Simplified Method to Study Stability of Pharmaceutical Preparations

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Abstract □ Data simulating zero-, first-, and simple second- and third-order kinetic reactions are employed at four elevated temperatures. The existence of a linear relationship is established between the logarithm of $t_{0.9}$ (the time required for the concentration of the reactant remaining to decompose to reach 90% of its original value) and the reciprocal of the corresponding temperature in absolute degrees. The independence of the result from the order of the reaction is reconfirmed using literature data. The application of the linear relationship to predict the shelflife of dosage forms is discussed.

Keyphrases □ Stability—pharmaceutical preparations, linear relationship between log $t_{0.9}$ and reciprocal of absolute temperature related to shelflife □ Shelflife—pharmaceutical preparations, predicted using linear relationship between log $t_{0.9}$ and reciprocal of absolute temperature

The classical method of determining the shelflife of a pharmaceutical preparation was to store it under conditions similar to those of the normal market and to study its stability for the expected period of storage in the market. If the preparation was unstable and was subsequently modified, the same time-consuming process had to be repeated for the modified form (1). Later, high temperature accelerated stability studies were employed to predict the shelflife of the drug in a considerably shorter time. The application of such kinetic studies dates back to 1950 for consumer products in the food (2) and pharmaceutical (3, 4) industries.

BACKGROUND

In general, accelerated stability studies involve the determination of the concentration of drug remaining to decompose as a function of time. The concentration or a function of it is linearized with respect to time. The effect of relatively high temperatures on the slope of these straight lines (the specific rate constants) is determined and utilized for the preparation of an Arrhenius plot. Prediction of the time required for the drug to decompose to 90% of its original concentration at 25°—the shelflife—is based on the Arrhenius plot.

Many factors have to be investigated in such a study (5) including pH, moisture, light, oxidation, chelation, solubility, excipients, trace metals, compatibility, closures, containers, and sterilization. Other variables are the solvent, dielectric constant, ionic strength, substrate pKa, substrate reactive groups, nucleophiles, acids, and bases (4). All such studies employ the proper experimental design, a reliable assay procedure, and the appropriate statistical evaluation of the results. From the findings, the formulator determines a set of conditions that best keep the quality, purity, identity, and strength of the drug (6). Numerous articles and reviews deal with different aspects of such studies (4, 6–12). To ensure the safety and efficacy of medicaments, accelerated stability studies are now an essential part of the activities of the pharmaceutical formulator and the hospital and clinical pharmacist.

Most investigators emphasize the determination of the exact kinetic path followed by the degrading drug compound. However, some ignored such exact determinations and obtained shelflife predictions using reference decomposition models based on kinetic concepts (3) in an attempt to reduce the length of time required for stability studies. Clark and Hudson (13) suggested the use of a stability chart and explained it for first-order reactions. An experimental design and its proper statistical analysis were constructed to predict shelflife when imprecise assay methods have to be employed (14). The design provides for the minimum number of time–temperature combinations that can be used.

For first-order degradation, a stability chart was developed utilizing the Arrhenius equation, the specific rate of degradation at two elevated temperatures, and the heat of activation (15). The overall first-order rate of hydrolysis of procaine solutions was studied, and a linear relationship was found between the logarithm of the half-lives and the reciprocal of the corresponding temperatures in absolute degrees (16). As a corollary to the half-life concept, the time taken for the drug to decompose to 90% of its original concentration can be designated as $t_{0.9}$. The linear relationship between log $t_{0.9}$ and the reciprocal of the corresponding temperature in absolute degrees may be employed in the prediction of room temperature stability in a first-order reaction (6). The relationship between $t_{0.9}$ and the first-order specific rate constant was studied (17), and this concept was used for the prediction of stability of parenteral solutions from considerations of first-order reaction kinetics (18).

