# Deaggregation Behavior of a Relatively Insoluble Substituted Benzoic Acid and Its Sodium Salt

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The deaggregation rates of a relatively insoluble benzoic acid derivative and its soluble sodium salt were studied. Based on the postulate that the deaggregation rate follows a first-order process, equations were developed to measure the rates quantitatively. A spectrophotometric procedure, which is simple and convenient for use routinely, is described. The surface area of the aggregates at the final state was estimated from a correlation between the absorbance measurements and the surface area determined with a Coulter counter. It was seen that encapsulating or tableting these compounds decreased the deaggregation rates. The addition of a nonionic polyol surfactant or the sodium salt of polymerized alkyl naphthalene sulfonic acid enhanced the rates. A relationship between the deaggregation rate and rate of solution of the acid in simulated intestinal fluid T.S. (U.S.P. XVI) is demonstrated. The biopharmaceutical significance of the deaggregation rate is discussed.

THE IMPORTANCE of particle size reduction of relatively insoluble drugs to enhance their gastric absorption has been emphasized during the past decade (1–4). The emphasis is based on the premise that the smaller the size, the faster the dissolution rate of the drug due to the increased surface area presented to the dissolving medium. This premise holds as long as the effective species undergoing dissolution are the primary particles. However, when dealing with insoluble drugs which are also difficult to wet and disperse, the effective species are generally not the primary particles but more often their aggregates.

The situation is further aggravated when these drugs are encapsulated or tableted. Very often the aggregates formed during the preparation of the dosage forms are difficult to disperse. The deaggregation or dispersion rate can, in these instances, be the rate-limiting step in the absorption sequence particularly in the initial phases.

The importance of deaggregation or dispersion in pharmaceutical systems has been recognized for a number of years (5) and recently re-emphasized (6–10). However, there has not been a systematic study carried out to evaluate the rate of deaggregation in pharmaceutical systems, except for two recent studies carried out by Lemberger and Mourad (11, 12). Their studies have dealt with the influence of a number of variables on the deaggregation behavior of oil and water emulsions.

A limited number of deaggregation studies were carried out in nonpharmaceutical systems. These have dealt with rates of systems at equilibrium. For instance, Hiemenz and Vold (13) studied the rates of flocculation and deflocculation of carbon black in hydrocarbon liquids, and Reich and Vold (14) of carbon black and ferric oxide in water. Gillespie (15) determined the rate constants of aggregation and deaggregation of monodisperse latexes in water. Parenthetically, there is also an extensive literature (13) on both the theoretical and experimental aspects of the associated phenomena of aggregation.

The present study was carried out to study quantitatively the deaggregation rates of a relatively insoluble benzoic acid derivative and its sodium salt from different dosage forms. Equations were developed based on the postulate that the deaggregation rate followed a first-order process. The effects of a wetting agent and a dispersant on the rate were evaluated. A correlation between the rates of solution and deaggregation was established.

## THEORETICAL CONSIDERATIONS

Smoluchowski (16) showed that deaggregation follows a first-order process. If, therefore, for a particular system there are a number of large aggregates $Na$, broken down to a number $b$, of small aggregates $N$, the following mechanism can be written to describe this occurrence:[1]

$$Na \xrightarrow{k} bN$$

$k$ is described as an apparent rate constant for the deaggregation process. The rate of disappearance of $Na$ with time ($t$) is then:

$$\frac{dNa}{dt} = -kNa \qquad \text{(Eq. 1)}$$

The following conditions can also be set: at $t = 0$, $Na = Na^0$, $N = 0$; at $t = t$, $Na = Na$, $N = N$; at $t = \infty$, $Na = 0$, $N = Ns$; where $Na^0$ is the concentration of large aggregates at zero time and $Ns$ is the concentration of small aggregates at the final state.

[1] The reaction is considered irreversible since under the experimental conditions employed in this study, the reverse reaction is negligible.

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Integrating Eq. 1 using the limits $t = 0$ and $t = t$, one obtains:

$$\ln \frac{Na}{Na^0} = -kt \qquad \text{(Eq. 2)}$$

Since the number of small aggregates $Ns$ at the final state is proportional to $Na^0$, one can set $Ns = bNa^0$. Then:

$$bNa = Ns - N \text{ or } Na = \frac{Ns - N}{b}$$

Equation 2 can now be written in terms of $Ns$ and $N$:

$$\ln \frac{Na^0}{Na} = \ln \frac{Ns}{Ns - N} = kt \qquad \text{(Eq. 3)}$$

or

$$2.303 \log \frac{Ns}{Ns - N} = kt \qquad \text{(Eq. 4)}$$

The applicability of Eq. 4 in this study is based on the hypothesis that the only species present in the system at any time are the large aggregates $Na$ and/or small aggregates $N$. This implies that the large aggregates $Na$ are broken down into the small aggregates without going through intermediate stages, or if these are formed, they are extremely short-lived. Experimental verification of the hypothesis was obtained by microscopic examination of samples removed at various times during the runs. To prevent shearing the aggregates during examination, the samples were removed with an inverted pipet and a drop was placed gently on a slide and viewed microscopically without a cover slip.

The number of aggregates $N$ present in the system at any time can be evaluated from the time dependence of any property which is directly proportional to $N$. In this study, absorbance was used since it could be conveniently determined without disturbing the system during the measurements.

Writing Eq. 4 in terms of absorbance $A$ and rearranging, one has:

$$\log \frac{As}{As - At} = \frac{k}{2.303} t \qquad \text{(Eq. 5)}$$

where $As$ is the absorbance at the final state and $At$ is the absorbance at time $t$.

If the deaggregation process follows Eq. 5, a plot of $\log As/As - At$ *versus* $t$ should be linear and the rate constant $k$ can be calculated by multiplying the slope of the resulting line by 2.303.

Vold (13), who used absorbance measurements in his studies of deaggregation rates of carbon black, has shown that although aggregates in many suspensions are of irregular shape, they are still likely to be spherically symmetrical if they are built up at random.

