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REPORT NUMBER 5

THE USE OF POLYMER UNZIPPING FOR THE DETECTION OF BIOLOGICALLY ACTIVE AGENTS

Fifth Progress Report

by

Edward B. Dismukes

December 6, 1965

US Army Edgewood Arsenal
CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
Edgewood Arsenal, Maryland 21010

Contract No. DA18-035-AMC-265(A)

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Birmingham, Alabama 35205

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FOREWORD

The research described in this report was authorized under Task 1A012501B02802, Basic Research in Life Sciences, Chemical. The work was conducted between June 15 and August 15, 1965. The experimental data are contained in Notebook 3763.

Acknowledgments

Most of the laboratory work described in this report was conducted by Dr. Edward R. Covington. The light-scattering experiments were performed by Mrs. Frances P. Dean.

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DIGEST

The over-all purpose of the research discussed in this report is to determine the feasibility of detecting biologically active agents by their initiation of the depolymerization of certain polymers in a rapid unzipping chain mechanism. Continued work on polymers of n-heptaldehyde has been done, since preliminary results indicated that these polymers were promising for use in detection. The uncapped type of polymer is far less stable than the capped type, and the unzipping of the latter might be initiated by the destruction of capping groups by biologically active agents, such as phosphorylating compounds.

The earlier preparation of an acetate-capped polymer of heptaldehyde has been repeated successfully. This polymer will be used in later, more extensive characterization studies, but it has already been found to have about the same degree of stability at room temperature in the solid state as the capped polymer investigated previously. Light-scattering and viscosity experiments have been conducted to gain additional insight on methods to characterize aldehyde polymers, but further work must be done before definite conclusions can be drawn from the results thus far obtained. Some new kinetic studies indicate that the rapid rates of depolymerization of uncapped polymers observed previously were not due to the effect of acid and thus probably were essentially a measure of the autodegradability of these polymers.

TABLE OF CONTENTS

THE USE OF POLYMER UNZIPPING FOR THE DETECTION OF BIOLOGICALLY ACTIVE AGENTS

I. INTRODUCTION AND SUMMARY

This is the fifth report on a study to determine the feasibility of detecting biologically active agents by their initiation of the chain unzipping of polymers. In contrast to earlier reports, which covered 3-month periods of work, this report covers only two months, from June 15 to August 15, 1965. Between August 15 and October 12, 1965, the work was interrupted until additional supporting funds could be made available.

Earlier reports have included a discussion of unsuccessful attempts to synthesize a polyketal by the addition polymerization of acetone by methods described in the literature. Since our work on polyacetone was terminated, a new publication on polyacetone[1] has appeared, and negative results similar to ours are reported. Thus, the wisdom of our terminating work on polyacetone appears to be confirmed.

Our earlier reports, especially Report 4 dated August 2, 1965, presented more promising results on polyacetals made by the addition polymerization of aldehydes. Most of our work on aldehyde polymers was done with poly(n-heptaldehyde). Although yields of this type of polymer were variable, adequate quantities were obtained for experimental evaluation. The most significant finding on polymers of heptaldehyde was that uncapped polymers, presumably with hydroxyl end groups, depolymerize far more rapidly than a capped polymer with acetate end groups. As a result of this finding, polyheptaldehyde appeared fairly promising for the proposed use in detection, which depends on the rapid depolymerization of a polymer after capping groups have been destroyed by the agent being detected.

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1. V. C. E. Burnop, Polymer 6, 411 (1965).

Although the depolymerization rates of the uncapped heptaldehyde polymers were unequivocally greater than that of a capped polymer, the rates for the uncapped polymers prepared by different methods differed considerably. The reason for the different rates is not known, since adequate methods were not known for characterizing the relevant properties of the aldehyde polymers, especially molecular weight.

The work described in the current report included experiments on the determination of the molecular weights of aldehyde polymers. It also included the synthesis of a new polymer sample and some new kinetic experiments. The work on methods for adequate polymer characterizations, such as molecular weight determinations, will be continued now that additional funds have been made available for the research.

