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

	$[B(A)_NB]$		
	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.

(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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(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).

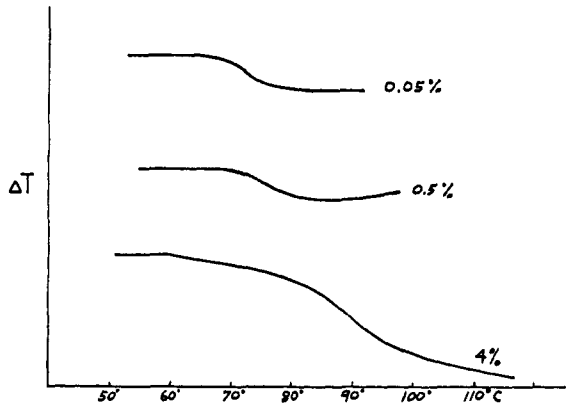

Figure 1. Thermograms for solutions of pepsin in water.

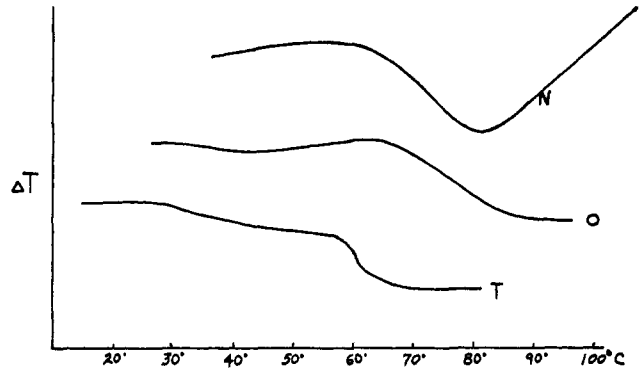

Figure 2. Thermograms for solutions of ovalbumin, trypsin, and deoxyribonucleic acid. O represents a 1% ovalbumin solution, T, a 0.5% trypsin solution, and N a 9% solution of deoxyribonucleic acid in water.

suggests that these systems may appropriately be examined by differential thermal analysis (DTA), a technique which, during the past few years, has come into extensive use as a sensitive tool for both the qualitative and quantitative study of thermally induced physical and chemical changes. A useful review by Smothers and Chiang[4] summarized much of the recent DTA work.

Early work on the application of DTA to organic and colloidal systems was undertaken by Vold and Vold[5] and by Brasseur and Champetier.[6] Morita[7] has examined some crystalline biopolymers and found characteristic thermograms for proteins and nucleic acids. Recently we have observed characteristic thermograms in aqueous solutions of proteins and deoxyribonucleic acid,[8] and in the present paper we wish to report the experimental procedure involved in these studies and to elaborate upon the theoretical interpretation of the results in terms of the kinetics and activation energy for the observed transitions. For our model systems we chose ovalbumin and pepsin, two proteins whose irreversible denaturation has been studied in detail.[2a,9] Because of the now well-established helix–coil transition of deoxyribonucleic acid (DNA), we were naturally interested in applying the technique to solutions of this substance.

Experimental Section

The proteins and calf thymus dexoyribonucleic acid were commercial materials of the highest purity available. No attempt at further purification was made. Solutions were prepared with deionized water, rather than buffer, for reasons which will become apparent later, and were run within a few hours of preparation. About 20–30 mg of the solution was sealed in a small, flat-bottomed glass vial and run in a commercial DTA apparatus[10] with the same volume of water as reference material in a similarly sealed vial. Heating rates were normally 20°/min although similar effects were observed at other heating rates.

Results

Some typical thermograms, resulting after steady-state heating conditions are established, are illustrated in Figure 1 for pepsin solutions of different concentrations. A number of features are apparent upon inspection of these curves.

1. All traces show a characteristic sigmoidal shape. Such thermograms appear in the DTA experiment when the system undergoes a change in heat capacity. In our experiment we always observed a downward transition with increasing temperature, a change indicative of an increase in the heat capacity of the system.

2. The transition normally occurs over a relatively narrow temperature range, seldom more than 20°.

3. The range in temperature over which the transition occurs increases along with the concentration of the protein solution, the higher the concentration the broader the range. It will be shown later that this broadening implies a decrease in the activation energy for whatever process is involved in this transition.

