

Determination of the kinetics of degradation of 13-*cis*-retinoic acid and all-*trans*-retinoic acid in solution*

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Abstract: The degradations of 13-*cis*-retinoic acid and all-*trans*-retinoic acid in an organic solvent were determined with an HPLC assay. The degradation curves at 70, 50 and 37°C all showed autocatalytic characteristics for both isomers. For this kind of complex reaction, the usual method cannot be used to estimate the shelf-lives and half-lives at room temperature. In this work a new method was developed to directly calculate the shelf-lives and half-lives. From this equation the activation energy was found to change as the multiple step reaction progressed.

Keywords: 13-*cis*-retinoic acid; all-*trans*-retinoic acid; autoxidation; chemical kinetics; shelf-life and half-life; Arrhenius equation.

Introduction

The stabilities of 13-*cis*-retinoic acid (*cis*) and all-*trans*-retinoic acid (*trans*) in the solid state have been studied [1]. The stabilities of these compounds in solution are also of interest. Several papers have investigated the solution stability of the retinoic acids. Kitano studied their stabilities in culture media, but did not mention if the experimental conditions included protection from light [2]. Mehta and Calvert tried to determine the stability of a *trans* eye-drop formulation at lower temperatures and no degradation was detected, when the samples were kept in the dark [3]. Oyler and coworkers characterized more than 10 major decomposition products from *trans* in 90% methanol and also measured the degradation curves of *trans* and the formation curves of the decomposition products at 80°C and 25°C, although they were not completed [4]. They found that degradation occurred by oxidative autocatalytic processes. As expected, the decomposition products are complicated because the conjugated double bond in the retinoic acid molecules may break down at any site. These complicated decomposition products create difficulty in studying the reaction mechanism to determine the reaction pathways. However, degradation curves are

needed to yield information about their stability. In this work, degradation curves under light protection condition at different temperatures were determined and evaluated for the estimation of the shelf-life and half-life at room temperature.

Materials and Methods

Materials

All the samples (including the solid *cis* and *trans* samples and the solvent) were supplied by Hoffmann-La Roche, Inc. (Nutley, NJ, USA). The solid sample lot numbers were 909046 for *cis* and 356019 for *trans*. They were stored at 4°C in amberized bottles under nitrogen atmosphere. The solvent was a mixture of medium chain length diglycerides.

All organic solvents were of HPLC grade. Purified water was prepared by a Millipore system.

Sample solutions

The stock solutions were prepared at 1 mg ml⁻¹ concentration level (*ca* 0.1%). For each sample preparation, about 0.3 ml stock solution was taken into an amber ampoule. The ampoules were sealed and placed in ovens at 70, 50 and 37°C and also at room temperature of about 22°C. At suitable time intervals, one

* Presented at the Seventh Annual Meeting and Exposition of the American Association of Pharmaceutical Scientists (Analysis & Pharmaceutical Quality Section), San Antonio, TX, USA, 15-19 November 1992.

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sample was collected and stored in the 4°C refrigerator for subsequent HPLC analysis. On the day of analysis, each solution was weighed into a 5 ml volumetric flask and the flask was filled to scale with the mobile phase of the HPLC system.

HPLC assay

The HPLC system used in this study was essentially the same as described before [1] with a minor modification. A Waters Associate HPLC apparatus (including a Model 6000A solvent delivery system and a Model 440 UV detector) was employed. In addition, another variable wavelength UV monitor was incorporated in the line. The injector was a Rheodyne model 7125 (20 μ l). The columns were a C₁₈ reversed-phase column, Hypersil ODS (4.6 mm \times 15 cm, 10 μ m), and a guard column. The mobile phase, acetonitrile–ammonium acetate (1%) (95:5, v/v), was delivered at a flow rate of 1.1 ml min⁻¹. The detection was performed at 360, 280 and 254 nm. Figure 1 shows the three chromatograms for a *cis* sample. The *cis* component had a retention time of 7 min. The early peaks were due to decomposition products. *Trans* samples had similar chromatograms, except that the *trans* component had a retention time of 11 min. The peak height at 360 nm was used for the quantitative degradation determination. No significant isomerization between these two isomers was observed while the samples were protected from light. All operations were performed in a darkened room illuminated with yellow light.

