minophen following oral administration of equivalent doses of *p*-acetaminophenyl decanoate. The availability of drug from this ester form was 34% of that produced by acetaminophen. Concomitant administration of *p*-acetamidophenyl decanoate, pancreatic lipase (3 Wilson units/mg), and calcium chloride increased the availability from 34 to 73% as calculated from the data reported in Table V.

To investigate the feasibility of using a combination of ester derivatives with pancreatic lipase and calcium ions to achieve a prolonged release of acetaminophen, *p*-acetamidophenyl acetate and *p*-acetamidophenyl dodecanoate, equivalent to 20 and 40 mg/kg of acetaminophen, respectively, were administered with 59 mg/kg of pancreatic lipase and 10 mg/kg of calcium carbonate. The acetate ester was included to provide the initial release, and the dodecanoate ester was selected because of its slower hydrolysis rate. The amounts of pancreatic lipase and calcium carbonate were determined to be adequate from preliminary studies.

The results of this combination using a short chain ester and an intermediate ester are shown in Table VI and in Fig. 1. At the 1-, 3-, and 5-hr intervals, the blood concentrations were somewhat higher than an average of 21 μg/ml; at 7 and 10 hr, they were slightly below. The control used in this study was identical to the experiment, except that the pancreatic lipase used in the dose was inactivated by moisture and heat. No statistical difference at a 90% confidence level was noted at the 1- and 3-hr intervals; however, at 5-, 7-, 10-, and 13-hr intervals, a significant difference was observed. The hydrolysis and subsequent absorption from *p*-acetamidophenyl dodecanoate were facilitated by the inclusion of lipase in the dose. The availability of acetaminophen was increased by a factor of 1.7 when the combination included the active enzyme preparation.

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Correlation of Kinetic Parameters and Thermal Behavior of Segmented Polyurethane Elastomers with Biological Responses

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Abstract □ Kinetic studies of thermal degradation of 16 segmented polyether polyurethane samples, containing various amounts of 3,4-diaminotoluene and dibutyltin diacetate as additives, were carried out by thermogravimetry. From a single dynamic thermogravimetric experiment, the temperatures of initiation of degradation, 10, 25, and 50% (w/w) of degradation, as well as the activation energies for degradation, were determined. The activation energies were computed from the thermogravimetric curves using Broido's graphical approximation method, which applies to first-order decomposition kinetics. The results of stepwise multiple linear regression analysis indicate that the biological responses to elastomer samples, such as tissue culture, hemolysis, intramuscular implant, intradermal irritation, systemic toxicity, and histopathological rating, and the cumulative biological response index are highly correlated with thermal stability and kinetic measurements of the materials.

Keyphrases □ Segmented polyurethane elastomers—correlation of kinetic parameters and thermal behavior with biological responses □ Polyurethane elastomers, segmented—correlation of kinetic parameters and thermal behavior with biological responses □ Biomaterials, segmented polyurethane elastomers—correlation of kinetic parameters and thermal behavior with biological responses

Segmented polyether polyurethane elastomers demonstrate superior properties of strength and flex-life (1) and are excellent candidates for biomedical applications (2–9). These polymers consist of at least two major segments in the repeating chain structural unit: a crystalline *hard* segment (urea), melting above 200°, and a *soft* segment (polyether glycol), melting below 50° (10–12). Both segments are polymeric and connected by urethane linkages. The final product is termed a polyether/urethane/urea or a segmented polyurethane.

The chemistry and techniques involved in the production of these polymer materials are well known (13, 14). In addition, several investigations considered the chemical, physical, mechanical, and biological properties (5), the dependence of morphological structure on the chain characteristics and cast conditions (15), the hemolytic activity (16, 17), and the toxicity (18, 19) of the polymeric material relevant to biological applications. However, no systematic studies have been reported that compare and correlate physical properties with biological responses for segmented polyurethane systems containing different kinds and amounts of additives.

The purposes of this paper, therefore, are to consider one system of segmented polyurethanes containing various quantities of additives and to illustrate the correlation between the measured thermal

stability and kinetic parameters with some biological responses[1]. The sample preparations were designed to give a wide range of biological responses and were not related to specific end-use conditions.