It is also apparent that absorbance can be used only if $A^0$ (absorbance at time zero) is much smaller than $As$ (absorbance at the final condition). Furthermore, the absorbance should be a linear function of the concentration of aggregates present in the system. These two conditions were met in this study. The absorbance at time zero was negligible and the linear relationship between the absorbance and concentration was checked by measuring the absorbance of the aggregates (at the final state) over a hundredfold dilution. Measurements were carried out with a Coleman junior spectrophotometer and repeated with a Beckman DU spectrophotometer. Beer's law was obeyed in each case.

From the absorbance measurements, neither the absolute size of the aggregates nor their concentration in the system can be determined. These measurements are simply an indirect estimate of the size and number of aggregates present, *i.e.*, the total surface area presented by the aggregates to the light beam. However, one may reasonably assume that if the volume of the dispersing liquid, concentration of the drug used, and primary particle size are fixed, the higher the turbidity (*i.e.*, higher the absorbance), the greater is the number of aggregates present in the system. Material balance would then require that the greater the number of aggregates present, the smaller would be their size.

The surface area of the aggregates present at the final condition was obtained from Coulter counter measurements. A correlation between the surface area of the aggregates at the final state and absorbance was established. This was done by taking samples of the aggregates having different absorbance values at the final state and measuring their surface area, using a Coulter counter. A working curve was then prepared by plotting the absorbance *versus* the surface area in cm.$^2$/Gm. The surface area of the aggregates at the final state for other runs was estimated from this curve. The Coulter counter measurements were carried out by diluting a volume of the "slurry" in normal saline solution.

Admittedly, some change could occur during the Coulter counter measurements. Nevertheless, it was felt that the data would be of sufficient value to estimate the relative aggregate sizes.

## EXPERIMENTAL

**Materials**—The benzoic acid derivative used in the studies was prepared by a special procedure to obtain the drug in a small but uniform particle size powder. The drug was further classified by passing through a No. 100 mesh screen (U.S. Standard) and collecting on a No. 120 mesh screen.

Capsules, tablets, and a suspension were prepared from the screened powder. The screened powder was hand filled into No. 1 and No. 00 hard gelatin capsules at a concentration of 250 mg. per capsule. The powder was also preblended with 0.5% of a nonionic polyol surfactant[2] and with 0.5% of the sodium salt of polymerized alkyl naphthalene sulfonic acid,[3] respectively, and made into capsules in a similar manner.

Compressed tablets were made containing 250 mg. of the acid, a disintegrant, a binder, and a filler. Another lot of tablets was prepared containing in addition 1.25 mg. of the polyol surfactant.

The suspension contained 250 mg. of the acid in 5 ml., a water-soluble gum, a preservative, a buffer, and a protective colloid.

The sodium salt was also sieved in the same manner as the acid and hand filled in a No. 1 capsule. A second lot of capsules was prepared containing in addition 0.5% of the polyol surfactant. Each capsule contained 275 mg. of the sodium salt, equivalent to 250 mg. of the acid.

[2] Marketed as Pluronic F-68 by the Wyandotte Chemical Corp., Wyandotte, Mich.

[3] Marketed as Daxad No. 11, Dewey and Almy Chemical Division, W. R. Grace Co., Cambridge, Mass.

The deaggregation studies were carried out in simulated gastric and intestinal fluid T.S. (U.S.P. XVI) without the enzymes. The enzymes were excluded as their turbidity interfered with the absorbance measurements. To compensate for the loss of wetting which the enzymes normally provide, 500 mg. of the polyol surfactant was added to 500 ml. of the test solution.

In carrying out the deaggregation studies with the tablets, it was necessary to add an amylolytic and a cellulolytic[4] enzyme to hydrolyze and solubilize the disintegrant and filler present in the tablet. Ten milligrams of each enzyme was dissolved in the 500 ml. of simulated intestinal fluid T.S., and the solution was filtered prior to use. In this way the turbidity caused by the filler and disintegrant was considerably minimized. It was not possible to determine the deaggregation rates of the tablets in the simulated gastric fluid T.S. since the amylolytic and cellulolytic enzymes have little activity at the low pH of the fluid.

**Methodology**—Five hundred milliliters of simulated intestinal or gastric fluid T.S. was added to an 800-ml. beaker placed in a constant-temperature bath set at 37° ± 0.5°. The solution was stirred at 70 r.p.m. and allowed to come to temperature. The design of the all glass stirrer used was such that it provided random agitation throughout the depth of the liquid, without creating a vortex. For all the studies, except those with the capsules, an empty capsule was dissolved in the test medium to provide a uniform background for the spectrophotometric measurements.

To study the deaggregation rate of the (loose) powders, 250 mg. of the acid (or 275 mg. of sodium salt) was sprinkled lightly on the surface of the agitated liquid. A stopwatch was started and readings were taken at appropriate intervals of time. Similarly, 5 ml. of the suspension containing 250 mg. of the acid was pipeted directly into 495 ml. of the agitated liquid. The capsules containing the drugs were added directly to the 500 ml. of simulated gastric fluid T.S. The stopwatch was started as soon as the gelatin dissolved and first traces of the drug appeared in the liquid, generally in about 2 min.

The gelatin shell composing the capsule was found to dissolve very slowly in the simulated intestinal fluid T.S. To minimize the difference in the time necessary to dissolve the capsule shell in the two fluids, a slightly different approach was used when studying the deaggregation rates of the capsules in the intestinal fluid. In this case, the capsule was first treated with 50 ml. of simulated gastric fluid T.S. (maintained at 37°) in a 100-ml. graduate. The graduate was gently rocked manually every 10 sec. At the end of 2 min., the contents of the graduate were emptied into 450 ml. of stirred simulated intestinal fluid T.S. also maintained at 37° ± 0.5°. This treatment of the capsule with the simulated gastric fluid T.S. effectively dissolved most of the gelatin leaving a very thin film. The remaining gelatin dissolved within a few minutes after the addition to the simulated intestinal fluid T.S. To neutralize the excess acid and maintain the pH of the simulated intestinal fluid T.S. at pH 7.5, 1.55 ml. of 2 *N* sodium hydroxide was added to it prior to adding the contents of the graduate. The treatment effectively cancelled any difference in the time necessary to dissolve the gelatin shell in the simulated gastric or intestinal fluid T.S.; the lag time in the two media could then be equated more easily.

