patterns for both norcaperatic acid and the isolated compound were identical. Peaks at m/e = 29, 43, 57, 59, 85, 99, 113, 126, 299, 308, and 326 were consistent with the structure of the molecule. The NMR spectra of both the isolated compound and norcaperatic acid were found to be identical and were in agreement with that reported by Miyata *et al.* (3) for norcaperatic acid. A ratio of 3:29 was obtained by the integration of the combined signals of the protons on the α and γ carbons and the protons on the aliphatic side chain. The absence of a methyl ester function in the infrared, mass, and NMR spectra excluded the possibility of the isolated compound being caperatic acid.

The potassium salt of the isolated compound was prepared as described by Miyata *et al* (3). The observed m.p. (173–174°) of the prepared salt was identical to that reported for the potassium salt of norcaperatic acid. A mixed m.p. with a sample of the reference compound resulted with no depression of the original m.p.

Quantitative elemental analysis of the prepared salt gave the following results.

Anal.—Calcd. for $C_{20}H_{35}KO_7$: C, 56.31; H, 8.27. Found: C, 55.97; H, 8.33.

RESULTS AND DISCUSSION

A 22% yield of norcaperatic acid was obtained from the ether extract of *P. fibrillosus*. The identification of the acid was established by its solubilities, its physical and chemical properties, and by direct comparison with reference compounds. This marks the first reported occurrence of norcaperatic acid in a *Polyporus* species.

The very high concentration of norcaperatic acid in *P. fibrillosus* is reminiscent of the reported concentration of agaricic acid (18%, dry weight basis) in *F. officinalis*. There appears to be no apparent function for these compounds in the respective fungi other than that of waste products. Nord (8) has suggested that many of the metabolites produced in yields exceeding functional requirements, or for which there is no apparent function, accumulate because some of the enzyme systems involved in the oxidative sequence become saturated with respect to their substrates.

The occurrence of norcaperatic acid in the genus *Polyporus* may possibly be restricted to only *P. fibrillosus* since Overholts (4) indicated that this species has no close relatives and numerous other phytochemical investigations (9, 10) have not revealed the presence of this type of acid in other *Polyporus* species.

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Keyphrases

Norcaperatic acid—*Polyporus fibrillosus*
α-Tetradecylcitric acid—isolated, identified
IR spectrophotometry—identity
Mass spectroscopy—identity
NMR spectroscopy—identity

Utilization of the Guggenheim Method in Kinetics

By PAUL J. NIEBERGALL and EDWIN T. SUGITA

The Guggenheim method for the evaluation of rate constants is shown to be applicable to a wide range of problems that are of pharmaceutical interest. These include reaction kinetics in which more than one product is produced from a common reactant, consecutive first-order reactions, dissolution followed by partitioning into a lipid phase, the use of dissolution kinetics to obtain drug solubility, and the analysis of drugs through kinetics.

FIRST-ORDER chemical reactions are frequently followed by directly measuring x, the concentration reacted, and then obtaining the rate constant by plotting the logarithm of $a - x$ *versus* time, where a is equal to the initial concentration of the reactant. Alternately, some physical property, P, which is proportional to concentration may be used, and a plot of log $(P - P_\infty)$ or log $(P_\infty - P)$ *versus* time is used to obtain the rate constant. One objection to this is that overemphasis is placed upon the initial concentration or the time infinity reading of the physical property. A further difficulty arises when either the initial concentration or the final reading of the physical property cannot be obtained. In order

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to overcome these difficulties, Guggenheim (1) proposed a method whereby measurements of concentration or of a physical property are made at equal time intervals, eliminating the need for measuring a or P_∞. The method was stated to be useful for "a unimolecular reaction, whether reversible or irreveresible," and the example given was for a simple irreversible unimolecular reaction. Textbooks of chemical kinetics continue to illustrate the use of the Guggenheim method using the same type reaction, and to the best of the authors' knowledge, this is the extent to which the Guggenheim method has been used in research. It has been found however, that the Guggenheim method is applicable to a much larger variety of kinetic systems than is generally realized, and these findings might be of interest to others.