Lordi and Scott (15) indicated that, at less than 10% degradation and within the limits of the experimental error involved in stability studies, it is not possible to distinguish between first-, zero-, and simple second-order kinetics using curve-fitting techniques. Consequently, they felt confident that assuming first-order kinetics in their work should result

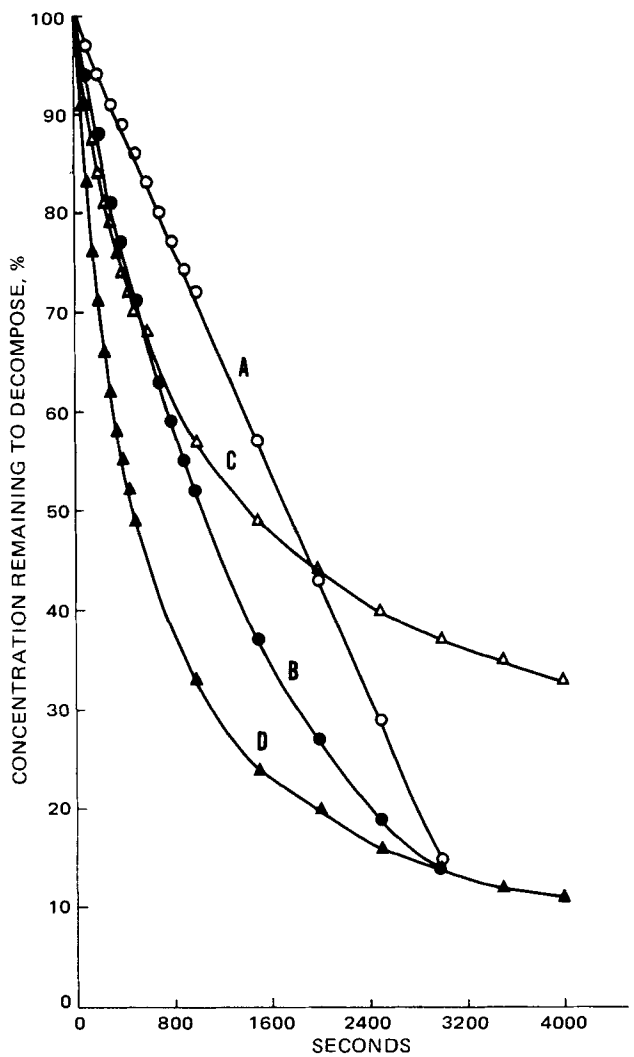

Figure 1—*Representative simulated concentration* versus *time data for zero-, first-, and simple second- and third-order reactions. Key: A, zero order; B, first order; C, third order; and D, second order.*

Table I—Straight Lines Obtained from Data Simulating a Zero-Order Kinetic Reaction[a]

Temperature[b]	Slope × 10^3	Intercept	Coefficient of Correlation[c]
40°	−3.64119	100.500	0.995915
	−4.00452	100.799	0.990657
50°	−8.03098	100.274	0.998321
	−8.03528	99.887	0.998078
60°	−14.39060	100.488	0.999175
	−14.21590	99.751	0.998575
70°	−28.75290	100.321	0.998592
	−28.53350	100.002	0.999313

[a] The dependent variable, Y, is the percent concentration remaining to decompose; the independent variable, X, is time in seconds. [b] The temperature is in centigrade degrees, and there are two replicates for each temperature. [c] All are significant at the 5% level of probability or higher.

in minimum error in the absence of a knowledge of the actual order of reaction.

The purpose of the present study was to investigate: (*a*) the presence of a linear relationship between log $t_{0.9}$ and the reciprocal of the corresponding temperature in absolute degrees regardless of the order of reaction and (*b*) the applicability of the results to the prediction of shelflife.

EXPERIMENTAL

In the first phase of this investigation, simulated concentration *versus* time data for zero-, first-, and simple second- and third-order reactions containing ±0.4–2% experimental error in the concentration terms were utilized (Fig. 1). Regression analysis of the data provided the formulas of the straight lines reported in Tables I–IV. For the different temperatures in each reaction order, the time required for the concentration to decrease to 90% of its value at zero time was determined from the respective straight-line equations. The logarithms of these values (log $t_{0.9}$) were plotted against the reciprocal of the corresponding temperatures in absolute degrees (Fig. 2).

Regression analysis provided the equations of the observed straight lines reported in Table V. The predicted $t_{0.9}$ at room temperature or the time required for the hypothetical substance to degrade at 25° to 90% of its original value was determined by substitution of the reciprocal of room temperature (in absolute degrees) multiplied by 10^3 for the independent variable. These values are also reported in Table V.

In the second phase of the investigation, available literature data were utilized to calculate log $t_{0.9}$ values. Standard kinetic equations for the particular order of reaction stated in the source were used for these calculations. The log $t_{0.9}$ values were plotted on the ordinate against the reciprocal of the corresponding temperatures (in absolute degrees multipled by 10^3 or 10^4) on the abscissa. The formula of the resultant straight line was obtained through least-squares regression analysis.

