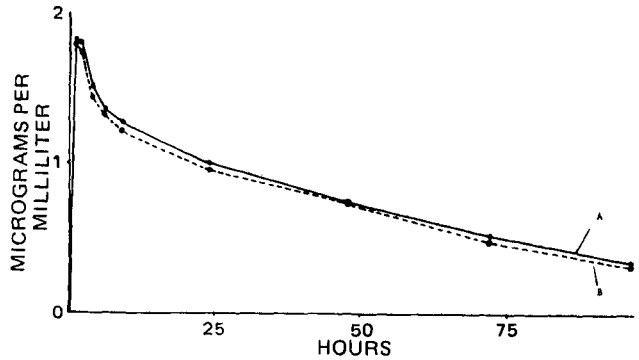

Figure 4—*Mean plasma warfarin concentrations in seven volunteers following a single oral dose of 15 mg of crystalline warfarin sodium. Key: A, UV method; and B, GLC method.*

lowed by a smooth, gradual decline from 24 to 96 hr, indicating slow drug disappearance from peripheral circulation. The estimated areas under the plasma concentration–time curves from 0 to 96 hr were 73.58 and 75.86 μg/ml × hr while the elimination half-lives were 50.9 and 51.4 hr as determined by the GLC and UV methods, respectively.

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Determination of Time Course of Tablet Disintegration II: Method Using Continuous Functions

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Abstract □ An analysis of the disintegration–dissolution sequence of drug release from a tablet leads to a mathematical expression relating disintegration to the dissolution profile of the tablet and the dissolution rate of the primary drug particles in the tablet. The equation describing the disintegration of an acetaminophen tablet is determined to demonstrate the application of the theory.

Keyphrases □ Disintegration, tablet—related to dissolution profile of tablet and dissolution rate of primary drug particles □ Dissolution—profile of tablet and rate of primary drug particles related to tablet disintegration

A method involving numerical analysis was described recently (1) that accounts for both the disintegration and dissolution aspects of drug release from a tablet. If the dissolution characteristics of the primary drug particles in the tablet are known, it is possible to determine the time course of the disintegration process from tablet dissolution data. This disintegration–dissolution analysis requires a numerical data analysis over many small time intervals. The purpose of the present paper is to extend this method to provide a model based on continuum mathematics.

THEORETICAL

The principle of the disintegration–dissolution analysis described previously (1) is to consider the processes of disintegration and dissolution to be discrete events over many small, equal time intervals. At any arbitrary interval, the fraction of drug in the tablet that has dissolved is considered to be the sum of the amounts dissolved from all disintegrated fractions, calculated using the cube-root equation for the time periods between the disintegration intervals and the arbitrary interval. The mathematical expression given that describes this phenomenon is:

$$M_n = \sum_{i=0}^{n} w_i\{1 - [1 - K(t_n - t_i)]^3\} \qquad \text{(Eq. 1)}$$

where M_n is the fraction of drug in the tablet dissolved after n time intervals and corresponds to time t_n. The fraction disintegrated at the ith interval is w_i, and K is the cube-root law dissolution constant. The term

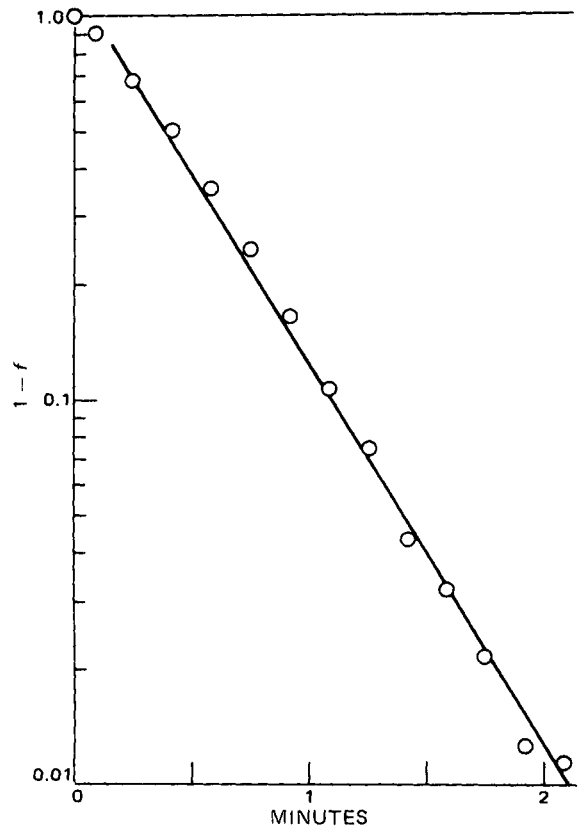

Figure 1—*Linearized plot of dissolution data for acetaminophen powder.*

in braces should be defined equal to one once it attains that value. The w_i can be obtained by applying Eq. 1 at each interval for the dissolution curve of the tablet. The cumulative fraction of drug disintegrated from the tablet, W_n, up to time t_n is given by:

$$W_n = \sum_{i=0}^{n} w_i \qquad \text{(Eq. 2)}$$

The disintegration profile for the tablet can be constructed by application of Eq. 2 at each time interval.