The segmented polyurethane elastomers used were prepared and cured from elastomer solution (1). The additives selected were 3,4-diaminotoluene (I) and dibutyltin diacetate (II). The amounts of I and II added to the polyurethane ranged from 0 to 6% (w/w), and the samples were not analyzed for I and II after preparation. Sixteen polyurethane samples containing various amounts of additives were prepared.

Six principal biological response tests, including tissue culture, hemolysis, intramuscular implant, intradermal irritation, systemic toxicity, and histopathological rating of the intramuscular implant site, were carried out in parallel on each preparation. The thermal stability and kinetic parameters of thermal degradation of the polyurethane samples were measured by thermogravimetry, using a dynamic (nonisothermal) degradation procedure under an inert nitrogen atmosphere. The correlations of the six principal biological responses with the overall activation energies for thermal degradation and other physical properties are discussed.

THEORETICAL

During the past few years, several methods using the nonisothermal approach have been developed to allow kinetic analysis of thermogravimetric data (20). The simple and sensitive approximation method proposed by Broido (21) was used in the present work. Broido's integral method applies only to first-order reactions, including the majority of simple pyrolyses (*i.e.*, for situations where the simple Arrhenius equation is applicable).

Modification of Broido's approach by using suitable assumptions and approximations allows a linear equation to be obtained. This equation is presumed to hold for uniform linear heating rates:

$$\log \log Z = -(E_a/2.303R)\,(1/T) + \text{constant} \qquad \text{(Eq. 1)}$$

where:

$Z = \Delta w_{\text{total}}/(\Delta w_{\text{total}} - \Delta w_t)$
Δw_{total} = total change in weight after completion of degradation
Δw_t = change in weight of active material at time, t

Thus, by applying Eq. 1, a plot of log log Z *versus* $1/T$ should yield a straight line if the reaction is first order. Then E_a can be evaluated from the least-squares slope. The calculations were performed by a computer.

EXPERIMENTAL

Materials—The starting material was a segmented polyether polyurethane polymer[2] offered as a 30% (w/w) solution in dimethylacetamide (III). Some physical properties of the elastomer solution are given in Table I.

Compounds I[3] and II[4] and the solvent III[5] were obtained commercially. All other solvents were reagent grade and were used without further purification. Table II lists some physical properties of I–III.

Apparatus and Conditions—The thermogravimetric instrument[6] consisted of a linear temperature programmer[7], a furnace[8], a thermogravimetric accessory[9], an RG electrobalance[10], and a potentiometric recorder[11]. The function of the systems were described previously (22, 23). Briefly, the experimental conditions set up for these studies were as follows: sample size, 10 ± 0.5 mg; electrobalance sensitivity, 0.01 mg; programming rate, 10°/min; recorder span, 10 mg full scale; chart speed, 20.3 cm (8 in.)/min; dynamic inert atmosphere, nitrogen gas, at a flow rate of 200 ml/min; and operational temperature range, room temperature to the completion of degradation (up to about 750°).

Table I—Physical Properties of Elastomer Solution

Physical Property	Description
Solubility	30% (w/w) solution of segmented polyether polyurethane in dimethylacetamide. The solution can be diluted with secondary solvents such as toluene, chlorobenzene, methylene chloride, chloroform, and tetrahydrofuran.
Viscosity	90,000–150,000 cps at 25°. The solution tends to increase in viscosity on standing. However, this viscosity increase can be reversed by stirring.
Density	0.96 g/cc
Flash point	70°
Hygroscopicity	It is extremely hygroscopic and must be protected from contact with atmospheric moisture.

Preparation of Polyurethane Samples—*Without Additives*—The elastomer solution was poured onto a glass plate, and the solvent was allowed to evaporate over a warming plate at 68° for 22 hr.

With Additive II Only—Compound II was added by pipet to the elastomer solution with continuous stirring. After thorough mixing, the mixture was poured onto a warming plate and the solvent was evaporated for 21 hr at 68°.

