tetraene isomerization was found to be some 40 kcal/mole above the normal state, so triplet mechanisms should be unimportant.

It should be noted that for the species V ($n = 0$ and 2) there are two degenerate singlet configurations. It is possible therefore, that configuration interaction may lower the energy of the singlet transition state for rotation. This energy lowering would have to be about 20 kcal/mole to affect our conclusions concerning the mechanism of isomerization for these molecules.

Acknowledgment. We thank Professor J. A. Pople for many valuable discussions and suggestions. This research was supported by grants from the U. S. Army Research Office, Durham, the National Institutes of Health, and the Max-Planck-Institut für Medizinische Forschung, Heidelberg.

The Monomer Distributions of Propylene Oxide–Maleic Anhydride Copolymers

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Abstract: The monomer distributions in the copolymer propylene oxide–maleic anhydride (PO–MA) prepared by two different homogeneous catalysts have been determined. Gas chromatographic techniques which accurately determine the relative concentrations of runs of propylene oxide up to length 11 have been employed to make the direct demonstration of the suitability of Markoffian statistics in describing the monomer distribution in low-conversion PO–MA copolymers. Analysis of the monomer distribution in high-conversion PO–MA copolymers indicates that the observed distribution may be a sum of two different distributions, each of which is Markoffian. This result is interpreted in terms of a chemical modification of the catalyst during the copolymerization.

A recent study of the monomer distribution in the copolymer propylene oxide–maleic anhydride (PO–MA), $—C(=O)CH=CHC(=O)O(CHCH_3CH_2O—)_N$, polymerized by various homogeneous catalysts, was performed by a nuclear magnetic resonance (nmr) analysis of the copolymer and a gas–liquid partition chromatographic (glpc) analysis of the glycol ethers obtained from hydrolysis of the copolymer.[1] The main conclusion of this study was that high-order Markoffian statistical descriptions of the monomer distributions fitted the observed glpc, nmr, and composition data for all catalyst systems investigated.

This paper describes the results obtained from analysis of PO–MA copolymers by an improved glpc technique. By using volatile derivatives of the glycol ethers obtained from hydrolysis of the copolymer, the relative numbers of runs of PO up to a length of 11 can be determined accurately. The previous glpc method only permitted detection of runs of PO up to a length of four and only up to a length of three with any accuracy. The present values for the shorter runs are in agreement with the previously reported experimental results. The relative concentrations of the longer runs in *low*-conversion copolymers confirm the conclusion that high-order Markoffian statistics are applicable to PO–MA monomer distributions. The relative concentrations of the longer runs of PO in *high*-conversion copolymers indicate that, within both $SnCl_4$ and $SbCl_5$ catalysts systems, at least two distinctly different catalyst types, each with a third-order Markoffian memory, may be generating copolymer chains.

Experimental Section

Glpc analysis was performed on an F & M Model 720 chromatograph operated with an injection port temperature of 300°, detector block temperature of 300°, flow rate of 185 ml/min, and bridge current of 150 mA. Response factors for the trimethylsilyl ether derivatives of the monomer, dimer, trimer, tetramer, pentamer, and hexamer of propylene glycol were obtained as follows. Monomer, dimer, and trimer glycols were obtained from commercial sources. Tetramer, pentamer, and hexamer were isolated from a synthetic glycol mixture on a 0.25 in. o.d. by 3-ft 2% SE-52 on 60–80 mesh gas pack F analytical glpc column programmed from 130 to 300° at the rate of 10°/min. Synthetic blends of the monomer through hexamer at various concentrations were made, and 20 mg of tetraethylene glycol dimethyl ether, used as an internal reference, was added to 100 mg of each blend. Volatile trimethylsilyl ether derivatives were formed by adding 50 μl of bis(trimethylsilyl)acetamide to 20 μl of each blend and allowing the mixtures to stand overnight at room temperature. (At least 4 hr is necessary to convert quantitatively all glycols up through the dodecamer to the corresponding trimethylsilyl ether derivative.) Ether derivatives were analyzed on a 0.25 in. × 6 ft 5% SE-33 on 60–80 mesh Diatoport-S column programmed from 75 to 350° at the rate of 7.5°/min. A calibration curve for each glycol was made by plotting the ratio of glycol peak area to internal standard peak area against mole per cent glycol. The slope of the line (extrapolated through zero) gave the relative response factor for each glycol.