THEORY

In order to extend the usefulness of the Guggenheim approach to first-order kinetics, it is necessary to realize that the type equation used by Guggenheim (1) to illustrate his method is in reality a particular solution to the more general equation type:

$$X = G' - G \exp(-kt) \qquad \text{(Eq. 1)}$$

in which X represents concentration[1] at time t and G', G, and k are constants. Assume that the concentration X is determined at times $t_1, t_2, \ldots t_n$, and that another set of concentration data is obtained at times $t_{1+\tau}, t_{2+\tau}, \ldots t_{n+\tau}$ in which τ is a constant time increment. This results in:

$$X_{t+\tau} = G' - G \exp[-k(t+\tau)] \qquad \text{(Eq. 2)}$$

Subtracting[2] Eq. 1 from Eq. 2 gives:

$$X_{t+\tau} - X = \Delta X = [G - G \exp(-k\tau)] \exp(-kt) \qquad \text{(Eq. 3)}$$

Taking logarithms yields:

$$\log(\Delta X) = \log[G - G \exp(-k\tau)] - kt/2.303 \qquad \text{(Eq. 4)}$$

The slope of the straight line is equal to $-k/2.303$. The intercept of the Guggenheim type plot is generally ignored. However, it can provide some very useful information for a variety of systems. Rearranging the intercept gives:

$$G = \frac{\text{antilog intercept}}{1 - \exp(-k\tau)} \qquad \text{(Eq. 5)}$$

Thus, once k is obtained from the slope of the Guggenheim plot, G can be obtained from the intercept and Eq. 5.

It should be noted that Eq. 1 reduces to the type equation generally used to illustrate the Guggenheim approach, if $G' = 0$. A very common situation of general pharmaceutical interest occurs when $G = G'$ and Eq. 1 becomes:

$$X = G - G \exp(-kt) = G[1 - \exp(-kt)] \qquad \text{(Eq. 6)}$$

Equations 5 and 6 form the basis for the present extension of the Guggenheim approach.

[1] Concentration is used in this paper for the sake of brevity. The equations are equally valid if changes in some physical property are followed in place of concentration.

[2] An interesting alternate solution to Eqs. 1 and 2, which obviates the log transform of Eq. 3 is given by Swinbourne, E. S., *J. Chem. Soc.*, **1960**, 2371.

EXAMPLES

Simultaneous Dissolution and Partitioning—A set of published data which can be used to illustrate the utility of Eqs. 5 and 6 is that of Niebergall *et al.* (2). The physical model involves dissolution into an aqueous phase followed by a first-order transfer into a lipid phase overlayering the aqueous phase. The integrated expression given was:

$$B = \frac{K}{K'}[1 - \exp(-K't)] \qquad \text{(Eq. 7)}$$

in which B represents the weight of drug in the aqueous phase and the constants K and K' are used to evaluate the rate constants k_1 and k_2. This equation is of the same general type as Eq. 6. In this publication, the Guggenheim method was used to obtain K'. However, the potential usefulness of the intercept of the plot was not realized, and a plot of B *versus* $[1 - \exp(-K't)]$ was used to obtain K. Treating the intercept of the data used for the authors' publication according to Eq. 5 yields the following comparison:

	k_1	k_2
Niebergall *et al.* (2)	0.00293	0.0316
Guggenheim approach	0.00292	0.0315

The validity of the Guggenheim plot of Eq. 7 was demonstrated by Figs. 3 and 4 of the authors' previous publication (2).

Two or More Products from a Single Reactant—Guttman and Meister (3) and Galleli and Kostenbauder (4) published studies concerning reactions in which a reactant produced two different products *via* a unimolecular process. The authors have unpublished data from their laboratories for the hydrolysis of penicillin that fits the same reaction pattern. In all instances, the integrated expressions for the appearance of products are of the form:

$$P_1 = \frac{k_1 R_0}{K}[1 - \exp(-Kt)] \qquad \text{(Eq. 8)}$$

$$P_2 = \frac{k_2 R_0}{K}[1 - \exp(-Kt)] \qquad \text{(Eq. 9)}$$

in which R_0 is the initial concentration of reactant, P_1 and P_2 are the concentrations of products, and $k_1 + k_2 = K$. In the literature cited (3, 4) and in the authors' penicillin studies, logarithmic plots of reactant concentration versus time were used to obtain K. Plots of P *versus* $[1 - \exp(-Kt)]$ were then used to evaluate the individual rate constants. Equations 8 and 9 are of the same form as Eq. 6, with the constant G equal to k_1R_0/K or k_2R_0/K, depending upon which product concentration is being followed. The authors therefore reanalyzed their data as a general test of the applicability of the Guggenheim approach to reactions in which more than one product is produced simultaneously from a single reactant *via* a first-order process. A plot of the data is shown in Fig. 1, and a comparison follows:

	k_1	k_2
Previous method	0.00274	0.0108
Guggenheim approach	0.00270	0.0108

In addition to requiring only one plot of the data instead of two, the Guggenheim method has the additional advantage of not requiring the loss of

