Plenum, New York, N.Y., 1972, p. 298.
(9) W. A. Phillips, W. W. Stafford, and J. Stuut, Jr., *Proc. Soc. Exp. Biol. Med.*, **143**, 733(1973).
(10) C. M. Metzler, "Compilation of Symposia Papers," 5th National Meeting, APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1970, p. 380.
(11) G. W. Snedecor and W. G. Cochran, "Statistical Methods," 6th ed., Iowa State University Press, Ames, Iowa, 1967, p. 258.
(12) D. G. Gallo, K. R. Bailey, and A. L. Sheffner, *Proc. Soc. Exp. Biol. Med.*, **120**, 60(1965).
(13) D. Perrier and M. Gibaldi, *J. Pharm. Sci.*, **62**, 225(1973).
(14) W. H. Johns and T. R. Bates, *ibid.*, **59**, 329(1970).
(15) C. L. Gemmill, *Arch. Biochem. Biophys.*, **54**, 359(1955).
(16) A. Albert and F. R. Keating, *Endocrinology*, **51**, 427(1952).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 6, 1973, from *Diabetes and Atherosclerosis Research, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication February 12, 1974.

An abstract of these studies appeared in *Circulation*, **46**, II-273(1972).

[x] To whom inquiries should be directed.

Succinylsulfathiazole Crystal Forms I: Preparation, Characterization, and Interconversion of Different Crystal Forms

M. A. MOUSTAFA, SAID A. KHALIL[x], A. R. EBIAN, and M. M. MOTAWI†

Abstract □ Some aqueous suspensions of succinylsulfathiazole exhibited physical instability which was manifested by crystal growth, caking, formation of cement-like precipitates, and difficult resuspendability. Therefore, the polymorphism of succinylsulfathiazole was studied as a probable important factor causing such instability. Methods of preparation of two polymorphs, two hydrates, two solvates, and an amorphous form of succinylsulfathiazole are described. Characterization of these forms was carried out using IR spectroscopy and X-ray crystallography. The interconversion of the crystal forms under various physical conditions was studied. The kinetics of transformation of Form I to the water-stable Form II in aqueous suspensions also are discussed. The half-life value of the transformation obtained from an Arrhenius plot ($\simeq$14 hr) was in fair agreement with the experimental value ($\simeq$16 hr) determined for suspensions stored at room temperature.

Keyphrases □ Succinylsulfathiazole—preparation, characterization, and interconversion of different crystal forms, kinetics, Arrhenius plots □ Polymorphism—preparation, characterization, and interconversion of succinylsulfathiazole crystal forms, kinetics, Arrhenius plots □ Crystal forms, succinylsulfathiazole—preparation, characterization, and interconversion

Aqueous suspensions of succinylsulfathiazole prepared from different solid batches were found to vary considerably in their physical stability. Some suspensions exhibited caking, crystal growth, difficult resuspendability, and cement-like precipitate formations which made the suspensions unsatisfactory for use. The physical stability of pharmaceutical preparations has been shown to be affected by the polymorphism of drugs in the same way as their biological availability is altered (1-6). The polymorphism of succinylsulfathiazole is examined in this report as being a probable important element of the physical stability of aqueous suspensions of this drug.

Succinylsulfathiazole was reported to be dimorphic, and methods of preparation of the two crystal forms were described previously (7). Shefter and Higuchi (4) also described methods of preparation of an anhydrous form, three hydrates, and a pentanol solvate of succinylsulfathiazole. Later, Mesley and Houghton (8) described the preparation of four crystal forms and an amorphous form of the same compound. Uncertainty concerning the number of crystal forms of this compound and the lack of reproducibility in preparing and characterizing them were observed. A more thorough investigation of this polymorphic system seemed necessary.

The present work is concerned with the preparation, characterization, interconversion, and kinetics of transformation of the various crystal forms of succinylsulfathiazole.

EXPERIMENTAL[1] AND RESULTS

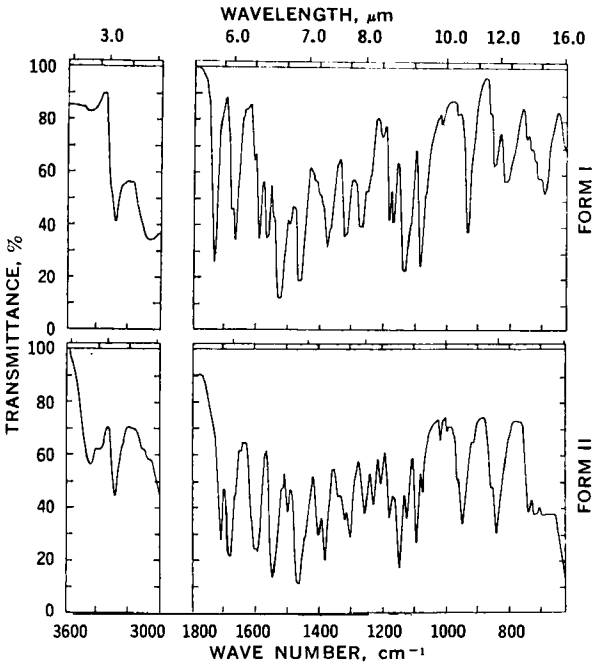
Materials—Three commercial samples of succinylsulfathiazole[2] were used during this investigation. The purity of the starting materials and the products of crystallization was checked by paper chromatography using the solvent system described by Steel (9). Solvents used for crystallization were of USP or BP quality.