With Both I and II Additives—Compound II was added by pipet to the elastomer solution with continuous stirring. Compound I was dissolved in the elastomer solvent III and then added to the mixture with continuous stirring. After thorough mixing, the product was poured onto a warming plate and the solvent was evaporated for 20.5 hr at 68°.

With Additive I Only—Compound I was dissolved in III and then added to the elastomer solution with continuous stirring. After thorough mixing, the solution was poured onto a warming plate and the solvent was evaporated for 20.5 hr at 68°.

In general, an evaporation time of 20–22 hr at 68° was sufficient to obtain a firm nonsticky film product.

Measurement of Density of Elastomer Samples—The density of elastomer films was measured at 25 ± 0.1° by a water-displacement method using a pycnometer. The method of calculation is similar to that presented previously (23).

Methanol Extraction (% RW)—Methanol was selected for the extraction of components from elastomer samples. The samples were cut into small pieces and exposed to 150 ml of methanol for 24 hr at room temperature with continuous stirring. The results are reported as percent residue weight (% RW).

Biological Response Tests—The six biological response tests employed for the evaluation of the formulations follow.

Hemolysis Assay (H)—Hemolytic activity was determined by spectrophotometric estimation of hemoglobin release from rabbit erythrocytes in saline solution in the presence and absence of polymer (5 g of polymer/10 ml of saline) at 37° in 1 hr. Percent hemolysis was calculated relative to the positive control (100% hemolysis).

Tissue Culture (TC)—The method of Guess *et al.* (24) was used. The tissue culture response was evaluated in terms of a re-

[1] The data reported here represent part of a study presently being pursued under the auspices of the Food and Drug Administration and directed toward the evaluation of biological test methods for preclinical screening of biomaterials.

[2] Biomer was a gift of Ethnor Medical Products, Division of Ethicon, Inc., Somerville, N.J.

[3] Fisher Scientific Co., St. Louis, Mo.

[4] J. T. Baker Chemical Co., Chattanooga, Tenn.

[5] ICN, K & K Laboratories, Plainview, N.Y.

[6] Fisher TG/DTA Series 100A, Fisher Scientific Co., Pittsburgh, Pa.

[7] Model 360.

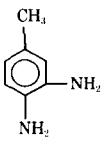
[8] Model 260.

[9] Model 120P.

[10] Cahn Instrument Co., Paramount, Calif.

[11] Servo/riter II, Texas Instruments, Houston, Tex.

Table II—Physical Properties of Solvent and Additives

Compound	Formula Structure	Formula Weight	Melting Point	Boiling Point	Density	Refractive Index (n_d)
Dimethylacetamide (III)	$CH_3CON(CH_3)_2$	87.12	−20.0°	165.0°	0.9366_6^{25}	1.4380^{20}
3,4-Diaminotoluene (I)		122.17	89.0–90.0°	265.0°	—	—
Dibutyltin diacetate (II)	$(C_4H_9)_2Sn(OOCCH_3)_2$	351.00	10.0°	142.–145.0°	1.3200	1.4820

sponse index (RI), reflecting zone size and percent lysis within the zone:

$$RI = ZI/LI \qquad \text{(Eq. 2)}$$

where ZI is the zone index, and LI is the lysis index. The values of ZI and LI are treated as independent values, however, in the calculations that yield the data presented in Table IV. The magnitude of ZI was defined as follows:

0 = no detectable zone around or under the sample
1 = zone limited to area under sample
2 = zone not greater than 0.5 cm in extension from the sample
3 = zone not greater than 1.0 cm in extension from sample
4 = zone greater than 1 cm in extension from sample but not involving the entire plate
5 = zone involving the entire plate

The magnitude of LI was defined as follows:

0 = no observable lysis
1 = less than 20% of zone lysed
2 = less than 40% of zone lysed
3 = less than 60% of zone lysed
4 = less than 80% of zone lysed
5 = greater than 80% lysis within zone

The normalization factors for tissue culture are (ZI + LI) × 10. The numerical values listed in Table III are the sum of four individual tests, including the sample material, cottonseed oil USP extract, polyethylene glycol 400 USP extract, and saline USP extract.

