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Thermolysis and Methanolysis of Poly(β-hydroxybutyrate): Random Scission Assessed by Statistical Analysis of Molecular Weight Distributions

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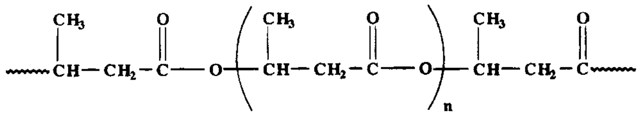
ABSTRACT: Samples of a bacterial polyester, poly(β-hydroxybutyrate), have been degraded thermally as a thin film on a thermocouple-controlled filament at 350 °C. In one series of these thermal experiments a short pyrolysis time (5 s) was used in order to ensure only partial pyrolysis, while in another series of thermal experiments the pyrolysis was allowed to proceed until all the sample had decomposed to volatiles. Samples from the same batch were also degraded in solution by methanolysis under acid conditions, while under reflux at 84 °C. In all cases, the yields and molecular weights of the products were assessed by gas chromatography. The number and weight distributions of the oligomeric products observed have been compared with those predicted statistically on the basis of random scissions. The results show that *total* thermolysis can be interpreted exclusively in terms of a random chain scission mechanism. However, the *partial* pyrolysis results are not consistent with random scission statistics; these results imply that there are some kinetically favored scissions occurring near the ends of the molecules. The product distributions from methanolysis are also somewhat inconsistent with those expected on the basis of random scission and may suggest that dimers hydrolyze more readily than higher molecular weight species.

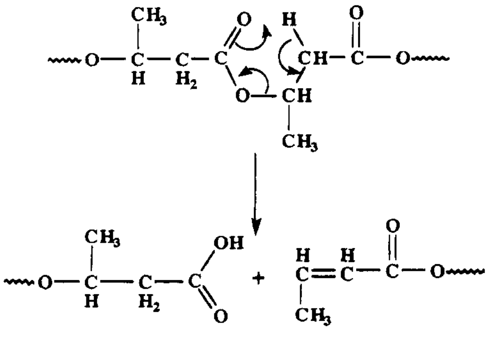
Introduction

Background to the Present Work. Poly(β-hydroxybutyrate), PHB, is an optically active polyester[1−3] with the following structure:



It is produced as an intracellular polymer by a large variety of micro-organisms.[4,5] First isolated and characterized in 1925 by Lemoigne,[6] this thermoplastic polymer is fully biodegradable by hydrolytic processes[7,8] or microbial activity.[9,10] PHB is currently made using a large scale fermentation process by Zeneca Bio Products and is sold under the trade name Biopol.

PHB is known[11−14] to be susceptible to thermal degradation at temperatures close to its melting point 180 °C. It is widely believed that this degradation occurs almost exclusively via a random chain scission mechanism involving a six-membered ring transition state,[11,13,15] as shown below:



Recent work[16] within the authors' laboratories has provided evidence that under certain conditions random chain scissions cannot be responsible exclusively for the formation of the observed degradation products. In particular, it was shown that primary products are involved in a significant number of secondary reactions and isomerizations and, indeed, that tetramer is formed principally as a result of such secondary reactions.

Aims of the Present Work. The starting point of the present work is to measure the yields of degradation products produced from the thermolysis and methanolysis of Biopol. The measured molecular weight distributions of these products can then be compared with those predicted statistically for purely random scission processes. Such comparisons thereby provide an indication of the extent to which the processes occur via random chain scission mechanisms.

Statistical Prediction of MW Distributions Resulting from Random Chain Scission. At time t, let α equal the fraction of bonds broken. This corresponds to the probability that a given bond is broken at time t. It follows that $(1 - \alpha)$ is the fraction of bonds not broken, and this similarly corresponds to the probability that a given bond has *not* broken at time t.

If we now consider the occurrence of a scission at some distance from the end of the molecule (e.g. after x units or $(x - 1)$ linkages), then the probability that there is a sequence of at least $(x - 1)$ unbroken linkages is $(1 - \alpha)^{x-1}$.