II. SYNTHESIS OF CAPPED POLYMERS OF n-HEPTALDEHYDE

Report 4 discussed the successful synthesis of an acetate-capped polymer (3763-72). The steps in this synthesis were: the polymerization of 0.15 mole of heptaldehyde with 0.0014 mole of potassium triphenylmethoxide catalyst in hexane at -75°C; the treatment of the unisolated, uncapped polymer with a mixture of pyridine and acetic anhydride to convert hydroxyl end groups to acetate end groups; and finally the isolation and the purification of the capped polymer by filtering the polymer-solvent mixture, heating the polymer in tetralin solution to degrade any residual uncapped polymer, and extracting any remaining impurities with ethanol in a Soxhlet apparatus.

Virtually the same procedure was followed to prepare another sample of capped heptaldehyde polymer. The product, designated 3763-97, represented a 9% yield from the amount of monomer used; its elemental analysis was 73.23% carbon and 12.06% hydrogen, in comparison with theoretical values of 73.68% carbon and 12.28% hydrogen (based on the analysis of the monomer).

The yield of 3763-97 was only one-half of that of 3763-77. At least part of the difference is attributable to the longer extraction time used to purify 3763-97, and the correspondingly greater amount of this product lost in the solvent. Part of the product lost was recovered and subsequently identified as polyheptaldehyde by its infrared spectrum. This material, designated as 3763-100, presumably had a lower molecular weight than 3763-97; and its elemental analysis of 71.66% carbon and 11.97% hydrogen indicated that it was less pure.

An unsuccessful attempt was made to prepare an acetate-capped polymer by starting the polymerization of the heptaldehyde monomer with n-butyllithium as the catalyst. Except for the use of butyllithium in place of potassium triphenylmethoxide, the procedure followed was the same as the one described above. Evidently, butyllithium is a relatively poor catalyst for the polymerization of heptaldehyde, since it previously gave a relatively low yield of uncapped polymer.

III. CHARACTERIZATION OF POLYMERS OF n-HEPTALDEHYDE

A. Molecular Weights

The determination of the molecular weights of uncapped polymers of heptaldehyde is complicated by the unstable nature of these polymers. Consequently, methods of determining molecular weights of heptaldehyde polymers have thus far been explored with capped polymers. Work based on light-scattering and viscosity determinations has been continued.

1. Light scattering

Light-scattering data were obtained with a Bryce-Phoenix photometer for Polymer 3763-72 in two solvents, tetrahydrofuran and carbon disulfide, and the results are presented in Figure 1. The data for tetrahydrofuran solutions were discussed in Report 4, but the data for carbon disulfide solutions were obtained since