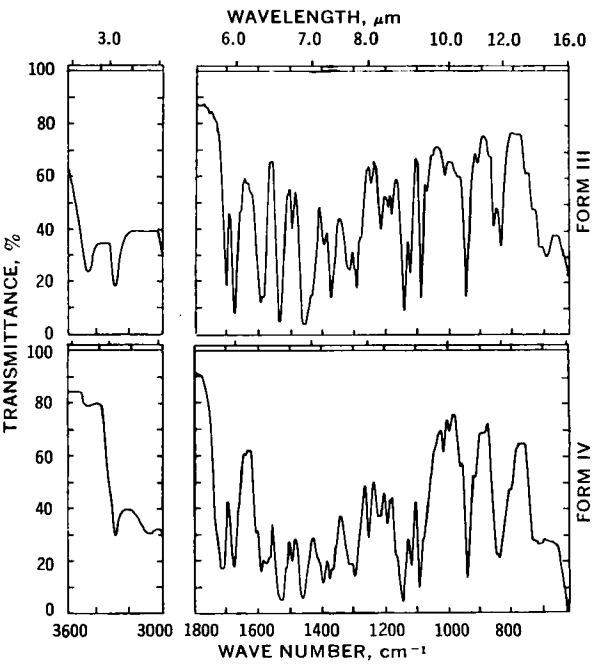
Preparation of Crystal Forms—The general procedure for the preparation of the different crystal forms involved crystallization from specific solvents. For this purpose, 0.2 g of the drug was dissolved in a suitable volume of an appropriate solvent to form a saturated solution at the boiling point of that solvent. The solution was allowed to cool slowly and stand at room temperature until most of the solid crystallized out. The crystals were then separated by filtration through a sintered-glass disk[3], dried in a current of air at room temperature (25°), and stored in a desiccator. Crystals prepared from aqueous solvents had to be dried between two filter papers. Optimum conditions for the preparation of the different crystal forms are summarized as follows.

[1] IR spectra were measured with a Perkin-Elmer double-beam grating IR spectrophotometer, model 237 B. X-ray diffraction measurements were made with a General Electric XRD-6 diffractometer under the following conditions: 3° beam slit and 0.2° detector slit, nickel-filtered CuKα radiation (30 kv, 11 mamp). A Unicam SP500 UV spectrophotometer was used to determine the molar absorptivity of the solution of the various crystal forms.

[2] Boots, England, Katwuk, Holland, and a Chinese brand, all supplied by Chemical Industries Development, Guiza, Egypt.

[3] Jena 39G3.





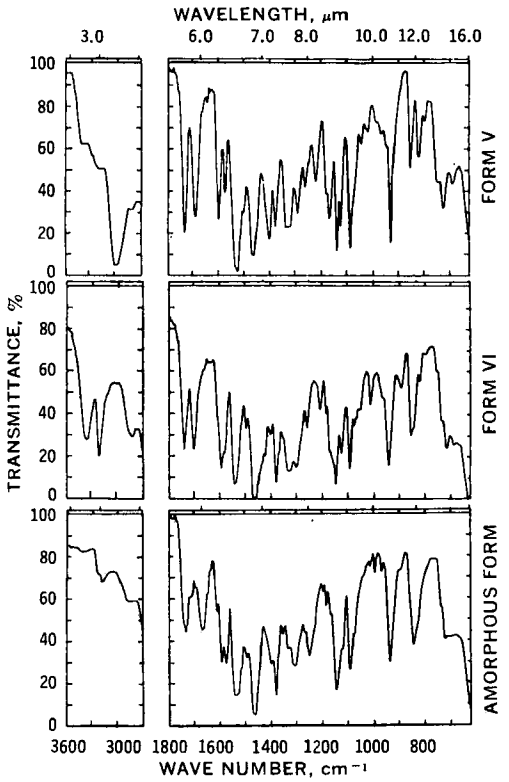


Figure 1—*IR spectra of succinylsulfathiazole crystal forms in mineral oil mulls.*

Form I was prepared by evaporation to dryness of a solution in acetone on a water bath. The same form was also encountered as one of the available commercial samples.