The probability that the xth linkage is broken equals α. Hence the probability that the scission produces an x-mer is $\alpha(1 - \alpha)^{x-1}$.

This probability must equal the ("*number*") fraction of x-mers, i.e.

$$\mathcal{N}_x = \alpha(1 - \alpha)^{x-1} \qquad (1)$$

We can express the number fraction in the following way:

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$$\mathcal{N}_x = \frac{N_x}{N} = \frac{\text{no. of } x\text{-mers}}{\text{no. of all molecules}}$$

A corresponding expression can be written as below for the weight fraction of x-mers, and assuming that at time t the $\overline{\mathrm{DP}}$ approximates to $1/\alpha$ (see Appendix), the weight fraction can be related to the number fraction as follows:

$$\mathcal{W}_x = \frac{\text{weight of } x\text{-mers}}{\text{weight of all molecules}} = \frac{N_x x M_u}{N(M_u/\alpha)}$$

where M_u is the MW of one unit. Thus

$$\mathcal{W}_x = \mathcal{N}_x x \alpha \tag{2}$$

or

$$\mathcal{W}_x = x\alpha^2(1-\alpha)^{x-1} \tag{3}$$

The fraction of bonds broken, α, is shown to be related to the weight and number fraction for each value of x by rearranging eq 2:

$$\alpha = \mathcal{W}_x/\mathcal{N}_x x \tag{4}$$

In order to assess the extent to which the experimental yields of oligomers are consistent with the distribution functions given by eqs 1 and 2, it is convenient to take logarithms in each case, so that

$$\ln \mathcal{N}_x = \ln \alpha + (x-1)\ln(1-\alpha) \tag{5}$$

and

$$\ln\left(\frac{\mathcal{W}_x}{x}\right) = 2\ln \alpha + (x-1)\ln(1-\alpha) \tag{6}$$

Thus if $\ln \mathcal{N}_x$ is plotted against $(x-1)$, the plot should be linear if the data satisfy the equation. Moreover, the value of α, calculated from the intercept, should agree with the value calculated from the slope.

Similarly, if $\ln(\mathcal{W}_x/x)$ is plotted against $(x-1)$, the linearity of the plot and the consistency of the α values calculated from the intercept and the slope should again confirm the random scission mechanism which underlies the equation.

On the other hand, lack of linearity in the plots, or inconsistent values of α, must be taken to indicate that a purely random scission mechanism is not adequate to interpret the data.

Experimental Section

Thermolysis. Thermolyses were performed using a thermocouple-controlled filament pyrolyzer[17] developed in the authors' laboratory. Samples (10 μg) of PHB were deposited uniformly from chloroform solution (1 g dm^{-3}) on the central 3 mm section of the pyrolysis filament. A graduated 5 μL syringe was used for this purpose. Each sample was pyrolyzed at 350 ± 1 °C. In one series of experiments the polymer was only partially pyrolyzed; in another series it was totally pyrolyzed. Degradation durations of 5 or 60 s were used respectively to achieve this.

Methanolysis. PHB (20 g) was dissolved in 200 cm^3 of 1,2-dichloroethane by refluxing at 84 °C for 1 h. To this solution was then added 80 cm^3 of a 5% w/w solution of sulfuric acid/methanol, and the reaction vessel was maintained under reflux for a further 3 h. After this period the reaction mixture was neutralized using a solution of sodium hydrogen carbonate and washed with 80 cm^3 of a saturated sodium chloride solution. Any degradation products present in the aqueous layer were extracted using three 25 cm^3 aliquots of 1,2-dichloroethane, and the extracts were added to the organic layer. The latter was then washed successively with 50 cm^3 of saturated sodium chloride solution, 50 cm^3 of saturated sodium hydrogen carbonate solution, and 50 cm^3 of saturated sodium chloride solution. The solution was finally dried using anhydrous magnesium sulfate, and concentrated to one-tenth of its volume by using a rotary evaporator.