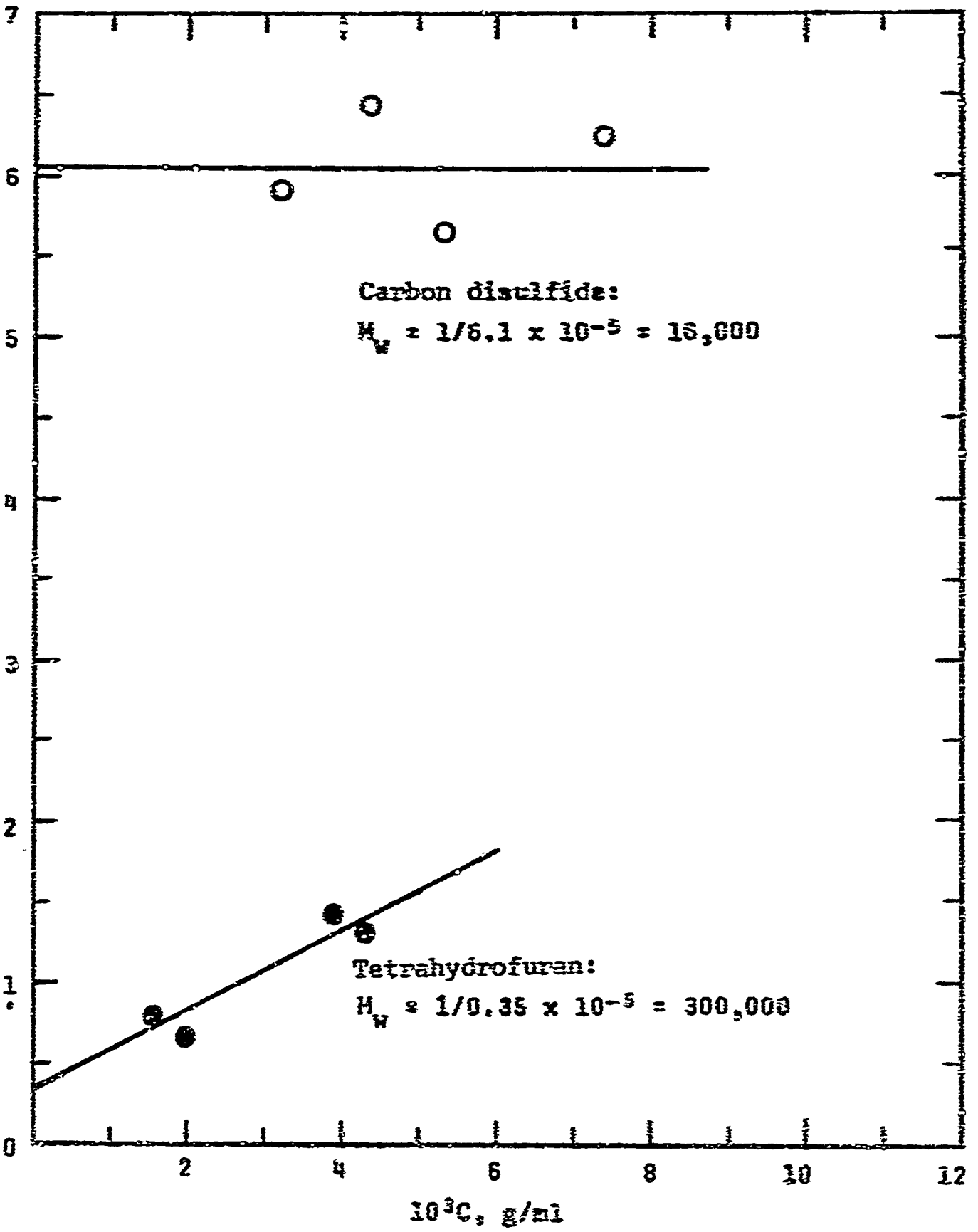

Figure 1. Light-Scattering Data of Acetate-Capped Polymer 3763-72 in Carbon Disulfide and in Tetrahydrofuran

$[CS_2: HC/\tau = 16.45 \times 10^{-5}(\Delta n/c)(\Delta n/\tau)$;
THF: $HC/\tau = 12.22 \times 10^{-5}(\Delta n/c)(\Delta n/\tau)]$

that report was issued. The values of Hc/τ shown in the figure for each type of solution were calculated from the equation given in the caption of the figure, with the (Δn/c) value equal to the average refractive index increment determined for four polymer concentrations and with the (Δn/τ) values calculated from data for individual polymer concentrations.

The reciprocal of the limiting value of Hc/τ at infinite dilution is theoretically equal to the weight-average molecular weight, M_w. Values of M_w calculated from the data for the two solutions - 16,000 and 300,000 - differ by more than an order of magnitude.* There are three possible explanations for this very large difference: (1) polymer decomposition might have caused a decrease in M_w during the 4-week interval between the two determinations; (2) the polymer might have been fractionated differently by the two solvents (each solvent failed to dissolve the polymer completely); and (3) the light-scattering data obtained with tetrahydrofuran as the solvent might be erroneous, due to the separation of solid polymer in the light-scattering cell after the solution had been filtered. In the experiments with tetrahydrofuran, some evidence of precipitation was noted and attributed to cooling as part of the volatile solvent evaporated. This effect is considered to be the most probable explanation for the difference in M_w values. Until further work on light scattering is done, the value of M_w obtained with carbon disulfide will be regarded as more reliable.