Propylene oxide–maleic anhydride copolymers were prepared using two different homogeneous catalysts, $SbCl_5$ and $SnCl_4$. Procedures for the copolymerization and hydrolysis have been given earlier.[1] The glycol ethers resulting from hydrolysis of the copolymers were derivatized and analyzed as above.

The 60-Mc/sec nmr spectra were obtained from a 15% by weight solution in $CDCl_3$ using a Varian A-60 spectrometer with an operating probe temperature of 35°.

Results

The 60-Mc/sec spectra of PO–MA copolymers prepared by two different homogeneous catalysts are pre-

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[1] R. J. Kern and J. Schaefer, *J. Am. Chem. Soc.*, 89, 6 (1967).

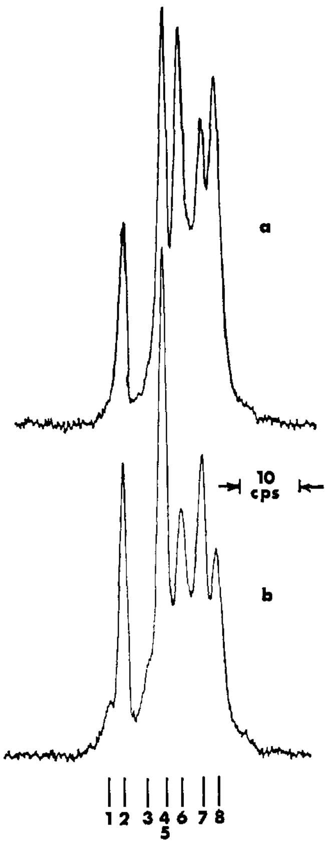

Figure 1. 60-Mc/sec spectra of methyl regions of PO–MA copolymers made from the catalyst systems (a) $SbCl_5$, 3:1, and (b) $SnCl_4$, 3:1. The ratio following the catalyst is the initial charge ratio of monomers. The PO methyl lines appear as doublets due to spin-spin coupling. The four doublets arise from the three distinct monomer triads in the chain: lines 1 and 3 from B*A*B; lines 2 and 4 and lines 5 and 7 from B*A*A (A*A*B); and lines 6 and 8 from A*A*A. The two pairs of lines arising from the triad B*A*A (A*A*B) reflect head-to-tail structural isomerism differences with this triad (A is PO and B is MA).

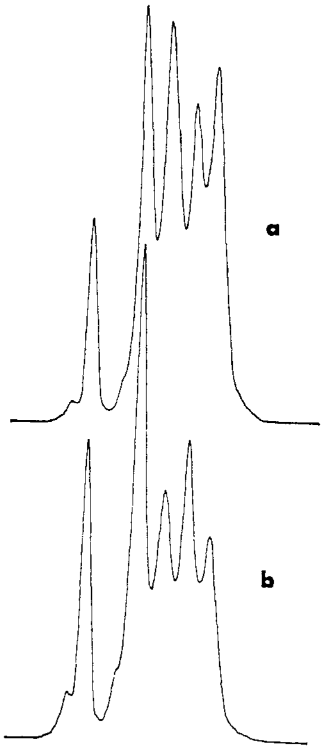

Figure 2. Simulated nmr spectra of methyl regions of PO–MA copolymers made from the catalyst systems (a) $SbCl_5$, 3:1, and (b) $SnCl_4$, 3:1, using a Dupont Curve Resolver Model 310. Areas of the various lines are given in Table IV.