A simple substitution of the proper form of the room temperature similar to that performed in the first phase of the investigation provided the predicted value of $t_{0.9}$ at room temperature. This $t_{0.9}$ value was utilized in calculating the specific rate constant for the reaction at 25° (k_n). The k_n was subsequently compared with the equivalent specific rate constant value (k_0) reported in the source. The two specific rate constants are reported in Tables VI–VIII.

The literature sources were selected from kinetic studies reported since 1936. Many such studies could not be used in this particular investigation; only those that clearly indicated the order of reaction and provided enough data at different temperatures suitable for application of the standard kinetic equations were employed.

Table II—Straight Lines Obtained from Data Simulating a First-Order Kinetic Reaction[a]

Temperature[b]	Slope × 10^5	Intercept	Coefficient of Correlation[c]
40°	−3.52478	1.99909	0.995818
	−5.29083	2.00263	0.526871
50°	−8.11233	2.00302	0.998097
	−8.06925	1.99924	0.997575
60°	−14.42210	2.00130	0.997718
	−14.60200	2.00134	0.999469
70°	−29.12050	2.00471	0.998278
	−35.06620	1.99769	0.881244

[a] The dependent variable, Y, is the logarithm of the percent concentration remaining to decompose; the independent variable, X, is time in seconds. [b] The temperature is in centigrade degrees, and there are two replicates for each temperature. [c] All are significant at the 5% level of probability or higher.

Table III—Straight Lines Obtained from Data Simulating a Second-Order Kinetic Reaction[a]

Temperature[b]	Slope × 10^6	Intercept × 10^3	Coefficient of Correlation[c]
40°	3.64495	9.90145	0.996234
	3.57739	9.94983	0.996151
50°	6.92413	10.06870	0.997432
	6.93258	9.42949	0.986778
60°	11.80850	10.32450	0.983003
	13.80450	8.57760	0.996930
70°	21.09650	9.38233	0.994166
	19.00590	10.98070	0.993933

[a] The dependent variable, Y, is the reciprocal of the percent concentration remaining to decompose; the independent variable, X, is time in seconds. [b] The temperature is in centigrade degrees, and there are two replicates for each temperature. [c] All are significant at the 5% level of probability or higher.

RESULTS AND DISCUSSION

The nature and experimental error of the simulated data of Tables I–IV are representative of studies performed on simple degradation reactions. The number of concentration *versus* time data points (15–18) is also a practical and reasonable sample. Since kinetic studies utilizing greater than four elevated temperatures are not very common, four temperatures of 40, 50, 60, and 70° were selected as an acceptable range. Furthermore, there were two replicates for each temperature, providing a better basis for the determination of error. The values of the coefficients of correlation were all significant at least at the 95% level of probability.

Each set of data (concentration *versus* time pairs for a certain order

Table IV—Straight Lines Obtained from Data Simulating a Third-Order Kinetic Reaction[a]

Temperature[b]	Slope × 10^8	Intercept × 10^5	Coefficient of Correlation[c]
40°	3.44370	10.02760	0.995974
	3.76499	9.96291	0.997786
50°	6.60210	12.14580	0.947648
	6.86911	10.30320	0.997576
60°	6.19608	15.50950	0.666163
	11.99770	10.05600	0.996267
70°	19.03500	10.91040	0.997737
	21.54420	9.47549	0.995440

[a] The dependent variable, Y, is the reciprocal of the square of the percent concentration remaining to decompose; the independent variable, X, is time in seconds. [b] The temperature is in centigrade degrees, and there are two replicates for each temperature. [c] All are significant at the 5% level of probability or higher.

Table V—Straight Lines Obtained When the Logarithms of the $t_{0.9}$ Values (Dependent Variable Y) Calculated from the Simulated Data Are Plotted against the Reciprocals of the Corresponding Temperatures in Absolute Degrees [Independent Variable $X = (1/T) \times 10^3$]

Order of Reaction	Slope	Intercept	Coefficient of Correlation[a]	Predicted $t_{0.9}$[b] at 25°
Zero	3.15635	−6.64371	0.998177	8.76×10^3
First	3.24416	−7.26522	0.991399	4.12×10^3
Second	2.86193	−6.64283	0.910650	9.03×10^2
Third	2.58861	−5.48260	0.988905	1.58×10^3

[a] All are significant at the 1% level of probability. [b] Time in seconds.