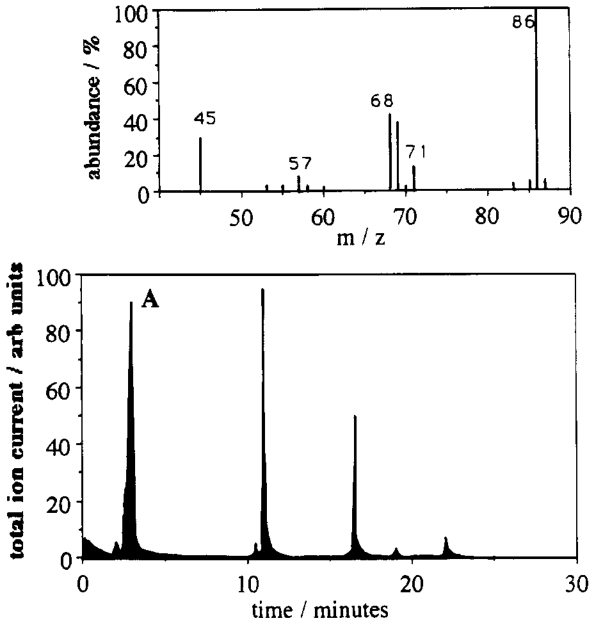


Figure 1. Lower: Total ion chromatogram of the products from the thermolysis of PHB. Top: Example of the mass spectra used to characterize the products (spectrum of chromatographic peak A).

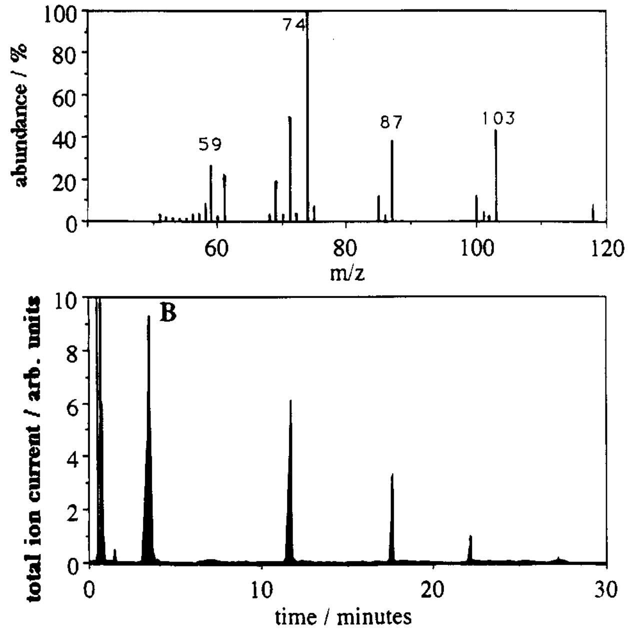
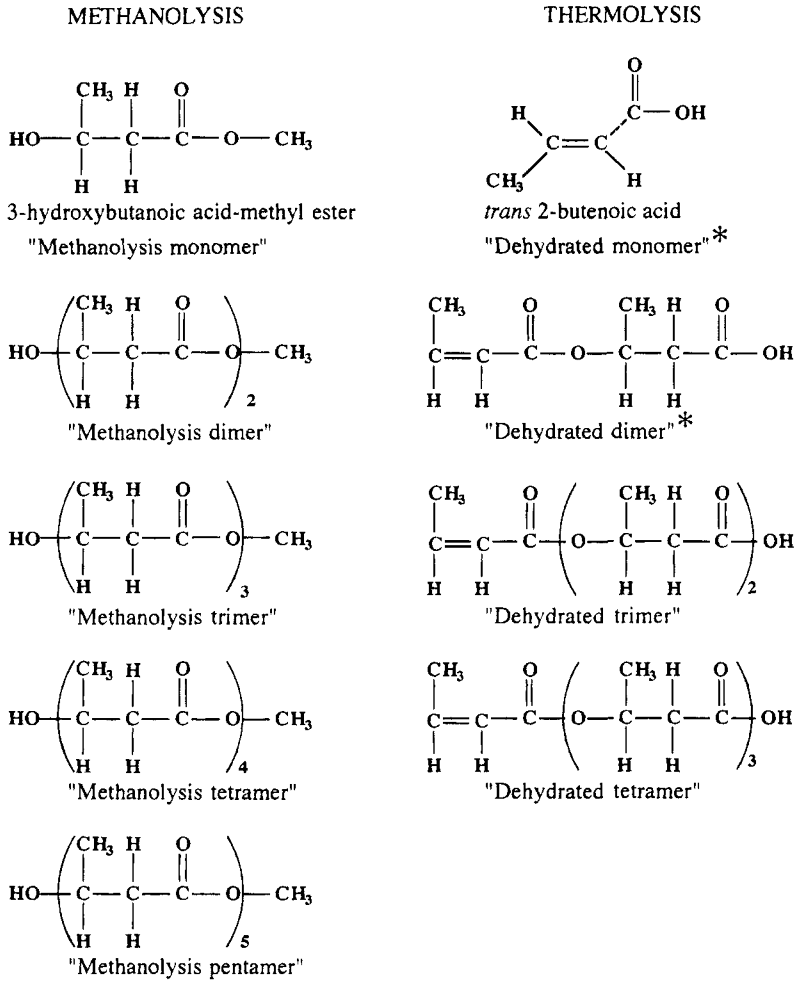


