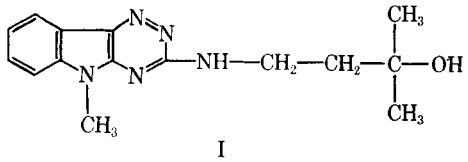
Physical–Chemical Evaluation of 3-(3-Hydroxy-3-methylbutylamino)-5-methyl-*as*. Triazino [5,6-*b*] Indole (SK&F 30097)

LOUIS J. RAVIN, ELIE G. SHAMI*, and ELISABETH RATTIE

Abstract □ X-ray diffraction, IR spectroscopy, and differential scanning calorimetry data are presented for the identification of two polymorphic forms and a hydrate of SK&F 30097. The relative rates of dissolution and the solubilities of the various forms were determined in artificial gastric fluid, water, and 50% ethanol solution. No appreciable difference in the dissolution rate was detected for the respective forms in artificial gastric fluid; however, Form II had a more rapid dissolution rate in the 50% ethanol solution. Dissolution studies in water indicated the formation of a hydrate. Protective colloids were shown to have an effect on the rate of hydrate formation. The change in particle-size distribution of Forms I and II in electrolyte suspension was investigated using the Coulter counter. The relative rate of crystal growth for Form I in the presence of protective colloids was determined. The data presented indicate that methylcellulose slows down the rate of crystal growth of Form I significantly.

Keyphrases □ 3-(3-Hydroxy-3-methylbutylamino)-5-methyl-*as*, triazino [5,6-*b*] indole (SK&F 30097)—identification □ Polymorphic, hydrated forms, SK&F 30097—determination □ Dissolution, solubility rates—SK&F 30097 □ X-ray diffraction—identification □ IR spectrophotometry—structure □ Differential scanning calorimetry—identification

The emphasis on drug availability in pharmaceutical research today requires that the physical–chemical properties of compounds be studied thoroughly prior to preliminary clinical trials. It has been demonstrated that the absorption and therapeutic efficacy of various drugs can be influenced by particle size, solubility, dissolution rate, and wettability (1–4). More recent literature reports stress the importance of the crystal form of a drug and its effect on drug availability (5, 6). The present study deals with the physical–chemical evaluation of a potential antiviral compound, 3-(3-hydroxy-3-methylbutylamino)-5-methyl-*as*, triazino [5, 6-*b*] indole, SK&F 30097 (Structure I).



I

Particular emphasis has been placed on the identification of two crystalline forms and a hydrate and the subsequent evaluation of their physical–chemical properties. Specifically, this study was concerned with an investigation of the relative dissolution behavior of the various forms in several solvent systems and an investigation of the rate of crystal growth in the presence and absence of protective colloids.

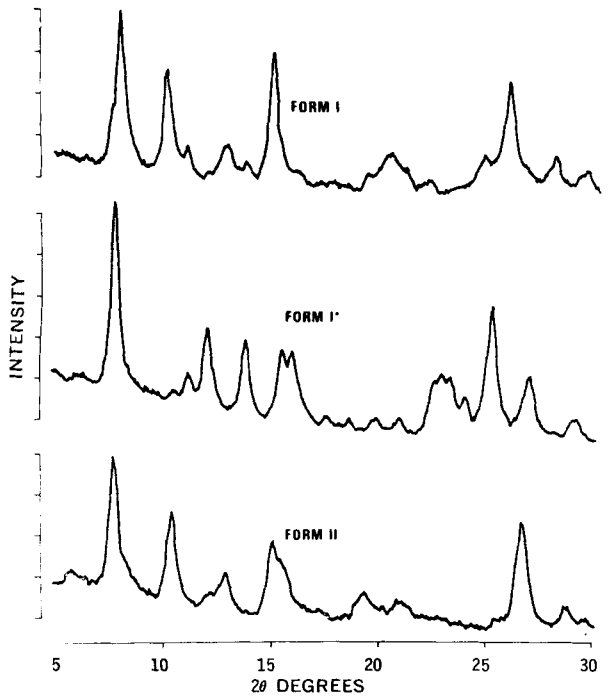


Figure 1—*X-ray diffractograms for Forms I, I*, and II of SK&F 30097.*

EXPERIMENTAL

Materials—A propylene glycol derivative;[1] polyvinylpyrrolidone;[2] carboxymethylcellulose USP; methylcellulose USP; artificial gastric fluid USP; and normal saline solution USP were used. SK&F 30097 Form I was prepared by dissolving SK&F 30097 in HCl solution and subsequently precipitating the compound slowly by adding NaOH solution. The resulting precipitate was filtered and allowed to dry in a vacuum desiccator over phosphorus pentoxide. SK&F 30097 Form I*[3] was prepared by suspending Form I in water and allowing it to be stirred overnight. The suspension was filtered and the filtrate was air-dried at room temperature. SK&F 30097 Form II was prepared by dissolving 1 g. of SK&F 30097 in 20 ml. of hot anhydrous methanol. The resulting solution was filtered and poured into anhydrous ethyl ether which had been previously dried over Linde molecular sieves[4] and cooled in liquid nitrogen. The mixture was shaken and set aside until precipitation was complete. The resulting precipitate was filtered and allowed to dry over phosphorus pentoxide. Extreme care must be taken to maintain anhydrous conditions to prepare Form II.