**Spectrophotometric Measurements**—The per cent transmission (from which absorbance was calculated) was measured using a Coleman junior spectrophotometer. The wavelength used was 650 mμ. A Manostat Varistaltic pump[5] circulated the fluid *via* a 3 mm. diameter Tygon tubing from the beaker through a flow-through cell located in the spectrophotometer. The diameter of the tubing and the pumping rate provided a continuous flow of the liquid from the beaker through the cell and back, without shear. Thus, measurements could be carried out directly without disturbing the system or necessitating pipeting samples, a procedure which was found to introduce considerable errors.

The force necessary to deaggregate the drugs was provided by the shearing action of the stirred liquid. In each instance, the stirring was continued until the absorbance measured was constant, indicating that the final state had been reached.

**Dissolution Studies**—Part of the same lot of the acid used in the deaggregation studies was used in the dissolution studies. A 250-mg. quantity of the drug as a loose powder was added rapidly to 500 ml. of simulated intestinal fluid T.S. containing 500 mg. of the polyol surfactant instead of pancreatin. The solution was maintained at 37° ± 0.5° and stirred at 70 r.p.m. with the same glass stirrer described previously. After the addition of the powder, 4-ml. samples were withdrawn from the system at measured time intervals. The sample was filtered through Millipore filters (pore sizes 0.45 μ), using a Swinney syringe adapter. The concentration of the drug in solution was measured spectrophotometrically in the ultraviolet region at 335 mμ after appropriate dilution with the solvent. All spectrophotometric measurements were made with a Beckman DU spectrophotometer. When the solubility and dissolution rates were measured with the drug in the capsules, these were pretreated as described with 50 ml. of simulated gastric fluid T.S. to dissolve the gelatin prior to its addition to the simulated intestinal fluid T.S. The polyol surfactant at the concentration added facilitated the wetting of the powders, without affecting appreciably the solubility of the drug in the medium.

## RESULTS AND DISCUSSION

The deaggregation profiles of the acid added to the simulated gastric fluid T.S. as a powder, suspension, and in two capsule sizes, are shown in Fig. 1. The profiles of these dosage forms and also of the tablet in the simulated intestinal fluid T.S. are shown in Fig. 2. These plots of logarithm of per cent transmission *versus* time provide a convenient comparison of the rates. They also compare the relative surface area of the aggregates present at the steady-state condition. The data presented in Figs. 1 and 2 indicate that there is no significant measurable difference in the deaggregation rates in the simulated gastric or intestinal fluid T.S. under the experimental condition used in these studies.

[4] Marketed as Mylase 100 and Cellase 1000, respectively, by the Wallerstein Co., Staten Island, N. Y.

[5] Manostat Corp., New York, N.Y.

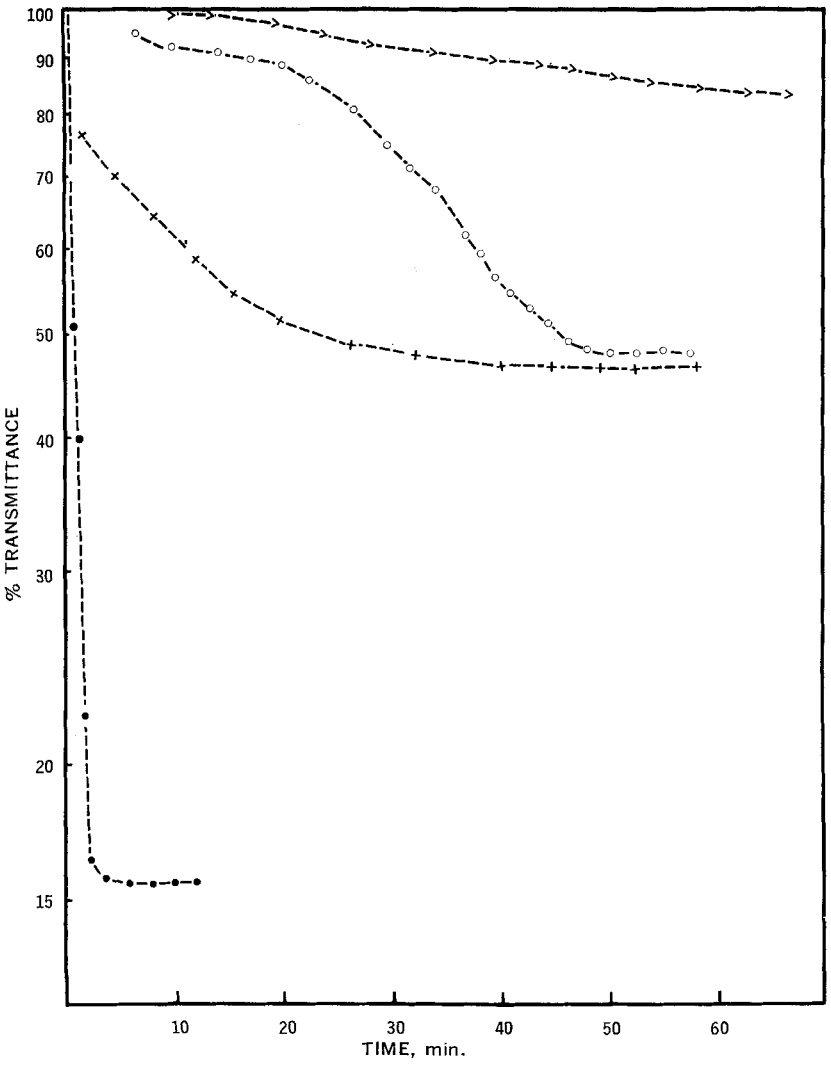


*Fig. 1—Plot of per cent transmittance vs. time for the dosage forms studied showing relative rates of deaggregation in simulated gastric fluid T.S. Key: ●, suspension; ×, loose powder; ○, No. 00 capsule; >, No. 1 capsule.*

The similarity of the plots also stresses the reproducibility of the experimental method used.