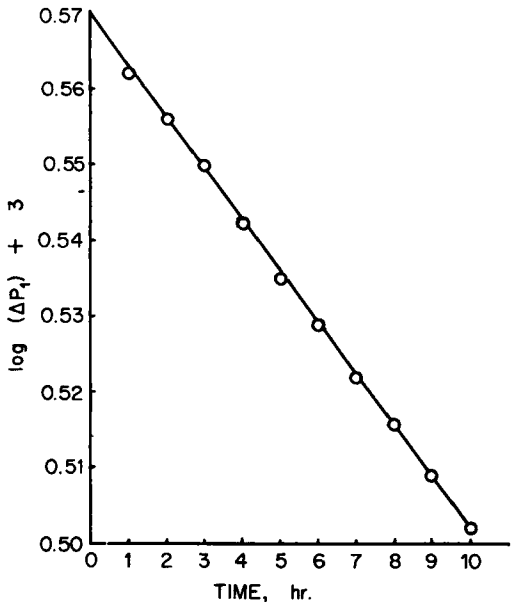

Fig. 1—Guggenheim plot illustrating a reaction in which two products are produced simultaneously via *first-order reactions from a single reactant.*

reactant to be followed. In fact, all of the rate constants can be obtained by following the appearance of only one product. It is preferable from the standpoint of good kinetic procedure to follow the concentration of reactant as well as the concentration of all products as a function of time, in order to insure a valid interpretation of the data. However, from a practical point of view, it is not at all uncommon to find that one or more of the species in question cannot be followed. This was true of the authors' penicillin data in which the penicillin assay was much less reliable than the assay for either of the two products, and many replications were needed to obtain a mean value for K. The authors' therefore calculated k_1 and k_2 using the Guggenheim approach on each of the two products which represented their most reliable data. The two values of k_1 and k_2 thus obtained agreed within 1%, and are being used as their most reliable estimates of the rate constants. The estimate obtained by following the loss of penicillin and the method described in the literature (3, 4) is thus being used only as a check of the validity of their proposed mechanism.

Consecutive First Order-Reactions—A common kinetic pattern which requires rather involved methods of analysis, is that in which a reactant A goes through an intermediate B to final product C with all processes being first order. Integrated expressions for the reactant and intermediate are:

$$A = A_0 \exp(-k_1 t) \quad \text{(Eq. 10)}$$

$$B = \frac{k_1 A_0}{k_2 - k_1} [\exp(-k_1 t) - \exp(-k_2 t)] \quad \text{(Eq. 11)}$$

in which the terms have their usual meanings. Jensen and Lamb (5) treated this type of system by following both A and B as a function of time. The rate constant k_1 was obtained by a logarithmic plot of Eq. 10. The rate constant k_2 was obtained using dimensionless parameters as described by Frost and Pearson (6) in which $\alpha = A/A_0$, $\beta = B/A_0$, $K = k_2/k_1$, and $\beta_{max.} = K^{K/(1-K)}$. A plot of β *versus* $1 - \alpha$ was made, and $\beta_{max.}$ obtained graphically. This enabled K to be obtained and k_2 evaluated by multiplying K by k_1. The rate constants can be obtained much more readily, and without the disadvantage of having to estimate one of the parameters graphically, by use of the Guggenheim approach. Dividing Eq. 11 by Eq. 10 gives:

$$\frac{B}{A} = R = K - K \exp(-K' t) \quad \text{(Eq. 12)}$$

in which $K = k_1/K'$ and $K' = k_2 - k_1$. Equation 12 is seen to be of the same general form as Eq. 6. Thus, if $\log (R_{t+\tau} - R)$ is plotted *versus* time, a straight line should be obtained. The slope of this line yields K' and through use of Eq. 5, K can be obtained from the intercept. Multiplying K by K' gives k_1 which can then be added to K' to give k_2. This reaction scheme was simulated on Pace TR-20 analog computer with the following results:

	k_1		k_2	
k_2/k_1	Computer	Guggenheim	Computer	Guggenheim
0.20	0.050	0.048	0.010	0.011
0.50	0.100	0.100	0.050	0.053
2.00	0.050	0.049	0.100	0.100

Plots of the data for $k_2/k_1 = 0.20$ using different values of τ are shown in Fig. 2. The slopes of the lines are positive, since $k_2 < k_1$. It was found that τ should be taken such that values of R are not used beyond $\beta_{max.}$. The method appears valid beyond this point, but there is a loss in accuracy, yielding constants that differ from the computer constants by 10–15%.