Intradermal Irritation Test (ID)—The USP XVIII method (25) was used. The intensity of gross visible inflammation and necrosis around the implant are primary factors. The intradermal irritation rating scale is as follows:

0 = no detectable irritation
0.5 = minimal irritation detected
1 = very slight but distinct irritation
2, 3, 4, 5 = increasing intensity of inflammation and wheal

A value of 5 represents a response greater than the positive control. A value of 4 for the positive control is normalized to 100%. The intradermal irritation values were calculated by the factor (0–5) × 25. The listed intradermal irritation values are the sum of three individual tests (*i.e.*, extracts of cottonseed oil USP, polyethylene glycol 400 USP, and saline USP).

Intramuscular Implant (IM)—The USP XVIII method (26) was used with the following exceptions: *(a)* the implant period was 7 days, and *(b)* clean, but not necessarily aseptic, technique and samples were employed.

The rating scale for the intramuscular implant test is analogous to that for the intradermal irritation test already presented.

Systemic Toxicity Test (ST)—The USP XVIII method (25) was used. The rating scale (0–5) of the systemic toxicity is the number of deaths after 7 days. The normalization factor is (0–5) × 20. The normalized values reported are the sum of intravenous and intraperitoneal administration.

Histopathological Rating (HR)—The method of Turner *et al.* (27) was used. The histopathological rating values are reported in terms of a 0–4 rating scale and normalized by a factor of (0–4) × 25. The magnitude of histopathological rating was defined as follows:

0 = not different from negative control
1 = minimal response
2, 3, 4 = increasing tissue reaction around implant; degree of necrosis is the primary factor

Cumulative Biological Response Index (CI)—CI values are the sum of the normalized values of response ratings for all tests. CI values represent a broadly based estimate of biological activity.

Molecular Weight Determination—The molecular weight distribution of an elastomer sample containing no additives was determined by gel permeation chromatography[12]. Calibration was based on polystyrene standards, and the sample was run in a dimethylformamide solvent.

Table III—Experimental Results of Biological Response Tests for Elastomer Samples

Sample	Tissue Culture (TC)[a]	Hemolysis (% H)	Intramuscular Implant (IM)	Intradermal Irritation (ID)[b]	Systemic Toxicity (ST)[b]	Histopathological Rating (HR)	Cumulative Biological Index (CI)
1	40	0	0	0	0	0	40
2	140	6	75	100	100	50	471
3	150	8	100	125	100	100	583
4	160	37	100	100	100	100	597
5	210	41	125	225	120	100	821
6	240	44	125	250	200	100	959
7	120	3	0	100	0	50	273
8	190	91	100	225	100	100	806
9	180	100	125	250	160	100	915
10	220	57	125	250	160	100	912
11	160	6	0	50	0	25	241
12	200	12	25	75	0	25	337
13	240	66	125	225	120	100	876
14	260	100	125	275	120	100	980
15	290	100	125	275	160	100	1050
16	180	20	0	0	0	25	225

[a] Sum of tissue culture response (ZI and LI) for material test samples and saline USP, cottonseed oil, and polyethylene glycol 400 extracts. [b] Sum of biological response to saline USP, cottonseed oil, and polyethylene glycol 400 extracts.

RESULTS AND DISCUSSION

Characterization of Segmented Polyether Polyurethane Elastomer—The samples used were prepared from an elastomer solution[13]. The molecular weight and molecular weight distribution were obtained by means of gel permeation chromatography. The molecular weight (based on polystyrene standards) calculated from the assumed mol. wt./size ratio *(Q)* of 25 are $\bar{M}_w$ (weight average) = 115,000, $\bar{M}_n$ (number average) = 51,200, and $\bar{M}_w/\bar{M}_n$ = 2.24.

Thermal Properties of Elastomer Samples—The thermal stability, as indicated by the amount degraded at specific temperatures, and the E_a values for thermal degradation of the 16 samples determined by the thermogravimetric technique are shown in Table IV. The total amount added listed in Table IV represents

[12] DeBell and Richardson, Inc., Enfield, Conn.
[13] According to the manufacturer, the product is based on the original DuPont formula for Lycra.

