Polymorphism and Drug Availability II

Dissolution Rate Behavior of the Polymorphic Forms of Sulfathiazole and Methylprednisolone

By W. I. HIGUCHI, P. D. BERNARDO, and S. C. MEHTA

The dissolution rate behavior of the two polymorphic forms of sulfathiazole and methylprednisolone has been studied. A correlation between the rates of reversion of the metastable forms to the stable forms during dissolution and the crystal growth rates of the stable form was found. Data obtained in a number of solvents showed that when the crystal growth rate of the stable phase was slow, there was little tendency for reversion during dissolution. These findings support the idea that the rate-determining step in the solvent mediated reversion of these polymorphs is the crystal growth of the stable phase. The recently presented mixture theory for dissolution rate was extended to explain the dissolution behavior of polymorphic mixtures. Experimental data on the effect of the agitation rate upon the dissolution rates of polymorphic mixtures agreed well with theory. It is proposed that this theory provides the explanation for the enigma of "the loss of sensitivity in distinguishing the difference in dissolution rates of polymorphs at high agitation rates."

FOR THERMODYNAMIC reasons (1) a drug exhibiting crystal polymorphism should possess different thermodynamic activities (or fugacities) depending upon its crystalline modification. Therefore, the drug release rate from any solid dosage form, *in vivo* or *in vitro*, may be strongly dependent upon this factor when the rate process is diffusion controlled. From the practical pharmaceutical standpoint, the existence of polymorphism may lead to good or bad consequences. The successful utilization of a polymorph of significantly greater thermodynamic activity than that of the stable modification may provide, in some instances, therapeutic blood levels for an otherwise inactive drug formulation. On the other hand, when the existence of multiple crystalline modifications goes unrecognized in a particular formulation, this may possibly result in too wide a range of dose-to-dose drug availability for the patient.

The purpose of this paper is to present some of the authors' studies on the dissolution rates of higher energy polymorphs of sulfathiazole and methylprednisolone. The data, when viewed in the light of the authors' crystal growth studies (2), provide a clear picture of what factors could be important in determining the physical kinetic stability of drug polymorphs. Furthermore, these studies appear to explain the mystery of the anomalous effect of agitation on the dissolution rate behavior of polymorphs (3–5).

Received April 28, 1966, from the College of Pharmacy, University of Michigan, Ann Arbor.

Accepted for publication September 20, 1966.

This investigation was supported by a grant from The Upjohn Co., Kalamazoo, Mich.

Presented to the Basic Pharmaceutics Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.

EXPERIMENTAL

General Considerations.—The compounds selected for this study were sulfathiazole and methylprednisolone.[1] These are both known to exhibit dimorphism under usual conditions. The designations I and II will be used for the thermodynamically stable and the unstable forms, respectively.

Various aqueous and organic media were considered for this work. This provided a wide range of solubilities and a variation in the intermolecular interactions at the crystal–solution interface.

It was decided to employ methods involving dissolution from a plane surface. Thus, the techniques as described by Milosovich (6) for fast dissolution rates and by Higuchi *et al.* (7) for slow rates were used in this work. These arrangements with controlled agitation were expected to provide situations and yield results that could be handled conveniently by mathematics.

Preparation of Stable and Metastable Forms.—Sulfathiazole crystal forms are I, which melts at 174°–175°, and II which melts at 200°–201°. The recrystallization of sulfathiazole in warm ethyl alcohol gave form I which was hexagonal plates. Form II was obtained by using the method of Grove and Keenan (8), *i.e.*, by supersaturating a normal propanol solution, filtering, and allowing it to recrystallize over a water bath at approximately 80°. Elongated rods resulted. It was found that form II could also be obtained by recrystallizing from *sec*-butyl alcohol at room temperature. A third method for obtaining form II was also used so that verification of the appropriate form could be made. This was accomplished by melting sulfathiazole on a hot bar and then allowing it to crystallize at approximately 90°.

The form I of methylprednisolone was obtained by recrystallization from acetone, while form II was prepared by recrystallization from *tert*-butyl alcohol as was noted by Shell (9).

[1] The authors thank Dr. L. C. Schroeter, The Upjohn Co., for supplying us with samples of this material.

Characterization of Respective Forms.—Several methods were used in determining the appropriate crystal forms.

The powder method using the nickel-filtered copper radiation of a Siemens Crystalloflex IV counter-tube X-ray diffractometer provided one means of characterization. This was done for both sulfathiazole and methylprednisolone. Note recordings in Figs. 1–3.

For sulfathiazole, melting point determinations were also conducted. The microscope method using a Zeiss microscope equipped with a Kofler hot stage was used and results agreed with those in the literature (8). The characterization of the methylprednisolone could not be accomplished by this method since decomposition occurred.