Results

The degradation curves for both *cis* and *trans* at 70, 50 and 37°C have been completed and they are shown in Figs 2 and 3, respectively. The degradation curves at room temperature were not complete because of room temperature fluctuation and time limitation. In the figures, α is the fraction decomposed. *Cis* and *trans* have the same kind of degradation curves, which are characteristic of an autocatalytic reaction. The degradation is slow during the induction period and accelerates afterwards. The induction periods at 70°C are so short that they can hardly be seen. At lower temperature, e.g. 37°C, the induction delay is substantial. The degradation curves of *cis* and *trans* at room temperature remain within the

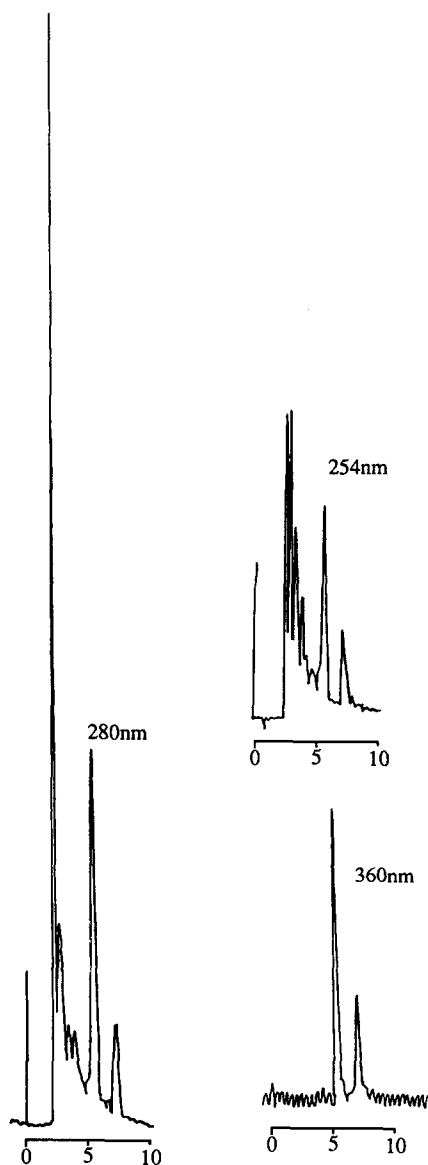


Figure 1
The chromatograms of a 13-*cis*-retinoic acid sample.

induction period for 150 days or longer. The difference between *cis* and *trans* is that *cis* has a much faster degradation rate than *trans*.

Usual method

To deal with this kind of curve, the usual method is to simulate the data with the first order model [5]. Take the middle part of the curve and fit in the first order rate equation to see if a straight line is obtainable. The slope of the straight line is the rate constant, the intercept represents the lag time, t_0 . A lag time estimates how long it takes for the reaction to start its propagation phase.

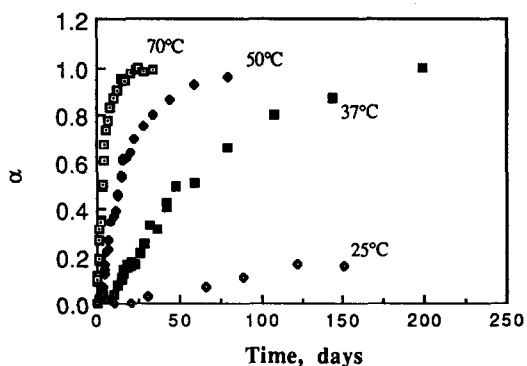


Figure 2
Degradation curves of 13-cis-retinoic acid in solution.

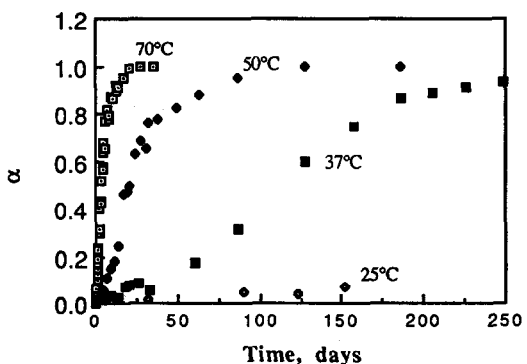


Figure 3
Degradation curves of all-trans-retinoic acid in solution.

This method has some shortcomings. Fitting the data with a first order model is somewhat arbitrary. There is no apparent break between the induction phase and the propagation phase on the degradation curves. It is hard to decide which part of the curve should be included to fit the model. For each curve in Figs 2 and 3, certain parts of the data could be fitted to a straight line. However, different parts of the data yielded somewhat different parameters in the rate equation. Practically, the data are chosen in order to achieve a best fit. Furthermore, although the shelf-life and half-life are calculated from the rate constant plus t_0 , and the rate constant at room temperature can be calculated with the Arrhenius equation, the t_0 at room temperature is not available. Therefore, there is no way to calculate the room temperature shelf-life and half-life, which are more important than rate constants for pharmacists.