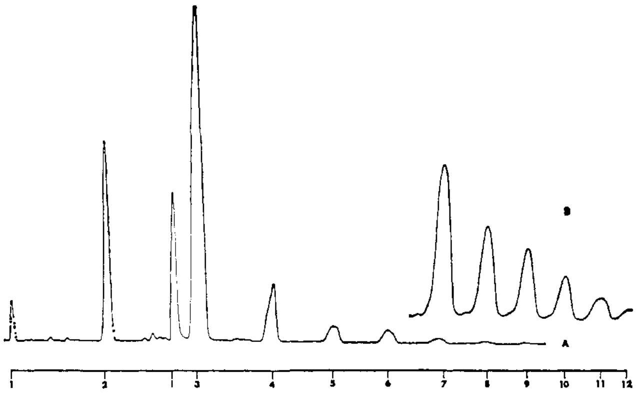

Figure 3. Chromatograms of the trimethylsilyl ether derivatives of the glycols obtained from hydrolysis of high-conversion PO–MA copolymer made from the catalyst system $SnCl_4$, 3:1. Insert B is at higher recorder gain than A. The numbers along the horizontal axis correspond to the numbers of PO units in the glycol. Tetraethylene glycol dimethyl ether is the internal standard (i). The unusual line shapes of some peaks are due to partial resolution of structural isomerism within the glycol.

sented in Figure 1. Line assignments[1] in terms of triads of monomers in the chain are given in the caption to Figure 1. The intensities of overlapping lines (and hence relative concentrations of triads) were estimated by fitting the methyl region of the PO–MA spectra using a Dupont Curve Resolver, Model 310. Accuracy of the method is comparable to that obtained by the more elaborate nonlinear regression analysis used earlier.[1] Examples of the fit are given in Figure 2.

Elution of the trimethylsilyl ether derivatives of the PO–MA glycol ethers from the SE-33 column appear as a series of regularly spaced peaks. Examples of the chromatograms are given in Figures 3 and 4. The relative molar response factors for the trimethylsilyl ether derivatives as a function of the number (*N*) of PO units in the glycol are given in Table I. For *N* greater than or equal to 4, the molar response factors are unity so that the relative concentrations of runs of PO greater than 3 are directly proportional to the areas of the cor-

Table I. Glpc Relative Molar Response Factors for Derivatized Glycols of Hydrolyzed Propylene Oxide–Maleic Anhydride Copolymers

Poly(propylene glycol)	Rel molar response factor[a]
Monomer	2.01 ± 0.14
Dimer	1.42 ± 0.07
Trimer	1.13 ± 0.05
Tetramer	0.95 ± 0.05
Pentamer	1.02 ± 0.03
Hexamer	1.01 ± 0.03

[a] Fletcher and Persinger have determined the relative molar response factors for trimethylsilyl ether derivatives of poly(ethylene glycol) on a similar column. For the monomer through pentamer their values are 1.47, 1.30, 1.00, 0.99, and 0.99: J. P. Fletcher and H. E. Persinger, presented at the Conference on Analytical Chemistry, Pittsburgh, Pa., 1967; *J. Polymer Sci.*, in press. The two systems are not identical, of course, but both show that, as the glycols become longer (and so more similar), the molar response factors become equal.

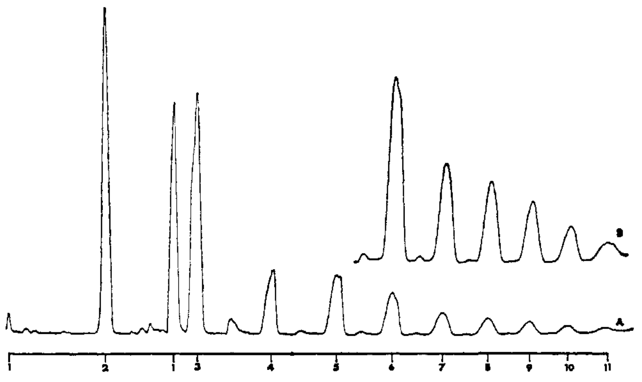

Figure 4. Chromatograms of the trimethylsilyl ether derivatives of the glycols obtained from hydrolysis of high-conversion PO–MA copolymers made from the catalyst systems $SbCl_5$ (A), 3:1, and (B), 6:1.