It is readily apparent from these plots that the suspension has the fastest rate in both media, and the aggregates formed have the largest surface area, and therefore the smallest aggregate size at the steady state. This is not surprising since the drug is deaggregated intentionally by homogenization during the manufacture of the suspension. The aggregates which form in the suspension on storage are generally readily redispersed in the test media.

The deaggregation rate of the acid in a No. 1 capsule is the slowest in both the simulated gastric and intestinal fluid T.S. and requires approximately 3 hr. to reach the steady-state condition. The surface area of the aggregates at this condition was similar to that shown for the loose powder. When the acid was packed in a No. 00 capsule, the rate was much faster. Although the steady state was reached sooner, the surface area of the aggregates was again the same as that of the loose powder.

The tablet showed a considerably faster initial rate than the capsules tested in the simulated intestinal fluid T.S.; however, the surface area of the aggregates at the steady state was much smaller. The presence of the disintegrants appeared to aid in breaking up the tablet readily, apparently exposing the granules; however, further deaggregation seemed to be difficult, probably due to the strong cohesive bonds formed between the primary particles during the granulation and compression processes.

The data shown in Figs. 1 and 2 are replotted in accordance with Eq. 5 in Figs. 3 and 4. The deaggregation rates of the acid added to the simulated gastric and intestinal fluid T.S. as a loose powder and as a suspension follow closely a first-order process. The apparent rate constant $k$, for the deaggregation in simulated gastric fluid T.S., calculated from the slope of each of these lines is given in Table I. The apparent rate constants for the deaggregation in simulated intestinal fluid T.S. are given in Table II.

The deaggregation process for the drug in the capsules, in both the simulated gastric and intestinal fluid T.S., shown in Fig. 4, seems to be more complicated. The curves for the capsules emerge from the abscissa with a very small slope which continues

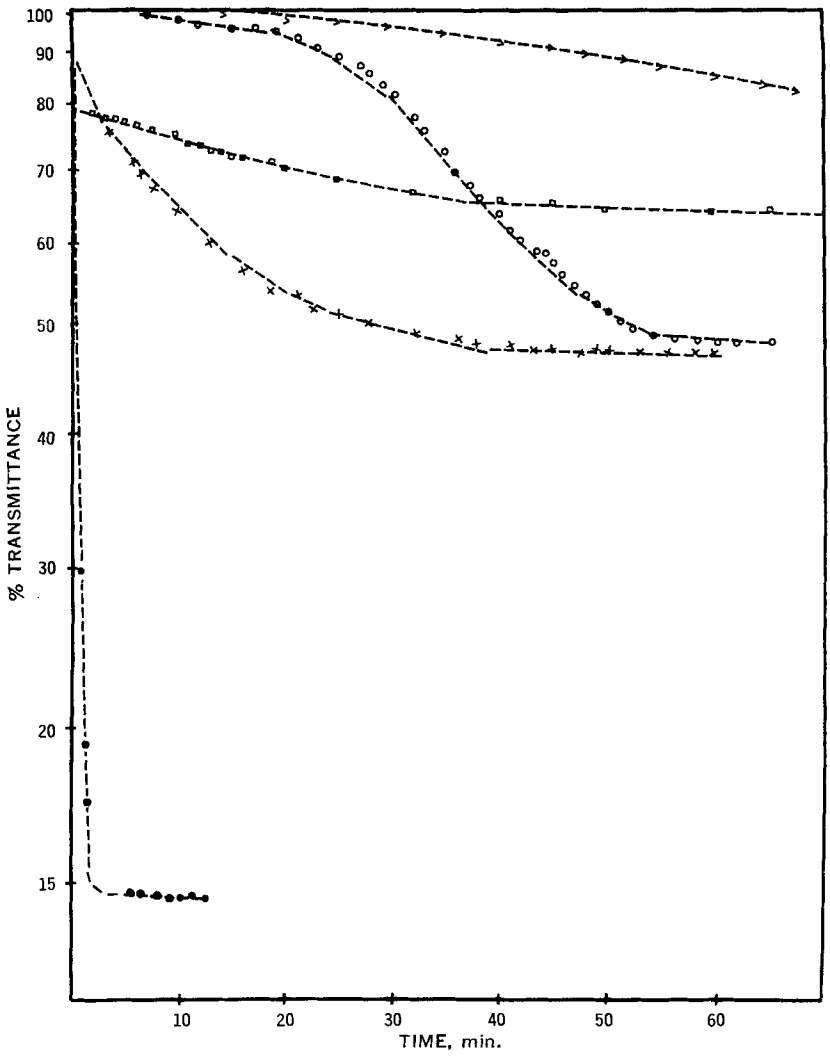


*Fig. 2—Plot of per cent transmittance* vs. *time for the dosage forms studied, showing relative rates of deaggregation in simulated intestinal fluid T.S. Key:* ●, *suspension;* ×, *loose powder;* □, *tablet;* >, *No. 1 capsule;* O, *No. 00 capsule.*

over a period of time; the curve then gradually bends upward and continues as a long straight line. There is, therefore, a lag time before the rate follows a first-order process. This lag time was apparently a function of the degree of compactness of the powder since it was longer for the smaller capsule. The rate was also approximately three times faster for the No. 00 than for the No. 1 capsule.

The observed deaggregation rate of the tablet in the simulated intestinal fluid T.S., shown in Fig. 4, was also complicated and indeterminate initially apparently due to the turbidity caused by the disintegrant and filler as they were released in the medium. However, after they were broken down and solubilized by the enzymes, the rate followed a first-order process fairly closely.

The apparent rate constant $k$, time necessary to reach the final state, and the estimated surface area at this state determined from Coulter counter measurements in the simulated gastric fluid T.S. are given in Table I and in the simulated intestinfluid T.S. are given in Table II.

The effects of compacting the powder in the capsules are evident from Table I. For example, the estimated surface areas for the aggregates of the acid at the final state are very close for the loose powder and two capsule sizes. However, the rate of deaggregation of the drug in the larger capsule was approximately two times faster than that in the smaller one. Similarly, the time to reach the final state was also faster for the drug in the No. 00 capsule.