Table IV—Physical and Thermal Properties of Elastomer Samples

Sample	Amount of I and II Added: I, wt. %	II, wt. %	Total (Moles/10 mg Sample) × 10^7	Density (d), g/cc	Residue Weight of Methanol Extractable Substance (% RW)	T_i	T_{10}	T_{25}	T_{50}	Activation Energy, E_a, kcal/mole
1	0	0	0	1.02	2.44	239.8°	316.2°	349.2°	391.0°	21.07
2	0	0.5	1.39	1.03	2.68	255.0°	311.0°	374.7°	401.4°	20.51
3	0	1.0	2.84	1.04	2.97	253.7°	308.0°	375.3°	399.2°	20.93
4	0	2.0	5.78	1.00	3.78	252.7°	291.0°	369.2°	397.6°	19.19
5	0	4.0	12.90	0.99	4.40	246.3°	276.4°	360.8°	395.6°	18.96
6	0	6.0	17.00	0.99	6.55	238.1°	289.8°	368.5°	396.5°	18.99
7	0.5	0	4.03	1.04	2.66	238.3°	316.9°	357.2°	377.0°	23.10
8	0.5	2.0	9.74	0.94	3.81	235.9°	289.3°	363.5°	389.2°	17.73
9	0.5	4.0	15.23	0.93	5.07	237.4°	298.6°	370.8°	385.6°	19.02
10	0.5	6.0	20.95	0.94	5.54	236.9°	289.0°	366.4°	381.5°	19.54
11	1.0	0	8.20	1.04	3.07	257.0°	322.8°	365.6°	387.8°	24.55
12	2.0	0	16.30	1.02	3.79	250.9°	320.1°	368.3°	390.8°	25.25
13	2.0	2.0	22.10	0.99	5.31	243.3°	287.5°	361.4°	391.0°	17.90
14	2.0	4.0	27.60	1.02	6.84	242.8°	283.2°	366.9°	393.2°	16.97
15	2.0	6.0	32.80	1.03	7.76	241.6°	281.0°	354.2°	392.2°	15.74
16	4.0	0	33.10	1.04	6.11	246.8°	318.4°	367.2°	390.5°	25.01

the sum of I and II in terms of number of moles/10 mg of sample. The four degradation temperatures are T_i, T_{10}, T_{25}, and T_{50}; T_i is the temperature of initiation of degradation, and T_{10}, T_{25}, and T_{50} represent the temperatures at which 10, 25, and 50% (w/w) of degradation occur.

The E_a values were evaluated from the slope of the plot of log log Z *versus* $1/T$ using Eq. 1. Figure 1 shows some typical examples (Samples 1, 6, 15, and 16) of such plots. The thermal degradation data of the elastomer samples follow the linear form of Eq. 1 very nicely; the average relative precision of the determinations is about 3.5%. The E_a values range from 16 to 25 kcal/mole, depending upon the kinds and quantities of additives in the sample.

For example, the E_a value for Sample 16, which contains the highest amount of I (4.0% added), is the largest (25 kcal/mole), while Sample 15, which consists of the highest quantity of II (6.0% added) and a medium amount of I (2.0% added), is the smallest (16 kcal/mole). Apparently, I and II affect the structural properties that contribute to activation energy but are not simply additive.

Physical Properties of Elastomer Samples—Density *(d)* data and the residue weight of methanol extractable substances (% RW) are also listed in Table IV.

In general, the % RW is directly related to the amounts of I and II added. However, it should be pointed out that the nonzero percent extracts (2.44%) observed for Sample 1 suggest that some unknown components other than the materials added are leached out of the polymers by methanol.

Biological Response Tests on Elastomer Materials—The experimental results of biological response tests are listed in Table III.

The results of no hemolytic activity and no other biological responses for the sample without additives (Sample 1) are consistent with the findings reported by other investigators (6, 16, 17). Generally speaking, % H is higher for those samples containing higher amounts of II added (*e.g.*, Samples 8, 9, 14, and 15). Samples 2, 3, 7, 11, and 12, which have the smallest quantities of either I or II added, have the lowest % H.