Table VI—Zero-Order Reactions: Straight Lines Obtained When the Logarithm of the $t_{0.9}$ Values (Dependent Variable Y) Calculated from Data Reported in the Literature Is Plotted against the Reciprocal of the Corresponding Temperatures in Absolute Degrees [Independent Variable $X = (1/T) \times 10^3$]

Slope	Intercept	r^a	$k_n{}^b$	$k_0{}^c$	Reference
4.531751	−9.499254	0.999263	1.980×10^{-7}	2.210×10^{-7}	1
5.233304	−13.868966	0.998346	2.075×10^{-3}	2.054×10^{-3}	20
5.879029	−18.157259	0.999911	5.500×10^{-5}	5.620×10^{-5}	21
5.015686	−15.824473	0.998027	2.000×10^{-1}	2.000×10^{-1}	21
4.847425	−14.074462	0.999887	1.310×10^{-2}	1.180×10^{2}	21
6.918985	−17.625532	0.999577	3.160×10^{-4}	1.850×10^{-3}	22
6.978310	−17.818506	0.999259	3.110×10^{-4}	1.800×10^{-3}	22
5.825150	−14.216891	0.999886	5.740×10^{-4}	3.280×10^{-3}	22

[a] All values of the coefficient of correlation are significant at the 5% level of probability. [b] The k_n is the specific rate constant at 25° calculated from $t_{0.9}$ at 25°. The $t_{0.9}$ was predicted using the appropriate straight-line equation. [c] The k_0 is the specific rate constant reported in the reference.

of reaction and a specific temperature) provided one of the reported straight lines (Tables I–IV) and one $t_{0.9}$ value, so a total of eight log $t_{0.9}$ values was available for each order of reaction. Four elevated temperatures were employed for the same order, and each two of the log $t_{0.9}$ values corresponded to a different elevated temperature. The observed relationship between the log $t_{0.9}$ values and the reciprocal of the temperatures in absolute degrees was linear in all of the different orders of reaction (Fig. 2).

The slope, intercept, and coefficient of correlation for each line are reported in Table V. The coefficients of correlation were significant at least at the 1% level of probability. The linear relationship was used to predict the $t_{0.9}$ for the particular reaction at room temperature. In accelerated stability studies of pharmaceutical dosage forms, this time is the desired shelflife.

The establishment of the existence of the linear relationship between the log $t_{0.9}$ and the reciprocal of the absolute temperature regardless of the order of reaction led to the second phase of the investigation in which available data from selected literature sources were used to provide the same linear relationship (Tables VI–VIII). By utilizing the linear relationship, the specific rate constant at room temperature was calculated for the particular reaction. This calculated specific rate constant was compared with the specific rate constant for 25° reported in the source, using the Student t test (19). The difference between the two was not significant.

The existence of a linear relationship between the log $t_{0.9}$ and the reciprocal of the absolute temperature independent of the order of reaction is demonstrated by the simulated and experimental data. In practice, therefore, one may maintain the degradation reaction at several high temperatures, carrying on concentration determinations at predetermined time intervals. A plot of concentration remaining to decompose (on the ordinate) against time (on the abscissa) is constructed for each elevated temperature. The time taken for the concentration to decrease to 90% of the original value is read, and its logarithm is plotted against the reciprocal of the corresponding temperature in absolute degrees. The shelflife at the desirable temperature is predicted from the observed straight line. Before this new method is fully and solely employed, however, it should be applied to available data from a dosage form for which the shelflife has already been determined by other more elaborate means. Such a precaution will be of assistance in evaluating the accuracy of the simple technique.

The k and x of the general kinetic formula $-dC/dt = kC^x$ (C = concentration remaining to decompose, x = order of reaction, and k = specific reaction rate constant) can be determined using a suitable computer and program. The log k values (dependent variable Y) plotted against the reciprocal of the corresponding temperatures in absolute degrees (independent variable x) will provide an Arrhenius plot (based on $k = Ae^{-E_a/RT}$) from which the k value at room temperature can be predicted. Knowing the x and the predicted k, one can then calculate the $t_{0.9}$ at room temperature.