Infrared spectra were also obtained to help characterize methylprednisolone forms I and II. The model 137, Perkin-Elmer spectrophotometer was used and data agreed with those in the literature (1).

TABLE I.—SOLUBILITIES AND ABSORBANCE MAXIMA

Solvent	Solubility at 30°, Gm./100 Gm.	$\lambda_{max.}$, mμ
Sulfathiazole		
Water	0.114	282
n-Propanol	0.277	291
sec-Butyl alcohol	0.149	291
95% Ethyl alcohol	1.02	288
75% (v/v) *n*-Propanol + 25% (v/v) ethyl alcohol	0.418	
40% (v/v) *sec*-Butyl alcohol + 60% (v/v) ethyl alcohol	0.545	
Methylprednisolone		
Water	0.0072	249
95% Ethyl alcohol	2.26	246
Decyl alcohol	0.269	243
75% (v/v) Water + 25% (v/v) ethyl alcohol	0.061	

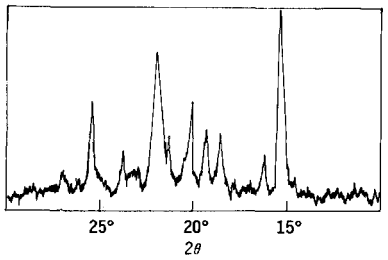

Fig. 1.—X-Ray recording of sulfathiazole form I.

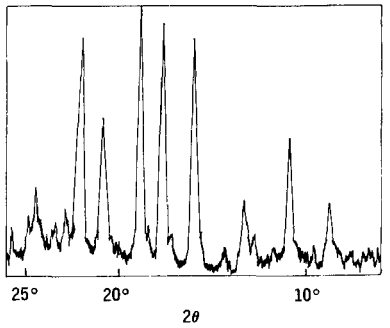

Fig. 2.—X-Ray recording of sulfathiazole form II.

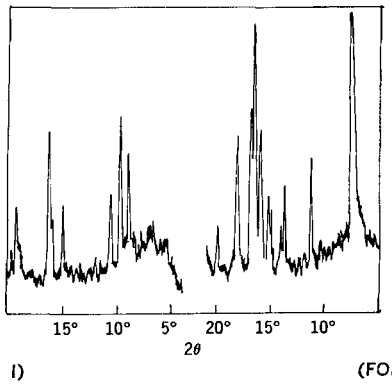

Fig. 3.—X-Ray recordings of methylprednisolone.

Solubility Determinations.—Excess sample was placed in the appropriate solvent and agitated in a water bath at 30.0° ± 0.1°. Samples were taken in duplicate at 12–24-hr. intervals. A preheated 10-ml. glass syringe was used to withdraw the samples which were filtered through a 0.45 μ membrane filter (Millipore HAWP 01300) by means of the syringe-Swinny-adapter assembly. The samples were then weighed and diluted for spectrophotometric assay. Reproducible data were obtained upon equilibration of the sample (Table I).

Apparatus and Procedure.—The apparatus and the procedure used were essentially those described by Milosovich (6). The sample was compressed by placing the powder in the die between the hardened steel block and a $^3/_8$ in. punch and compressing using a Carver press at approximately 2000 lb. pressure. The die had been previously ground to eliminate the tapered shoulder. This, therefore, ensured a constant surface area. The die containing the sample was mounted in the holder and placed into the dissolution solvent at zero time. The amount dissolved as a function of time was measured automatically by a Varian model G-10 recorder attached to a Beckman DU spectrophotometer through which the solution from the dissolution rate beaker was continuously circulated.

For the study of the dissolution rates of methylprednisolone in water, a second apparatus was necessary as the low water solubility of this compound demanded time intervals of 2 to 8 hr. The apparatus used was that described by Higuchi, Mir, and Desai (7). The tablet was made in the same manner as before and then the open end of the die was stoppered with a cork. This was placed in a Plexiglas holder which was mounted on the beaker cover. This was then placed into a 600-ml. water-jacketed beaker containing 200 ml. of water at 37°. A stainless steel stirrer, attached to a Bodine synchronous motor and mounted on the beaker cover, provided the necessary agitation. The speeds utilized were 10, 50, and 150 r.p.m. At time zero the entire unit was introduced into the solvent. Samples were pipeted out at specified times and assayed spectrophotometrically.

RESULTS AND DISCUSSION

Solvent Effects with Sulfathiazole.—Some typical dissolution rate results of the pure forms of sulfathiazole are given in Fig. 4. In all solvents the form I data were always linear as expected over the entire time period of the runs. With the form II experiments the results varied. Only in the two solvents, *n*-propanol and *sec*-butyl alcohol, were the rates for form II constant during the entire run. In many of the solvents initial constant rates were observed, but these decreased after some time and approached those for form I (see, *e.g.*, the II-in-ethanol and the II-in-50% ethanol–hexane curves in Fig. 4). In pure water and in water–ethanol mixtures containing up to 25% (v/v) ethanol, the form II rates and the form I rates could not be distinguished, even for initial rates. Conversion of II to I must be extremely rapid in water.