2. Viscometry

Previously reported viscosity data for carbon tetrachloride solutions of Polymer 3763-72 are included in the table on the following page. Since values of the specific viscosity,

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* In view of the scatter in the experimental data, there is an uncertainty of at least 25% in each value, but the two values are still unmistakably different.

Specific Viscosities of Acetate End-Capped Polymer 3763-72 in Various Solvents at 30°C

c', g/100 ml	$(\eta/\eta_0-1)^a$		
	CCl_4[b]	$CHCl_3$[b]	THF
0.526			0.080[b]
0.500		0.183	0.030[c]
0.400	0.070		
0.261			0.043[b]
0.250		0.052	0.042[c]
0.200	0.026		
0.131			0.021[b]
0.125		0.032	0.022[c]
0.100	0.014		

[a] η/η_0 = ratio of capillary flow times of solution and solvent.

[b] These data were obtained with a Ubbelohde No. 70 viscometer.

[c] These data were obtained with a Ubbelohde No. 50 viscometer.

(η/η_0-1), did not differ appreciably from zero, an accurate value of the intrinsic viscosity could not be obtained by extrapolating $(\eta/\eta_0-1)/c'$ to $c'=0$. However, the estimated value of $[\eta]$ was in the range of 0.02 to 0.1.

Recent data have revealed a greater viscosity effect by this polymer in chloroform than in carbon tetrachloride, as shown by comparative data in the table. Values of $(\eta/\eta_0-1)/c'$ were calculated and extrapolated, yielding an apparent intrinsic viscosity value of 0.26. However, the slope of $(\eta/\eta_0-1)/c'$ versus c' was negative, an anomalous result of the type encountered with polyelectrolytes.[2] Thus, the apparent value of $[\eta]$ may not be theoretically significant.

Two sets of data for Polymer 3763-72, obtained with two different viscometers and listed in the last column of the table, showed a viscosity effect whose magnitude was intermediate in comparison with that in carbon tetrachloride and that in chloroform. One set of values, plotted in Figure 2, shows that $[\eta]$ is 0.16, about the same as the result from the other set of values.

Viscosity data were also obtained for another capped polymer, 3763-97 (see Section II), and the results are included in Figure 2. For this polymer, the slope is negative rather than positive, which would be theoretically more acceptable. It is doubtful that the approximate equality of the apparent values of $[\eta]$ for Polymers 3763-72 and 3763-97 means that both have the same molecular weight. Data at $c' = 0.5$ g/100 ml indicate that 3763-97 has a lower molecular weight.

Additional work on solution viscosities will be necessary before any conclusions can be reached about solvents that are suitable for obtaining molecular-weight information based on intrinsic viscosities.

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2. F. W. Billmeyer, Jr., "Textbook of Polymer Science," Interscience Publishers, New York, 1962, p 88.