Form II was not recovered from any solvents used for crystallization. However, suspension of any of the other crystal forms in water effected transformation to Form II in a time varying from a few hours to several weeks. Two of the available commercial samples of succinylsulfathiazole existed as Form II.

Form III was prepared by crystallization from water, methanol, or ethanol. Precipitation from a solution in sodium hydroxide by the addition of hydrochloric acid also gave Form III.

Form IV was prepared by evaporation on a water bath of a solution in acetone until the first crystals separated. The solution was then left to stand at room temperature.

Form V was prepared by crystallization from *n*-butanol, and Form VI was prepared by crystallization from *n*-pentanol. An amorphous form was also prepared by melting any of the crystal forms (≃200°) followed by slow cooling of the melt.

Characterization of Crystal Forms—The IR spectra of Forms I–VI and the amorphous form in mineral oil mulls are shown in Fig. 1, and characteristic bands and absorbance ratios are presented in Table I. X-ray diffraction patterns of Forms I and II (being the most frequently encountered in commercial preparations) are shown in Fig. 2. The IR spectra and X-ray diffraction patterns show distinct differences which can be used for the characterization of the crystal forms.

Interconversion of Crystal Forms—*Crystallization*—Any crystal form can be converted to another by crystallization from the appropriate solvent as described under *Preparation of Crystal Forms.*

Heating—Heating at a temperature above the melting point (≃200°) followed by slow cooling of the melt resulted in a transformation of any of the crystal forms to the amorphous form. In the transformation of Forms V and VI, solvent was given off at 120–140° and Form I was produced. Form I subsequently transformed to the amorphous form at its melting point.

Suspension in Water—Suspension of all forms in water resulted in a transformation to Form II. Form III was detected as an intermediate in all cases except for the transformation of Form I, which changed directly to Form II. The time required for complete transformation varied according to the particle size of the crystals and the temperature of the aqueous suspension. Heating, shaking, and grinding under water accelerated the transformation to Form II.

Crystal growth accompanied the transformation of commercially available micronized succinylsulfathiazole Form I to Form II. This resulted in the formation of long needles which, by interlocking together, formed a cement-like cake. Photomicrographs of some stages in the transformation of Form I to Form II are shown in Fig. 3.

A summary of the course of interconversions is illustrated in Fig. 4.

Determination of Solvent—Solutions of the various crystal forms (4.23×10^{-5} *M*, assuming the molecular weight of succinylsulfathiazole is 355.4) in 0.1 *N* sulfuric acid were prepared. Absorbance values of these solutions were measured at 258 nm. Calculation was made as to the number of molecules of solvent associated with each molecule of succinylsulfathiazole in those cases where the presence of the solvent of crystallization was suspected (Table II); similar treatment was previously reported (4).

Table I—Characteristic Absorbance Bands of Succinylsulfathiazole Crystal Forms by IR Spectroscopy[a]

Form	800–900 cm^{-1}	1100–1200 cm^{-1}	1200–1300 cm^{-1}	1500–1600 cm^{-1}	3200–3500 cm^{-1}	Other Characteristic Bands and Absorbance Ratios (r)
I	2b (825, 852)	doublet (1172, 1185)	b (1270), s	b (1530), doublet (1568, 1595)	b (3300)	A_{1730}/A_{1670} = 1.333
II	b (845), s	b (1150), 2s*	3b (1210, 1235, 1260)	2b (1548, 1595)	2b (3295, 3450)	A_{1700}/A_{1670} = 0.825, no b between 1700 and 1750
III	2b (845, 865)	3b (1130, 1150, 1185)	3b (1225, 1250, 1295)	2b (1540, 1600)	2b (3320, 3480)	No b between 1700 and 1750
IV	b (842), s	3b (1125, 1150, 1198), s	2b (1225, 1260)	2b (1535, 1595), s	b (3300)	—
V	2b (825, 855)	3b (1130, 1142, 1170), s	3b (1225, 1265, 1295)	2b (1530, 1575)	b (3220)	—
VI	2b (855–895), s	b (1150), 2s*	2b (1210, 1260)	2b (1540, 1595)	2b (3350, 3450)	—
Amorphous	b (848)	b (1148), 2s*	b (1252), s	b (1540), doublet (1575, 1595)	b (3310)	—

[a] Figures in parentheses are the specific absorption maxima; b = band, s = shoulder, and * denotes shoulders on either side of the band.