X-Ray Diffraction Procedure—A diffractometer (General Electric XRD-5) was used. The sample was packed into a planchet having a depression 1.5 mm. deep and 22 mm. in diameter. The flat side of the spatula was used to pack the powder and smooth it so that a uniform level surface was presented to the X-ray beam. Coarse or lumpy powder was first ground in a mortar or mechanical grinder. The instrument variables of the diffractometer were set as follows: (*a*) 1° beam slit and 0.1° detector slit; and (*b*) CuKα radiation, 40 kv., 16 ma., Ni filtered. Intensities were measured by recording the time necessary to count a fixed number of particles.

IR Procedure—The IR spectra for the various forms of SK&F

[1] Pluronic F-68, Wyandotte Chemical Co., Wyandotte, MI 48193
[2] Plasdone C, Antara Chemical Co.
[3] Monohydrate of SK&F 30097 will be designated by Form I*.
[4] Linde Division, Union Carbide Corp., New York, NY 10017

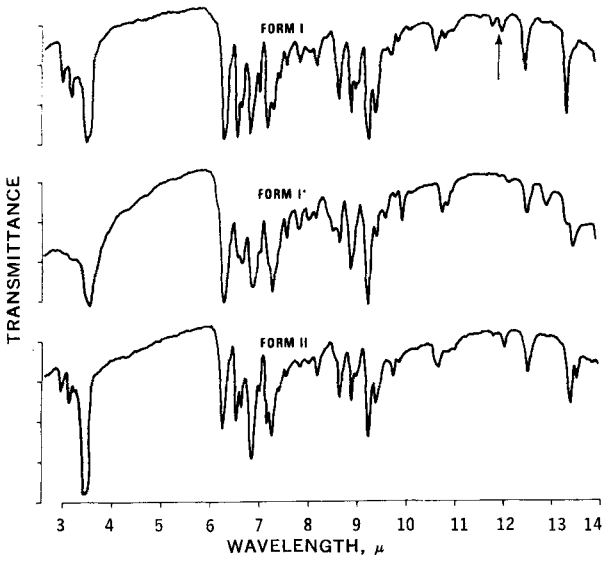


Figure 2—*IR spectra of Forms I, I*, and II of SK&F 30097.*

30097 were determined using a spectrophotometer (Perkin-Elmer model 21). The compounds were run in a mineral oil mull.

Differential Scanning Calorimetry Procedure—A differential scanning calorimeter (Perkin-Elmer DSC-1B) was used. Samples of indium were used to calibrate the instrument, and dry nitrogen at 20 ml./min. was used as the carrier gas. Since the preliminary work was qualitative, the samples were not accurately weighed. The rate of heating used was 20°/min. at a chart speed of 2 in./min.

Dissolution-Rate Studies—The procedure of Milosovich (7) was utilized to run the dissolution experiments under conditions of constant-surface and diffusion-layer thickness. Essentially, 100 mg. of powder was compressed into a disk in a tablet die at a compression force of approximately 5000 p.s.i. The tablet die holding the disk was placed in a plastic holder, and this assembly was placed into 700 ml. of artificial gastric fluid or 50% ethanol solution in a 1-l. glass-jacketed beaker. The solution was maintained at 37° by circulating water from a constant-temperature bath. The stirring rate was maintained at 200 r.p.m. during the experiment. Ten-milliliter samples were removed periodically and replaced by 10 ml. of the appropriate solvent. The samples were analyzed spectrophotometrically using a recording spectrophotometer (Cary model 15).

Dissolution-rate studies were also carried out according to the method of Shefter and Higuchi (8). Five hundred milligrams of the appropriate form was added to 500 ml. of distilled water maintained at 25° in a glass-jacketed 1-l. beaker thermostatically controlled by circulating water from a constant-temperature bath. The suspension was stirred at a rate of 200 r.p.m. Samples were withdrawn periodically and filtered through a syringe fitted with a Swinney filter adapter containing a 0.45-mμ Millipore filter disk. The resulting

