, Fluka) and polypeptide samples (only polyether is extracted).

Solubility in benzene or dimethylformamide was determined by shaking 20—30 mg of the copolymer with 2—3 ml of the solvent at 25°C for 16—20 h.

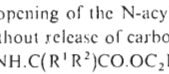
RESULTS AND DISCUSSION

In the course of the condensation polymerization of N-carboxyanhydrides of phenylalanine with hexamethylenediamine as initiator in absolute benzene it was demonstrated that the monomer was fully converted to the polymer, *i. e.*, oligopeptide (OP). For the product which after subsequent aminolysis with hexamethylenediamine remained dissolved in benzene (30—90%), the concentrations of primary amino groups ([—NH_2]), carboxylic groups ([—COOH]), intrinsic viscosity ([η]), and number average molar mass $(\bar{M}_n)_{OP}$ were determined independently of the endgroup concentration. The number average functionality, $\bar{F}_n$ (ref.[25]), was calculated using the relation $\bar{F}_n = [—NH_2]\,(\bar{M}_n)_{OP}$, *i.e.*, functionality of the telechelic polymer in the sense of amino endgroups. Using $\bar{F}_n$, the number average degree of chain extension was calculated[25], $\bar{E}_n = 2/(2 - \bar{F}_n)$, giving the average number of segments of the respective telechelic polymer in the copolymer prepared by coupling the polymer with an exactly bifunctional extender at the equimolar ratio of reactive groups (used in the coupling) assuming a quantitative and irreversible character of the coupling reaction.

It was found that by using the initiator in an amount of 15—20 mol% (related to the monomer), polymers or oligomers with $\bar{E}_n$ about 10 were obtained (precursors 1 and 4

in Table I) with a negligible content of carboxylic groups. They are considered to be endgroups, *i.e.* at the end of the macromolecular chain $\sim CO.NHC(R^1R^2)CO.OH$, and their origin may be attributed to the hydrolytic role played by the traces of moisture during propagation, or to the hydrolysis (with simultaneous decarboxylation) of residual oxazolidine end groupings in the isolation of oligomers (extraction with water). Their occurrence in side groupings also cannot be ruled out, if the cyclic growth centre were opened by the initiator (during propagation or additionally) without decarboxylation and with formation of *e.g.* the acylurea grouping[10] (inside or as the end grouping),

On the contrary, in the reference sample (No 2, Table I) which after the polymerization was heated with ethanol instead of hexamethylenediamine a higher content of carboxylic groups is typical, because the opening of the N-acyl-2,5-dioxo-1,3-oxazolidine ring in the alcoholysis may occur without release of carbon dioxide[26], and thus lead to the formation, along with $\sim CO.NH.C(R^1R^2)CO.OC_2H_5$, of the end grouping



which may also be regarded as indirect evidence of the assumed cyclic endgroups in the polymerization mixture. This also shows that the conditions of aminolysis in the samples taken for comparison were sufficient: if, namely, cyclic groupings were present in the oligomers at an important concentration also after the aminolysis with diamine, these groupings would by hydrolyzed in the extraction of the products with water, and the content of carboxylic groups would become comparable with their concentration in the alcoholyzed sample. Moreover, N-acyl-2,5-dioxo-1,3-oxazolidine rings could not be proved by IR spectra in the checked oligopeptides (samples Nos 1, 3, 4).

Hence, the $\bar{F}_n$ value lower than two found with the oligopeptides discussed here should be attributed to the expense of chain terminations other than $-NH_2$, —COOH and N-acyl-2,5-dioxo-1,3-dioxazolidine. Assuming that these terminations resemble the rings mentioned above and using characteristics in Table I and the computational analysis of ($\bar{M}_n$, [N]), precursor 1 can be described as a mixture of bifunctional tetramer, bifunctional pentamer (number of peptide structural units per one hexamethylenediamine unit) and monofunctional tetramer (including the cyclic termination) in the ratio 5 : 5 : 2, precursor 2 can be described as a mixture of bifunctional hexamer with monofunctional pentamer and hexamer in the ratio 10 : 1 : 1, *i.e.* each time with a content of