The present method does not depend on the x and k values, thus

Table VII—First-Order Reactions: Straight Lines Obtained When the Logarithm of the $t_{0.9}$ Values (Dependent Variable Y) Calculated from Data Reported in the Literature Is Plotted against the Reciprocal of the Corresponding Temperatures in Absolute Degrees [Independent Variable $X = (1/T) \times 10^3$]

Slope	Intercept	r^a	$k_n{}^b$	$k_0{}^c$	Reference
3.015920	−8.579299	0.999999	3.0687×10^{-3}	3.0684×10^{-3}	16
2.622350	−9.845875	0.999999	1.1845	1.1843	16
8.165610	−9.824702	0.996643	2.890×10^{-19}	3.420×10^{-19}	23
8.206471	−9.779591	0.994406	1.910×10^{-19}	2.060×10^{-19}	23
6.019405	−14.514888	0.999872	2.230×10^{-7}	2.218×10^{-7}	24
6.144731	−14.794646	0.997903	1.620×10^{-7}	1.587×10^{-7}	24
6.020490	−14.551780	0.999641	2.410×10^{-7}	2.349×10^{-7}	24
5.995196	−14.580394	0.999971	3.132×10^{-7}	3.134×10^{-7}	24
6.363474	−14.507285	0.999911	1.540×10^{-8}	1.566×10^{-8}	24
9.319568	−12.422928	0.997851	1.540×10^{-20}	1.650×10^{-20}	25
11.429101	−13.839593	0.999985	3.390×10^{-26}	3.400×10^{-26}	26
10.423916	−14.578298	0.999994	4.370×10^{-22}	4.380×10^{-22}	26
11.083506	−13.981022	0.999997	6.770×10^{-25}	6.930×10^{-25}	26
5.584305	−15.876697	0.997936	3.944×10^{-9}	3.867×10^{-4}	20
3.669914	−10.715582	0.999936	7.180×10^{-8}	7.300×10^{-8}	21
5.053777	−14.238390	0.998874	5.460×10^{-9}	5.600×10^{-9}	21
5.690677	−15.997422	0.999990	2.290×10^{-9}	2.310×10^{-9}	21
4.601365	−12.726488	0.999702	5.530×10^{-9}	5.730×10^{-9}	21
6.870275	−15.120840	0.999450	1.260×10^{-9}	1.420×10^{-9}	27
6.734594	−15.115829	0.998960	3.560×10^{-9}	4.770×10^{-9}	27
5.310601	−11.074757	0.960368	1.930×10^{-8}	1.490×10^{-8}	27
5.375524	−14.484671	0.998470	8.360×10^{-9}	8.110×10^{-9}	28
6.177557	−16.004070	0.999848	5.640×10^{-10}	7.920×10^{-10}	29
4.302462	−13.836206	0.999243	7.440×10^{-6}	7.920×10^{-6}	30
6.560143	−16.412022	0.998516	2.710×10^{-7}	2.750×10^{-7}	31
6.986699	−16.127803	0.999439	5.220×10^{-9}	7.570×10^{-9}	31
7.442154	−12.877465	0.998183	8.710×10^{-14}	5.180×10^{-14}	32

[a] All values of the coefficient of correlation are significant at the 5% level of probability. [b] The k_n is the specific rate constant at 25° calculated from $t_{0.9}$ at 25°. The $t_{0.9}$ was predicted using the appropriate straight-line equation. [c] The k_0 is the specific rate constant reported in the reference.

Table VIII—Second-Order Reactions: Straight Lines Obtained When the Logarithm of the $t_{0.9}$ Values (Dependent Variable Y) Calculated from Data Reported in the Literature Is Plotted against the Reciprocal of the Corresponding Temperatures in Absolute Degrees [Independent Variable $X = (1/T) \times 10^3$]

Slope	Intercept	r^a	$k_n{}^b$	$k_0{}^c$	Reference
1.400958	−15.418121[d]	0.999849	3.000×10^{-35}	4.510×10^{-35}	33
5.292292	−13.129828	0.999300	2.660×10^{-8}	3.989×10^{-8}	34
3.021353	−11.461445	0.996671	2.370×10^{-2}	1.090×10^{-2}	35
4.230676	−11.960942	0.999335	6.570×10^{-6}	5.320×10^{-6}	35
2.630486	−13.012160	0.999570	1.720×10^{4}	1.715×10^{4}	36
4.804216	−11.873939	0.996270	6.410×10^{-8}	6.760×10^{-8}	35
4.092603	−12.020604	0.999447	2.190×10^{-5}	2.010×10^{-5}	35