Table II—Solvent Determination in Succinylsulfathiazole Crystal Forms by Measuring Their Molar Absorptivity at 258 nm

Form	Expected Solvent Molecule per Molecule Drug	Absorbance of 4.23×10^{-5} M Solution (Experimental)	Calculated Absorbance for Equimolar Solution of Crystals Containing Solvent	Solvent Found, Molecule Solvent/ Molecule Drug
Amorphous	—	0.797	0.797	
I	—	0.794	0.797	
IV	—	0.800	0.797	
III	1 molecule water	0.750	0.758	1
II	2 molecules water	0.716	0.723	2
V	1–2 molecules butanol	0.597	0.609	3/2
VI	0.5–1 molecule pentanol	0.688	0.684	2/3

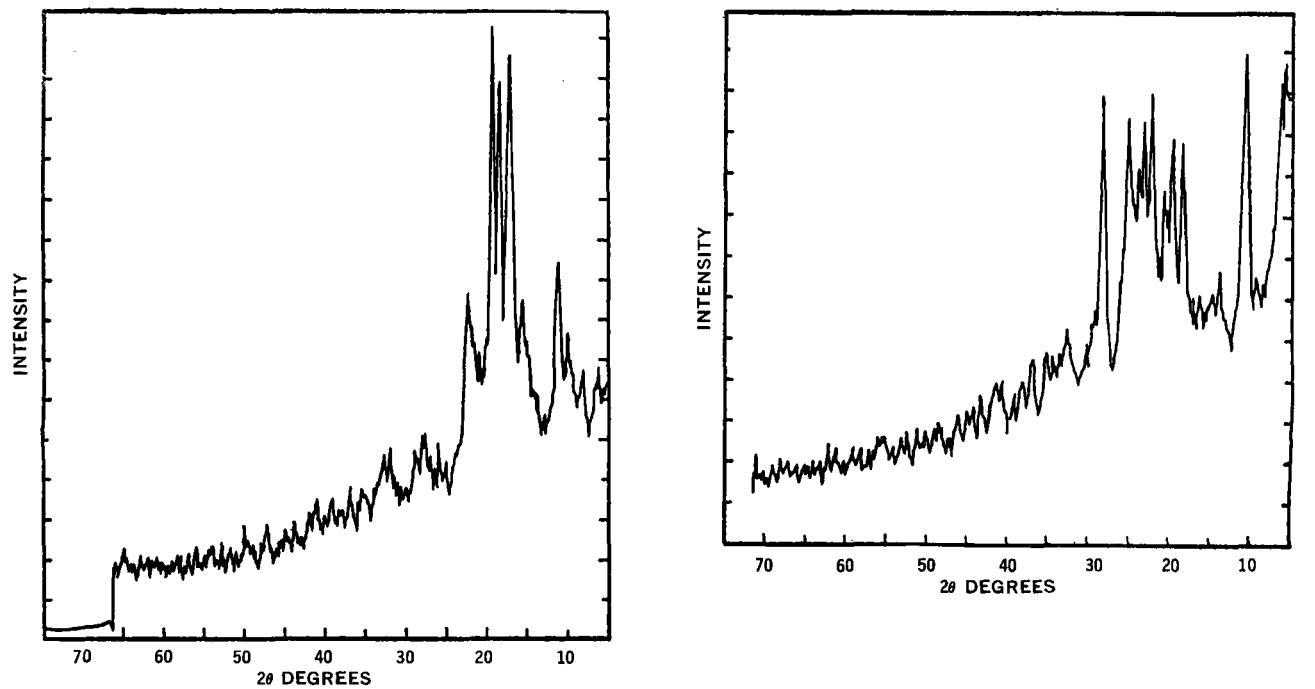


Figure 2—*X-ray diffraction data of succinylsulfathiazole crystal Form I (left) and Form II (right).*