TABLE I

Preparation and characteristics of oligopeptidic precursors (OP). $[I]_0$ starting concentration of hexamethylenediamine as initiator (mol %, related to monomer), s mass yield of the product soluble in benzene, [N] nitrogen content, $\bar{M}_G = 2/([-NH_2] + [-COOH])$

Precursor No	N-Carboxy-anhydride	$[I]_0$ mol %	t h	s %	$[\eta]$ dl g^{-1}	$[-NH_2]$ mol kg^{-1}	$[-COOH]$ mol kg^{-1}	[N] %	$\bar{M}_G$ g mol^{-1}	$(\bar{M}_n)_{OP}$ g mol^{-1}	$\bar{F}_n$	$\bar{E}_n$
1	L-Phe	16·1	150	33·0	0·13	2·454	0·047	11·27	800	750	1·84	13
2	L-Phe	18·1	216[a]	38·5	0·11	1·667	0·270	—	—	1 050	1·75	8
3	L-Phe	90·7	96	78·8	0·37	5·877	0·358	13·99	320	450	2·64	—
4	DL-Phe	16·8	168	66·7	0·13	1·820	0	10·76	1 100	990	1·80	10
5	DL-Phe	91·2	120	87·4	0·57	5·380	0·422	13·80	355	410	2·29	—
6	L-Leu	91·8	120	41·3	0·25	4·531	0·144	15·88	425	380	1·72	7

[a] Reaction terminated with alcoholysis (ethanol, 10% volume of the reaction mixture: 50°C).

16·7% of the monofunctional component. Their theoretical values $\bar{M}_n$ 778, [N] 11·26% and $\bar{F}_n$ 1·83, and $\bar{M}_n$ 999, [N] 10·86% and $\bar{F}_n$ 1·83, respectively, are in good agreement with experimental data.

If a large amount of hexamethylenediamine ($[I]_0$ about 90 mol.% calculated per monomer) is used in the initiation of the condensation polymerization of L-phenylalanine or DL-phenylalanine, the polymer obtained at a low $(M_n)_{GP}$ and high $[-NH_2]$ appears to have a functionality higher than two. The relatively high [—COOH] and the unexpectedly high intrinsic viscosity seem noteworthy (Table I). To elucidate this, one may suggest, rather arbitrarily, that under the given experimental conditions aminolysis plays a considerable role already during the propagation, giving rise to the acylurea grouping mentioned above, or that the release of CO_2 from the labile and transitional carbamic carbonyl is suppressed by the formation of an ammonium salt. Such salts might anomalize the intrinsic viscosity of the polymer and distort data on molar masses, and thus also the calculated functionality. Synthetic polypeptides based on phenylalanine are known to have a tendency to form strong intermolecular associates (microgels in solutions) whose presence distorts measurement of the physico-chemical parameters[27,28].

The product of the condensation polymerization of L-leucine in the presence of a high concentration of the chosen initiator was found to possess, after additional heating with hexamethylenediamine, more favourable characteristics of a telechelic oligomer than similar samples obtained with phenylalanine (Table I), so that the chemical structure of N-carboxyanhydrides of α-amino acids may affect partial reactions in the polymerization and the structure of the reaction products. Bearing this in mind, and also with respect to the expected higher solubility[29], the condensation copolymerizations of N-carboxyanhydrides of DL-phenylalanine and L-leucine were carried out (Table II). Low concentrations of hexamethylenediamine as the initiator, up to 5 mol% calculated per the mixture of monomers, lead to polymers with a high $(\bar{M}_n)_{OP}$ and a very low average functionality of macromolecules as reactive extenders (probably with a wide molar mass distribution); these were regarded as unsatisfactory without further discussion. Again, however, it was found that at $[I]_0 > 20$ mol.%, $\bar{F}_n > 2$. It may be admitted that calculations of the functionality should be taken with certain reserve, bearing in mind the accuracy of determination of $[-NH_2]$ and of molar masses. This reserve, however, should not be exaggerated as the correlation $2/[G]$ *vs* $(\bar{M}_n)_{GP}$ has a linear character for the samples used ($[G] = [—NH_2] + [—COOH]$) (Fig. 1); the slope of the correlation straight line higher than unity as found for oligopeptides with $\bar{F}_n > 2$ corroborates the supposed existence of more than two primary amino groups in the oligopeptide molecule.