To make the transition from the numerical analysis type of expressions of Eqs. 1 and 2 to equations involving analytical functions, Eq. 1 should first be generalized as:

$$M_n = \sum_{i=0}^{n} w(t_i) f(t_n - t_i)\,\Delta t \qquad \text{(Eq. 3)}$$

where w is the differential disintegration function and Δt is the length of the time interval. The function f describes the dissolution of the primary drug particles. The term in braces in Eq. 1 is $f(t_n - t_i)$ for the Hixson–Crowell cube-root model for particle dissolution.

In the limit as $\Delta t \to 0$ and $n \to \infty$, Eq. 3 becomes the convolution integral:

$$M(t) = \int_0^t w(\theta) f(t - \theta)\, d\theta = w(t) * f(t) \qquad \text{(Eq. 4)}$$

where M now becomes a continuous function of time t, θ is an integration variable, and $*$ represents convolution. The convolution integral is well characterized and is dealt with on the basis of operational calculus (2). Because $w(t)$ is the differential disintegration function, the cumulative fraction of the tablet disintegrated as a function of time, $W(t)$, is given as:

$$W(t) = \int_0^t w(t)\, dt \qquad \text{(Eq. 5)}$$

If Laplace transforms are taken for the convolution equation $M(t) = w(t) * f(t)$, then the relationship for the transforms is:

$$\overline{M}(s) = \overline{w}(s)\overline{f}(s) \qquad \text{(Eq. 6)}$$

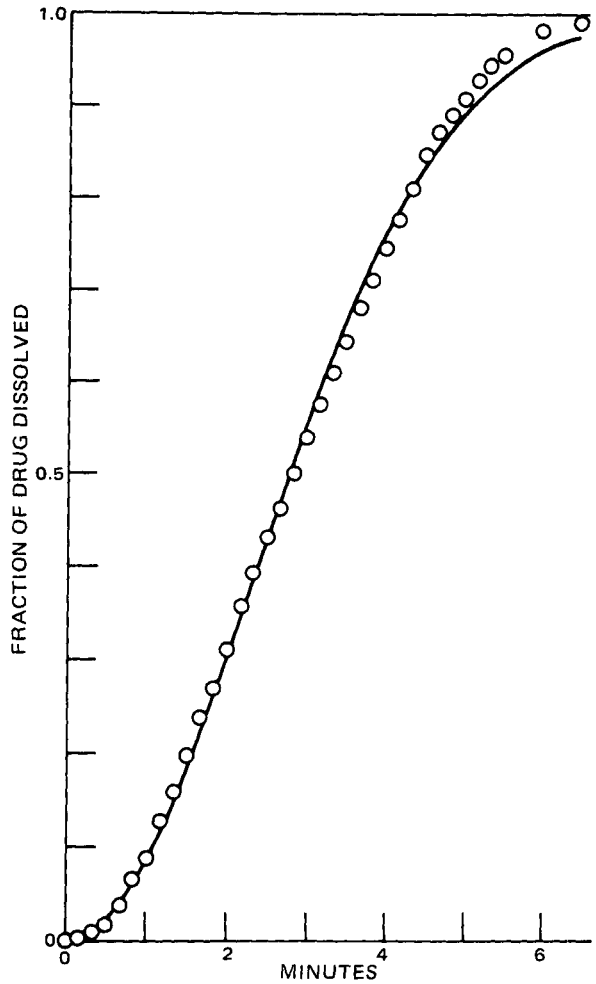

Figure 2—*Dissolution of the acetaminophen tablet (points). The curve is the Weibull function with* a = *11.15 and* b = *1.996.*

The bar over the function and the s represent the Laplace transform for the function. A rearrangement of Eq. 6 yields:

$$\overline{w}(s) = \frac{\overline{M}(s)}{\overline{f}(s)} \qquad \text{(Eq. 7)}$$

Equation 7 indicates that the function w can be obtained from the inverse transform of the ratio of the Laplace transforms of M and f.