In all cases where the initial greater form II rates were observed, the ratios of the initial form II rate to that for form I in the same solvent were found to be essentially the same in all solvents. This ratio = 1.70 is the same as that reported by Milosovich for the sulfathiazole polymorphs in ethanol. According to the Noyes-Whitney relation, this ratio should correspond to the ratio of the solubilities of the two forms.

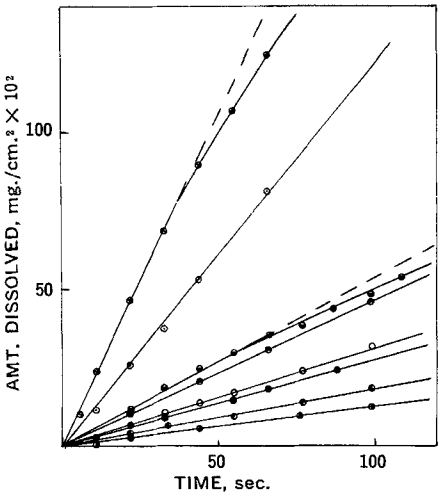

Fig. 4.—Dissolution rate of sulfathiazole (form I and form II) in various solvents. Key: ◑, I in *sec*-butyl alcohol, ◐, II in *sec*-butyl alcohol; ●, I in *n*-propanol; ◓, II in *n*-propanol; ○, I in 50% (v/v) mixture of ethyl alcohol and hexane; ◒, II in 50% (v/v) mixture of ethyl alcohol and hexane; ⊙, I in 95% ethyl alcohol; ⊖, II in 95% ethyl alcohol.

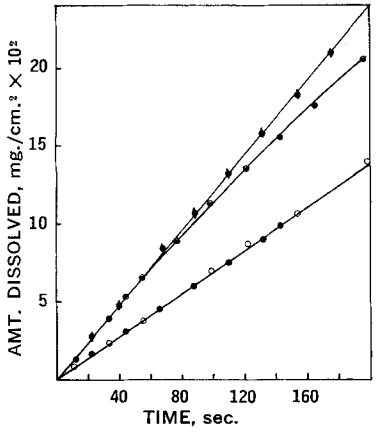

Fig. 5.—Effect of 0.1% gelatin on dissolution of sulfathiazole, form II in 40% (v/v) water + 60% (v/v) ethyl alcohol. Key: ●, form I in solvent; ○, form I in solvent + 0.1% gelatin; ◒, form II in solvent; ◓, form II in solvent + 0.1% gelatin.

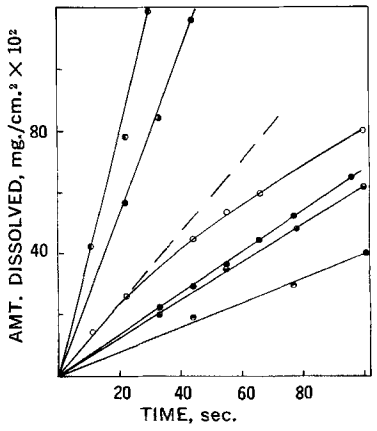

Fig. 6.—Dissolution rate of methylprednisolone (I and II) in various solvents. Key: ◓, I in decyl alcohol; ◒, II in decyl alcohol; ●, I in 50% (v/v) water–ethyl alcohol; ○, II in 50% (v/v) water–ethyl alcohol; ◑, I in ethyl alcohol; ◐, II in ethyl alcohol.

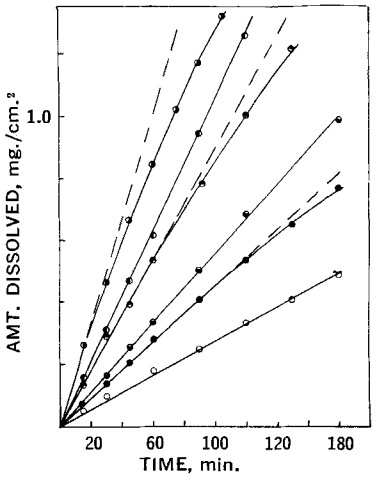

Fig. 7.—Dissolution rates of methylprednisolone (form I and form II) in water at 37° at agitation speeds of 10 r.p.m., 50 r.p.m., and 150 r.p.m. Key: ○, I at 10 r.p.m.; ●, II at 10 r.p.m.; ◒, I at 50 r.p.m.; ◓, II at 50 r.p.m.; ◐, I at 150 r.p.m.; ◑, II at 150 r.p.m.