Oligopeptides with the amino endgroups and $\bar{F}_n$ 1·72–1·96 (samples 1, 4, 6, 9 and 12 in Tables I and II) were used in coupling telechelic poly(oxyethylene) terminated with isocyanate groups with $\bar{F}_n$ 1·69–1·94 (samples 15–19 in Table III). The respective reaction ability of the polyether precursor[22] was checked. The joining reactions were carried out at the equimolar ratio of the reactive groups. The reaction solutions became more thick (dense) during the reaction; after 3–7 days, when the consistence did not change any further (thixotropic or sirupy mixtures), isopropylamine was added (sudden thinning of the solution was observed, but no turbidity

TABLE II

Preparation and characteristics of oligopeptide precursors (OP). $[Phe]_0$ mole fraction of DL-phenylalanine-N-carboxyanhydride in the initial mixture with L-leucine-N-carboxyanhydride, $[I]_0$ initial concentration of hexamethylenediamine as initiator (mol. %, related to the mixture of monomers), *s* mass yield of the product soluble in benzene, [Phe] mole fraction of the respective peptide constitutional structural unit in the copolymer (related to the sum of units), [N]-nitrogen content, $\bar{M}_G = 2/([-NH_2] + [-COOH])$

Precursor No	$[Phe]_0$	$[I]_0$ mol. %	*t* h	*s* %	$[\eta]$ dl g^{-1}	$[-NH_2]$ mol kg^{-1}	$[-COOH]$ mol kg^{-1}	[N] %	$\bar{M}_G$ g mol^{-1}	$(\bar{M}_n)_{OP}$ g mol^{-1}	[Phe]	$\bar{F}_n$	$\bar{E}_n$
7	0·451	1·0	120	82·0	0·31	0·178	0	10·69	11 230	4 496	0·496	0·79	2
8	0·451	3·5	115	43·6	0·19	0·559	0·012	10·55	3 500	2 300	0·532	1·29	3
9	0·451	15·8	120	63·1	0·12	1·889	0·047	12·00	1 030	1 040	0·437	1·96	50
10	0·451	24·0	120	88·4	0·13	2·558	0·040	12·31	770	880	0·493	2·20	—
11	0·622	24·3	90	89·3	0·12	2·237	0·025	11·90	880	1 100	0·668	2·46	—
12	0·622[a]	15·0	90	84·3	0·13	1·873	0·043	11·57	1 040	1 000	0·678	1·87	15

[a] Mixture of L-Phe and D-Phe 3 : 1.

appeared). In the following filtration through the glass filter S4 a small amount of clear gel appeared in some samples; the products were not explicitly crosslinked, because in many cases they could be dissolved in a new dose of chloroform. From the filtered clear copolymer solutions, white or yellowish brittle compounds were obtained by evaporation and characterized by the determination of intrinsic viscosity, molar mass, content of basic groups and solubility in water (solvent of the polyether component), benzene, and DMF. All samples could be dissolved in chloroform and dichloracetic acid; in all cases the molar mass and intrinsic viscosity increased compared with the respective precursors, but the increase was not pronounced.

TABLE III

Characteristics of telechelic poly(oxyethylene) (PO); [N] – nitrogen content, $\bar{M}_{NCO} = 2/[-NCO]$, $\bar{F}_n = [-NCO] \cdot (\bar{M}_n)_{PO}$

Precursor No	$[\eta]$ dl g^{-1}	[N] %	[—NCO] mol kg^{-1}	$\bar{M}_{NCO}$ g mol^{-1}	$(\bar{M}_n)_{PO}$ g mol^{-1}	$\bar{F}_n$	$\bar{E}_n$
13	0·09	5·99	2·095	955	1 010	2·12	—
14	0·13	5·94	1·960	1 020	1 070	2·10	—
15	0·14	5·98	2·065	970	940	1·94	33
16	0·31	1·55	0·552	3 620	3 350	1·85	13
17	0·43	1·29	0·460[a]	4 350	4 000	1·84	13
18	0·45	1·29	0·460[a]	4 350	4 150	1·91	22
19	0·77	0·54	0·238	8 390	7 900	1·88	17

[a] According to [N].