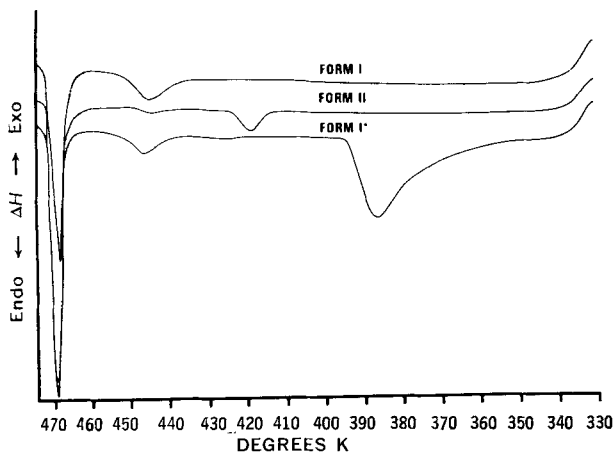


Figure 3—*Thermograms for Forms I, I*, and II of SK&F 30097.*

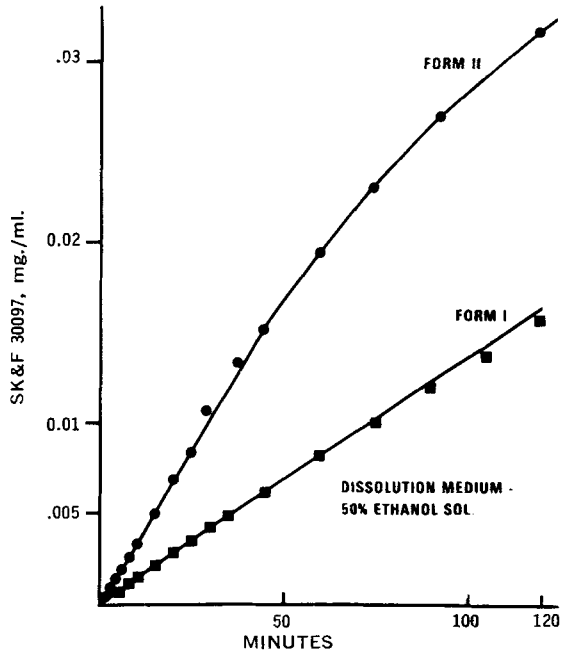


Figure 4—*Dissolution behavior of Forms I and II in 50% ethanol solution.*

clear solutions were analyzed spectrophotometrically (Cary mode 15 recording spectrophotometer). Additional experiments were done in the presence of 0.01% concentration of various protective colloids such as methylcellulose, carboxymethylcellulose, polyvinylpyrrolidone, and propylene glycol derivative.[1] The size of the crystals in the dissolution experiment was not controlled. The same lots of chemical for the various forms of SK&F 30097 were used throughout the study.

Crystal Growth Studies—Crystal growth studies were carried out using a modified procedure of Carless *et al.* (9). A saturated solution of SK&F 30097 was prepared by suspending the compound in normal saline solution containing 0.01% pro-