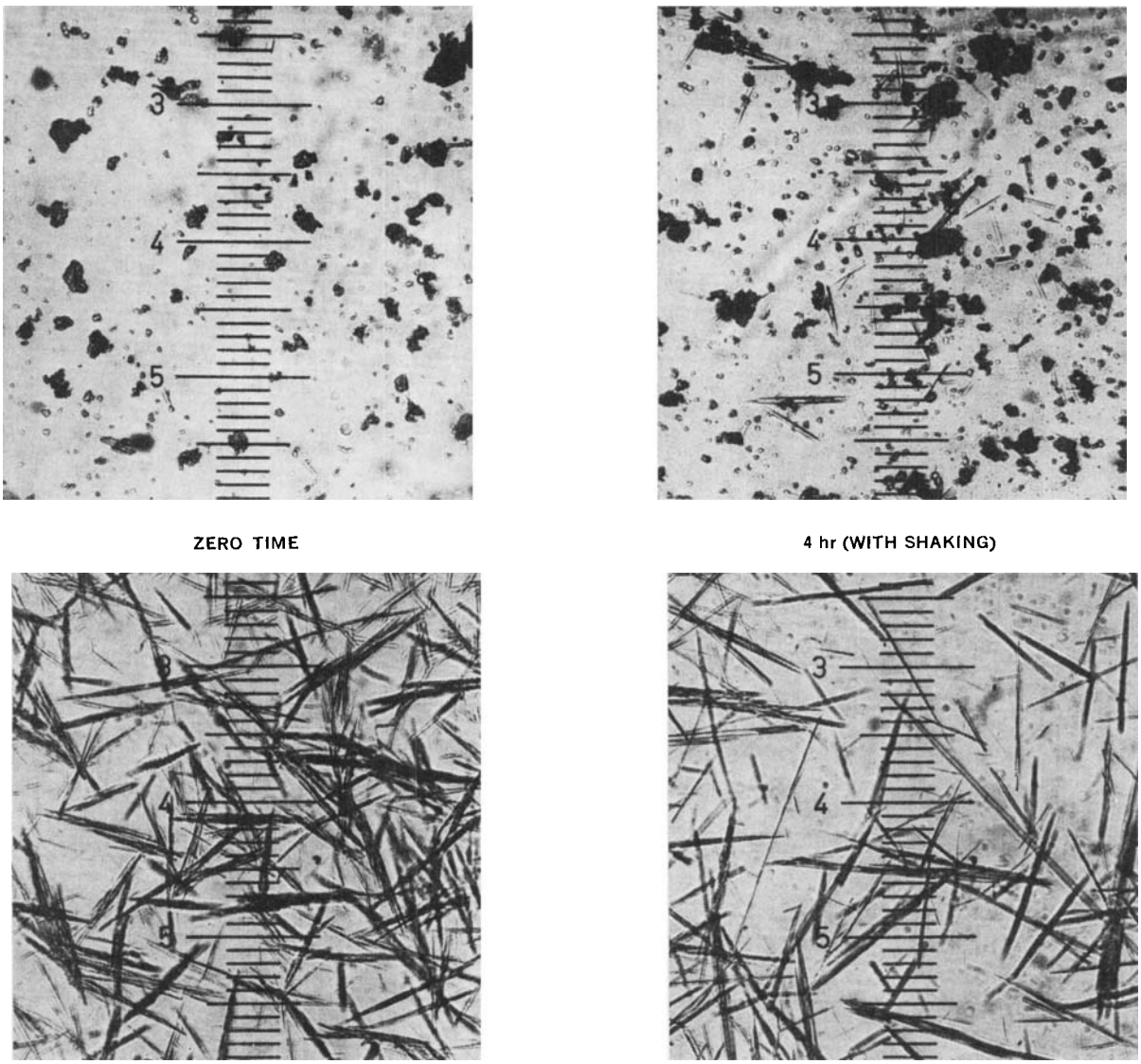


Figure 3—*Photomicrographs representing various stages in the crystal growth accompanying the transformation of succinylsulfathiazole Form I to Form II in aqueous suspension (1 small micrometer division = 100 μm).*

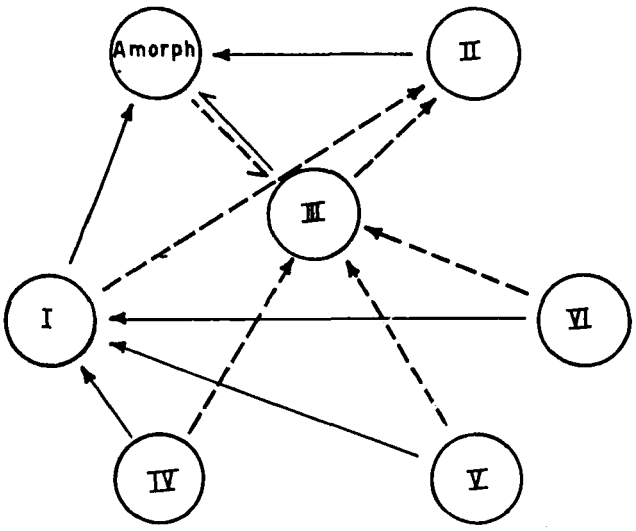


Figure 4—*Interconversion of succinylsulfathiazole crystal forms. Key: ——, heating; and ---, suspension in water.*

Kinetics of Transformation of Form I to Form II—The transformation of succinylsulfathiazole Form I to Form II in aqueous suspensions is of importance because of its effect on the stability of pharmaceutical preparations containing this compound. Therefore, the rate of transformation under normal conditions was studied. For this purpose, the quantitative IR method previously used to study the kinetics of interconversion of various sulfamethoxydiazine crystal forms (10) was employed. This method utilizes an application of the absorbance ratio procedure (11) in which specific bands characteristic of each crystal form are selected from the IR spectrum. The bands 1730 cm^{-1} for Form I and 1700 cm^{-1} for Form II were used, and the band at 1670 cm^{-1} common to both forms of succinylsulfathiazole was selected as an internal marker for absorbance ratio measurements.