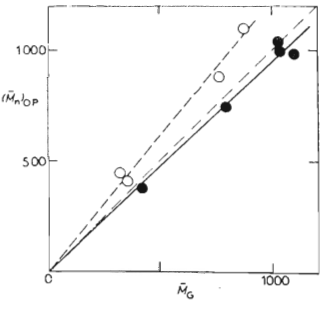


FIG. 1

Correlation of molar masses (g mol^{-1}) of oligopeptides calculated from [—NH_2] and [—COOH], $\bar{M}_G$, and determined by the V.P.O method, $(\bar{M}_n)_{OP}$. Range $\bar{F}_n$: ○ 1·7 to 2·0, ● > 2·0

The expected chemical joining of the precursors, *i.e.* of the two components of the reaction mixture, could be estimated judging by the behaviour of products in the extraction with water: with one exception only, the samples were partly or completely soluble in water, while the starting peptide precursors are insoluble in water; the content of the oligopeptide component in the fraction insoluble in water is always lower than in the initial reaction mixture of precursors. The coupling reaction leads to an essential drop in the concentration of amino groups, *i.e.* the experimentally determined value in the product, $[-NH_2]_C$, is a fraction of the initial value, $[-NH_2]$. $[OP]_0$, where $[OP]_0$ is the fraction of the oligopeptide in the reaction mixture (Table IV). The preliminary correlation $(\bar{M}_n)_C$ *vs* $(\bar{M}_n)_{C,a}$ (*i.e. vs* the sum $(\bar{M}_n)_{OP}$ and $(\bar{M}_n)_{PO}$) allows a conclusion (Fig. 2) that as a rule, only diblocks or triblocks were formed by the coupling reaction, and that the content of segmental copolymers $(OP-PO)_m$ with $m \geq 2$ may in some cases be low or a minority one. Although one should bear in mind the high sensitivity of the coupling reaction to the ratio of the reaction groups (their equimolarity depends on the accuracy of analytical determination in both components) and to the functionalities of the precursors different from 2, such a low efficiency of the reaction still could not be expected. Explanation based on the formation of cyclic structures in the formation of block copolymers similarly to the cyclization of the telechelic "living" polystyrene[30] under the given conditions[31,32] cannot be excluded, but does not to seem very plausible. Also, some sort of masking of the reactive amino groups, *e.g.* in the form of carbonates, cannot be considered as the reaction in the presence of a tert-amine led to a comparable result. Another likely cause of the low degree of extension could be seen in the formation of ammonium salts in the preparation of the peptide precursor, if the role and stability of ~NH.COOH in the polymerization of N-carboxyanhydrides of α-amino acids is higher[19] than estimated earlier. If in the assumed telechelic oligopeptide

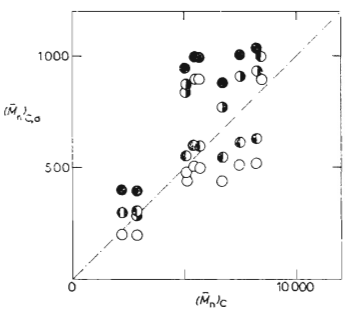


FIG. 2

Correlation of determined relative molecular masses of copolymers, $(\bar{M}_n)_C$, with calculated relative molecular masses of joined precursors, $(\bar{M}_n)_{C,a}$: ○ $(\bar{M}_n)_{OP} + (\bar{M}_n)_{PO}$, ◑ $(\bar{M}_n)_{OP} + 2(\bar{M}_n)_{PO}$, ◐ $2(\bar{M}_n)_{OP} + (\bar{M}_n)_{PO}$, ● $2(\bar{M}_n)_{OP} + 2(\bar{M}_n)_{PO}$

diamine also were incorporated in the form of an ammonium salt, *e.g.*,

$$H_2N(CH_2)_6NH\text{–}[\text{–}CO.O(R^1R^2)NH\text{–}]_z\overset{(-)}{C}OO\ldots\overset{(+)}{H_3}N(CH_2)_6\overset{(+)}{N}H_3\ldots\overset{(-)}{O}CO.NH\sim NH_2$$