Multiple Correlations of Physical Properties and Biological Responses—To determine which variables best predict the biological responses, a stepwise multiple linear regression analysis was carried out. This analysis included 10 properties: percent 3,4-diaminotoluene (I) added, percent dibutyltin diacetate (II) added, total amount of I and II added, E_a, T_i, T_{10}, T_{25}, T_{50}, density *(d)* and residue weight of methanol extractable substance (% RW) as independent variables, and the seven biological responses as dependent variables. The results are given below.

$$(TC) = 31.93\,(RW) + 43.03 \qquad (R = 0.844) \quad \text{(Eq. 3)}$$

$$(\%\ H) = -7.20\,(E_a) + 1.54\,(\text{total added}) - 315.70\,(d) + 483.53 \qquad (R = 0.936) \quad \text{(Eq. 4)}$$

$$(IM) = -7.37\,(E_a) - 1.99\,(T_{10}) + 2.80\,(T_{25}) - 195.90 \qquad (R = 0.975) \quad \text{(Eq. 5)}$$

$$(ID) = 24.54\,(\text{dibutyltin diacetate}) - 13.62\,(E_a) + 376.46 \qquad (R = 0.913) \quad \text{(Eq. 6)}$$

$$(ST) = 24.11\,(\text{dibutyltin diacetate}) + 3.65\,(T_{50}) - 1401.08 \qquad (R = 0.920) \quad \text{(Eq. 7)}$$

$$(HR) = -2.10\,(T_{10}) + 1.79\,(T_{25}) + 47.43 \qquad (R = 0.930) \quad \text{(Eq. 8)}$$

$$(CI) = 54.96\,(\text{dibutyltin diacetate}) - 12.14\,(T_{10}) + 9.68\,(T_{25}) + 606.85 \qquad (R = 0.956) \quad \text{(Eq. 9)}$$

Each coefficient of the independent variables in Eqs. 3–9 is significant at the $\alpha = 0.05$ level or better. Equation 3 indicates that tissue culture response is correlated with the residue weight (percent) of methanol extractable substance. In other words, the data suggest that the tissue culture reaction results directly from leachable materials existing in the polymer matrix. The multiple correlation coefficient is 0.844.

For percent hemolysis, Eq. 4, a multiple correlation coefficient of 0.936 is obtained. Since the percent hemolysis determination is performed at 37° and the total exposure time of the polymer to aqueous solutions is no greater than 90 min, it may be somewhat surprising that E_a is so highly correlated to percent hemolysis assay. However, the E_a value may be considered as a measure of thermal stability; the greater the E_a value, the more stable is the material. A simple correlation coefficient of −0.788 is obtained for the E_a–% H relationship, showing that the easier it is for the plastic to decompose, the greater is the hemolysis. Furthermore, evaporation of additives from the polymer in the process of thermal analysis is not distinguishable from decomposition of the polymer so that the E_a value calculated from the thermogravimetric curve is certainly influenced by the presence of additives.

Correlation of percent hemolysis assay with the total amount of additives is to be expected, since at the conditions of hemolysis it would appear that the additives could be easily leached out and contribute to cellular destruction.

Density appears as the third variable in Eq. 4. The simple correlation coefficient of d–% H is −0.587. There is little or no simple correlation between density and the total amount of additives or between density and E_a ($r = 0.427$).

The multiple correlation coefficient corresponding to Eq. 5 for intramuscular implant is 0.975, the highest value observed for all seven dependent variables analyzed. The simple correlation coefficients for IM–E_a and IM–T_{10} are −0.858 and −0.899, respectively. These results strongly suggest that the intramuscular implant response is highly dependent on the thermal stability of elastomer samples. The easier the material is degraded, the higher is the tissue reaction around the implant.