The absorbance ratios are calculated as follows.

From the IR spectrum of Form I, $A(1670\ cm^{-1}) = \log I_0/I = \log 66.5/9.5 = 0.845$, and $A(1730\ cm^{-1}) = \log I_0/I = \log 83/6 = 1.141$. The absorbance ratio is $A(1730\ cm^{-1})/A(1670\ cm^{-1}) = 1.141/0.845 = 1.350$. The means of four runs (IR spectrum was recorded for four samples taken from the same mull) (10) = 1.333 (Table I).

Following the same procedure, the following values were calculated: Form I, $A(1730\ cm^{-1})/A(1670\ cm^{-1}) = 1.350$; Form II, $A(1700\ cm^{-1})/A(1670\ cm^{-1}) = 0.846$; the mixture of Form I and

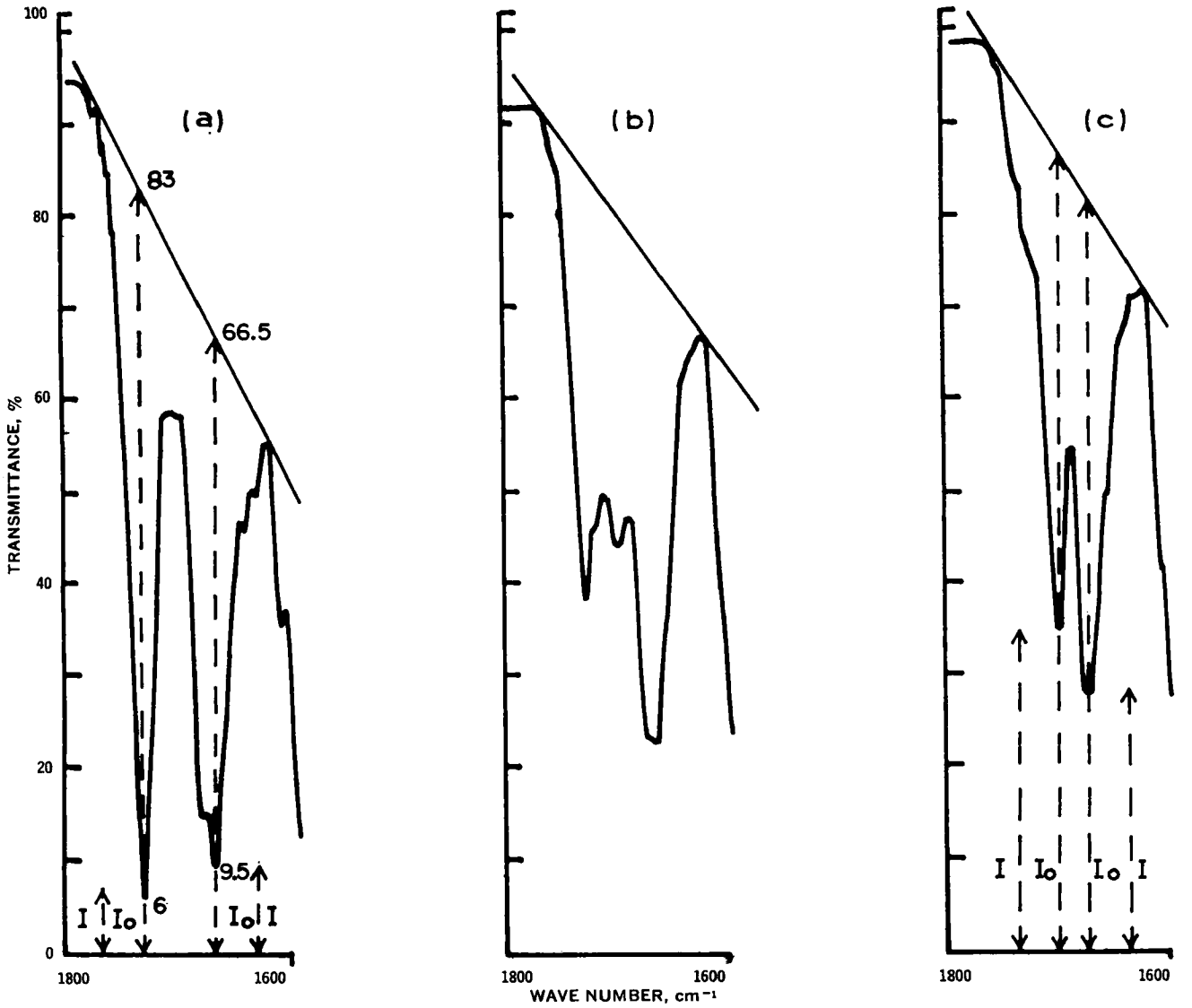


Figure 5—*Measurement of absorbance ratio of succinylsulfathiazole. Key: a, Form I; b, 40:60 mixture of Form I and Form II; and c, Form II.*

Form II (40:60), $A(1730\ cm^{-1})/A(1670\ cm^{-1})$ = 0.680; and the mixture of Form II and Form I (60:40), $A(1700\ cm^{-1})/A(1670\ cm^{-1})$ = 0.505. In these measurements, the baseline minimums for each form and mixture were measured from 1770 to 1630 cm^{-1}, respectively (Fig. 5). Additional absorbance ratios for mixtures of Form I and Form II and of Form II and Form I were recorded and plotted against the concentration of the respective crystal form (Fig. 6).