Figure 2. Lower: Total ion chromatogram of the products from the methanolysis of PHB. Top: Example of the mass spectra used to characterize the products (spectrum of chromatographic peak B).

Product Measurement and Characterization. Degradation products obtained from the methanolysis and the thermolysis were separated and measured by gas chromatography and characterized by gas chromatography–mass spectrometry.

Chart 1



METHANOLYSIS

3-hydroxybutanoic acid-methyl ester
"Methanolysis monomer"

"Methanolysis dimer"

"Methanolysis trimer"

"Methanolysis tetramer"

"Methanolysis pentamer"

THERMOLYSIS

trans 2-butenoic acid
"Dehydrated monomer"*

"Dehydrated dimer"*

"Dehydrated trimer"

"Dehydrated tetramer"

Degradation products larger than *pentamer* obtained from *methanolysis* have too low a vapour pressure to be detected under the conditions used.

Degradation products larger than *tetramer* obtained from *thermolysis* have too low a vapour pressure to be detected under the conditions used.

* *Cis* and *trans* isomers exist for these components, *trans* being the most abundant. The isomers of trimer and tetramer are not resolved under the g.c. conditions used.

Gas Chromatography and Mass Spectrometry. The gas chromatography apparatus used for the analysis of pyrolysis products was a Carlo Erba HRGC 5300 Mega series instrument incorporating an FID detector. The instrument was fitted with a 25 m DB1 bonded, nonpolar, fused silica megabore column (internal diameter 0.53 mm), through which flowed nitrogen carrier gas at 15.5 $cm^3\ min^{-1}$. The temperature of the detector unit was set at 270 °C, and that of the pyrolysis chamber at the head of the gc column at 170 °C. Hydrogen and air flow rates for the FID detector were maintained at 25 and 350 $cm^3\ min^{-1}$, respectively. The gc column temperature program was set at 40 °C isothermal for 2 min and then 10 °C/min to 250 °C, where it was maintained for a further 10 min.

Data acquisition was performed using an IBM-compatible PC fitted with Baseline 810 chromatography software (Millipore, Watford, U.K.).

An in-line VG-TS 250 mass spectrometer was used to characterize gas chromatographic peaks. Examples of total ion current chromatograms obtained from thermolysis and methanolysis are shown in Figures 1 and 2, respectively.

Characterized Degradation Products. Degradation products were identified by gc-ms as in Chart 1.

Results

Calculation of Weight and Number Fractions. Since the gas chromatographic peak areas obtained with a FID detector are closely proportional to the masses of the components, it is possible to calculate the weight fraction of each oligomeric species directly from the peak area data. The *relative* number fractions can be calculated from the peak area and the corresponding value of x for each species, and in the case of total pyrolysis or thermolysis, those relative values can then be converted to number fractions, by scaling them so that the sum of all the number fractions is equal to unity.