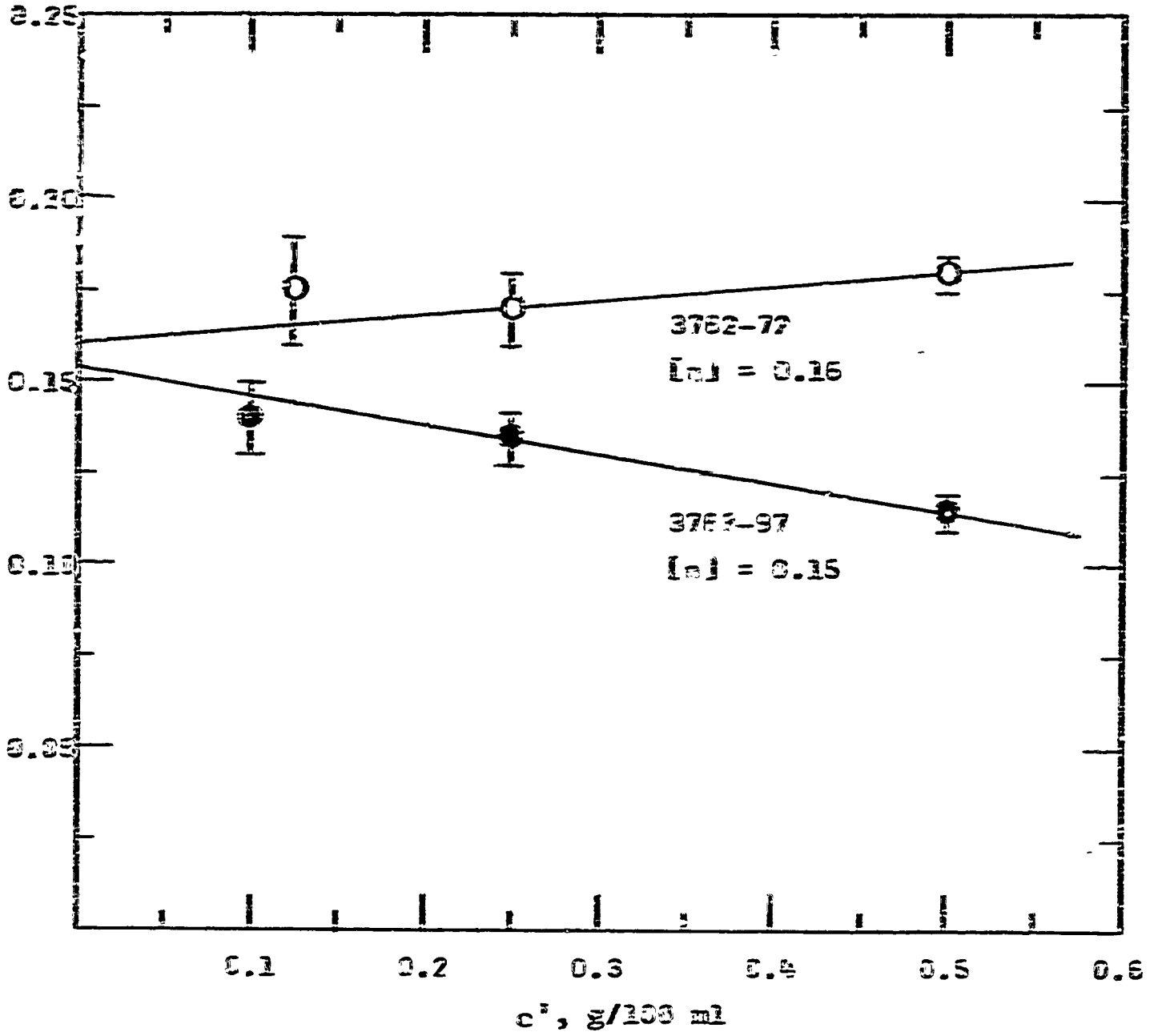

Figure 2. Viscosity of Tetrahydrofuran Solutions of Acetate-Capped Polymers of n-Heptaldehyde

B. Depolymerization Kinetics

The rapid rates of depolymerization previously observed for uncapped heptaldehyde polymers in carbon tetrachloride could not be attributed altogether to the autodegradability of this type of polymer. Rapid depolymerization might have been caused in part by acid impurities, which cause the cleavage of aldehyde polymer chains and the acceleration of depolymerization under some conditions.[3]

In order to evaluate the possible effect of acid impurities in the past experiments, we performed two experiments with heptanoic acid added to carbon tetrachloride solutions of an acetate-capped polymer. Since this polymer (3763-72) was found to be stable when acid was not intentionally added, any acceleration in its rate of depolymerization with the acid would be attributable to acid-induced chain cleavage.