[a] All values of the coefficient of correlation are significant at the 5% level of probability. [b] The k_n is the specific rate constant at 25° calculated from $t_{0.9}$ at 25°. The $t_{0.9}$ was predicted using the appropriate straight-line equation. [c] The k_0 is the specific rate constant reported in the reference. [d] In this equation, X is $(1/T) \times 10^4$ instead of $(1/T) \times 10^3$.

minimizing the time and effort involved in accelerated stability studies without sacrificing scientific rigor. This approach is of particular advantage to small laboratories and hospital pharmacy manufacturing units where a suitable computer and program may not be available. The procedure is applicable only where the degradation reaction is a thermal process in which the use of an Arrhenius plot is justified.

The physicochemical properties of the particular medicinal substance and the possibilities available to the formulator dictate the range of elevated temperatures employed. The statistical reliability of the result can be increased by using a greater number of elevated temperatures. The reliability is also increased if the lower end of the temperature range is as close to the room temperature as possible. The accuracy of the result is indeed greater if the number of log $t_{0.9}$ and $1/T$ pairs is preferably about eight to 12. The procurement and analysis of the samples need not be carried beyond the point at which the concentration remaining to decompose becomes less than 80% of its original value. This possibility is particularly helpful in cases where the product of degradation is insoluble in the reaction mixture vehicle.

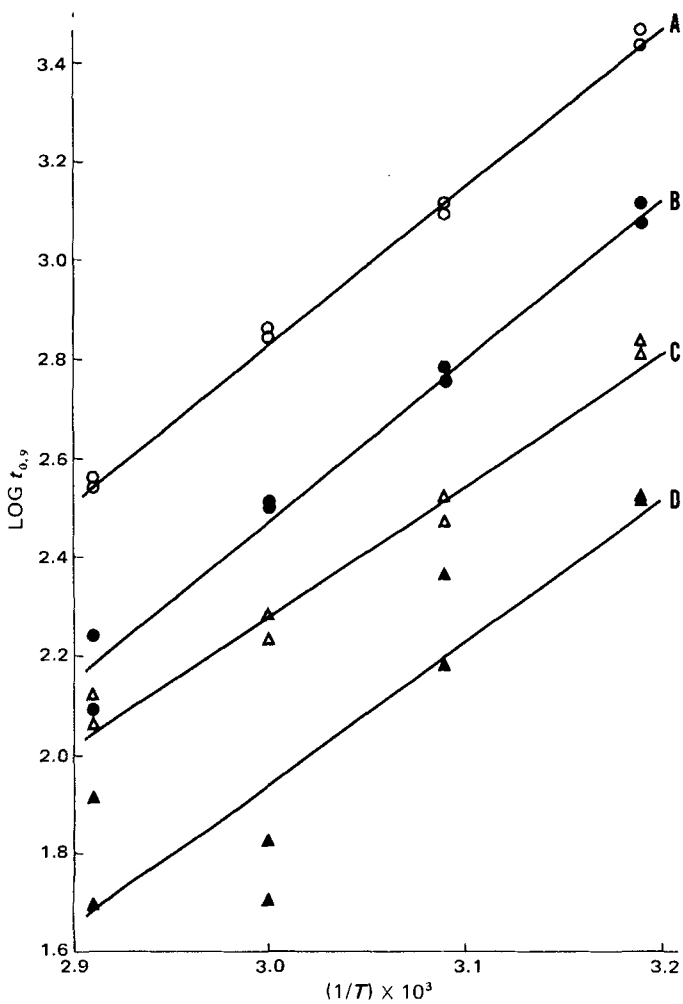

Figure 2—*Log $t_{0.9}$ values obtained from the simulated data and plotted against the reciprocal of corresponding temperatures (T in absolute degrees). Key: A, zero order; B, first order; C, third order; and D, second order.*

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Simple GLC Determination of Ethylene Oxide and Its Reaction Products in Drugs and Formulations

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Abstract □ Convenient rapid GLC methods for the estimation of residual ethylene oxide, ethylene chlorohydrin, and ethylene glycol in ethylene oxide-sterilized bulk drugs and formulations prepared therefrom are described. Ethylene oxide was chromatographed using a porous polymer column; ethylene chlorohydrin and ethylene glycol were chromatographed using either polyethylene glycol 400 or a porous polymer column. All three residuals were determined from the same sample preparation for each type of drug or formulation examined. Recoveries of each of the three residuals were greater than 95% for all samples examined. Detection limits, at moderate electrometer sensitivities, were 2 μg/g or ml for ethylene oxide and ethylene chlorohydrin and 5 μg/g or ml for ethylene glycol. The most likely interferences are discussed.