The function M is a continuous function that describes the fraction of drug in a tablet dissolved with time. To obtain this function on a practical basis, an empirical equation can be fit to experimental tablet dissolution data. An equation found to be useful is the Weibull function (3):

$$M(t) = 1 - e^{-(t^b/a)} \qquad \text{(Eq. 8)}$$

The adjustable parameters are a and b, the scale factor and the shape factor, respectively. The Weibull function normally contains a lag time, but this term has been omitted because it is usually zero for tablet dissolution experiments.

The function f describes the dissolution of the primary drug particles in the tablet; it is initially zero and approaches unity as the particles completely dissolve. This function can be developed from a model that describes dissolution of powders, *e.g.*, the term in braces in Eq. 1 is $f(t_n - t_i)$ developed from the cube-root law, or it may be an empirical function fit to powder dissolution data. The latter approach is taken for the present work because a smooth function that facilitates the solution of Eq. 4 may be chosen. Functions f that arise from the powder dissolution models usually have a discontinuity in the first derivative at the time of complete dissolution, making the mathematics more complex. For the present, an exponential function is assumed to describe the particle dissolution:

$$f(t) = 1 - e^{-kt} \qquad \text{(Eq. 9)}$$

where k is an adjustable parameter.

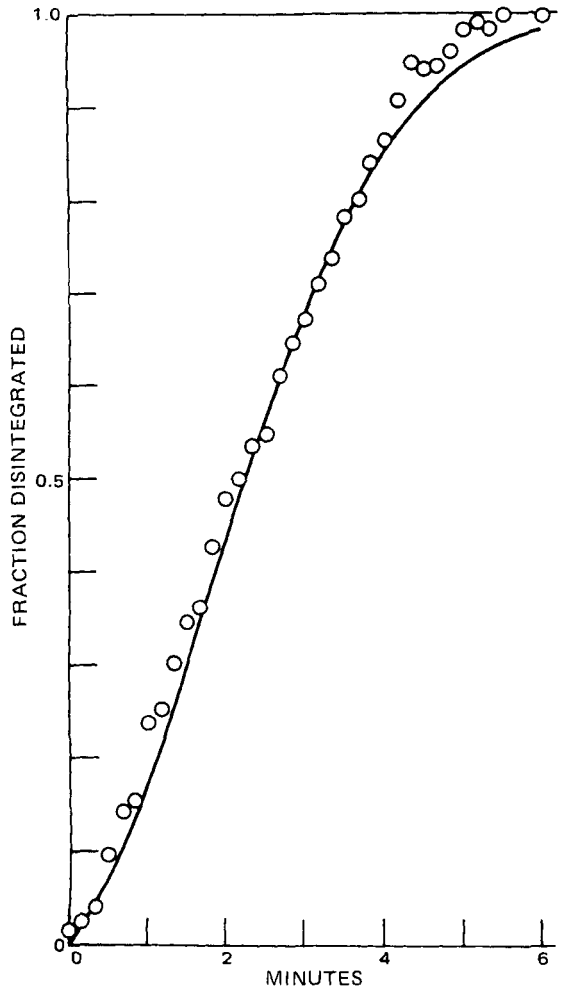

Figure 3—*Disintegration profile (curve) determined with Eq. 14. The points are the results of the numerical method using Eq. 9.*

If the Laplace transform of Eq. 9 is substituted into Eq. 7 and rearranged:

$$\overline{w}(s) = \frac{1}{k} s^2 \overline{M}(s) + s\overline{M}(s) \qquad \text{(Eq. 10)}$$

Thus:

$$w(t) = \frac{1}{k} L^{-1}[s^2\overline{M}(s)] + L^{-1}[s\overline{M}(s)] \qquad \text{(Eq. 11)}$$

where L^{-1} represents the inverse transform. Because of the fundamental relationship between the derivative of a function (denoted by a prime) and the transform, if $M'(0) = 0$ and $M(0) = 0$, Eq. 11 becomes:

$$w(t) = \frac{1}{k} M''(t) + M'(t) \qquad \text{(Eq. 12)}$$

Integration of Eq. 12 yields:

$$W(t) = \frac{1}{k} M'(t) + M(t) \qquad \text{(Eq. 13)}$$

If M from Eq. 8 is substituted into Eq. 13:

$$W(t) = 1 - \left(1 - \frac{bt^{b-1}}{ka}\right) e^{-(t^b/a)} \qquad \text{(Eq. 14)}$$

Equation 14 thus represents the disintegration profile for a tablet whose dissolution curve is represented by the Weibull function with parameters a and b and whose particles dissolve according to Eq. 9 with parameter k.