The data in Table II for the deaggregation measurements in simulated intestinal fluid T.S. also emphasize the same aspects. They also show that while the deaggregation rate for the tablet was relatively fast compared to the smaller capsule, and the final state was reached in only 50 min. *versus* 180 min. for the capsule, the estimated surface area of the aggregates at the final state was considerably smaller than that obtained with the other dosage forms in the same medium.

It is difficult to assess *a priori* which of these would govern the rate of absorption. However, one can assume that in the initial phases the deaggregation rate, lag time, and time to reach the final state would be more important than the final surface area of the aggregates, for the simple reason that

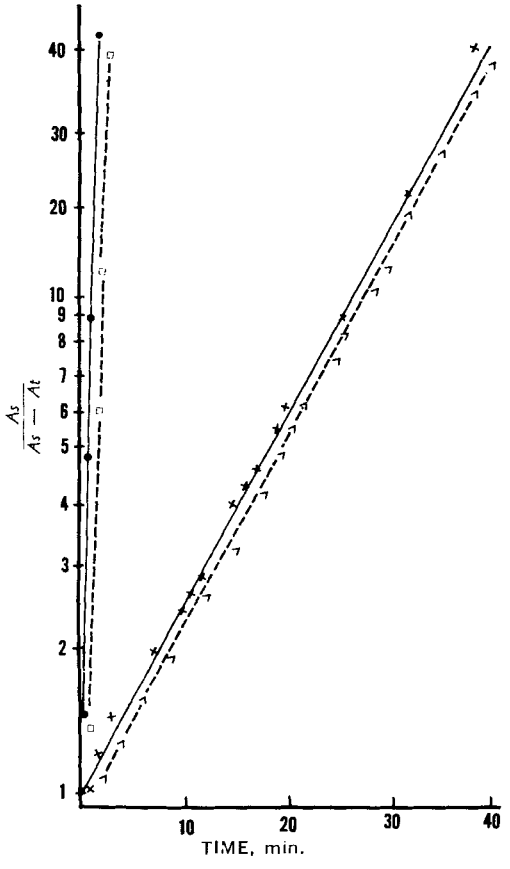


*Fig. 3—Plot showing deaggregation rates of the acid as a loose powder and in suspension in simulated gastric fluid T.S. and intestinal fluid T.S. Key: ●, suspension in simulated intestinal fluid T.S.; □, suspension in simulated gastric fluid T.S.; ×, loose powder in simulated intestinal fluid T.S.; >, loose powder in simulated gastric fluid T.S.*

they make the drug available earlier for dissolution in the gastrointestinal fluids at the absorption sites. The surface area would become more important after the initial exposed drug is dissolved and the rate of solution becomes dependent only on the area. Ideally, the fastest dissolution and absorption would occur in a preparation which is deaggregated rapidly, and in which the surface area of the aggregates at the final state is large. The drug in a suspension has these characteristics and would

TABLE I—COMPARISON OF THE DATA FOR DEAGGREGATION OF THE ACID IN SIMULATED GASTRIC FLUID T.S.

| Dosage Form | Lag Time, min. | $k$, min.$^{-1}$ | Time to Reach Final State, min. | Estimated Surface Area of the Aggregates at the Final State, cm.²/Gm. |
|---|---|---|---|---|
| Powder | 0 | 1.94 | 38 | 5,500 |
| Suspension | 0 | 41.0 | 3.5 | 44,500 |
| No. 1 capsule | 51 | 0.28 | 170 | 5,400 |
| No. 00 capsule | 34 | 0.74 | 48 | 5,400 |

TABLE II—COMPARISON OF THE DATA FOR DEAGGREGATION OF THE ACID IN SIMULATED INTESTINAL FLUID T.S.

| Dosage Form | Lag Time, min. | $k$, min.$^{-1}$ | Time to Reach Final State, min. | Estimated Surface Area of the Aggregates at the Final State, cm.²/Gm. |
|---|---|---|---|---|
| Powder | 0 | 1.94 | 42 | 6,000 |
| Suspension | 0 | 41.3 | 3 | 45,000 |
| No. 1 capsule | 53 | 0.25 | 180 | 6,000 |
| No. 00 capsule | 39 | 0.65 | 54 | 6,000 |
| Tablet | 5 | 0.41 | 50 | 3,000 |

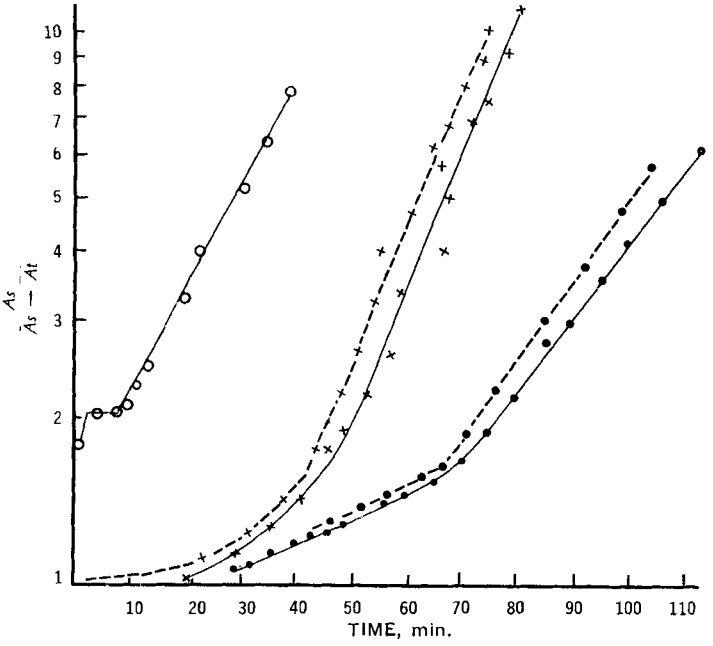


*Fig. 4—Plot showing the deaggregation rates of the acid dosage forms in simulated gastric fluid T.S. and intestinal fluid T.S. Key: ○, tablet; ●, No. 1 capsule; ×, No. 00 capsule; ——, simulated intestinal fluid T.S.; – – –, simulated gastric fluid T.S.*

therefore be absorbed more rapidly, since the drug is exposed to the dissolving medium at the site of absorption rapidly and the dissolution rate would be fast because of the large surface area present.