In the case of partial pyrolysis, the above procedures for calculating the weight and number fractions must be modified to take account of the unpyrolyzed material. Since the fractional (weight) conversion can be found experimentally (40% in the present study), the weight fractions could then be calculated by making allowance for the 60% residue. It was then necessary to calculate the number fractions by a more indirect method, which

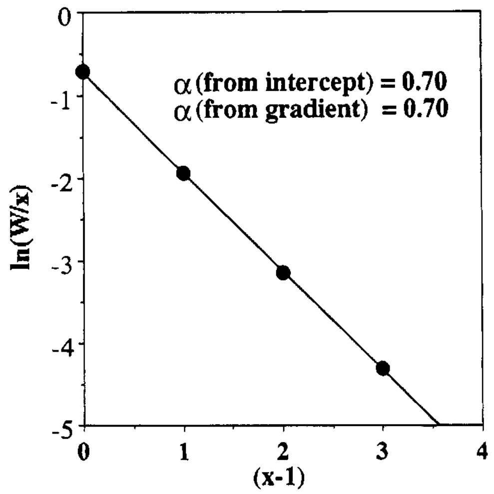


Figure 3. log plot of the weight fraction data obtained from the total pyrolysis study.

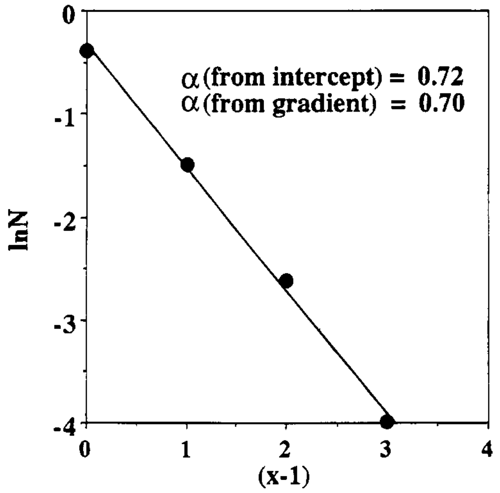


Figure 4. log plot of the number fraction data obtained from the total pyrolysis study.

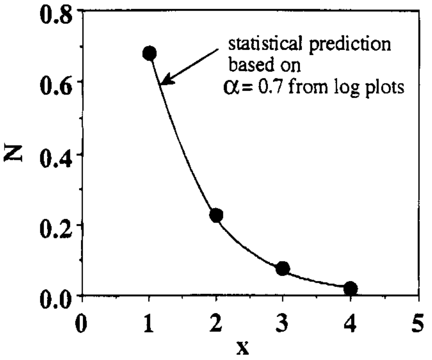


Figure 5. Number distribution of the products from the total pyrolysis study, compared with the curve predicted on the basis of the α values from the log plots: (●) experiment, (—) theory.

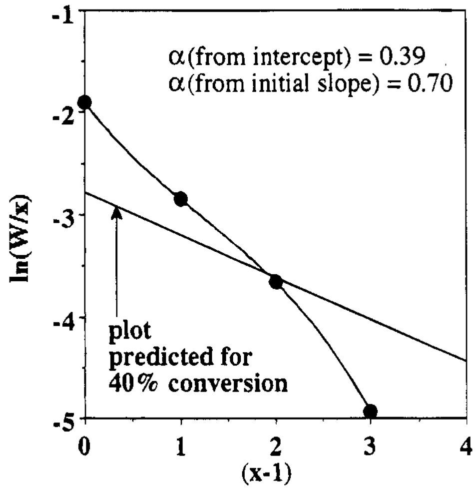


Figure 6. log plot of the weight fraction data obtained from the partial pyrolysis study, compared with the plot predicted from the measured 40% weight conversion to volatiles.

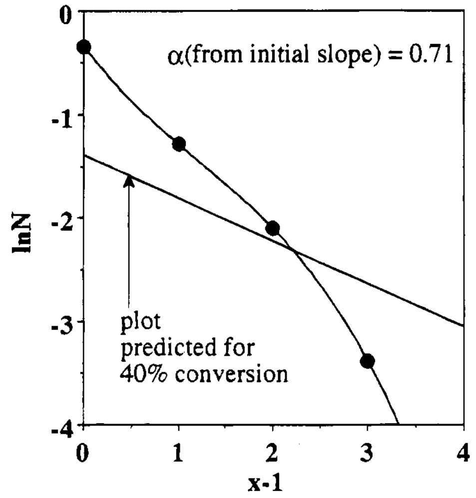


Figure 7. log plot of the number fraction data obtained from the partial pyrolysis study, compared with the plot predicted from the measured 40% weight conversion to volatiles.

will be explained when discussing the details of these results.