Equation 6 indicates that intradermal irritation is a function of percent II added and the E_a values. The simple correlation coefficient between the percent II added and the E_a value is −0.736, indicating that E_a is inversely related to the percent II added; the simple correlation coefficients for ID–% II and ID–E_a are 0.873

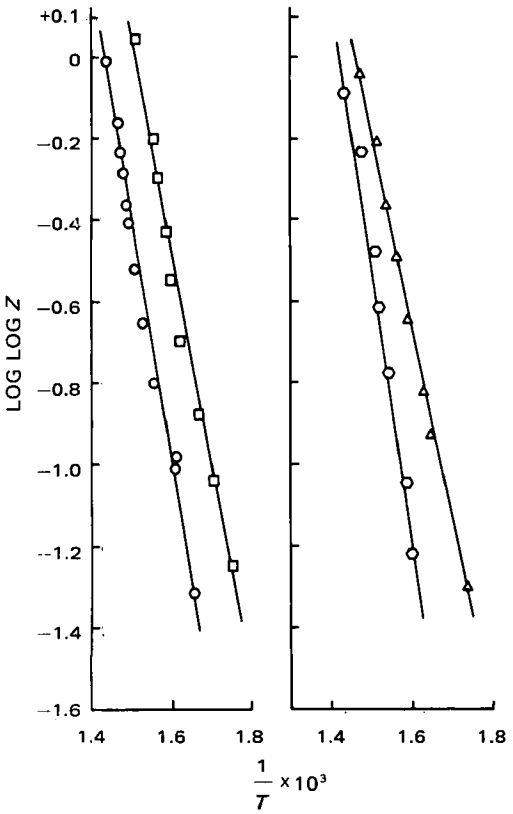


Figure 1—*Typical examples showing the determination of activation energies for thermal degradation of segmented polyether polyurethane elastomers by Broido's (21) method. Key:* ○, *Sample 1, no additives;* □, *Sample 15, 2% 3,4-diaminotoluene and 6% dibutyltin diacetate;* ○, *Sample 16, 4% 3,4-diaminotoluene only; and* △, *Sample 6, 6% dibutyltin diacetate.*

and −0.825, respectively. The higher the percent II added, the lower is the E_a required for degradation (hence the less stable the material) and the higher is the intradermal irritation response. Therefore, it is obvious that intradermal irritation is also directly related to the thermal stability of the elastomer samples. The multiple correlation coefficient is 0.913.

The systemic toxicity is correlated with the percent II added and T_{50} (Eq. 7, R = 0.920). However, the simple correlation coefficient for ST–% II is 0.851; but for ST–T_{50}, it is only 0.358.

The multiple correlation coefficient corresponding to Eq. 8 for histopathological rating is 0.930. But only two variables, T_{10} and T_{25}, are found in Eq. 8. A simple correlation coefficient of −0.866 was obtained for the T_{10}–HR relationship, indicating that T_{10} is negatively correlated to histopathological rating.

In general, T_{10} is considered as a measure of the degradation of active material free from the influence of contamination or sorption of volatile components or solvent on the material. It is, therefore, a better measurement of the thermal stability of the material than T_i (T_i is very sensitive to impurities). The stability of the material may indeed play an important role in the histopathological rating response, since implantation was continued for 1 week. Thus, the lower the value of T_{10}, the easier the material can decompose to products that increase the histopathological rating value.

Furthermore, since the histopathological rating and total amount of additives have a simple correlation coefficient of only 0.254, the additives apparently are relatively ineffective in producing the histopathological response. The data suggest that the additives may strongly influence the thermal stability of the material and thereby indirectly affect the histopathological rating response through the leaching process in the body of the animal.

Equation 9 expresses the multiple correlation of cumulative biological index to the amount of II added, T_{10} and T_{25} (R = 0.956). Since the cumulative biological index is a composite of six major tests, it may be viewed as an overall response. The amount of II added strongly contributes to this overall response. The simple correlation coefficient between II and the cumulative biological index is 0.88. The simple correlation coefficient relating percent I added to the cumulative biological index is only −0.07, a negligible value. Thus, it appears that the amount of II added rather than the amount of I added is largely responsible for the cumulative biological response. However, the average amount of II added for all samples was larger by a factor of two than the amount of I added, and this may in part account for its presence in Eq. 9. The terms T_{10} and T_{25} are related to the cumulative biological response index in a manner similar to the histopathological rating, as already discussed.