Effect of Varying τ—Values of τ greater than one half-life ($t_{0.5}$) should tend to give more accurate results using the Guggenheim approach, since the greater τ, the greater the value of ΔX at a given time. However, it may not always be practical to take $\tau \gg t_{0.5}$. The authors investigated the effect of varying τ in a number of systems. In general, for all systems studied, it was found that rate constants

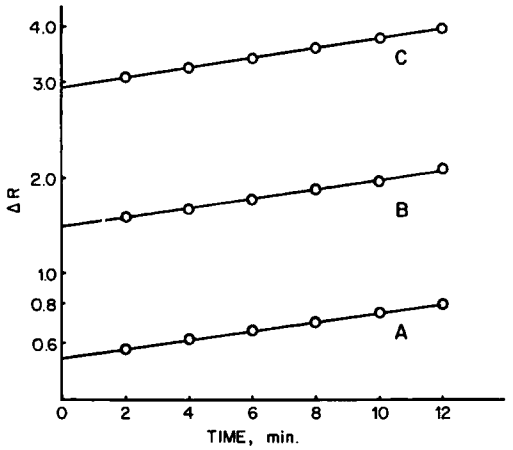

Fig. 2—Guggenheim plot for a series of consecutive first-order reactions in which $k_2/k_1 = 0.20$ *at varying values of* τ. *Key:* A, $\tau = t_{0.25}$; B, $\xi = t_{0.50}$; C, $\tau = t_{0.75}$.

evaluated using $t_{0.25}$ through $t_{0.75}$ differed by less than 5%. This is illustrated in Fig. 2 for the series first-order reaction in which $k_2/k_1 = 0.20$. The choice of τ appears to have no effect upon the slope of the Guggenheim plot. The following estimates of k_1 were obtained:

τ	k_1
$t_{0.25}$	0.047
$t_{0.50}$	0.048
$t_{0.75}$	0.047

Since this is computer simulated data, it is rather idealized. However, it does point out the fact that even if the time interval cannot be equal to one half-life or more, the Guggenheim method should not automatically be discarded as a means of evaluating kinetic data.

Dissolution Rate Estimation of Drug Solubility—Assuming constant surface area, the dissolution rate of a drug may be expressed as:

$$dC/dt = k(C_s - C) = kC_s - kC \quad \text{(Eq. 13)}$$

in which C is the concentration of drug at time t, C_s is taken to be the equilibrium solubility of the drug, and k is the dissolution rate constant. This equation can be integrated through use of an integrating factor and the fact that at time zero, $C = 0$ to give:

$$C = C_s - C_s \exp(-kt) \quad \text{(Eq. 14)}$$

This has the same general form as Eq. 6, and a plot of log (Δ C) *versus* time should yield a straight line with slope equal to $-k/2.30s$. The intercept of the straight line can be used to evaluate C_s through the use of Eq. 5. This was done for the dissolution of salicylic acid pellets of constant surface area in a pH 2.00 buffer at 30°. The value for C_s that was obtained using the Guggenheim approach was 2.25 g./l. as compared to 2.24 g./l. determined by allowing the dissolution to proceed to equilibrium. This information is included only to illustrate a potential application of the Guggenheim approach. Further work must be done to completely evaluate this application.

Kinetic Analysis of Drugs—A second potential application of the Guggenheim method would be the kinetic analysis of drugs (7). For example, if a drug for which no reliable assay exists, degrades *via* a first-order process into a readily assayable product, the integrated expression for the concentration of product as a function of time is:

$$P = A_0[1 - \exp(-kt)] \quad \text{(Eq. 15)}$$

in which P is the concentration of product at time t, A_0 is the initial concentration of the reactant, and k is the observed first-order rate constant. This equation has the same general form as Eq. 6, and the Guggenheim method should be applicable for the estimation of A_0. The authors' tried this for the analysis of penicillin by following the appearance of penicillinic acid at 322 mμ using a Beckman model DU spectrophotometer. The estimated penicillin concentration was 4.06×10^{-4} M as compared to the actual concentration of 4.00×10^{-4} M. Complete details of the procedure utilized are not being given at this time, and the data mentioned are included only to illustrate a potential usefulness for the Guggenheim method. Further studies into the practicality and desirability of using this method for the assay of drugs are currently underway.

These are but a few examples of the variety of kinetic patterns which are capable of being analyzed using the Guggenheim approach. The results obtained thus far suggest that the Guggenheim method should be applicable to any kinetic equation that is of the same form as Eq. 6. Further application for example, might be the analysis of a zero-order process followed consecutively by a first-order process, such as zero-order drug release followed by first-order drug absorption. The fact that the Guggenheim approach is applicable to a given system does not mean that it necessarily is the method of choice. The authors feel however, that in the past the Guggenheim approach has not been utilized to its full capabilities, and have attempted to point out ways in which it might be utilized.

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Keyphrases

Guggenheim method—kinetic utilization
First-order kinetics—Guggenheim method
Consecutive first-order reaction—Guggenheim method
Drug degradation—Guggenheim method