These findings correlate with the authors' studies (2) on the crystal growth behavior of sulfathiazole form I. No measurable crystal growth of form I was found in *n*-propyl alcohol and in *sec*-butyl alcohol up to supersaturation ratios of 2.2 and 5.5, respectively. These supersaturations are far above the maximum supersaturation ratios (~1.7) available during the dissolution of form II in the present experiments. On the other hand, appreciable form I crystal growth rates were observed at supersaturation ratios below or near 1.7 in those solvents that have exhibited reversion in the present studies. Water and ethanol exhibited relatively rapid crystal growth rates at low supersaturations, and in the present studies these same solvents provided the most rapid reversion of II to I. Thus, one finds that reversion appears to occur rapidly in those solvents that allow relatively rapid crystal growth of the stable form and slowly in those solvents that require a relatively high supersaturation for the growth of the stable form.

In Fig. 5 the effect of 0.10% gelatin in 60% ethanol–water on the dissolution behavior of sulfathiazole can be seen. There appeared to be a retardation of the reversion of II to I. As the dissolution rate of I and the initial dissolution rate of II were not affected by the presence of gelatin, one can conclude that solution diffusional processes were not affected, and therefore gelatin must interfere at the crystal intreface. More studies with additives are planned for the future.

Solvent Effects with Methylprednisolone.—Some of the results in various solvents are presented in Figs. 6 and 7. The data given in Fig. 6 were obtained with the automatic recording apparatus. In the time ranges encountered here, the methylprednisolone form II data obtained in decyl alcohol did not show noticeable curvature with time. With 50% water–ethanol as the solvent, appreciable reversion of II to I was observed in less than 1 min. With ethanol as the solvent, the results were not conclusive as the dissolution was extremely rapid and disintegration of the tablet face was observed in some of the runs.

Because of the low solubility of methylprednisolone in water, the data given in Fig. 7 were obtained by the manual sampling procedure. The experiments were carried out at three agitation rates and much longer times were involved. It can be seen that, in water at 37°, reversion of II to I also occurred, although more slowly as reflected in these data.

If the results obtained in ethanol are excluded from consideration, these findings also correlate with the crystal growth data for form I in the same solvents (2). Thus, as was found with the sulfathiazole system, reversion was observed in those solvents (*e.g.*, water, water–ethanol mixtures) in which the crystal growth rates of form I were relatively rapid. In those solvents (*e.g.*, decyl alcohol) where the crystal growth rates of form I were extremely slow even at high supersaturation ratios, reversion was not observed in the experiments.

From these limited studies it appears that polymorphic reversion of those crystalline phases that are generally regarded as kinetically stable in the absence of solvent contact may be frequently controlled by the solvent-mediated crystal growth kinetics of the more stable phase and *not* by the dissolution rate of the less thermodynamically stable polymorph. This is perhaps not surprising if the crystal growth rates are controlled by a cooperative process such as two-dimensional nucleation. In these instances the reverse process, dissolution, likely would not be the reciprocal of growth but instead follow an energetically more comfortable route such as "peeling off" of molecular layers initiated at the corners and edges of the crystals.

Dissolution Rate from a Mixture of Two Polymorphs (Theory).—In order to be able to describe the results of the experiments with mixtures of polymorphs and of the authors' studies on the unusual effects of agitation on dissolution rates of polymorphs, a discussion of a quantitative theory is presented. This theory is an extension of that discussed recently (7) for the dissolution behavior of general polyphase mixtures.

Figure 8 schematically shows the three stages (from left to right) during the dissolution process. It is assumed that the mixture is initially uniform and reversion does not occur during dissolution. As dissolution of the drug begins by diffusion across the effective liquid diffusion layer of thickness h, only form II dissolves initially because C', the solution concentration at the tablet boundary–solution interface, is still greater than, C_s^{I} the solubility of form I. Thus, at time zero, $C' = C_s^{II}$, the solubility of form II. At later times

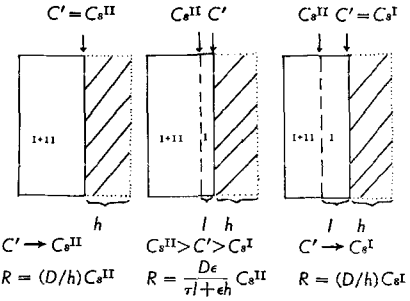

Fig. 8.—Mixture theory model.

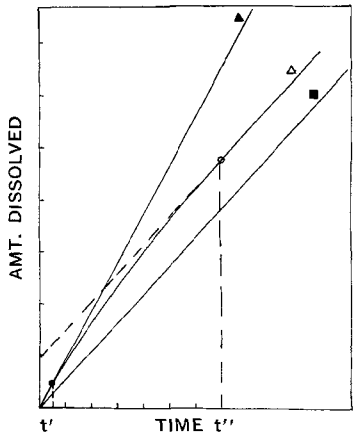

Fig. 9.—Comparison of dissolution rate of the two polymorphs and a mixture according to theory. Key: ▲, II; △, mixture; ■, I.