New method

In order to evaluate the room temperature

shelf-life and half-life for autocatalytic reactions, a new equation was developed:

$$\ln t_n = \ln \frac{F(\alpha_n)}{Z} + \frac{E}{R} \frac{1}{T} \tag{1}$$

The derivation of the equation is included in the Appendix. In this equation, t_n is the time corresponding to the particular fraction α_n on the degradation curve at temperature T . R is the gas constant and E the activation energy. Z is the preexponential constant in the Arrhenius equation. $F(\alpha_n)$ is the value of the function $F(\alpha)$ at $\alpha = \alpha_n$ (see Appendix).

The application of the above equation to the degradation curves in Figs 2 and 3 is demonstrated in Figs 4 and 5 for cis and trans, respectively. At a particular α_n , for example $\alpha_n = 0.1$, three $t_{0.1}$ values can be read from the three degradation curves at 70, 50 and 37°C in Fig. 2 for cis. These three $t_{0.1}$ data are plotted according to the equation and fitted by a linear regression. At another α_n , the same process is repeated. Nine straight lines corresponding to α_n from 0.1 to 0.9 are plotted and their linear equations are listed in Fig. 4 from bottom to top. Trans degradation curves in Fig. 3 are treated in the same way and the results are shown in Fig. 5. As seen in the plots and the linear correlation coefficients, they are all

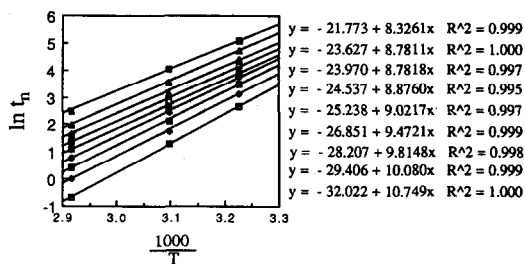


Figure 4
Application of the new equation to cis degradation curves in Fig. 2.

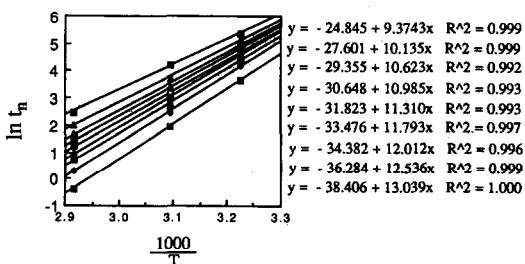


Figure 5
Application of the new equation to trans degradation curves in Fig. 3.

Table 1
Shelf-life and half-life data measured and calculated for 13-*cis*-retinoic acid and all-*trans*-retinoic acid in solution

Temp. (°C)	Cis		Trans	
	Meas.	Calc.	Meas.	Calc.
Shelf-life, $t_{0.1}$ (day)				
70	0.50	0.505	0.68	0.676
50	3.6	3.51	7.0	7.12
37	14.3	14.2	39.0	38.7
22	86.0	82.7	—	209
Half-life, $t_{0.5}$ (day)				
70	3.0	2.90	3.4	3.16
50	13.6	14.8	20.5	24.3
37	50.0	47.7	117	106
22	—	328	—	676

excellent straight lines for both *cis* and *trans* at any α . Since a straight line is obtained at any α_n , the shelf-life at room temperature can be calculated from the linear equation at $\alpha_n = 0.1$, and the half-life at room temperature can be calculated from the linear equation at $\alpha_n = 0.5$. Table 1 lists the data measured and calculated at all the temperatures studied.

Discussion

It is somewhat surprising that straight lines are obtained when the new equation is applied to the experimental data. From this fact, it is concluded that $F(\alpha_n)$ is independent of temperature, since Z is assumed to be a constant. But, as seen in the Appendix, $F(\alpha_n)$ is a function of α and some constants, including rate constants, which usually depend on temperature. The fact that the experimental data do behave in a linear fashion indicates that the dependency of $F(\alpha_n)$ on temperature is very small.

The shape of the decomposition curves are found to be similar to that described by the Ng equation [6]: $d\alpha/dt = kf(\alpha)$, in which $f(\alpha) = \alpha^{1-x}(1-\alpha)^{1-y}$. An attempt was made to determine the constants, k , x and y in the Ng equation using the data in Figs 2 and 3. Unfortunately, their first derivatives, the values of $d\alpha/dt$, are too scattered to locate the α_m point, which must be known before a straight line can be made to evaluate these constants [6]. Although the Ng equation was put forward to describe solid state autocatalytic reactions, it is possible that this equation may also describe the degradation curves of the autocatalytic reaction in solution, since this equation was established based on the shape of

the degradation curve, not the physical state of the reactants. In spite of the fact that the constants in the Ng equation cannot be determined, it is expected that this new equation will generate a straight line when it is applied to the data in Figs 2 and 3. Because $f(\alpha)$ does not contain any rate constants, it is temperature independent.