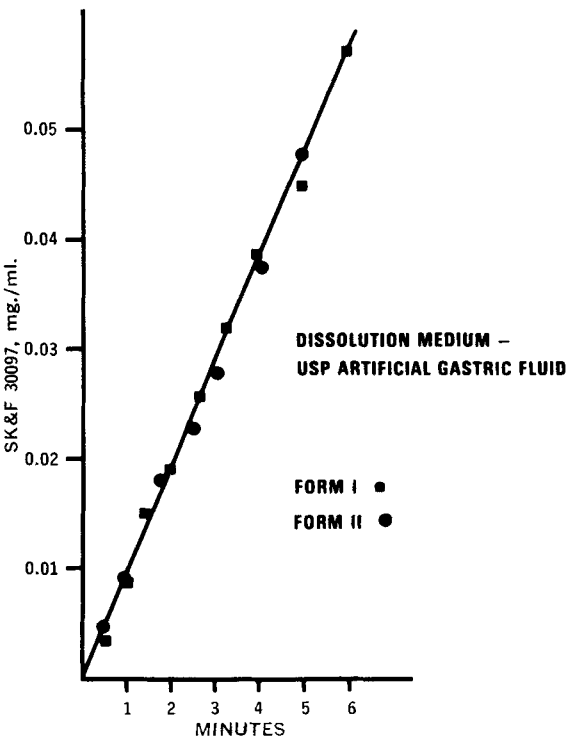


Figure 5—*Dissolution behavior of Forms I and II in artificial gastric fluid.*

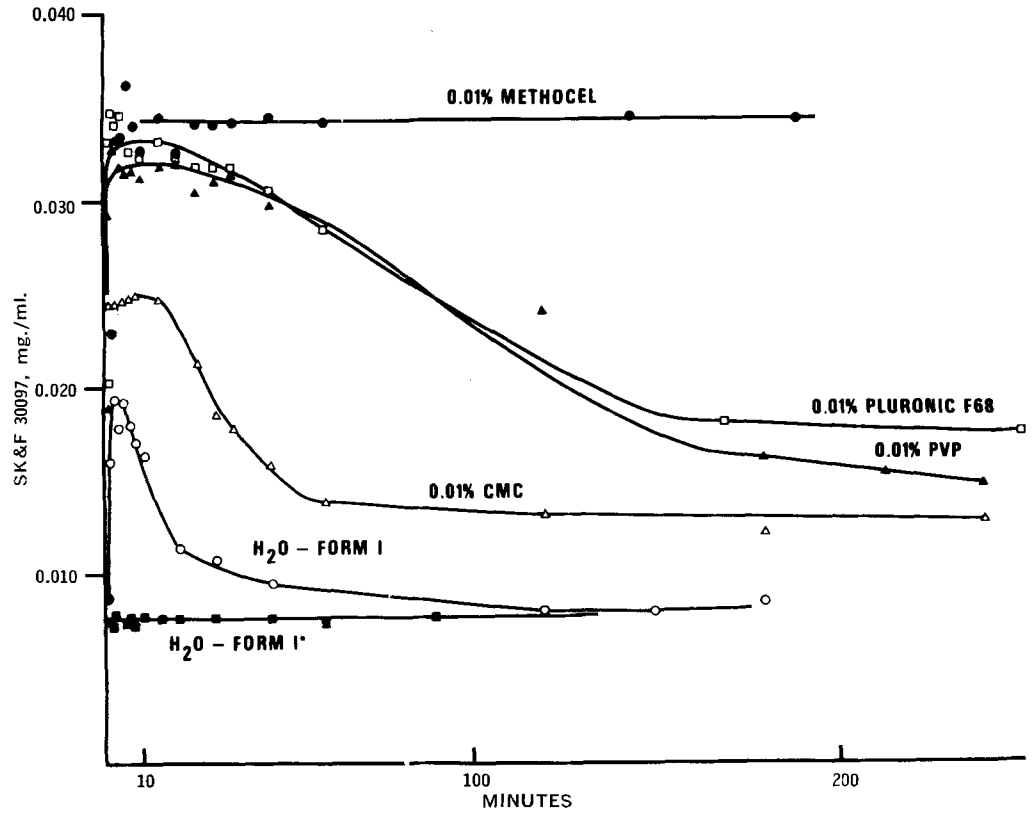


Figure 6—*Dissolution behavior of Forms I and I* in water in the presence of various protective colloids.*

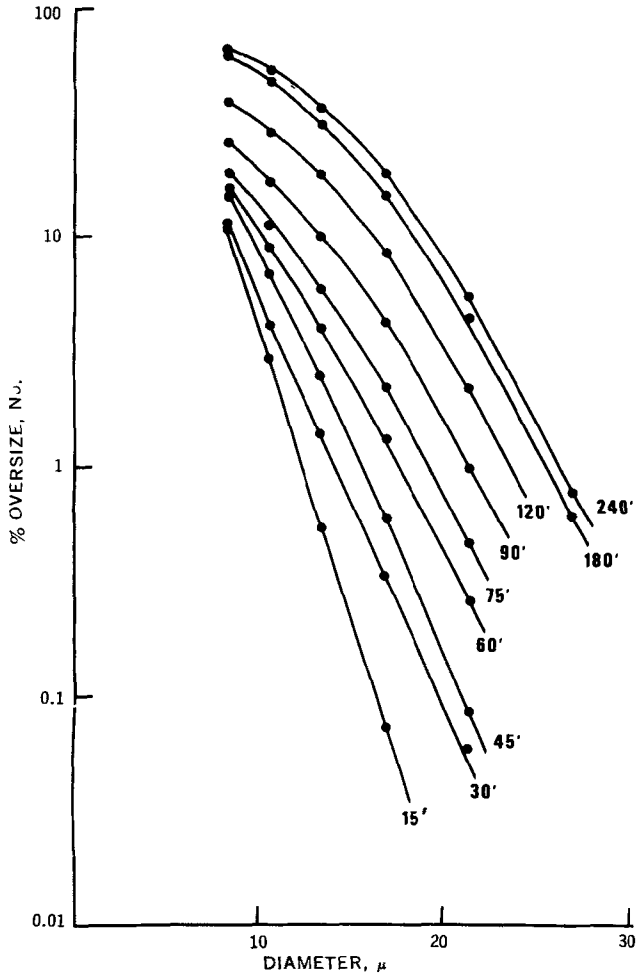


Figure 7—*A plot of change in particle size* versus *diameter at various time intervals for an aqueous suspension of Form I.*

pylene glycol derivative[1] as dispersing agent. The resulting suspension was stirred overnight and subsequently filtered. The filtrate was ready for use in the crystal growth studies. The seed crystals were prepared by suspending 100 mg. of the appropriate form of SK&F 30097 in 50 ml. of saturated solution. The suspension was sonified for 5 min. in an ultrasonic bath[5] to ensure deaggregation of the particles. Four milliliters of the seed suspension was pipeted into 70 ml. of electrolyte solution in a 100-ml. screw-cap bottle. The bottles were placed in a water bath set at 25° and rotated. Samples were removed at fixed intervals, and the contents were transferred to a jacketed glass vessel at 25° for crystal-size counting with the Coulter counter (model B). A 100-μ aperture tube was used during these studies. The tube had previously been calibrated with lycopodium having an average particle size of 27 μ. Experiments were carried out with Form I in electrolyte solution alone and in electrolyte solution containing propylene glycol derivative[1] and 0.01% concentrations of methylcellulose, carboxymethylcellulose, and polyvinylpyrrolidone, respectively. Form I* and Form II were studied in electrolyte solution only.