$C_s^{II} > C' > C_s^{I}$. Finally, in the steady-state, $C' \simeq C_s^{I}$, and both forms I and II dissolve at rates proportional to their percentages in the original mixture.

Mathematically, one may write for the dissolution rate per unit tablet area, R,

$$R = \frac{dw}{dt} = (D/h)C' = \frac{D\epsilon}{\tau l}(C_s^{II} - C') \quad \text{(Eq. 1)}$$

where

C' = concentration at the tablet–solution interface,
C_s^{II} = solubility of the form II,
ϵ = porosity of the form I residue layer,
τ = tortuosity of the form I layer,
h = diffusion layer thickness,
l = layer thickness of the form I layer,
w = amount dissolved per unit area of tablet at time, t,
D = diffusion coefficient.

Solving for C', one obtains

$$C' = \frac{C_s^{II}}{\left(1 + \frac{\tau l}{\epsilon h}\right)} \quad \text{(Eq. 2)}$$

whence

$$R = \frac{D\epsilon}{\tau l + \epsilon h} C_s^{II} \quad \text{(Eq. 3)}$$

R is also related to l and t by

$$R = H_{II}\frac{dl}{dt} \quad \text{(Eq. 4)}$$

where H_{II} is the amount of form II per unit volume in the original mixture.

Both Eqs. 3 and 4 will be examined in the time period up to the time, t'', when form I begins to dissolve. After $t = t''$ when a steady-state is reached, neither Eq. 3 nor Eq. 4 will correctly give the total dissolution rate. Instead one must write

$$R = \frac{D}{h} C_s^{I} \text{ for } t > t'' \quad \text{(Eq. 5)}$$

Therefore, when $0 < t < t''$,

$$H_{II}\left(\frac{dl}{dt}\right) = \frac{D\epsilon}{\tau l + \epsilon h} C_s^{II}$$

Integrating,

$$H_{II}\int_0^l dl(\tau l + \epsilon h) = \int_0^t C_s^{II} D\epsilon \, dt$$

or

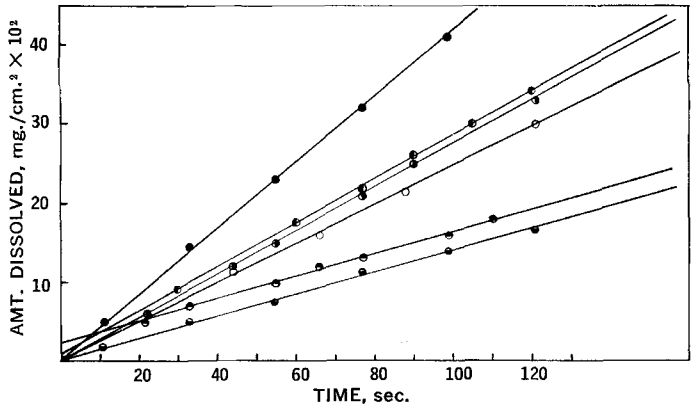

Fig. 10.—Dissolution rates of sulfathiazole (form I and form II and mixture of 80% I and 20% II) at two agitation speeds in *n*-propyl alcohol. Key: ◒, form I (*A*); ◓, mixture (*A*); ○, form II (*A*); ◑, form I (*B*); ◐, mixture (*B*); ●, form II (*B*); *A*, diffusion layer thickness = 5.62 × 10^{-3} cm.; *B*, diffusion layer thickness = 2.94 × 10^{-3} cm.

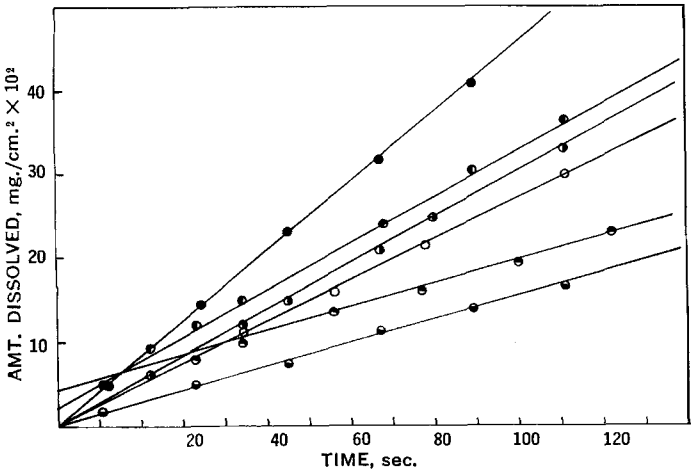

Fig. 11.—Dissolution rates of sulfathiazole (form I, form II, and mixture of 50% I and 50% II) at two agitation speeds in *n*-propyl alcohol. Key: ◒, form I (*A*); ◓, mixture (*A*); ○, form II (*A*), ◑, from I (*B*); ◐, mixture (*B*); ●, form II (*B*); *A*, diffusion layer thickness = 5.62 × 10^{-3} cm.; *B*, diffusion layer thickness = 2.94 × 10^{-3} cm.