It is noted that the slopes of the straight lines in Figs 4 and 5 change as α changes. From the equation, the slope of the straight line yields the activation energy. The plots of the activation energies calculated from the slopes versus α for both *cis* and *trans* are shown in Fig. 6. It is found that the activation energy is a function of α . This would be expected for multiple-step reactions. The activation energy measured is the sum of each individual reaction:

$$E_{\text{obs}} = r_1 E_1 + r_2 E_2 + \dots + r_i E_i \quad (2)$$

$$r_1 + r_2 + \dots + r_i = 1. \quad (3)$$

E_i and r_i are the activation energy and fraction

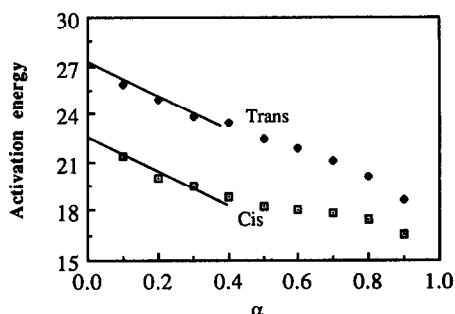


Figure 6
Plots of the activation energies versus α for both *cis* and *trans*. The activation energies were calculated from the slopes of the straight lines in Figs 4 and 5.

of the individual reaction i in the multiple-step reaction. These individual reactions are not simultaneous. Some of them finish early while others later. For example, the induction reaction (the first reaction, see Appendix) may have reached 50% completion before the chain branching reaction (the last reaction) starts. At a particular time t_n , i.e. a particular fraction α_n , each r_i has a different value. At smaller t_n and α_n , the values of r_i of the reactions that occur first are larger, while those of reactions that occur later are smaller. At larger t_n and α_n , the values of r_i of the reactions that occur first become smaller, while those of reactions that occur later become larger. Because r_i is a function of α , E_{obs} changes as α changes. The relationship is not linear in Fig. 6, but the activation energy of the induction reaction can be estimated as α is extrapolated to 0:

$$\alpha = 0, r_1 = 1, E_{\text{obs}} = E_1. \quad (4)$$

The new equation has a similar form to the Arrhenius equation. While the Arrhenius equation deals with the rate constant, this equation deals with time t_n . Actually, this new equation may be considered to be a supplement to the Arrhenius equation. The Arrhenius equation can be utilized only if the degradation data can be simulated with a certain rate law. In the cases where the degradation data cannot be fitted into a simple equation, such as the autocatalytic reaction, no rate constant exists. The Arrhenius equation is therefore not applicable. However, the new equation may be used to obtain the required stability information.

Hansen and co-workers have developed the same equation under the restrictions that the reaction is zero-order and that t_n is in the range of the induction period [7]. With the availability of highly sensitive isothermal microcalorimetry techniques, the initial portion of the degradation curve, approximated by zero order kinetics, is measurable in many cases, even at room temperature storage conditions. In some cases like the solutions of retinoic acids, microcalorimetry was not sufficiently sensitive to measure the zero order slope. In the past, this equation has been applied to zero order and first order reactions, but under accelerated high temperature conditions in order to obtain the necessary sensitivity [8]. Extrapolation to storage temperature then required the assumption that the Arrhenius

slope remained constant. In this work as seen in the Appendix, this equation was derived without consideration of any particular rate law. It could indeed be applied to simple situations such as zero order and first order reactions, besides more complex cases, and t_n was not limited to a certain part of the reaction, but any point on the $\alpha-t$ curve. This equation covers more complex reactions than the Arrhenius equation for the calculation of only shelf-life and half-life.

The new equation is useful for experimental design. It can be used to predict the time intervals to withdraw samples. If the rate law is not known for a new compound, it is hard to design the time intervals for the collection of samples. To obtain a homogenous curve, repetitive preparation of samples is required. As seen in both Figs 2 and 3, the data densities on the degradation curves at 70 and 50°C are homogenous, because the samples at 70 and 50°C were prepared two or three times to supplement the data on the 70 and 50°C curves, since less than 40 days are required to start over. The data for the degradation curves at 37°C are not homogenous, however. Too many data were collected before 30 days and too little afterwards. We did not have time to supplement the data at 37°C, since more than 150 days are needed. If the new equation were known at that time, the data at 70 and 50°C could have been used to predict the time intervals for sample withdrawing at 37°C. The data densities on the two 37°C curves could have been improved.