4. The most concentrated solution, the 4% pepsin solution, produces a thermogram with two regions in which transitions from a base line occur. In the first only a gradual increase in heat capacity takes place, while in the second a more rapid transition occurs at a higher temperature, about 85°.

The phenomena illustrated in Figure 1 are not restricted to pepsin solutions but are characteristic of solutions of some other biopolymers. In Figure 2 we reproduce some of our thermograms for solutions of ovalbumin (curve O) and trypsin (curve T). The sigmoidal transition for the 1% ovalbumin commences at about 60° but is preceded by a shallow endotherm which begins at about 30°. With the 0.5% trypsin solution two distinct regions are again discernible; after the establishment of the initial base line, there is a gradual fall-off to a new plateau followed by a rapid transition at about 60° to a new base line.

Figure 2 also reproduces a thermogram typical of those resulting when solutions of calf thymus deoxyribonucleic acid are heated. The thermogram labeled "N" is for a 9% solution in deionized water. While the sensitivity of the DTA apparatus used in our experiments is great enough to record a sigmoidal shaped curve for a 0.1% solution, the smallness of the resulting transition makes it impossible to calculate any kinetic data for these lower concentrations. The thermograms of the DNA solutions characteristically show an increase in heat capacity in the range of 70–80°, a temperature range in or near which much other experimental work[11]

(4) W. J. Smothers and Y. Chiang, "Handbook of Differential Thermal Analysis," Chemical Publishing Co., New York, N. Y., 1966.
(5) R. D. Vold and M. J. Vold, *J. Phys. Chem.*, **49**, 32 (1945).
(6) P. Brasseur and G. Champetier, *Bull. Soc. Chim.* France, **13**, 265 (1946).
(7) H. Morita, *Biopolymers*, **4**, 215 (1966).
(8) H. W. Hoyer, *Nature*, **216**, 997 (1967).
(9) (a) E. J. Casey and K. L. Laidler, *Science*, **111**, 110 (1950); (b) P. S. Lewis, *Biochem. J.*, **20**, 965, 978 (1926).
(10) Dupont No. 900 differential thermal analyzer with calorimeter cell.

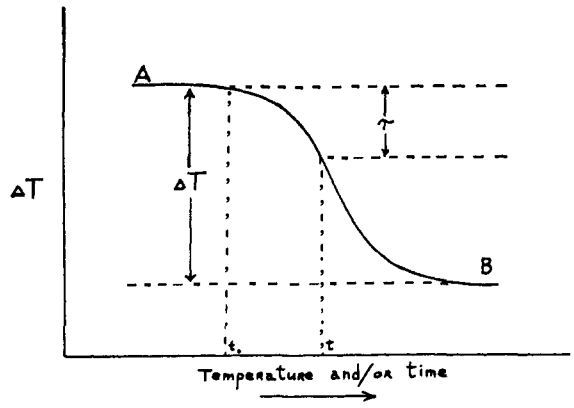

Figure 3. Idealized sigmoid-type thermogram; see text for explanation.

suggests that the DNA molecule in aqueous solution undergoes a helix–coil transition. In agreement with this other evidence, the transition which we observe during DTA experiments with solutions of DNA is shifted to lower temperatures if the solution also contains strongly hydrogen-bonding substances such as urea or ethylene glycol.

Although the DNA thermogram resembles a portion of a normal endotherm, it actually portrays a change in heat capacity since the lower "base line" shows no tendency to level off but continues to rise, at least to 125°, as high as we have followed these systems. The difference in slope of the upper and lower "base lines" implies a difference in the rate of change of heat capacity of the system before and after the transition.

The irreversible nature of the thermal transition for the pepsin and ovalbumin solutions was demonstrated by reruns of the reacted samples. In every case the repeat thermogram failed to show the sigmoidal shape observed initially, in accordance with the irreversible nature of the denaturation process for these two proteins. When such reruns were made of the DNA gels, some sigmoidal character did reappear but the extent was much smaller than in the initial run. Apparently, too, the amount of recovery of the DNA depended upon the time which elapsed between trials since greater recovery was observed when more time elapsed. Interesting though this recovery phenomenon may be, we have not yet been in a position to undertake any quantitative study of it.

Discussion

Much recent work has centered around the theoretical interpretation of the exothermic or endothermic peak occurring during the DTA experiment. Kissinger,[12] Borchardt and Daniels,[13] and Piloyan, *et al.*,[14] have shown how the kinetics and activation energy for a reaction may be obtained from such studies, and Rogers and Smith[15] have presented a method for estimating the preexponential factor from DTA thermograms.