Total Thermolysis. The log plot of the weight fraction data for total pyrolysis is shown in Figure 3, and that for the number fraction data in Figure 4. The linearity of the plots and the totally consistent results for the four α values show that when total pyrolysis to volatiles has occurred, the product distributions correspond precisely to those expected for a random scission mechanism. Using the value $\alpha = 0.70$, as determined from these log plots, the corresponding number distribution function is shown in Figure 5, with the superimposed experimental points. The weight distribution function also shows a perfect fit with the experimental points, and it was considered unnecessary to reproduce it here.

Partial Thermolysis. The log plots of the weight and number fractions for partial pyrolysis are shown in Figures 6 and 7. The weight fractions were calculated as described above, but the number fractions were estimated by normalizing with a total number chosen so that the deviation from theory was comparable with that displayed by the weight fraction data. (The effect of this is to invalidate the calculation of α from the intercept of the log plot of the number fraction, though the use of the gradient is still relevant.) Also shown on Figures 6 and 7 are the statistically predicted log plots for the situation in which the sum of the monomer to tetramer yields correspond to 40% of the toal products. The discrepancy between the experimental and predicted log plots, the lack of linearity of the experimental plots, and the impossibility of deducing a unique value of α, all lead to the conclusion that the degradation mechanism cannot be exclusively by random scission. This is shown even more dramatically by the plots

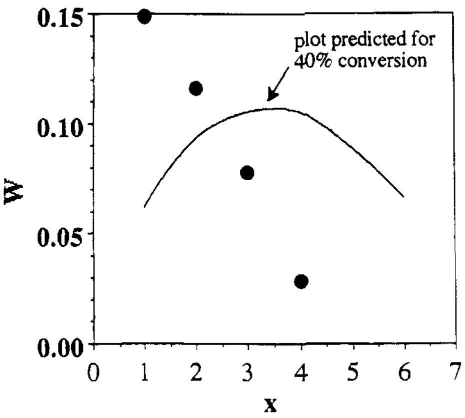


Figure 8. Weight distribution of the products from the partial pyrolysis study, compared with the plot predicted from the measured 40% conversion to volatiles: (●) experiment; (—) theory.

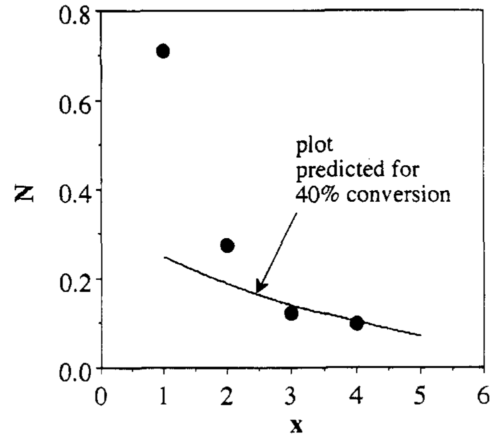


Figure 9. Number distribution of the products from the partial pyrolysis study, compared with the plot predicted from the measured 40% conversion to volatiles: (●) experiment; (—) theory.

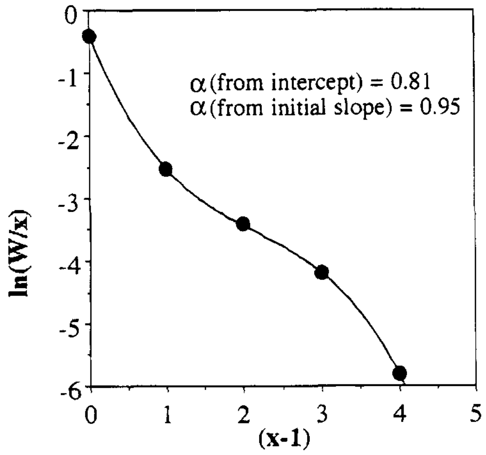


Figure 10. log plot of the weight fraction data obtained from the methanolysis study.

of the actual weight and number distributions (Figures 8 and 9). The experimental points are quite inconsistent with the theoretical curves.