responding peaks. For N equal to 1, 2, or 3, the appropriate correction factor must be applied. Tabulated values for the relative concentrations of runs in high- and low-conversion copolymers are presented in Tables II and III. Since no consecutive MA units are allowed,[1] nmr observed triads can be predicted from the distribution of runs of PO determined by glpc. These predictions are given in Table IV and are in agreement with the experimentally determined values.[2]

From the run concentrations $[B(A)_NB]$, conditional probabilities can be calculated. $P(B/B)$, $P(BA/B)$, $P(BAA/B)$, ..., $P(BA_N/B)$ are the conditional probabilities that the next unit will be B given that the previous units are B, BA, BAA, ..., BA_N, respectively. Since it is known experimentally that consecutive B units do not form, $P(B/B) = 0$. Then

$$P(BA/B) = [BAB]$$

$$P(BAA/B) = [BAAB]/(1 - [BAB])$$

$$P(BA_N/B) = [B(A)_NB]/(1 - \sum_{M=1}^{N-1}[B(A)_MB])$$

Conditional probabilities for the low-conversion $SnCl_4$- and $SbCl_4$-catalyzed PO–MA copolymers are given in Table V.

Molecular weights of the copolymers ranged between 2100 and 3400 as determined by vapor-phase osmometry. Attempted distillation of the copolymers produced no low molecular weight components. Gel permeation chromatography showed no extremely high or low molecular weight components and gave molecular weights in agreement with the vapor-phase osmometry result.

Table II. Relative Molar Concentrations of Closed Runs of Propylene Oxide in Low-Conversion PO–MA Copolymers[a,b] $[B(A)_NB]$

Catalyst		$SnCl_4$				$SbCl_5$		
Initial PO:MA charge ratio		3:1				3:1		
Conversion, %		2				4		
Final PO:MA charge ratio		3:1				3:1		
	$N = 1$	0.025	$N = 7$	0.066	$N = 1$	...	$N = 7$	0.101
	2	0.219	8	0.051	2	0.109	8	0.094
	3	0.228	9	0.040	3	0.128	9	0.087
	4	0.133	10	0.031	4	0.108	10	0.087
	5	0.101	11	0.022	5	0.105	11	0.080
	6	0.085	12	...	6	0.101	12	...

[a] The monomer-to-catalyst molar concentration ratio is on the order of 2000:1. [b] Reproducibility indicates errors in concentrations of runs of PO are less than ±5% for all N less than 10. For N equal to 10–12, errors are less than ±10%. The upper bound on the error is reached when the concentrations of the runs are low.

Table III. Relative Molar Concentrations of Closed Runs of Propylene Oxide in High-Conversion PO–MA Copolymers $[B(A)_NB]$[a]

Catalyst	$SnCl_4$	$SnCl_4$	$SbCl_5$	$SbCl_5$	$SbCl_5$
Initial PO:MA charge ratio	12:1	3:1	12:1	6:1	3:1
Conversion, %	58	77	41	68	76
Final PO:MA charge ratio	45:1	3:1	20:1	15:1	3:1
$N = 1$	0.00	0.068	0.006	0.011	0.014
2	0.115	0.264	0.277	0.291	0.290
3	0.385	0.520	0.207	0.203	0.281
4	0.099	0.078	0.074	0.091	0.090
5	0.080	0.028	0.124	0.156	0.114
6	0.101	0.021	0.107	0.113	0.082
7	0.060	0.0082	0.058	0.052	0.042
8	0.047	0.0043	0.053	0.036	0.032
9	0.038	0.0033	0.044	0.023	0.022
10	0.028	0.0020	0.026	0.015	0.013
11	0.021	0.0010	0.014	0.010	0.011
12	0.015	...	0.008	...	0.006

[a] Error estimates are the same as those given in Table II.

Table IV. Predicted Nmr Triads in Propylene Oxide–Maleic Anhydride Copolymers from Relative Concentrations of Runs of Propylene Oxide as Determined by Glpc[a–c]

Catalyst	$SnCl_4$	$SbCl_5$
Initial PO:MA charge ratio	3:1	3:1
B*A*B (calcd)	0.024	0.003
B*A*B (obsd)	0.03	0.01
B*A*A, A*A*B (calcd)	0.644	0.500
B*A*A, A*A*B (obsd)	0.65	0.50
A*A*A (calcd)	0.332	0.497
A*A*A (obsd)	0.32	0.49

[a] High-conversion samples. [b] Error estimated as ±0.01. [c] Concentrations in moles liter^{-1}.