Besides this commonly observed exothermic or endothermic peak, there is a second type of thermogram which may occur during the DTA experiment. This is the sigmoidal curve illustrated in Figure 3 which occurs when the sample undergoes an alteration in heat capacity and not, as is characteristic of the first type, a change in enthalpy. In this figure temperature and/or time is plotted along the abscissa, and ΔT, the difference between the temperature of the sample and the inert reference, along the ordinate. In many experiments it is of little consequence whether the temperature recorded along the abscissa is that of the sample or the inert reference.

In the idealized sigmoidal type thermogram of Figure 3, the upper base line is labled "A," the lower "B," and the difference between the two "ΔT." At any particular time, say t, this difference will be designated by τ in the discussion which follows. If the upper line is characteristic of a substance or state A and the lower of another substance or state B, then it can readily be shown that α, the fraction of A converted into B, is just the quotient of τ and ΔT.

In the absence of any thermal reaction producing or absorbing heat, the temperature difference between the sample and the inert reference when steady-state conditions prevail is given by[16]

$$T_s - T_r = \frac{a}{e}[M_rC_r - M_sC_s - M_{sh}C_{sh}] \quad (1)$$

where a is the heating rate (°C/sec), e is the heat-transfer coefficient (g cal/(°C sec)), M is the mass in grams, C is the specific heat, cal°/(°C g), and the subscripts r, s, and sh refer to reference substance, sample, and sample holder, respectively. Since similar equations apply to states A and B, it follows that

$$\Delta T = T_A - T_B = \frac{aM}{e}[C_B - C_A] \quad (2)$$

where M now represents the total mass of the sample. At any time t, the quantities of the sample in states A and B will be given by $(1 - \alpha)M$ and by αM, where α is the degree of conversion of A to B and therefore

$$\tau = \frac{aM}{e}[(1 - \alpha)C_A + \alpha C_B - C_A] = \frac{aM\alpha}{e}[C_B - C_A] \quad (3)$$

or

$$\alpha = \tau/\Delta T \quad (4)$$

The rate of a reaction may be expressed in terms of α by the equation

$$\frac{d\alpha}{dt} = A_0(1 - \alpha)^n \exp\left(-\frac{E}{RT}\right) \quad (5)$$

where A_0 is a constant, E the activation energy, T the absolute temperature, and n the order of the reaction.

Since the temperature is a function of the time, it is not possible to integrate eq 5 directly into a form suitable for evaluating the activation energy. However, all of the transitions which we have observed occur over a narrow temperature range of 5–20° so that the temperature during the transition is constant to better

(11) R. F. Steiner and R. F. Beers, Jr., "Polynucleotides," Elsevier Publishing Co., New York, N. Y., 1961, pp 206–208.
(12) H. E. Kissinger, *J. Res. Natl. Bur. Std.*, 57, 217 (1956).
(13) H. J. Borchardt and F. Daniels, *J. Am. Chem. Soc.*, 79, 41 (1957).
(14) G. O. Piloyan, I. D. Ryabchikov, and O. S. Novika, *Nature*, 212, 1229 (1966).
(15) R. N. Rogers and L. C. Smith, *Anal. Chem.*, 39, 1024 (1967).
(16) "Background Report on Mathematical Development-Differential Calorimetry," E. I. du Pont de Nemours & Co., Instrument Products Div., Wilmington, Del., 1965.

than ±3%. It should be possible therefore to approximate the exponential integral by

$$\int_0^t \exp\left(-\frac{E}{RT}\right)dt = k \exp\left(-\frac{E}{RT}\right)t$$

where k is a correction factor which will depend upon the activation energy and the small range of temperatures over which the integration is taken. We are not, however, concerned with k except in so far as it permits us to set down a suitable expression from which the activation energy may be obtained. With the above assumption, eq 5 becomes

$$\int_0^\alpha \frac{d\alpha}{(1-\alpha)^n} = A_0 kt \exp\left(-\frac{E}{RT}\right) \qquad (6)$$

Taking logarithms, it is apparent that the graphing of $\ln[(1/t)\int d\alpha/(1-\alpha)^n]$ *vs.* $1/T$ should result in a linear plot whose slope will be $-E/R$.