TABLE II.—COMPARISON OF DIFFUSION LAYER THICKNESS TO INTERCEPT. SULFATHIAZOLE IN *n*-PROPYL ALCOHOL

Mixture Compn.	Diffusion Layer Thickness h, cm.	Ratio of h	Intercept	Intercept Ratio
50% II	5.62×10^{-3}	1.91	4.3	1.87
	2.94×10^{-3}	1	2.3	1
20% II	5.62×10^{-3}	1.91	1.7	1.85
	2.94×10^{-3}	1	.91	1

$$l^2 + \frac{2\epsilon h l}{\tau} = \frac{2C_s^{II} D\epsilon}{H_{II}\tau} t \qquad \text{(Eq. 6)}$$

If now one considers the situation when $C' \simeq C_s^{II}$ and defining t' as the maximum time up to which the dissolution will be essentially that from pure form II, one obtains from Eq. 6,

$$l = \frac{C_s^{II} D}{H_{II} h} t'$$

since

$$\frac{2\epsilon h l}{\tau} \gg l^2$$

Arbitrarily t' may be defined as the time up to which the dissolution rate is still within 99% of the pure form II rate. In this case

$$t' = \frac{0.01\ \epsilon h^2 H_{II}}{\tau\ C_s^{II}\ D} \qquad \text{(Eq. 7)}$$

The results of these theoretical considerations are graphically illustrated in Fig. 9. Here t' is shown as the point where deviation of the mixture curve from the pure form II curve begins to become important, and t'' is the point at which the rate of dissolution of the mixture becomes equal to the rate for pure form I as given by Eq. 5.

Finally, let us now obtain an equation for the intercept of the extrapolation of the linear portion of the mixture curve on the ordinate. If w″ is the amount dissolved at t'', one has then

$$\int_0^{w''} dw = \int_0^{l''} H_{II} dl \qquad \text{(Eq. 8)}$$

where

$$l'' = \frac{\epsilon h}{\tau}\left(\frac{C_s^{II} - C_s^{I}}{C_s^{I}}\right) \qquad \text{(Eq. 9)}$$

Integration of Eq. 8 gives

$$w'' = \frac{H_{II}\epsilon h}{\tau}\left(\frac{C_s^{II} - C_s^{I}}{C_s^{I}}\right)$$

Therefore, the intercept I will be given by

$$\frac{D}{h} C_s^{I}\, t'' + \mathrm{I} = w'' \qquad \text{(Eq. 10)}$$

where t'' is given by Eqs. 6 and 9. One has then

$$\mathrm{I} = \frac{H_{II}\epsilon h\,(C_s^{II} - C_s^{I})^2}{2\tau\ C_s^{I} C_s^{II}} \qquad \text{(Eq. 11)}$$

for the intercept.

Dissolution Rates from Mixtures, Effect of Agitation.—The effect of the agitation rate upon the dissolution rate, as predicted by the above theory, may be examined through the use of Eq. 11. Let us first note that Eq. 11 may be written

$$\mathrm{I} = Kh \qquad \text{(Eq. 12)}$$

where K is a constant for a given two-phase mixture of constant composition. The values of the diffusion layer thickness, h, at different agitation rates may be determined from the dissolution rate experiments with either of the pure polymorphs employing the Noyes-Whitney relation. If Eq. 12 is correct, the ratio of two h values should be the same as the ratio of corresponding intercepts.

Figures 10 and 11 present the experimental dissolution rate data in *n*-propanol at 30° for two mixtures of the sulfathiazole polymorphs obtained at two agitation rates corresponding to $h = 2.94 \times 10^{-3}$ cm. and $h = 5.62 \times 10^{-3}$ cm.

The intercepts were estimated as shown from the extrapolation of the linear portions of the data. These are given in column 4 of Table II. The ratio of the intercepts given in column 5 may be compared with the corresponding ratio of the h values given in column 3. The agreement between the h ratios and the intercept ratios is very satisfactory supporting the correctness of the theory.