RESULTS AND DISCUSSION

X-Ray Diffraction and IR Spectroscopy—The X-ray diffraction patterns and the IR spectra for Forms I, I*, and II are shown in Figs. 1 and 2. Distinct differences in the profiles are evident. These distinguishing features can be utilized for the identification and possibly for the analysis of the various forms in mixtures.

Differential Scanning Calorimetry—The thermal behavior of the various forms of SK&F 30097 is illustrated in Fig. 3. The thermogram for Form II shows three endothermic transitions; the first at 143° corresponds to a transformation of Form II to Form I, the second at 165° corresponds to a transformation of Form I to a form that has not been isolated under the conditions of this study, and the third at 183° corresponds to melting. The thermogram for Form I* shows an endotherm at approximately 110° suggesting the presence of water, an endotherm at 165°, and melting at 183°. When Forms I* and II are cooled and reheated, the endotherms at 110 and 143° disappear; the endotherm at 165° is present, along with the endotherm corresponding to melting. In all cases, there

[5] L&R Manufacturing Co., Kearney, N. J.

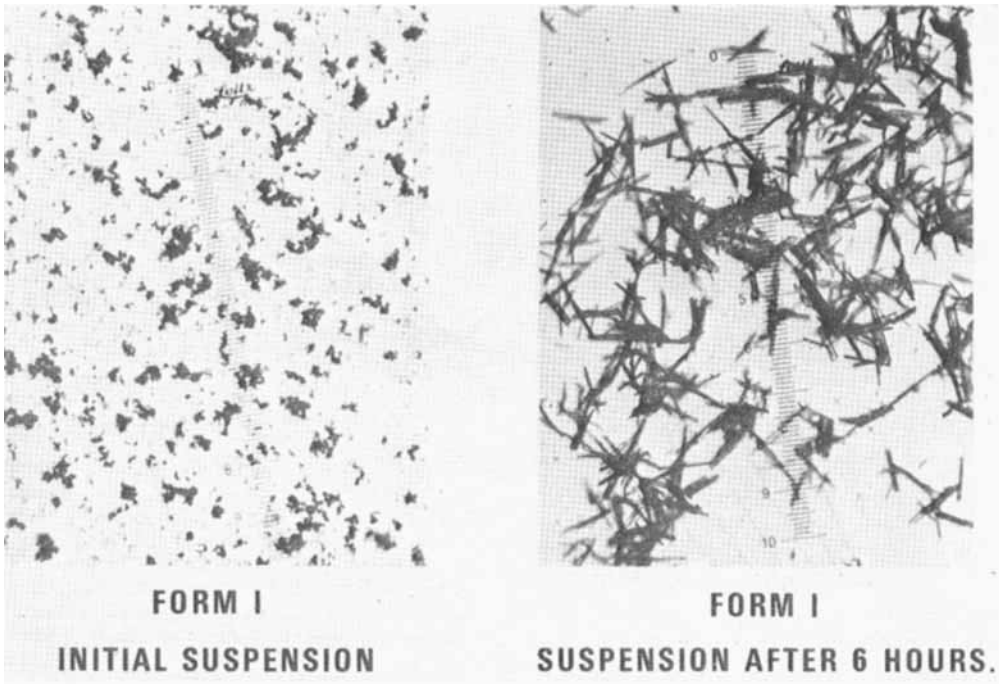


Figure 8—*Photomicrographs showing change in crystal size for a suspension of Form I. Each scale division equivalent to 6 μ.*

appears to be an apparent transformation to another form; however, it has not been isolated or prepared during this study.

Dissolution-Rate Studies—The dissolution behavior of Forms I and II under conditions of constant-surface and diffusion-layer thickness in 50% ethanol solution is illustrated in Fig. 4. As expected, Form II, which is less stable thermodynamically, had a more rapid dissolution rate. After approximately 50 min., the slope of the line for Form II deviates from linearity. This deviation suggests that Form II is being converted to Form I during the dissolution process.

Figure 5 shows the results of a dissolution-rate study for Forms I and II in artificial gastric fluid. There does not appear to be any difference in the dissolution behavior of either form in this medium. On the basis of this information, it was postulated that no appreciable difference in the *in vivo* availability of the various forms would be expected after oral administration. Subsequent studies in beagle dogs indicated that there was no statistical difference in the plasma blood levels obtained after the oral administration of Forms I and II in capsules and in a solution of the hydrochloride salt (10).