Keyphrases □ Ethylene oxide—and reaction products, GLC analysis, sterilized bulk drugs and formulations □ GLC—analysis, ethylene oxide and reaction products in sterilized bulk drugs and formulations □ Sterilizers—ethylene oxide and reaction products, GLC analysis in bulk drugs and formulations

The determination of residual levels of ethylene oxide and its reaction products, ethylene chlorohydrin and ethylene glycol, has received a great deal of attention. However, most work has dealt with the estimation of the residual levels in plastic medical devices and foods (1, 2).

Essentially all recently published procedures depended on GLC for separation and quantitation. Differences in the methods were primarily in the sample preparations (3). Natural and synthetic polymers have the capacity to absorb large quantities of ethylene oxide, and various methods have been proposed to measure accurately ethylene oxide, ethylene chlorohydrin, and ethylene glycol in these materials (4). However, drugs and pharmaceutical formulations have received scant attention. A GLC procedure was described for the determination of residual ethylene oxide in steroids that involved distillation and thermal conductivity detection (5).

Recent work centered on the use of porous polymer packings (Chromosorb 101 or Porapak) for ethylene oxide and of polyethylene glycols for ethylene chlorohydrin and ethylene glycol.

To test large numbers of samples, a method was desired that would require minimal sample preparation and analyst time without compromising the requirements for quantitative results. Since the measurement of residual levels is more of a limits test than a precise quantitation for a potency measurement, simple sample preparations and minimum chromatographic time were desired. In this paper, procedures for water-insoluble bulk drugs, aqueous suspensions, aqueous solutions, and ointments are described. For each material or formulation discussed, a single sample preparation was suitable for the quantitation of ethylene oxide, ethylene chlorohydrin, and ethylene glycol.

For quantitation of ethylene oxide, a Porapak R column was employed; a polyethylene glycol 400 column was used for the other two compounds. The use of the two columns was advantageous, since both were operated at the same temperature. With a dual-column dual-electrometer instrument, all three residuals could be determined simultaneously.

EXPERIMENTAL

Instrument—A gas chromatograph[1] equipped with a flame-ionization detector and U-shaped glass columns, 180 cm × 3 mm i.d., was used. The column packings used were Porapak R[2] (80–100 mesh) for ethylene oxide determinations and 15% Carbowax 400 on 80–100-mesh Gas Chrom Q[3] for ethylene chlorohydrin and ethylene glycol measurements. The carrier gas was helium at a flow rate of approximately 60 ml/min, with hydrogen and air flows adjusted for maximum flame response.

The oven temperature was approximately 110° for both columns. A Porapak R column at 170° was also used for ethylene chlorohydrin and ethylene glycol. The flash heater and the detector were operated about 10 and 30° above the column temperature, respectively. The instrument sensitivity was range 10 and attenuation 1, 2, or 4, depending upon sample concentration.

Reagents and Chemicals—Distilled water, hexane[4], ethylene oxide[5] (liquid), ethylene chlorohydrin[5], and ethylene glycol[6] were used. The hexane was washed with distilled water prior to use.

Reference Preparations—The ethylene oxide reference solution was prepared by diluting an ethylene oxide stock solution with distilled water to a concentration of 30 μg/ml. The stock solution of ethylene oxide was prepared by adding about 0.5 ml of liquid ethylene oxide to a 50-ml volumetric flask containing about 25 ml of distilled water. The flask was weighed before and after addition of the ethylene oxide; the increase in weight was the amount of ethylene oxide dissolved. The ethylene oxide solution was diluted to volume and mixed prior to dilution.

A combined ethylene chlorohydrin and ethylene glycol reference solution was prepared by dilution of a stock solution containing both compounds to a concentration of 30 μg/ml each. The stock solution was

[1] Model 402, Hewlett-Packard Corp., Avondale, Pa.
[2] Waters Associates, Milford, Mass.
[3] Applied Science Labs, State College, Pa.
[4] Burdick and Jackson, Muskegon, Mich.
[5] Eastman Organic Chemicals, Rochester, N.Y.
[6] Matheson, Coleman and Bell, Norwood, Ohio.