The effect on the deaggregation rate of adding 0.5% of the polyol surfactant to the acid powder is shown in Fig. 5. The presence of the surfactant slowed the initial rate, possibly due to the tackiness it induced in the powder; however, the rate was increased perceptibly in the later stages. The exact mechanism by which the surfactant increased the rate was not clear and is under investigation at the present time. It could be due simply to the enhanced wetting of the powder by the liquid or coating of the surface of the particles, preventing their adhesion.

It is also seen from Fig. 5 that the sulfonate salt at the same concentration also increased the deaggregation rate of the powder in the simulated intestinal fluid T.S. The sodium salt of polymerized alkyl naphthalene sulfonic acid cannot be classified as a true wetting agent (17) since it does not reduce the interfacial tension between the powder and the

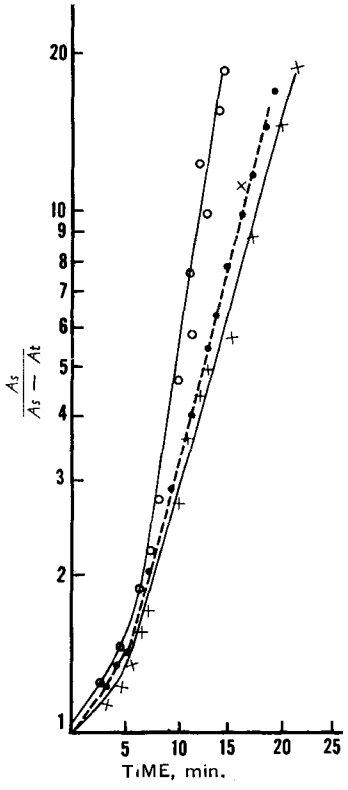


*Fig. 5—Plot showing the effect of 0.5% nonionic polyol surfactant on the deaggregation rates of the acid as a loose powder in simulated gastric fluid T.S. and intestinal fluid T.S., and the effect of 0.5% sodium salt of polymerized alkyl naphthalene sulfonic acid on the rate of powder in simulated intestinal fluid T.S. Key: ●, loose powder with 0.5% polyol surfactant in simulated gastric fluid T.S.; ×, loose powder with 0.5% polyol surfactant in simulated intestinal fluid T.S.; ○, loose powder with 0.5% sulfonic acid salt in simulated intestinal fluid T.S.*

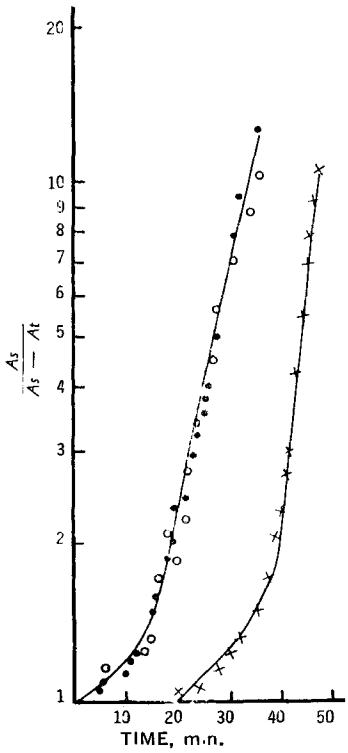


*Fig. 6—Plot showing the effect of 0.5% sulfonic acid salt and polyol surfactant on the deaggregation rate of the acid in No. 1 capsule. Key: ×, No. 1 capsule with 0.5% sulfonic acid salt in simulated intestinal fluid T.S.; ●, No. 1 capsule with 0.5% polyol surfactant in simulated intestinal fluid T.S.; ○, No. 1 capsule with 0.5% polyol surfactant in simulated gastric fluid T.S.*

TABLE III—EFFECT OF 0.5% OF THE POLYOL SURFACTANT AND OF THE SULFONATE SALT ON THE DEAGGREGATION OF THE ACID IN SIMULATED GASTRIC FLUID T.S. AND INTESTINAL FLUID T.S.

| Dosage Form | Lag Time, min | $k$, min.$^{-1}$ | Time to Reach Final State, min. | Estimated Surface Area of the Aggregates at the Final State, cm.$^2$/Gm. |
|---|---|---|---|---|
| Powder + 0.5% polyol surfactant (gastric and intestinal T.S.) | 4 | 2.76 | 23 | 15,000 |
| Powder + 0.5% sulfonate salt (intestinal T.S.) | 4 | 2.92 | 20 | 26,000 |
| No. 1 capsule + 0.5% polyol surfactant (gastric and intestinal T.S.) | 12 | 1.01 | 40 | 13,000 |
| No. 1 capsule + 0.5% sulfonate salt (intestinal T.S.) | 37 | 2.3 | 52 | 24,000 |

liquid. Its action is not completely understood, but it seems (17) that it induces like charges on the individual particles so that they repel each other, thus preventing strong interparticulate bonds being formed.

The addition of 0.5% of the polyol surfactant and of the sulfonate salt to the acid in the capsule also has a significant effect on the deaggregation rate. The data are shown in Fig. 6. It is evident that the polyol surfactant effectively decreases the lag time, probably due to its wetting properties and also increases the deaggregation rate. The sulfonate salt also increases the deaggregation rate; however, its effect on the lag time is not so great as that of the surfactant. The surface area of the aggregates for the drug containing 0.5% sulfonate salt in No. 1 capsule was also larger than that for the polyol surfactant at the same concentration. The data are summarized in Table III.

**Deaggregation Rates of the Sodium Salt**—Only simulated gastric fluid T.S. was used to evaluate the deaggregation characteristics of the sodium salt since it is soluble in the simulated intestinal fluid T.S. It should be pointed out that the deaggregation rates measured are actually those of the nascent acid, since the sodium salt is rapidly converted to the acid when it is exposed to the simulated gastric fluid T.S.