The solubility and dissolution-rate data obtained with suspensions of Forms I and I* are shown in Fig. 6. This figure shows the concentration of drug attained in solution as a function of time in the presence of an excess of the solid phase under constant agitation. The anhydrous Form I dissolves much faster than the corresponding hydrated Form I* and yields concentrations supersaturated with respect to the stable anhydrous form.

Since protective colloids are known to retard various nucleation

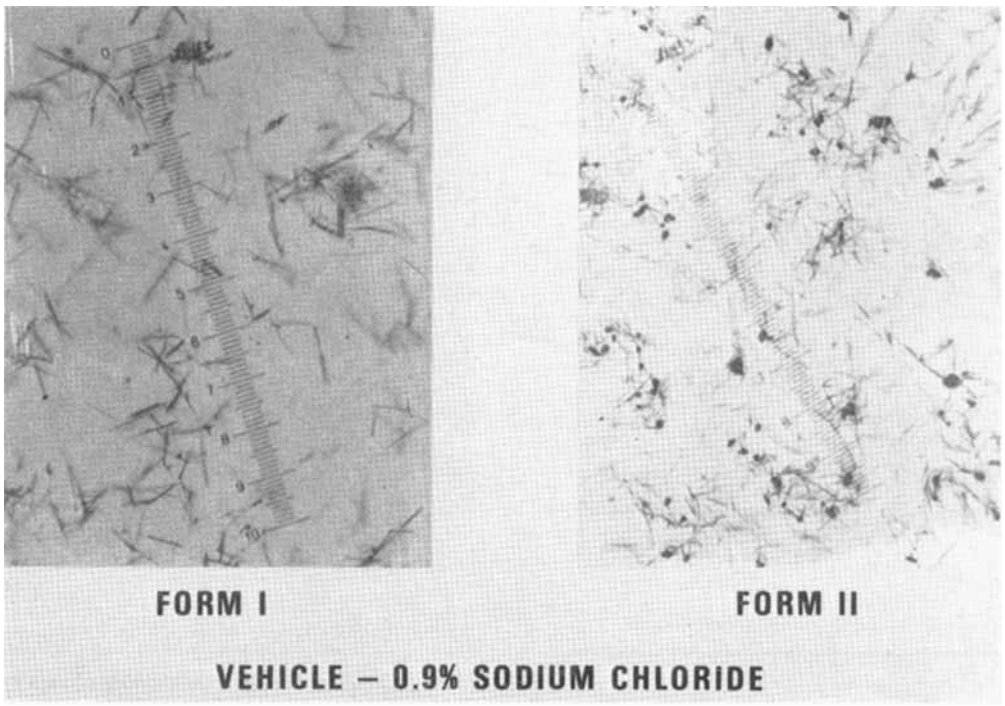


Figure 9—*Photomicrographs depicting crystal growth for Forms I and II after 5 min. in 0.9% sodium chloride solution.*

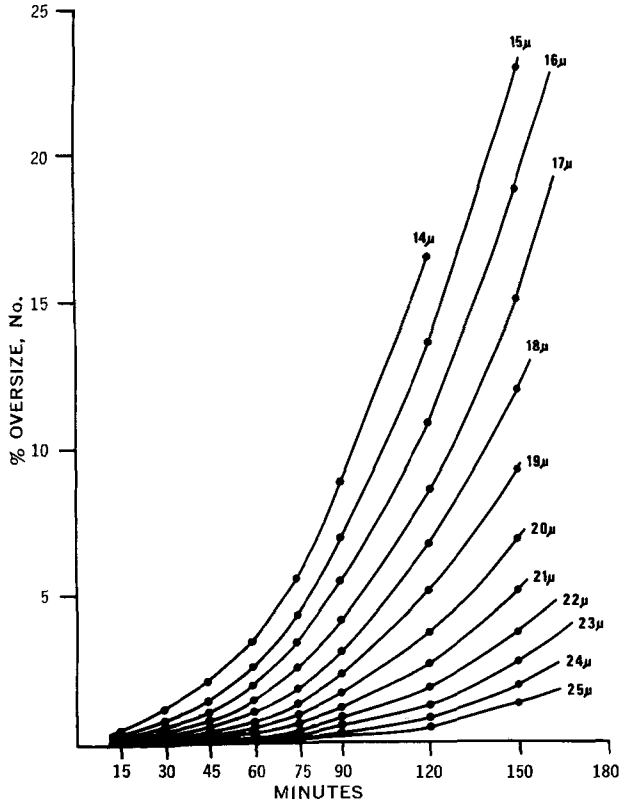


Figure 10—*Change in cumulative count with time for an aqueous suspension of Form I.*

phenomena, their effect on the transformation in this system was investigated. Studies were carried out with propylene glycol derivative,[1] carboxymethylcellulose, polyvinylpyrrolidone, and methylcellulose to determine their influence on the rate of transformation of Form I to Form I*. The dissolution behavior for Form I in 0.01% concentrations of the protective colloids is also shown in Fig. 6. It is apparent that the dissolution process is altered in the presence of the various protective colloids. The slightly higher solubility in the presence of these agents may be attributed to an interaction with the respective colloid. Methylcellulose had a