Stepwise multiple linear regression analysis has shown that the magnitude of various biological responses is highly correlated to the thermal and physical properties of the polymer system as well as to the amounts of dibutyltin diacetate added to the polymer system in arbitrarily excessive quantities. Since the segmented polyether polyurethane elastomer *per se* showed very low biological response and was present in the same absolute amount in each preparation, it was concluded that its contribution to biological response in the presence of additives was limited to its influence on the leachability of the additives, the biological response being highly correlated with the total amount of additives and the thermal properties of the complex polymer system.

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Decomposition of Amitriptyline Hydrochloride in Aqueous Solution: Identification of Decomposition Products

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Abstract □ The decomposition of amitriptyline hydrochloride upon autoclaving in a buffered solution (pH 6.8) was investigated. Three major decomposition products [3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene, dibenzosuberone, and 3-(2-oxoethylidene)-1,2:4,5-dibenzocyclohepta-1,4-diene] were detected and identified by chromatographic and spectroscopic techniques. Evidence is presented that the latter two compounds are formed by further oxidation of 3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene, and a possible decomposition pathway is outlined.

Keyphrases □ Amitriptyline hydrochloride—identification of decomposition products, aqueous solution, mechanisms □ Decomposition—amitriptyline hydrochloride in aqueous solution, identification of products, mechanisms □ Antidepressants—decomposition of amitriptyline hydrochloride in aqueous solution, identification of products, mechanisms

The antidepressant drug amitriptyline hydrochloride may be formulated as a parenteral solution and sterilized by filtration according to BP 1974. Although Henwood (1) showed that amitriptyline base breaks down into dibenzosuberone and Bouche (2) indicated that anthraquinone was formed when the base was boiled in alkaline potassium permanganate solution, there are little published data concerning the stability of the drug.

A project was initiated to assess the drug's instability in aqueous solution and to identify the decomposition products produced during autoclaving and long-term storage. Preliminary experiments showed that decomposition occurred when solutions of amitriptyline hydrochloride in water or phosphate buffers were autoclaved for 30 min at 115–116° in the presence of excess oxygen. The purpose of this paper is to describe the isolation of the decomposition products by TLC and GLC and their identification using mass spectrometry, NMR, and other spectroscopic techniques.

EXPERIMENTAL

Materials—The following chemicals were used: amitriptyline hydrochloride[1], dibenzosuberone[2], ethyl iodide[3], potassium chloride[4], potassium dihydrogen phosphate[4], ether[4], *n*-propanol[4], carbon tetrachloride[4], ethylene dichloride[4], benzene[4], hexane[4], chloroform[4], ethanol[4], methanol[4], ethyl acetate[4], and acetone[4].

Degradation of Amitriptyline Hydrochloride Solution—A 0.5% (w/v) amitriptyline hydrochloride solution was prepared in 0.2 *M* phosphate buffer solution (pH 6.8); 2-ml quantities were distributed in 10-ml ampuls to ensure excess oxygen and sealed. The ampuls were autoclaved at 115–116° for up to 6 hr to produce sufficiently large quantities of the degradation products for subsequent identification by various physicochemical techniques.

TLC—The plates (20 × 20 cm) were coated with silica gel G[5], 250 μm thick, and activated by heating at 110° for 2 hr. They were stored in a desiccator prior to use.

The contents of a heated ampul were extracted with 200 μl of ether, and 50 μl was spotted on a plate in 10-μl portions. Ascending development was carried out over 15 cm with benzene–carbon tetrachloride (7:3 v/v). The spots were detected visually in transmitted light and also by spraying with 4% (v/v) formaldehyde solution BP in concentrated sulfuric acid and viewing under UV light. Dragendorff reagent and 0.4% (w/v) 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid were also used as spray reagents to detect nitrogen- and carbonyl-containing compounds, respectively. The R_f values were determined for the various degradation products (Table I).

[1] Merck Sharp and Dohme.
[2] Authentic specimen, British Pharmacopoeia Commission.
[3] Koch Light Ltd.
[4] Analar grade reagent, B.D.H. Ltd.
[5] Type 60, E. Merck, Darmstadt, West Germany.