RESULTS AND DISCUSSION

In the report where the disintegration profile was determined by a numerical technique (1), the method was applied to an acetaminophen tablet prepared with a direct compaction formulation which included a monodisperse sample of the drug. The same data are used for the present analysis. The dissolution behavior of the acetaminophen powder sample is shown in Fig. 1, plotted as the linearized form of Eq. 9. The straight line is the least-squares fit and yields a good correlation coefficient ($r^2 = 0.995$). A nonlinear regression of Eq. 9 with the data gives $k = 1.824$.

Figure 2 shows the data points for the dissolution profile of the acetaminophen tablet. The continuous line is the Weibull function for $a = 11.15$ and $b = 1.996$. These parameters were determined by a nonlinear regression of the Weibull function to the data. The fit is quite good, although the function is below the data at large times.

The disintegration profile constructed from Eq. 14 with the above-mentioned values for a, b, and k is given in Table I and as the continuous curve in Fig. 3. The shape is sigmoidal. The profile determined before had slight curvature but was sufficiently linear that a straight line was fitted to it and utilized for simulation purposes. Data for the profile determined previously are included in Table I for comparison. Because W in Fig. 3 appears to have more curvature than the profile determined previously, it is of interest to evaluate the possible reasons for this change. Two functions used in the present report that differ from the earlier work are the exponential function (Eq. 9) instead of the cube-root law and the Weibull function (Eq. 8) instead of the actual data.

To evaluate whether the use of the exponential function contributed to the discrepancy, Eq. 9 was used in Eq. 3 and the numerical analysis technique was applied using the actual data to generate the disintegration profile (Table I). These results closely follow those of the previous work using the cube-root law. The results of this calculation are also shown in Fig. 3. It is apparent from Figs. 2 and 3 that the problem resides in the Weibull function at higher times where it does not fit the experimental dissolution data with a high degree of accuracy. With this in mind, the agreement between the discrete and continuous mathematical treatments is quite satisfactory.

Equation 13 arises primarily from the application of Eqs. 7 and 5 to the exponential description of the dissolution of primary particles (Eq.

Table I—Fraction Dissolved and Fraction Disintegrated from an Acetaminophen Tablet

		W		
Minutes	M	Discrete Case, Cube Root (1)	Discrete Case, Exponential	Continuous Function (Eq. 14)
0.00	0.000	0.018	0.015	0.000
0.17	0.004	0.028	0.025	0.019
0.33	0.009	0.043	0.039	0.042
0.50	0.017	0.109	0.096	0.070
0.67	0.038	0.156	0.142	0.103
0.83	0.065	0.158	0.154	0.137
1.00	0.088	0.251	0.237	0.176
1.17	0.128	0.255	0.253	0.217
1.33	0.159	0.306	0.303	0.258
1.50	0.198	0.347	0.346	0.303
1.67	0.237	0.356	0.362	0.348
1.83	0.269	0.428	0.427	0.392
2.00	0.311	0.480	0.479	0.438
2.17	0.356	0.497	0.501	0.483
2.33	0.393	0.531	0.536	0.525
2.50	0.431	0.540	0.549	0.568
2.67	0.462	0.614	0.613	0.609
2.83	0.501	0.648	0.649	0.646
3.00	0.540	0.669	0.674	0.684
3.17	0.576	0.708	0.711	0.719
3.33	0.610	0.734	0.738	0.749
3.50	0.644	0.781	0.783	0.779
3.67	0.681	0.795	0.801	0.807
3.83	0.711	0.837	0.840	0.831
4.00	0.746	0.860	0.864	0.854
4.17	0.777	0.906	0.907	0.874
4.33	0.810	0.949	0.949	0.891
4.50	0.847	0.931	0.941	0.908
4.67	0.872	0.933	0.945	0.922
4.83	0.891	0.950	0.960	0.934
5.00	0.909	0.978	0.983	0.945
5.17	0.929	0.986	0.991	0.954
5.33	0.945	0.976	0.984	0.962
5.50	0.955	0.995	0.999	0.969
6.00	0.982	0.994	0.997	0.983
6.50	0.991			0.991

9) along with the restrictions on M of $M(0) = 0$ and $M'(0) = 0$. Within these limitations, Eq. 13 is a rather general relationship between the disintegration and dissolution of a tablet. Any function M that fits the tablet dissolution data and meets these restrictions may be substituted into Eq. 13 to give an explicit expression for the disintegration.