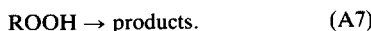
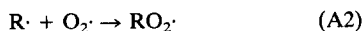
Acknowledgement — Support for this work by Hoffmann-La Roche Inc. is gratefully acknowledged.

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Appendix

Derivation of a new equation for shelf-life and half-life evaluation



Above is a general model for an autooxidative reaction [9]. Where $\text{R}' \cdot$ is the radical present in the solution. Equation (A1) is the induction reaction, equations (A2) and (A3) are the propagation reactions, equations (A4), (A5) and (A6) the chain termination reactions, equation (A7) is the chain branching reaction. Actually, an autooxidative reaction is more complex than this model. For example, the chain branching products may contain some radicals, which will participate in the above reactions. For the purpose of developing a general equation, this simpler model is employed.

Since what is measured is the retinoic acid concentration, $[\text{RH}]$, the rate equation should be written as:

$$-\frac{d[\text{RH}]}{dt} = k_1[\text{RH}] + k_3[\text{RH}][\text{RO}_2 \cdot] \quad (\text{A8})$$

In order to solve equation (A8), an expression of $[\text{RO}_2 \cdot]$ in terms of $[\text{RH}]$ is needed. Applying steady state assumption to $[\text{RO}_2 \cdot]$,

$$k_2[\text{R} \cdot][\text{O}_2] = k_3[\text{RH}][\text{RO}_2 \cdot] + k_4[\text{RO}_2 \cdot]^2 + k_5[\text{R} \cdot][\text{RO}_2 \cdot] \quad (\text{A9})$$

Equation (A9) contains a new variable, $[\text{R} \cdot]$. The steady state assumption is applied to $[\text{R} \cdot]$,

$$k_1[\text{RH}] + k_3[\text{RH}][\text{RO}_2 \cdot] = k_2[\text{R} \cdot][\text{O}_2] + k_5[\text{R} \cdot][\text{RO}_2 \cdot] + k_6[\text{R} \cdot]^2 \quad (\text{A10})$$

In principle, equation (A8) can be solved in terms of $[\text{RH}]$ and rate constants k_1 , k_2 , k_3 , k_4 , k_5 and k_6 . Practically, because equations (A9) and (A10) contain square terms, it is difficult to solve $[\text{R} \cdot]$ and $[\text{RO}_2 \cdot]$ in a complete expression. Hence a function f is used to represent this expression. Equation (A8) can be written

$$-\frac{d[\text{RH}]}{dt} = kf([\text{RH}], k_i), \quad (\text{A11})$$

where k is an assigned rate constant for the equation (A11), $f([\text{RH}], k_i)$ is a function with the variable, $[\text{RH}]$, and some rate constants, k_i .

For simplicity, α is used instead of $[\text{RH}]$,

$$\frac{d\alpha}{dt} = kf(\alpha) \quad (\text{A12})$$

$$\alpha = \frac{[\text{RH}]_0 - [\text{RH}]}{[\text{RH}]_0} \quad (\text{A13})$$

Exchanging dt and $f(\alpha)$,

$$\frac{d\alpha}{f(\alpha)} = k dt \quad (\text{A14})$$

Integrating equation (A14),

$$F(\alpha) = k t \quad (\text{A15})$$

Because the exact form of $f(\alpha)$ is unknown, its integration form is represented with $F(\alpha)$, $F(\alpha) = \int d\alpha/f(\alpha)$.

Equation (A15) indicates that when α is at a particular value, α_n , $F(\alpha)$ is a constant, $F(\alpha_n)$. That means the product of the apparent rate constant k and t_n , the time corresponding to α_n , can be calculated from $F(\alpha_n)$,

$$k t_n = F(\alpha_n) \quad (\text{A16})$$

For example, $\alpha_n = 0.1$, $t_{0.1}$ is shelf-life; $\alpha_n = 0.5$, $t_{0.5}$ is half-life,

$$k t_{0.1} = F(0.1) \quad (\text{A17})$$

$$k t_{0.5} = F(0.5) \quad (\text{A18})$$

The Arrhenius equation can be used to replace the k in equation (A16),

$$k = Z e^{-E/RT}$$

$$t_n = \frac{F(\alpha_n)}{Z} e^{E/RT} \quad (\text{A19})$$

The logarithm of equation (A19) can be used to evaluate the shelf-life and half-life:

$$\ln t_n = \ln \frac{F(\alpha_n)}{Z} + \frac{E}{R} \frac{1}{T} \quad (\text{A20})$$

[Received for review 30 October 1992]