In one experiment, the solution contained 0.05 g/ml of polymer and 0.003 g/ml of acid; in the other experiment, the solution was prepared by diluting the first polymer-acid solution with an equal volume of water-saturated carbon tetrachloride. Infrared spectra of the polymer solutions were recorded at intervals over a period of almost 18 hours. There was no evidence of any depolymerization in either solution. The infrared spectra showed that the concentration of added acid in both experiments was far greater than that of any acid impurity in previously studied solutions of uncapped polymers. Consequently, the rapid depolymerization rates of the latter apparently were not due to the effect of acid or to the combined effects of acid and water.

Polymer 3763-72 has now been stored for 6 months in a vial at room temperature. No evidence of increasing depolymerization has been noted during this time. Actually, heptaldehyde is detectable in the sample by odor, but it has always been present as an impurity. In contrast to this stable capped polymer, uncapped polymers depolymerize so completely under the same conditions that they become liquids overnight.

Polymer 3763-97, another end-capped polymer, has been stored under the conditions described above, and it is apparently unchanged after 3 months.

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3. G. Delzenne and G. Smets, Makromol. Chem. 18/19, 82 (1956).

IV. CONCLUSIONS AND PLANS FOR FUTURE WORK

The earlier preparation of a acetate-capped polymer of heptaldehyde has been repeated successfully. This polymer will be used in later, more extensive characterization studies, but it has already been found to have about the same degree of stability as a solid at room temperature as the capped polymer investigated previously. Light-scattering and viscosity experiments have been conducted to gain additional insight on methods to characterize aldehyde polymers, but further work must be done before definite conclusions can be drawn from the results thus far obtained. Some new kinetic studies indicate that the rapid rates of depolymerization of uncapped polymers observed previously were not due to the effect of acid and thus probably were essentially a measure of the autodegradability of these polymers.

Our immediate plans for further work call for the preparation of some new samples of uncapped polymers of heptaldehyde and for a study of the viscosities of various solutions of these polymers. We plan to use solvents such as carbon tetrachloride, chloroform, carbon disulfide, and tetrahydrofuran, and to measure the viscosity of each solution as a function of time at 30°C and at a lower temperature, probably near 0°C. The rates at which solution viscosities approach solvent viscosities should indicate how stable the polymers are in various solvents at the two temperatures. The rate data will be of interest as basic kinetic information, but they will also be used to select conditions which do not cause rapid depolymerization and which may thus be used to obtain information on molecular weights.

We expect to be able to evaluate the intrinsic viscosities of several uncapped polymers in one or more solvents, but only at a low temperature. If we are able to evaluate intrinsic viscosities of uncapped polymers, we will then evaluate the intrinsic viscosities of several capped polymers under the same conditions; determine the molecular weights of the stable capped polymers by light-scattering measurements at room temperature;* and finally correlate intrinsic viscosities and molecular weights by means

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* We are not equipped to conduct light-scattering experiments except at room temperature; thus, we probably will not be able to do light-scattering experiments directly on uncapped polymers.

of the Staudinger equation, $[\eta] = KM^{a}$. The constants K and a in the Staudinger equation should be equally valid for capped and uncapped polymers, and they should permit us to calculate molecular weights of uncapped polymers from intrinsic viscosities.

After finding a way to evaluate molecular weights of uncapped polymers, we will then attempt to develop methods to characterize the end groups of heptaldehyde polymers, of both capped and uncapped types. We do not believe that all end groups are either acetate or hydroxyl groups, since some may be residues of the polymerization catalyst. To characterize end groups, we plan to use both infrared spectra and chemical analyses.

Once we have adequate methods to determine molecular weights and characterize end groups of uncapped polymers, we will study the influence of these properties in depolymerization rates. We believe that differences in one or both of these properties will be found to affect depolymerization.

We will subsequently conduct detailed studies on depolymerization as a function of extrinsic factors, such as solvent, light, and oxygen.