Another ramification of Eq. 13 is that it would be possible to determine the disintegration profile in real time if k is known by using the output signal from the spectrophotometer or potentiometer that is monitoring the tablet dissolution as input for an analog computer programmed for Eq. 13. The dissolution and disintegration profiles could thereby be determined simultaneously.

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Binding of 3-Aminoacridinium and 7-Aminoquinolinium Monocations to Double-Stranded DNA: Evidence for Nonlinear Binding Isotherm in Intercalative Region

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Abstract □ The complexations of the singly charged cations of 3-aminoacridine and 7-aminoquinoline by double-stranded, calf thymus DNA were studied by electronic absorption spectrophotometry. At high ratios of total DNA phosphate concentration to total probe concentration (the region associated with intercalative binding), four DNA phosphate units are associated with each bound monocation. However, a binding isotherm based on four DNA phosphate groups in a single binding site does not yield a reproducible equilibrium constant for the binding process. Rather, the sequestration of the monocations by two binding sites, each containing two DNA phosphate units, yields a satisfactory equilibrium expression. This result suggests that the intercalative mode of binding by DNA is not a simple one-step process, which is in agreement with previous kinetic studies. In the region of low DNA phosphate concentration to monocation concentration (the external binding region), the binding is described adequately by a linear isotherm.

Keyphrases □ 3-Aminoacridinium monocations—binding to double-stranded DNA, equilibrium expressions developed □ 7-Aminoquinolinium monocations—binding to double-stranded DNA, equilibrium expressions developed □ Binding—3-aminoacridinium and 7-aminoquinolinium monocations to double-stranded DNA, equilibrium expressions developed □ DNA, double stranded—binding to 3-aminoacridinium and 7-aminoquinolinium monocations, equilibrium expressions developed

Numerous investigators have examined the binding of aminoacridines and aminoquinolines to double-stranded and denatured deoxyribonucleic acid (DNA) (1–8). The aminoacridines have received particular attention because of their well-known antibacterial activity, which has been attributed to their binding by bacterial DNA (9–11). The ability of aminoacridinium compounds to induce frameshift mutations by causing a deletion or insertion of a single nucleotide in the complementary chain of a replicating chromosome is believed to be due to the strong or intercalative binding of these compounds to nucleic acid (12–14).

Aminoquinolines, particularly the 4-amino and 8-amino analogs, have been employed extensively as antimalarial agents (15). Their antimalarial activity has been said to be due to their interaction with the DNA of infectious plasmodia found in malaria-infested mammals (16).

BACKGROUND

The various interactions of aminoacridines and aminoquinolines with nucleic acids can be classified into two groups: intercalation and external binding (6, 11). Intercalation or strong binding of the aminoacridine or aminoquinoline involves insertion of these compounds, to varying degrees, into the DNA molecule and results in some disruption of the latter (1–3). The intercalative binding of drugs or probes to double-stranded DNA is strongest with compounds possessing three fused aromatic rings, linearly annulated, as in the aminoacridines (6–17). A decrease in the size of the aromatic portion of the intercalating probe results in weaker intercalative binding, as demonstrated by DNA–aminoquinoline complexes (18).

Previous studies (19–23) utilizing temperature-jump relaxation kinetics indicated that the intercalation of the singly charged cations of proflavine and acridine orange may occur in two kinetically discrete steps at high DNA phosphorus to probe ratios. However, this behavior has not been established for all aminoacridine cations. External binding to the double-stranded DNA polyanion occurs after all intercalative binding sites are saturated. The interaction apparently occurs mainly between the cationic probe molecule and the negatively charged DNA phosphate groups and, as such, is primarily an electrostatic interaction (6).

Albert (9) demonstrated that it is the cation of aminoacridine that is necessary for bacteriostasis. The inference is that the cationic form is a necessary condition for intercalation of aminoacridines and aminoquinolines. Therefore, electrostatic forces are believed to provide the initial driving force behind the formation of the DNA–probe complex, prior to intercalation of the probe into the DNA molecule, as well as a substantial portion of the forces that maintain the integrity of the complex (6, 9, 18). However, electrostatic forces are said to be less important in intercalative binding than in the binding of cationic drugs or probes to the exterior of the DNA molecule (5, 6).

Data obtained from investigations of the complexation of small cations to DNA are usually treated according to a model developed by Scatchard (24) to describe the binding of small molecules to proteins. This model was later modified and adapted by other investigators to describe binding to nucleic acids (5, 6, 11, 25).

As a consequence of the pharmacological and physicochemical significance of the complexation of aminoquinolines and aminoacridines to double-stranded and denatured DNA, as well as of an interest in im-