Discussion

A Markoffian statistical distribution of order p of the monomer distribution in PO–MA copolymers has the property that $[B(A)_{N+1}B]/[B(A)_NB]$ equals a constant less than or equal to 1 for all $N \geq p$ where $[B(A)_NB]$ is the relative concentration of a closed run of A, N

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(2) In PO–MA copolymers in which consecutive B units do not appear, A*B*A triads can be obtained from the known B*A*B, A*A*B (B*A*A), and A*A*A triads and so do not provide another independent check on the consistency of the data.

Table V. Conditional Probabilities for Low-Conversion PO–MA Copolymers, $P(\mathrm{BA}_N/\mathrm{B})$[a]

Catalyst	$SnCl_4$				$SbCl_5$			
Initial PO:MA charge ratio	3:1				3:1			
Conversion, %	2				2			
Final PO:MA charge ratio	3:1				3:1			
	$N = 0$	0	$N = 6$	0.28	$N = 0$	0	$N = 6$	0.19
	1	0.025	7	0.31	1	...	7	0.23
	2	0.22	8	0.30	2	0.11	8	0.28
	3	0.30	9	0.43	3	0.14	9	0.4
	4	0.25	10	0.6	4	0.14	10	0.5
	5	0.26	11	1	5	0.16	11	1

[a] The experimental errors for the conditional probabilities with $N = 2$ and 3 are ±0.01 and ±0.03, respectively, for the $SnCl_4$-catalyzed copolymer and about half of these values for the $SbCl_5$-catalyzed copolymer. The error increases rapidly for $N > 3$ because of an accumulated error in the denominator of the expression for the conditional probabilities in terms of the observed run concentrations.

Table VI. Ratios of Relative Concentrations of Closed Runs of Propylene Oxide in Propylene Oxide–Maleic Anhydride Copolymers

	$[\mathrm{B(A)}_{N+1}\mathrm{B}]/[\mathrm{B}(A)_N\mathrm{B}]$						
Catalyst	$SnCl_4$	$SnCl_4$	$SnCl_4$	$SbCl_5$	$SbCl_5$	$SbCl_5$	$SbCl_5$
Initial PO:MA charge ratio	12:1	3:1[a]	3:1[b]	12:1	6:1	3:1[c]	3:1[d]
$N =$ 1	...	3.8	8.8	44	27	20	...
2	3.4	2.0	1.0	0.75	0.70	0.97	1.2
3	0.26	0.15	0.58	0.36	0.44	0.32	0.85
4	0.81	0.37	0.76	1.6	1.7	1.3	0.97
5	1.27	0.75	0.84	0.86	0.73	0.72	0.96
6	0.59	0.38	0.77	0.55	0.46	0.51	1.0
7	0.78	0.52	0.77	0.92	0.70	0.77	0.93
8	0.80	0.73	0.80	0.82	0.63	0.68	0.93
9	0.74	0.60	0.77	0.60	0.65	0.67	1.0
10	0.75	0.50	0.69	0.55	0.65	0.75	0.92
11	0.71	...	...	0.58	...	0.56	...

[a] 77% conversion. [b] 2% conversion. [c] 76% conversion. [d] 4% conversion. All other samples are high conversion. Estimated errors can be obtained from the footnote to Table II.

units long.[1] The relative concentrations of runs of PO and their ratios given in Tables II and VI, respectively, demonstrate within experimental error the applicability of Markoffian statistics of third order in the description of $SnCl_4$- and $SbCl_5$-catalyzed low-conversion PO–MA copolymers. This conclusion is consistent with the conditional probabilities in Table V. For $N > 2$, all the conditional probabilities are the same, within experimental error.[3,4] (The drift toward higher values of the conditional probabilities for large N reflects the greater difficulty in detection in that range and an accumulated error.)