The results of determinations with the sodium salt in the simulated gastric fluid T.S. are plotted in accordance with Eq. 5 in Fig. 7. It is seen that the loose powder again showed the fastest rate. By encapsulating the drug in a No. 1 capsule, the rate was considerably slowed. It was observed during the determinations that after the removal of the gelatin shell from the capsule, the salt exposed to the simulated gastric fluid T.S. was converted to the acid. The action of simulated gastric fluid T.S. on the exposed salt formed an acid mantle around the unconverted sodium salt, maintaining the shape of the capsule and preventing the liquid from reaching the inner core. It is also obvious from Fig. 7 that the addition of the polyol surfactant had a limited effect on the rate when the salt was encapsulated. It should be pointed out that of all the determinations, the deaggregation rate of the sodium salt with the polyol surfactant in the No. 1 capsule was the least reproducible. The reason is probably that a nonuniform acid mantle forms on repeated runs when the gelatin is removed, exposing uneven pockets of the surfactant at the surface.

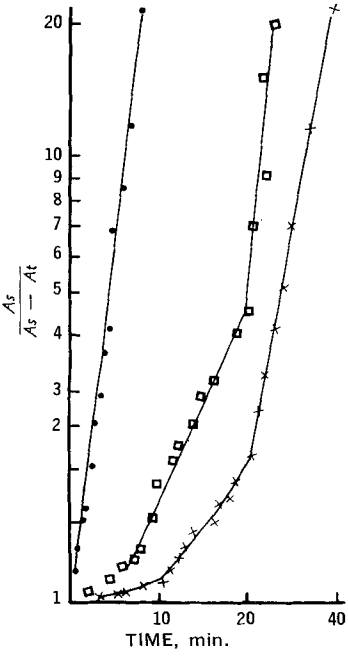


*Fig. 7—Plot showing the deaggregation rate of the sodium salt in simulated gastric fluid T.S. as a loose powder, in No. 1 capsule, and in No. 1 capsule with 0.5% polyol surfactant. Key: ●, loose powder; □, No. 1 capsule with 0.5% polyol surfactant; ×, No. 1 capsule.*

TABLE IV—COMPARISON OF THE RATE CONSTANT, LAG TIME, AND TIME TO REACH STEADY STATE FOR THE SODIUM SALT IN SIMULATED GASTRIC FLUID T.S.

| Dosage Form | Lag Time, min. | $k$, min.$^{-1}$ | Time to Reach Final State, min. |
|---|---|---|---|
| Powder | ... | 9.21 | 9 |
| No. 1 capsule | 18 | 6.12 | 30 |
| No. 1 capsule + 0.5% polyol surfactant | ≈15 | 8.0 | 24 |

The rate constant, calculated from the slope of these lines, lag time, and time to reach the steady state are summarized in Table IV. The data presented in Table IV stress the influence which the surfactant had on the deaggregation rate, time to reach the final state, and lag time when the salt was encapsulated.

## DISSOLUTION STUDIES

The results of rate of solution studies carried out with the acid as a loose powder and in different dosage forms are shown in Fig. 8. It is obvious that the drug in suspension has the fastest dissolution rate and reaches the plateau level in approximately 8 min. On the other hand, the acid in a No. 1 capsule shows the slowest rate. These results appear to correlate with the deaggregation rates, which is to be expected, as it is well established that the dissolution rate is dependent on the surface area exposed to the dissolving media. Since the suspension deaggregates very rapidly, the surface area presented is much greater than that of the capsules or other dosage forms studied.

The dissolution behavior of the acid in a tablet also reflects its deaggregation pattern. The tablets broke up readily in the medium exposing some of the drug to the dissolving medium. This gave a comparatively fast dissolution rate in the initial phases. However, as the exposed drug dissolved, the rate of solution at the later time became solely dependent on the surface area of the large aggregates present, and the dissolution rate was considerably slowed.

The effect on the dissolution rate of adding 0.5% polyol surfactant to the acid powder, tablet, and No. 1 capsule is demonstrated in Fig. 9. It is apparent that the presence of polyol surfactant increased significantly the dissolution rate of the acid as a loose powder and in the No. 1 capsule. The increase in the dissolution rate of the powder and capsule can again be attributed to the faster

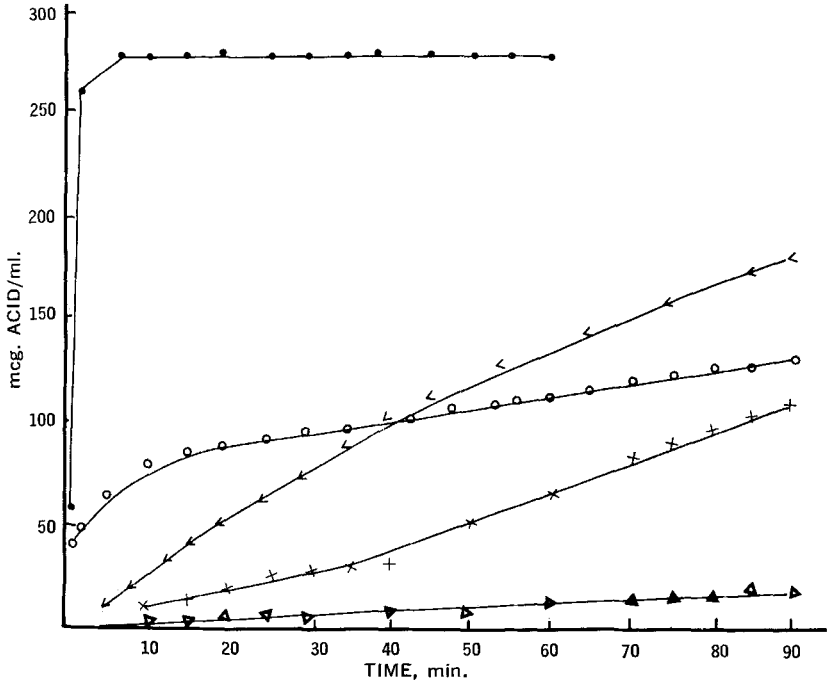


*Fig. 8—Plot showing the dissolution rates of various dosage forms of the acid. Dissolution rate measured in simulated intestinal fluid T.S. Key: ●, suspension; ○, tablet; <, loose powder; ×, No. 00 capsule; ▶, No. 1 capsule.*