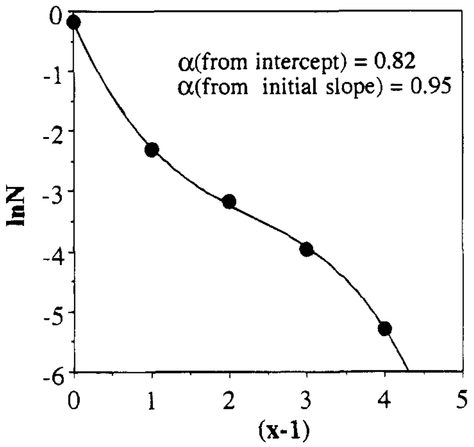


Figure 11. log plot of the number fraction data obtained from the methanolysis study.

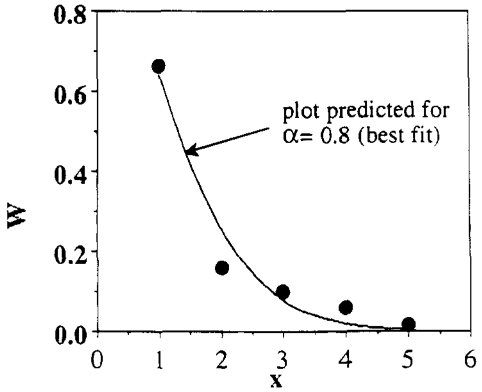


Figure 12. Weight distribution of the products from the methanolysis study, compared with the best fit statistical plot assuming α = 0.8: (●) experiment; (—) theory.

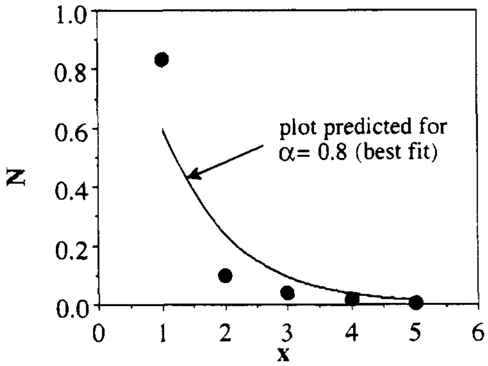


Figure 13. Number distribution of the products from the methanolysis study, compared with the best fit statistical plot assuming α = 0.8: (●) experiment; (—) theory.

Methanolysis. The log plots for the methanolysis data (Figures 10 and 11) provide some degree of correspondence for the α values, but both plots appear to display some sigmoid deviation from linearity. Also, Figures 12 and 13 show that although the observed distributions can be fitted reasonably by plots based on the random distribution functions, the fits are not precise. From these observations we suggest that the methanolysis process may occur in a largely random manner, but perhaps not exclusively so.

Conclusions

The conclusions from the statistical analysis of the results obtained in this study can be summarized as follows:

(a) In the *total pyrolysis* of PHB to oligomeric products, the weight and number distributions of the latter are totally consistent with those predicted on the basis of random scission degradation. This not only provides a clear message about the mechanism in the final stages of thermolysis but also confirms the validity of the statistical analysis for application in the present situation.

(b) In the *partial (40%) pyrolysis* of PHB, the weight and number distributions of the oligomeric products are not consistent with those predicted statistically on the basis of random scission. The deviations indicate in particular that there are pro rata greater yields of monomer and dimer than would be produced by purely random scission processes. Thus it appears that there are some kinetically favored scissions occurring near the ends of the molecules, possibly involving back-biting reactions of labile groups on the chain ends. This conclusion supports some previous findings of the authors,[16] obtained from studies of the dependence of pyrolysis product yields on film thickness.