Figure 12 presents the dissolution rate data obtained for the 50–50 mixture of methylprednisolone polymorphs at three agitation rates. Also presented, for reference purposes, are the pure form I

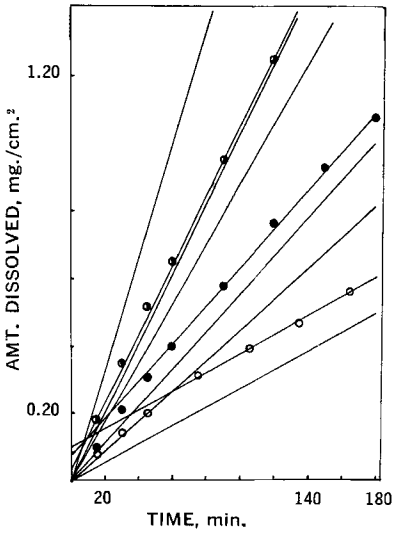

Fig. 12.—Dissolution of 50% I and 50% II mixture of methylprednisolone in water at agitation speeds of 10, 50, 150 r.p.m. Key: ○, mixture at 10 r.p.m.; ●, mixture at 50 r.p.m.; ◑, mixture at 150 r.p.m. Form II lines are those predicted if no reversion occurred.

TABLE III.—50% I AND II METHYLPREDNISOLONE MIXTURE IN WATER AT 37°

Agitation Speed, r.p.m.	Diffusion Layer Thickness h, cm.	Ratio h/h 150	Intercept	Ratio Intercept: Intercept 150
10	9.09×10^{-3}	3.68	.51	3.40
50	4.43×10^{-3}	1.79	.28	1.87
150	2.47×10^{-3}	1.00	.15	1.00

and form II data (taken from Fig. 9). Table III compares the ratio of the h values to the intercept ratios from the mixture data. Again one finds that the agreement of theory and data is excellent.

So far we have only examined the data with theory through the use of Eq. 11. Another look at the data, this time through Eq. 7, is helpful. Equation 7 predicts that the place where the dissolution data begins to deviate from the pure polymorph II line is proportional to the square of h. Therefore, for a given mixture, at low agitation rates, the data should follow the pure form II curve for a much longer time than at high agitation rates. An examination of the data given in Figs. 10–12 shows that the dependence of t' upon h^2 is essentially quantitatively followed in all instances.

The assumption that appreciable concomitant reversion did not occur in the runs with these mixtures should be a good one. Pure sulfathiazole form II in *n*-propanol showed no tendency to revert (Fig. 4). Pure methylprednisolone form II in water did show (Fig. 7) some reversion during dissolution. However, the mixture effect itself with the methylprednisolone 50–50 mixture appeared to be much larger during the times under consideration than the little reversion that did occur with pure form II. Therefore, the agreement of both the sulfathiazole and the methylprednisolone data with theory in the many aspects leaves little doubt that the theory is quantitatively sound.

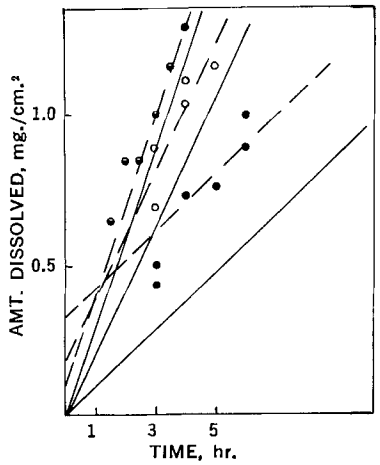

Fig. 13.—Interpretation of the dissolution rate data of Hamlin *et al.* Key: ●, form II at 0 r.p.m.; ○, form II at 6 r.p.m.; ◒, form II at 12 r.p.m.

Discussion of Some Previously Published Studies.—Hamlin, Nelson, Ballard, and Wagner first reported (3) the unusual effect of agitation upon the dissolution rate of a metastable polymorph. The material they investigated was methylprednisolone form II. These investigators found that upon increasing the agitation rate, it became increasingly difficult to distinguish the difference in the dissolution rates of form I and form II. Subsequently, Levy (4) and then Taylor and Wurster (5) observed similar effects with metastable methylprednisolone and prednisolone, respectively.

We propose that the answer to this mystery may be provided by the results of the authors' present studies. The following is the suggested description of the "anomalous" dissolution rate behavior.

First, it is necessary to have the reversion tendency of II to I neither very great (*e.g.*, sulfathiazole II in water at 30°) nor very small (*e.g.*, methylprednisolone in decyl alcohol at 30°) in the time range of interest. Methylprednisolone tablets in water at 37° (Fig. 9) appear to be in this "moderate" tendency category when the dissolution rate experiment is carried out for several hours under usual agitation conditions.

Then when the dissolution rate experiment is started, the initial rate will be dominated by the form II solubility, C_s^{II}. After some time, however, because of the reversion of II to I a layer of I will form if the rate of reversion is fast enough compared to the leaching-out rate of II. If the rate of reversion is not fast enough, the little form I that crystallizes out will probably flake out into the medium.