then after the deactivation of the residual isocyanate groups by the addition of isopropylamine at the end of the coupling reaction, fragmentation of the chain would take place due to the substitution (and release) of the diamine. The system could contain a large number of ammonium salts of this type. If we admit only the presence of one and two linear peptide structural units in molecules, then the number of ammonium salts is 269 with 173 elementary structures, and this number increases permutationally with increasing lergth of the chains. Computational analysis has revealed that a very good fit in the molar mas and in the nitrogen content for the chosen real samples (Nos 1 and 4, $\bar{F}_n = 1{\cdot}8$) and for theoretically modelled mixtures is achieved if the salts form only oligopeptides with one cyclic end grouping, which is absurd. This reasoning has been additionally verified by experimental check-up in which no release of a significant amount of CO_2 from the selected oligopeptide in the acid medium could be demonstrated. Thus, the only cause of the low efficiency of the

TABLE IV

Preparation and characteristics of copolymers (C). $[OP]_o$ fraction of oligopeptide in the initial mixture with poly(oxyethylene) (PO), g insoluble fraction in water; nitrogen content, [N], and the content of peptide units in the copolymer, [OP] (calculated from [N] of copolymer and precursors), are related to the insoluble fraction

OP No	PO No	$[OP]_o$ mass. %	t h	$[\eta]$ dl g^{-1}	$[-NH_2]_C$ mol kg^{-1}	$(M_n)_C$ g mol^{-1}	g	[N] mass. %	[OP]
1	17	12·57	67	0·91	0·025	5 100	81·80[a]	2·10[a]	13·40
4	17	16·24	240	0·64	0·020	5 700	2·32	8·68	78·10
6	17	[c]7·27	124	0·72	0·020	5 100	100	1·66	2·50
9	15	52·22	168	0·30	0·053	2 900	83·63	9·06	51·20
9	16	22·10	72	0·38	–	6 700	2·45	10·18	82·60
9	17	15·73	72	0·62	0·006	7 750	2·38	12·31	92·71
9	18	16·21	120	0·81	0·024	8 250	0	—	—
9	19	11·19	235	1·21	0·017	8 500	0·04	—	—
12	14	52·79	120	0·17[b]	0·018	2 200	73·46	8·44	43·99
12	17	15·82	72	0·61	0·015	5 500	33·99	3·95	18·33

[a] In the extraction in a Soxhlet apparatus (three times 8 h) the following values were reached (mass %): g 84·90, [N] 2·56, [OP] 12.8. [b] In a repeated experiment in presence of triethylamine (0·02 mol/kg of reaction product) the same value was reached.

coupling reaction may consist in an important content of monofunctional oligopeptides in the respective precursor, *i.e.* oligopeptides terminated with an unidentified group, not reacting with —NCO or not giving an adduct by reacting with —NCO. This is also corroborated by the much lower $(\bar{M}_n)_C$ values compared with the data on $(\bar{M}_G)_C$ calculated as the fraction $1/([—NH_2]_C + 0{\cdot}5[—COOH][OP]_0)$.

The experimental aim to use a simple polymerization of N-carboxyanhydrides of α-amino acids with initiation and aminolysis by means of diamine did not result in the expected formation of telechelic oligopeptides (with amino endgroups) as suitable precursors for the synthesis of alternating multiblock copolymers of the type $(A\text{-}B)_m$, *i.e.* as bifunctional extenders of other reactive telechelic polymers. Obviously, the reaction system α-amino acid N-carboxyanhydride – amine is too complicated, does not yield a homogeneous product of condensation polymerization[7], and is of little use for the purpose. With the optimal amount of hexamethylenediamine used for the initiation (10–20 mol.%), the procedure gives rise to oligopeptides which – unlike analogous polyamides[22] – in the reaction with polyoxirane terminated with isocyanate groups yield only copolymers of the A-B, A-B-A, B-A-B type with (sometimes) admixtures of copolymers $(A\text{-}B)_m$ at $m \geqq 2$. However, these findings too are a valuable contribution to the synthesis of biodegradable polymeric materials.

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