Earlier studies assigned third-order Markoffian statistical descriptions to the high-conversion copolymers produced by these catalysts.[1] Within the accuracy of those experiments, third-order statistics adequately described the monomer distributions. (Actually, second order was sufficient for $SbCl_5$-catalyzed material.) However, from Table III and Figures 3 and 4, the relative concentrations of runs of PO with N = 4–7 for the high-conversion copolymers do not show the same regular decrease in concentration as the corresponding runs in the low-conversion copolymers. These deviations from a regular decrease are well outside the experimental error indicated in Tables I and II. For $N > 7$, both high- and low-conversion copolymers contain runs of PO which decrease regularly in concentration with increasing N, within experimental error.

The deviations from a third-order Markoffian description can be explained by assuming the high-conversion material is actually generated by an eighth-order process. The physical situation required to produce such a process might assign control of propagation to very large numbers of monomers combined and ordered in a large reaction sphere. Such an involved explanation is rather unlikely.

A plausible mechanism for generating a non-Markoffian monomer distribution is a copolymerization in which there is an equilibrium of different catalytic states.[5] A description of the observed deviations in terms of this mechanism is unlikely since a regular decrease in run concentrations is observed for large N, and also the size and kind of the deviations are relatively insensitive to change in the charge ratio (ratio of reactants) as shown in Table III. Differences between Markoffian and this kind of a non-Markoffian description exist for all N and are strongly dependent on charge ratio.[6]

Since even the high-conversion copolymer was not carried to such high degrees of conversion that the charge ratio was changing by orders of magnitude, monomer distribution irregularities arising from severe compositional drift during the copolymerization are not present.[7]

The deviations can be explained by assuming that within both $SnCl_4$ and $SbCl_5$ catalyst systems the observed monomer distributions are a non-Markoffian sum[8] of two different monomer distributions arising from two different, independent types of catalysts, each generating a different Markoffian distribution. Table VII contains a numerical example of how the sum of closed runs from two Markoffian distributions can give rise to the kinds of deviations from Markoffian statistics which are observed.[9] In Table VII, distribution I is third-order Markoffian with closed runs decreasing regularly by a factor of 0.75 beginning with

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(3) B. D. Coleman, *J. Polymer Sci.*, 31, 155 (1958); B. D. Coleman and T. G Fox, *ibid.*, A1, 3183 (1963).

(4) The same end units must be counted in using conditional probabilities to determine the order of applicable statistics. Thus if three units affect propagation, third-order Markoffian statistics are appropriate. Yet $P(\mathrm{BAA/B})$ does not, in general, equal $P(\mathrm{BAAA/B})$.

(5) B. D. Coleman and T. G Fox, *J. Chem. Phys.*, 38, 1065 (1963).

(6) J. Schaefer, *Macromolecules*, 1, 111 (1968).

(7) A high-conversion pth-order Markoffian process must be considered a non-Markoffian average over many low-conversion, Markoffian-like processes. However, as long as the reactivities of the monomers and the extent of conversion are such that homopolymer is not formed and the monomer charge ratio changes by no more than a factor of 3 or 4, the deviations in run concentrations from a single, average, pth-order Markoffian description are small (on the order of a per cent of each run) compared to experimental error and can be ignored.

(8) W. Feller, "An Introduction to Probability Theory," John Wiley and Sons, Inc., New York, N. Y., 1957, p 379.

(9) With one exception, the large concentration of $\mathrm{B(A)_5B}$ runs in high-conversion PO–MA produced by $SbCl_5$ may be associated with end effects in the chain or possibly some nonpolymeric source.