In order to provide some idea of the fit of the experimental data to eq 6, we include in Figure 4 plots for a 9% DNA gel and a 1% pepsin solution. In both cases the points cover a range of conversion from 10 to 90%. Our data for the thermal denaturation of the protein and DNA solutions gave linear plots for $n = 1$ and yielded the values of the activation energies shown in Table I. While these are consistently lower than

Table I. Activation Energies for the Thermal Denaturation of Proteins and DNA

Protein	Concn, %	Activation energy, kcal/mole DTA	Lit
Pepsin	4.0	16	62[9a]
	1.0	39	...
	0.5	39	56[9a]
	0.05	52	80[9a]
			55.6[2a]
Ovalbumin	1.0	19	...
	0.5	44	...
	0.1	79	132[9b]
Deoxyribonucleic acid	8–9	26 ± 3	...

the published data, they do illustrate the marked dependence of the activation energy upon the protein concentration as originally reported by Casey and Laidler,[9a] the lower the concentration the higher the activation energy. This, however, is immediately apparent in a qualitative sense from the shape of the curves in Figures 1 and 2 and from eq 6, since an increase in the temperature region over which the transition occurs must lead to a higher value of the activation energy.

If the transition measured by the DTA experiment is the denaturation of the protein, and the irreversibility which we observed for these systems would seem to substantiate this, then the sigmoidal transition to a state with a higher heat capacity is not unexpected. Denaturation, with its concomitant substitution of a disordered structure for an ordered one, must result in increased freedom of motion of those portions of the molecule which were previously restricted. This increased freedom of motion must manifest itself on the macroscopic level as an increase in the heat capacity of the system.

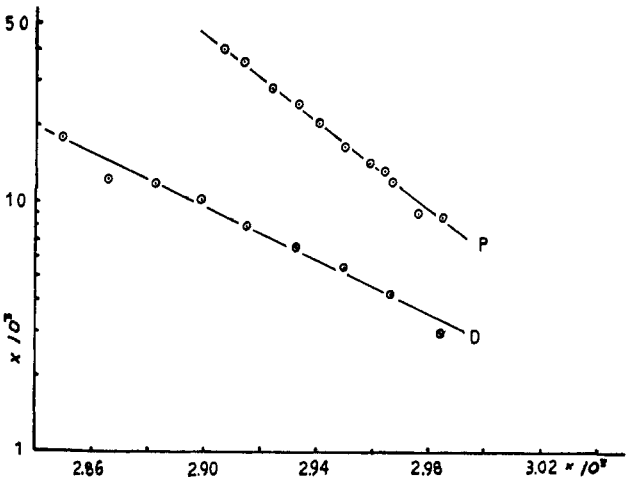

Figure 4. Plot of $[-\log(1-\alpha)]/t$ along the ordinate *vs.* $1/T$ along the abscissa for a 9% DNA gel (D) and a 1% pepsin solution (P). To avoid crowding, the points for the pepsin solution have been displaced vertically by log 2.

Initially we had expected to obtain thermograms with peaks typical of enthalpic changes associated with the rupture of hydrogen and other stabilizing bonds in the secondary structure. The absence of these peaks can readily be rationalized; after the destruction of the bonds in the secondary structure, hydrogen bonds will re-form between the protein or nucleic acid molecules and the water molecules, and the net enthalpy change should be close to zero. Thus, Tanford[17] points out that the $-C{=}O\cdots H{-}N$ hydrogen bonds of proteins should not differ greatly in strength from $-C{=}O\cdots H_2O$ and $H_2O\cdots H{-}N$ hydrogen bonds. However, the shallow endothermic depression in the thermogram for the concentrated ovalbumin solution indicated a net endothermic reaction, implying either (1) the energies of the protein–water and the protein–protein interactions are not exactly equal, and/or (2) not all of the protein–protein bonds which are broken reappear as protein–water bonds. It is interesting and suggestive that this shallow endotherm precedes the sharp sigmoidal transition.

Several reasons may be advanced for the differences between our values for the activation energies and the literature values. Perhaps the most important is that the DTA experiment yields an average value for the over-all transition while those of Casey and Laidler,[9a] for example, are for the initial stages of the denaturation process. It is therefore possible that the two techniques may measure somewhat different processes.