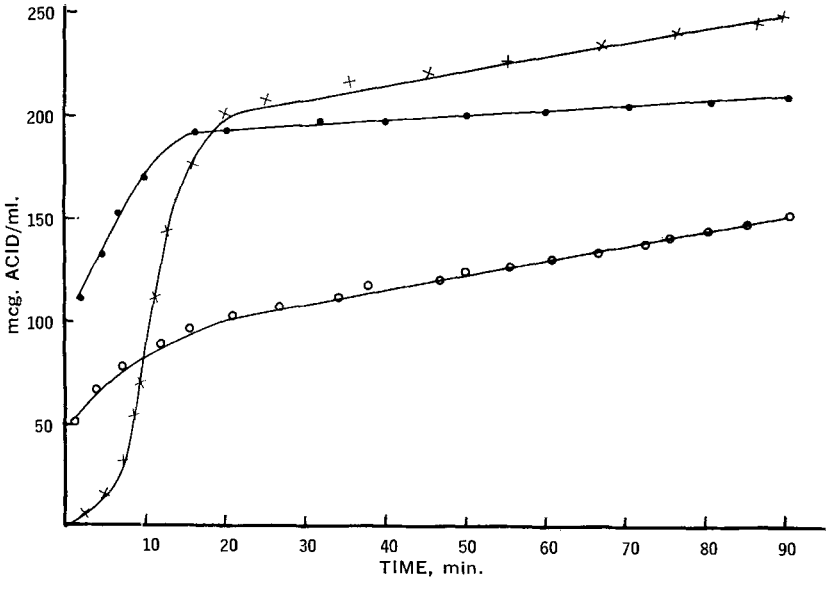


*Fig. 9—Plot showing the effect of 0.5% polyol surfactant on the dissolution rates of the acid as a tablet, in a No. 1 capsule, and as a loose powder. Dissolution rates measured in simulated intestinal fluid T.S. Key: ○, tablet with 0.5% polyol surfactant; ×, loose powder with 0.5% polyol surfactant; ●, No. 1 capsule with 0.5% polyol surfactant.*

deaggregation rates induced by the surfactant in these dosage forms. The effect of tackiness, due to the surfactant, in the initial phases of the dissolution of the powder was also evident.

The effect of the surfactant on the deaggregation rate of the tablet was small, and this was reflected in the small increase in the rate of solution. Possibly higher concentrations of the surfactant are necessary to break up and disperse the aggregates.

## BIOPHARMACEUTICAL IMPLICATIONS

The study emphasizes the importance of deaggregation rates. If the absorption is dependent on the dissolution rates, which in turn are dependent on the rates of deaggregation, the study would predict that the highest and earliest blood levels would be obtained with the suspension followed in order by the sodium salt, the acid as a loose powder, in the No. 00 capsule, in the tablet, and last the No. 1 capsule. It is probable that the addition of a surfactant to the acid to increase the deaggregation rate would also increase the absorption rate.

The absorption profiles in man of the acid and sodium salt are currently being investigated in these laboratories. Preliminary results of these investigations support the findings of these studies, and will be published elsewhere.

Recently Wagner and his co-workers (10) studied the effect of dosage form on serum levels of indoxole. They found that the drug administered as a suspension produced a greater serum level response than did the hard gelatin capsules, although both were prepared from the same lot of drug with identical small particle size. They state that the most likely explanation is that the indoxole particles were less agglomerated when administered in suspension form than when given in capsules, and the surface area of indoxole presented for dissolution in the gastrointestinal fluids at the absorption sites must have been greater with the suspension.

In a recent publication, Higuchi *et al.* (6) cited various other examples where the deaggregation rates were the controlling factors in absorption.

It would appear that in dealing with insoluble drugs which are difficult to wet and disperse, the rate of deaggregation from different dosage forms should be routinely determined, just as the rate of solution, particle size, and polymorphic forms are investigated.

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# Mechanistic Toxicology of Triethyl Citrate in Mouse Fibroblast Cells by Liquid Scintillation Techniques

By MARILYN GOLAZ, W. L. GUESS, and JOHN AUTIAN*

**Mouse fibroblast cells, strain L-929, in static tube culture were exposed to 6, 9, and 12 mmole doses of triethyl citrate (TEC) for periods up to 78 hr. At 1.5, 48, and 78 hr. after TEC exposure, the cells were pulsed with $^{14}C$-labeled purine and pyrimidine metabolic precursors in an attempt to differentiate the site of purine–pyrimidine metabolism interference. U.V. spectrophotometric analyses were run concurrently with liquid scintillation analyses. The mechanism of inhibition appeared to be initial mitochondrial involvement, with secondary DNA repression.**

SINCE ALL pharmacology and toxicology begins ultimately at the cellular level, it seems only logical to elucidate mechanisms involving basic biochemical pathways by way of cellular techniques, and project or apply these findings to the whole organ or body. The advantages of cell culture in a variety of applications are many. Highly specialized cells, differentiating cells, or abnormal cells may be used so that drug effects may be seen under widely varying circumstances. Another convenience is that quantitative evaluation of activity is possible, whereas pharmacodynamic quantitization in the intact animal is often impossible due to the nature of the compound or of the system. Using cell culture techniques, one can test solids, liquids, and gases, and evaluate changes both to the cells and to the growth medium (1–3).

Recently in this laboratory, Rosenbluth *et al.* (2), found that triethyl citrate (TEC), a commonly used plasticizer for polyvinyl chloride resin, was a growth inhibitor to mouse fibroblast cells, strain L-929, in static tube culture at 35° at several dose levels. Through a series of simple experiments, he was able to eliminate chelation of calcium ions as the mechanism of triethyl citrate toxicity. By correlating parachor and other physiochemical values assigned to triethyl citrate, it was postulated that TEC is probably a physically toxic compound. Triethyl citrate is relatively lipid soluble. Being

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