Assuming that a substantial deposit of I does form, some of the remaining II would leach out into the solution, and some would contribute to the deposit by further reversion. The net effect after a lag time should then be the same as having dissolution from a mixture of the two forms. That this situation is plausible may be seen by comparing the later time data of Fig. 7 with the early time data of Fig. 12.

At this point one may refer to Eq. 7. At high agitation rates h will be small. Therefore, the data in this case will, very quickly, become very like the data for pure form I. On the other hand at very low agitation rates, even though comparable reversion may have occurred, the dissolution rate

TABLE IV.—INTERCEPT AND h RATIOS CALCULATED BY THEORY FOR DATA OF HAMLIN *et al.*

Agitation, r.p.m.	Redrawn Slope of Form I	Slope from Lit.	D/h cm. hr.$^{-1}$	h (cm.)	Ratio $h:h_{12}$	Intercept	Ratio Intercept: Intercept$_{12}$
0	.096	.091 ± .021	.818	.022	3.06	.33	3.00
6	.208	.203 ± .027	1.77	.012	1.67	.18	1.64
12	.294	.276 ± .011	2.50	.0072	1.00	.11	1.00

may follow the expected pure form II behavior for much longer times.

The methylprednisolone form II data of Hamlin *et al.* (3) have been replotted in Fig. 13 and compared with theory (dotted lines) assuming a constant K value (Eq. 12). The corresponding h ratios and the intercept ratios are given in columns 6 and 8 of Table IV.

Because the use of a constant K value is subject to question here, the agreement of the theory with data must be regarded as only qualitatively satisfactory. However, the authors believe that the present arguments are the most satisfactory ones for explaining this phenomenon.

A quantitative model that can account for simultaneous reversion and dissolution is presently being investigated. Results of these studies will be reported at a later date.

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Enzyme Inhibitors XVI

Mode of Binding of Some 9-(2-Hydroxyalkyl)-6-(substituted)-purines to Adenosine Deaminase

By HOWARD J. SCHAEFFER and CHARLES F. SCHWENDER

The syntheses of a variety of 6-substituted-9-(2-hydroxybutyl)-, 9-(2-hydroxyheptyl)-, and 9-(2-hydroxyoctyl)purines have been completed. Those compounds with a 6-amino or a 6-methylamino group were inhibitors of adenosine deaminase, the compounds with a 6-amino group being more active than those compounds with a 6-methylamino group. For a series of 9-substituted adenines, the decreasing order of binding was: 9-(2-hydroxypropyl) > 9-(2-hydroxyethyl) ≅ 9-(2-hydroxybutyl) >9-(2-hydroxyheptyl) > 9-(2-hydroxyoctyl). From the data, it is concluded that there is a specific binding site for the terminal methyl group in 9-(2-hydroxypropyl)adenine and that the hydroxyl binding site and the main hydrophobic site of adenosine deaminase cannot be bridged by adenine derivatives which are substituted at the 9-position by straight-chain alkyl group bearing a hydroxyl group on carbon 2.

RECENTLY EVIDENCE was presented that there exists on the enzyme, adenosine deaminase, a nonpolar area which is involved in binding, by means of hydrophobic interactions, the alkyl group of some 9-alkyladenines (1). In addition, evidence was presented that if a hydroxyl group is attached to the alkyl group of a 9-alkyladenine, the hydroxyl group may either increase or decrease the binding of the inhibitor to the enzyme depending on the position of the hydroxyl group on the alkyl chain (1, 2). On the basis of these studies, it was suggested that in the reversible complex between a 9-(hydroxyalkyl)adenine and adenosine deaminase there is only one hydroxyl binding site on adenosine deaminase, and that this site on the enzyme is an area two to three carbon atoms removed from the site on the enzyme which binds the 9-position of the purine nucleus of the inhibitor (2). In order to study further the binding sites of this enzyme, it is apparent that it should be possible to prepare compounds which would occupy the hydrophobic binding region as well as the hydroxyl binding site of adenosine deaminase; such compounds should be bound more tightly to the enzyme. Compounds which may be able to occupy both binding sites on this enzyme are the 9-(2-hydroxyalkyl)-6-substituted purines. The present paper describes the synthesis and enzymatic evaluation of these compounds as inhibitors of adenosine deaminase.

Received September 14, 1966, from the Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo 14214.
Accepted for publication October 18, 1966.
This investigation was supported by grant T-337A from the American Cancer Society, by research career program award 5-K3-CA-18718 from the National Cancer Institute, and by training grant 5-T1-GM-555 from the Division of General Medical Services, U. S. Public Health Service, Bethesda, Md.

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The compounds which were selected for synthesis are the 6-substituted purines which are substituted at the 9-position by either a 2-hydroxybutyl, a 2-hydroxyheptyl, or a 2-hydroxyoctyl group. The general method of synthesis is a modification of the