A second reason may involve our decision to work in unbuffered systems at this initial stage of our research. As is well known, buffer systems maintain constant pH by virtue of the interaction of a weak acid or weak base with a salt. Such interaction must produce enthalpy effects during the heating process of the DTA experiment, effects which could swamp out the changes in heat capacity accompanying the denaturation process. A few attempts at studying the thermal denaturations in buffered solutions produced erratic results, and we therefore abandoned such systems. As we gain

(17) C. Tanford, "Physical Chemistry of Macromolecules," John Wiley and Sons, Inc., New York, N. Y., 1961, p 130.

familiarity and confidence in the technique, it should become possible to reexamine once again the possibility of working in buffered systems.

As a third possible reason, it should be pointed out that the derivation of eq 6 assumes the absence of enthalpy effects, the appearance of which would distort the sigmoidal shape and introduce an error whose magnitude is difficult to estimate.

It is, however, apparent from the studies that have been made to date that the technique of differential thermal analysis provides a sensitive tool for examining thermally induced order–disorder transitions occurring in solutions of biopolymers. While the present paper has been primarily concerned with the application of this technique to protein solutions, we have also observed and reported upon transitions in solutions of deoxyribonucleic acid.

Acknowledgment. The author gratefully acknowledges financial support for this work by the Research Foundation of the City University.

Relative Rates of Methylene Radical Reactions with Silicon–Hydrogen, Silicon–Deuterium, and Carbon–Hydrogen Bonds in the Methylsilane System[1a]

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Contribution from the Chemistry Department, New Mexico State University, Las Cruces, New Mexico. *Received September 14, 1967*

Abstract: A study of the reactions of methylene radicals with methylsilane and methyltrideuteriosilane is reported. The reactions were studied by photolyses at 3660 Å of methylsilane–diazomethane, methylsilane–*n*-butane–diazomethane, methyltrideuteriosilane–diazomethane, and methyltrideuteriosilane–*n*-butane–diazomethane mixtures in the presence and absence of oxygen. Singlet methylene radicals were found to insert into the Si–H bonds 8.9 times faster than the C–H bonds of methylsilane, indicating that Si–H insertion is one of the fastest methylene radical reactions known. The isotope effect for Si–H insertion was determined to be 1.15 which is somewhat smaller than earlier measurements of C–H insertion isotope effects. Evidence for the decomposition of chemically activated dimethylsilane and ethylsilane produced by insertion at quite high pressures is presented.

Recently it has been shown by the gas-phase photolysis of diazomethane at 3660 Å in the presence of excess monosilane that methylene radicals insert into the Si–H bonds of monosilane and also abstract H atoms.[2] Previous work[3] on the liquid-phase photolysis of diazomethane in the presence of compounds containing Si–H bonds gave a higher yield of products resulting from the reaction of methylene radicals with Si–H bonds (presumably insertion although abstraction cannot be ruled out[2]) than with C–H bonds. Kramer and Wright found a minimum ratio of 100 for the ratio of Si–H/C–H "insertion" in ether solvent.[3]

A gas-phase study at high pressures of the reactions of methylene radicals with methylsilane should allow intramolecular comparisons to be made of the Si–H insertion and abstraction rates relative to C–H insertion. It should be possible to study the Si–H and C–H insertion reactions independent of abstraction by the addition of a suitable scavenger for the radicals produced by abstraction. Evidence for the chemical removal of doublet- and triplet-state radicals by oxygen is provided by a number of workers.[4,5] Previous studies of diazomethane and ketene photolysis systems indicate that in the presence of oxygen the C—H insertion and C=C addition reactions of excited singlet-state methylene radicals are not markedly altered, whereas the ground-state triplet methylene radical reaction products are efficiently scavenged.[6–9] Thus, one might expect that the photolysis of diazomethane–methylsilane mixtures in the presence of oxygen would yield volatile products resulting only from the insertion of singlet methylene radicals into the Si–H and C–H bonds of methylsilane. The products resulting from the radicals produced by abstraction and scavenged by oxygen would presumably be of higher molecular weight.[6–9] A comparison of Si—H insertion rates to those for C=C addition and C—H insertion[10,11] is of particular interest.

(1) (a) This work was supported in part by the NSF under Grant No. GP-6124 and in part by PRF under Grant No. 511-G2. (b) New Mexico State University Physical Science Laboratory Predoctoral Fellow.

(2) J. W. Simons and C. J. Mazac, *Can. J. Chem.*, **45**, 1717 (1967).

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