Table VII. Relative Molar Concentrations of Closed Runs of Propylene Oxide in a Simulated PO–MA Copolymer

	$[B(A)_N B]$		
	Distribution I	Distribution II	Sum of distributions I and II
$N =$ 1	...	0.068	0.068
2	0.020	0.250	0.270
3	0.028	0.500	0.528
4	0.021	0.075	0.096
5	0.016	0.011	0.027
6	0.012	0.0016	0.014
7	0.008	0.0002	0.008
8	0.006	0.00003	0.006
9	0.004	...	0.004
10	0.003	...	0.003
11	0.002	...	0.002

$N = 3$. Distribution II is similar except closed runs decrease by a factor of 0.15 beginning with $N = 3$. The sum of these two distributions has a monomer distribution similar to that of the high-conversion $SnCl_4$-catalyzed copolymer given in Table III. The same treatment can be used to simulate a monomer distribution similar to that of the high-conversion $SbCl_5$-catalyzed copolymer. Of course, these simulations, and in particular the numbers arising from them, are not unique but simply offer a possible, reasonable interpretation of the available data.

For a single catalyst type generating PO–MA chains by a third-order Markoffian scheme, five species or states differing in their reactivity are present.[1] This allows BAAAB sequences to form more frequently than BAB or BAAB sequences. These reactive species are independent (since the chain statistics are Markoffian) but are connected by propagation steps of the chain. They can arise either from various combinations of monomers in the completed chain somehow associated with the catalyst, or from the growing chain and combinations of unreacted monomers (considered a part of the chain they are about to enter) in a coordination sphere of the catalyst. Analysis of the monomer distribution does not allow a choice between these two possibilities. If the catalyst is chemically modified during the copolymerization, then the reactivities of the various combinations of monomers with this new catalyst type can change, producing an entirely different Markoffian monomer distribution.

By this two-distribution interpretation, the high-conversion copolymer consists predominantly but not exclusively of chains produced by a type of catalyst not present in the early stages of the copolymerization. (It is possible that both catalyst types are independently active during the later copolymerization period, each producing Markoffian chains.[10]) The later catalyst type produces mostly closed runs of three PO units (especially in the $SnCl_4$ system) but also both shorter and longer runs according to a third-order Markoffian scheme. Evidence for this explanation is available from a study of the structural isomer distribution which shows that the head-to-tail, head-to-head, etc., structures of high- and low-conversion copolymers are different, suggesting they were generated by different types of catalysts.[11]

Acknowledgment. The authors wish to thank Dr. T. G Fox for several helpful suggestions.

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(10) The low-conversion $SnCl_4$-catalyzed copolymer may have a small contribution from the later catalyst type because of the somewhat high value for $[B(A)_3B]$. See Table VI.

(11) J. Schaefer, R. J. Katnik, and R. J. Kern, *Macromolecules*, **1**, 101 (1968).

Denaturation Kinetics of Biopolymers by Differential Thermal Analysis

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Abstract: When aqueous solutions of biopolymers such as ovalbumin, trypsin, pepsin, and deoxyribonucleic acid are heated in a differential thermal analysis apparatus, sigmoid-shaped thermograms result. Such behavior is characteristic of systems undergoing a change in heat capacity during the heating process. An interpretation of the thermograms is given based upon the assumption that they represent order–disorder transitions of the biopolymers. Activation energies can be calculated from such thermograms, but the values obtained for the protein solutions are consistently lower than those published for the thermal denaturation of such solutions. For the DNA solution a value of 26 ± 3 kcal is obtained. Differential thermal analysis seems to be a promising technique for studying the thermally induced order–disorder transition in solutions of biopolymers.

It is well known that aqueous solutions of many proteins may be denatured by heating. Eyring and Stearn[2a] and Jolly[2b] have summarized the early work. Pollard,[3] in a review concerned with thermal effects on proteins and nucleic acids, points out that such denaturation is usually associated with an unfolding of the secondary and tertiary structure of the biopolymer and with the formation of a new form or forms which are generally much less accurately structured. This response of protein solutions to thermal treatment

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(1) Currently (1967–1968) Fulbright Fellow at the Fysisk-Kemisk Institut, DTH 206, Lyngby, Denmark.

(2) (a) H. Eyring and A. E. Stearn, *Chem. Rev.*, **34**, 253 (1939); (b) M. Jolly, *Progr. Biophys.*, **5**, 168 (1955).

(3) E. C. Pollard, *Advan. Chem. Phys.*, **7**, 201 (1964).