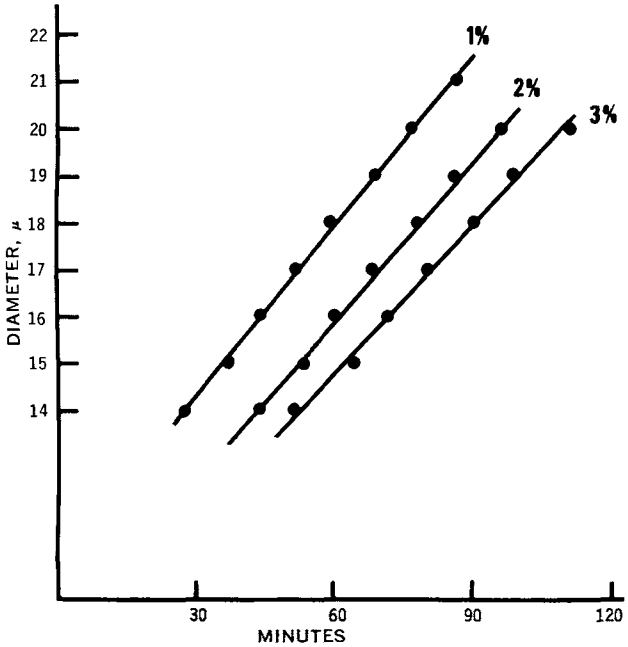


Figure 11—*Rate of growth of Form I in aqueous suspension.*

Table I—Relative Rates of Crystal Growth for Suspensions of SK&F 30097, Form I

Protective Colloid 0.01%	μ/min. × 10^{-4} Above 1%	Above 2%	Above 3%
Propylene glycol derivative	1040	930	880
Propylene glycol derivative plus:			
Carboxymethylcellulose	840	720	640
Polyvinylpyrrolidone	970	870	820
Methylcellulose	3	3.1	3

significant effect on the rate of hydrate formation and, after 48 hr., there was no apparent decrease in solubility similar to that observed with the other additives. Seed crystals of the hydrate form added at this point did not induce the conversion to the hydrate in this system.

Crystal Growth Studies—It became apparent during the preliminary studies that the particle size of Forms I and II increased rapidly when they were placed in aqueous suspension. This change is due to the conversion to Form I*. It was also observed that protective colloids slowed down this process. The dissolution-rate studies reflect these observations. As a result, crystal growth studies were undertaken with the various forms in the presence and absence of protective colloids. Figure 7 illustrates data from a typical crystal growth study with Form I where the change in particle-size distribution is plotted against diameter at various time intervals. There was an increase in particle size for intervals up to 6 hr., and then there was no apparent increase after this time. Photomicrographs of the suspension initially and after 6 hr. are shown in Fig. 8. It is apparent that there is a significant increase in particle size with time. Propylene glycol derivative[1] was used as the dispersing agent during these studies. Since this agent had some effect on the nucleation of Form I, which in turn may affect its crystal growth rate, additional studies were done in its absence. The rate of crystal growth for Forms I and II was extremely rapid.

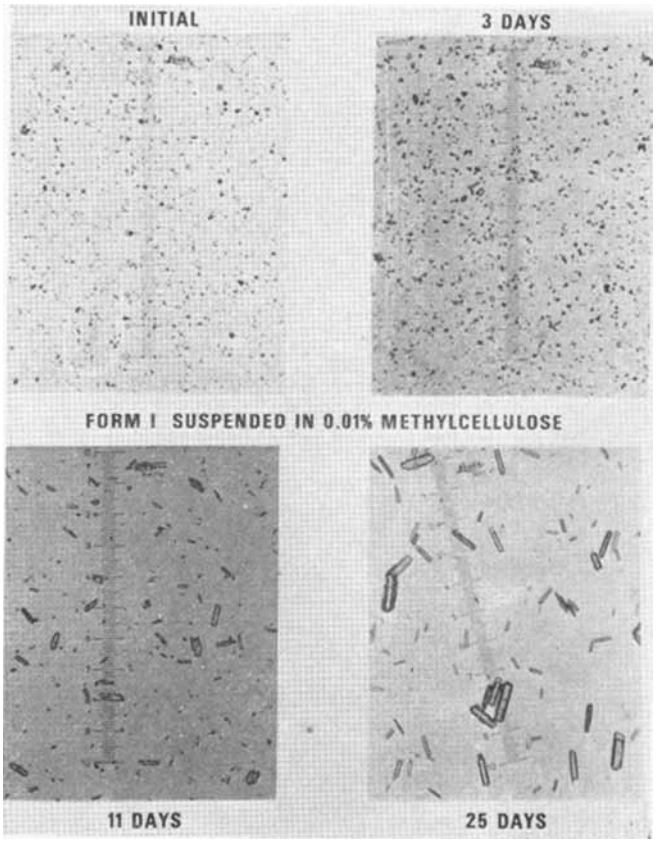


Figure 12—*Photomicrographs showing change in particle size with time for a suspension of Form I in the presence of 0.01% methylcellulose.*

These phenomena are illustrated in Fig. 9, which contains photomicrographs of these forms after 5 min.