(c) *Methanolysis*: If it had not been established from the total pyrolysis study that total concordance with statistical predictions can be observed, the methanolysis log plots could possibly have been accepted in terms of scatter on plots essentially in agreement with random scission statistics. However, the authors believe that the sigmoid curvature in the log plots may well be real and that the deviations in the distributions from the best fit statistical curves are probably significant. The only common abnormal feature of both the weight and number distributions is that the yield of dimer seems to be less than that expected on the basis of purely random scission. A possible implication of this is that the dimeric species hydrolyzes rather more readily than higher oligomers.

We now consider the extent to which the above conclusions are in accord with those obtained in other studies of the thermal degradation or methanolysis of this polymer.

Kunioka and Doi,[11] who studied the thermolysis of PHB copolymers, concluded that the mechanism involved random chain scission at ester groups. Grassie *et al.*[12-14] also proposed this as the predominant mechanism, but noted that the early stages of the process were delayed, and attributed this to additional reactions involving the end groups of the polymer molecules. These results are consistent with our finding that total thermolysis results are completely in accord with random scission statistics, but that the partial pyrolysis results show some deviations. We think it possible that the deviations involve reactions of the end groups, but Grassie's view that these are condensation reactions does not explain the trend observed in our results. It seems more probable that some intramolecular exchange mechanisms are involved, and this proposal is consistent with that put forward by Abate *et al.* in their thermolysis study.[18]

With respect to methanolysis, Seebach *et al.*[19] showed that the H_2SO_4-catalyzed methanolysis of PHB leads to large yields of the methyl ester of hydroxybutanoic acid, together with a mixture of oligomeric methyl esters, as observed in the present work. Assuming that the methanolysis process leads to random chain scissions, Ballistreri *et al.* have developed methods for estimating sequence distributions in bacterial copolyesters.[20,21] However, their HPLC analyses of the methanolysis products do not appear to follow a totally smooth distribution, and their results may well be displaying similar deviations from the statistical distribution to those found in the present work. It must nevertheless be emphasized that the deviations which we report are not very large and would permit the general conclusion that methanolysis gives rise predominantly to random scission.

Acknowledgment. The authors would like to thank Zeneca Bio Products for financing the research program. C.F. studied the methanolysis as part of his final year degree project.

Appendix

Proof that $\overline{\mathrm{DP}} = 1/\alpha$. Let there be U units in the whole sample.

Assumption 1: *If the original sample were a single molecule, there would be $(U - 1)$ linkages.* If a fraction α of these were broken, the number broken would be $\alpha(U - 1)$.

Since one scission produces two molecules, two scissions produce three molecules, three scissions produce four molecules, etc., then $\alpha(U - 1)$ scissions produce $\alpha(U - 1) + 1$ molecules

$$\overline{\mathrm{DP}} = \frac{\text{no. of units}}{\text{no. of molecules}} = \frac{U}{(\alpha(U-1)+1)}$$

Since U is very large, this reduce to

$$\overline{\mathrm{DP}} = 1/\alpha$$

Assumption 2 *(alternative to assumption 1): The sample has a MW distribution.* If the original sample had $\overline{\mathrm{DP}} = D_0$ there would be U/D_0 molecules present; i.e. there would be $[(U/D_0) - 1]$ fewer bonds available for scission.

The total number of bonds available for scission is

$$\left(U - 1 - \frac{U}{D_0} + 1\right) \quad \text{i.e.} \quad \left(U - \frac{U}{D_0}\right)$$

Thus if a fraction α are broken, the number of scissions is $\alpha[U - (U/D_0)]$.

As before, it can be shown that the number of molecules resulting is one more than the number of scissions, i.e.

$$\alpha\left(U - \frac{U}{D_0}\right) + 1$$

Therefore

$$\overline{\mathrm{DP}} = \frac{U}{\alpha\left(U - \frac{U}{D_0}\right) + 1} \approx \frac{U}{\alpha\left(U - \frac{U}{D_0}\right)} = \frac{1}{\alpha\left(1 - \frac{1}{D_0}\right)}$$

Since the initial degree of polymerization, D_0, is large, the last equation reduces to

$$\overline{\mathrm{DP}} = 1/\alpha$$

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