The rate of transformation of Form I to Form II was studied by preparing 1% (w/v) suspensions of Form I in distilled water and placing them in thermostated water baths at 22, 37, 40, 55, 60, and 70 ± 0.1°. Samples of each suspension were taken at various time intervals and filtered, and the residue was dried by pressing between two filter papers. The concentration of Form II in the residue was determined from the calibration curve (Fig. 6), and the concentration of Form I was calculated by the difference. Results of the transformation of Form I at various temperatures are shown in Fig. 7.

DISCUSSION

Succinylsulfathiazole exists in the solid state in one of six or more crystal forms or in an amorphous state. Forms I and IV are anhydrous, Forms II and III are hydrates, and Forms V and VI are solvates of butanol and pentanol, respectively.

In the present study, the methods of preparation of succinylsulfathiazole crystal forms (4, 7, 8) were often difficult to reproduce. No evidence could be found in favor of the claim that one crystal form was stable below 35° and the other was stable above that temperature (7). The suggestion (7) that Form II (mp 180°) changed through solid phase transformation to Form I (described as a pseudopolymorph of Form II, mp 125°) is against expectations based on thermodynamic considerations, which would favor a transformation in the opposite direction—*viz.*, from a lower melting-point crystal form to a higher melting-point form. Under the conditions of the present study, crystallization from 25% eth-

Table III—Kinetic Parameters of Transformation of Succinylsulfathiazole Form I to Form II in Aqueous Suspension[a]

Rate Constant ($K \times 10^4$), min^{-1}					
22°	37°	40°	55°	60°	70°
6.33	33.6	40.30	203.82	361.80	959.42

[a] K at 25° = 8.5 × 10^{-4} min^{-1}, $t_{1/2}$ at 25° = 13.6 hr (by extrapolation), and Ea = 21.05 kcal/mole.

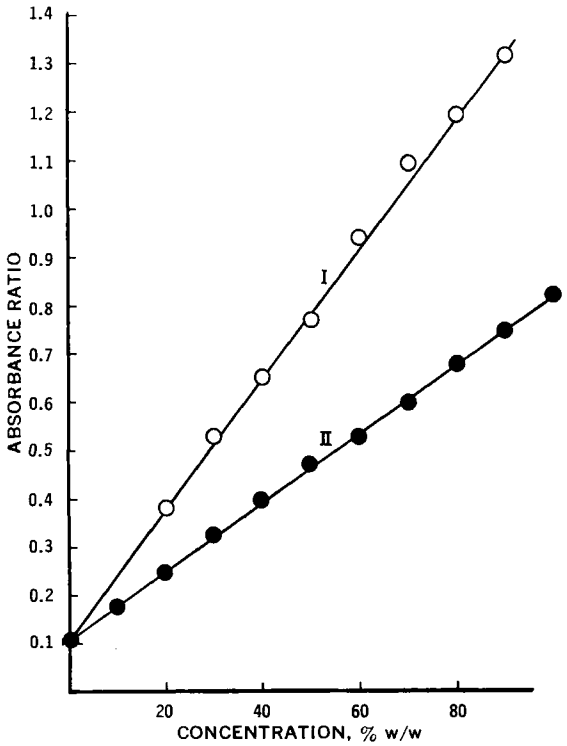


Figure 6—*Calibration curve of succinylsulfathiazole. Key:* ○, *Form I in presence of Form II using the ratio* A(*1730 cm*$^{-1}$)/A(*1670 cm*$^{-1}$); *and* ●, *Form II in presence of Form I using the ratio* A(*1700 cm*$^{-1}$)/A(*1670 cm*$^{-1}$).

anol (7) gave Form III, which did not undergo any transition below its melting point.

Shefter and Higuchi (4) used 25% ethanol to prepare a monohydrate (Form II), which probably matches Form III of the present study. The same form was also recovered from various solvents in addition to an alkali-acid treatment. The water-stable hydrate (Form I) of Shefter and Higuchi (4) matched Form II of the present work. The preparation of a pentanol solvate (4) and its conversion through partial melting at 127–136°, which probably accompanied loss of solvent to a form that melted at 191° (probably Form I of the present study), was in agreement with the present findings.