The overall rate of crystal growth for Form I of SK&F 30097 was derived according to the method of Edmundson and Lees (11). A plot of percentage number cumulative frequency oversize against time for diameters from 14 to 25 μ is shown in Fig. 10. This size represents the faster growing end of the distribution obtained from Fig. 7. Horizontal lines corresponding to various percentage cumulative counts were made. The intercepts of these lines at the 1, 2, and 3% levels for the various time intervals give the equivalent diameter, which is then plotted against time. Figure 11 shows the rate plot for a typical crystal growth study. The slope of the lines represents the rate of growth of the crystals expressed as an increase in diameter per unit time. Table I contains values showing the relative rates of crystal growth of Form I in the presence and absence of various protective colloids.

The crystal growth in the presence of methylcellulose was approximately 300 times slower than in the presence of the other colloids. Photomicrographs showing the change in particle size with time in the presence of methylcellulose are shown in Fig. 12. There was no apparent increase in crystal size after 3 days; however, after 8 days, there was some evidence of growth. The fact that the Coulter counter measurements indicated a growth rate of 3×10^{-4} μ/min. indicates the extreme sensitivity of this instrument to change in crystal size with time.

SUMMARY

1. The presence of two polymorphic forms and a hydrate of SK&F 30097 has been confirmed by X-ray diffraction, IR spectroscopy, and differential scanning calorimetry.

2. Form II, which was less stable thermodynamically, had a more rapid dissolution rate than Form I in 50% ethanol solution; however, in artificial gastric fluid, there was no apparent difference.

3. Forms I and II readily formed the hydrate in aqueous suspension; protective colloids were shown to affect the rate of hydrate formation.

4. Coulter counter measurements were used to follow the change in particle-size distribution for Form I in electrolyte solution. The relative rates of crystal growth were determined in the presence of various protective colloids. Methylcellulose retarded the rate of crystal growth of Form I significantly.

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* Present address: Endo Laboratories, Garden City, L. I., N. Y.

Thermodynamics and Kinetics of Covalent Addition of Bisulfite Ion to Pyrimidinium Ions

IAN H. PITMAN* and MARK A. ZISER

Abstract □ Equilibrium and rate constants have been calculated for the reversible covalent addition of bisulfite ion to 2-aminopyrimidinium ion and to its 1-methyl and 4-methyl derivatives. 2-Amino-4,6-dimethylpyrimidinium ion did not appear to add bisulfite ion under the experimental conditions. The 1:1 covalent adducts had very low solubility in aqueous buffers around pH 4. This was consistent with their being zwitterions. Rate-determining steps in adduct formation appeared to involve attack of both bisulfite ion and sulfite ion on the pyrimidinium cation. In the reverse reactions, the zwitterionic adducts appeared to decompose by both nonbase-catalyzed and specific base-catalyzed reactions.

Keyphrases □ Bisulfite ion, kinetics, thermodynamics—covalent addition to pyrimidinium ions □ Covalent addition—bisulfite ion to pyrimidinium ions □ 2-Amino-1,6-dihydropyrimidinium-6-sulfonate—synthesis □ 2-Amino-1,6-dihydro-4-methylpyrimidinium-6-sulfonate—synthesis □ UV spectrophotometry—identification

Many of the known reactions of sodium bisulfite with organic and inorganic molecules have been studied quantitatively, and a considerable amount of thermodynamic and kinetic data is available for use when considering the inclusion of sodium bisulfite as an antioxidant in drug formulations (1). However, very little quantitative data are available on the reversible covalent additions of bisulfite ion to nitrogen-containing heteroaromatics such as pyridines (2), pyrimidines (3, 4), pteridines (5–8), and quinazolines (9, 10), although many drugs belong to these classes of compounds. The occurrence of the addition reaction in a drug formulation would reduce the effective concentration of the bisulfite ion, and the covalent adduct may have different chemical reactivity to that of the augend.

The present study was undertaken to determine the effects of temperature and pH on the kinetics and thermodynamics of addition of bisulfite ion to 2-aminopyrimidinium ion (I; R = R′ = H), 2-amino-1-methylpyrimidinium ion (II), 2-amino-4-methylpyrimidinium ion (I; R = H, R′ = Me), and 2-amino-4,6-dimethylpyrimidinium ion (I; R = R′ = Me). Studies on addi-