Mesley and Houghton (8) recommended dissolution of succinylsulfathiazole in sodium hydroxide solution and precipitation with dilute hydrochloric acid to prepare Form B. The present results

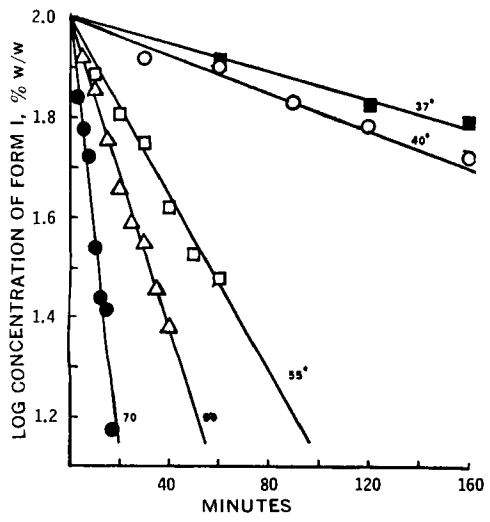


Figure 7—*Transformation of succinylsulfathiazole Form I to Form II in aqueous suspension at various temperatures.*

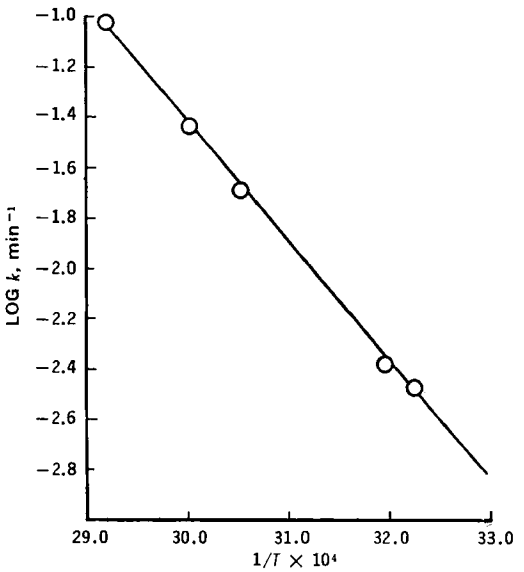


Figure 8—*Arrhenius plot of the transformation of succinylsulfathiazole Form I.*

would agree with this recommendation for identification purposes, the crystal form produced being Form III. Although Mesley and Houghton (8) suggested other treatments such as evaporation at room temperature of solutions of Forms A and D in acetone for the preparation of their Form B, similar treatments in the present study produced Form I. Heating Form A of Mesley and Houghton's (8) study resulted in its transformation to their Form D, a transformation that could not be reproduced in the present investigation. However, some crystal forms, namely Forms IV, V, and VI, changed to Form I by heating at about 150°; Forms II and III transformed through partial melting and resolidification to the amorphous form. In all cases, transformation to the amorphous form occurred when any crystal form was melted (melting point of all forms 191–193°) and allowed to resolidify slowly from the melt. The preparation of the amorphous form by crystallization from alcoholic solvents (8) is also questionable, since most alcoholic solvents used in the present study gave other crystal forms.

Molar absorptivity studies of the various crystal forms in 0.1 *N* sulfuric acid (Table II) suggested that Form II existed as a dihydrate, that Form III existed as a monohydrate, and that butanol and pentanol in a ratio of approximately 1 molecule solvent/molecule succinylsulfathiazole accompanied Forms V and VI, respectively, probably as adducts or clathrate forms. The relative ease with which solvent is given off by heating above its boiling point and below the melting point of the crystalline drug, as shown by the evolution of bubbles from an oil mount on a microscope hot stage, is further evidence for the existence of butanol and pentanol solvates in the clathrate form. Shefter and Higuchi (4) also reported the existence of a pentanol solvate containing 0.9 molecule solvent/molecule succinylsulfathiazole.

IR spectroscopy proved to be a valuable technique for both the identification and quantitative estimation of succinylsulfathiazole crystal forms. Use of the calibration curve of Form II (Fig. 6) gave more accurate results than those obtained when the calibration curve of Form I was used. This is probably due to the fact that both absorption bands used in measuring the absorbance ratio characteristic of Form II merge together from a common background, represented by the tangent to both bands between the points at 1630 and 1770 cm^{-1}. The band at 1730 cm^{-1}, characteristic of Form I, merges separately. Therefore, a greater error is anticipated when the latter band is used for the quantitative determination of Form I in mixtures. Accordingly, Form II was measured in all samples and Form I was found out by difference.

Log concentration–time plots for the decrease in concentration of Form I in aqueous suspensions of succinylsulfathiazole are shown in Fig. 7. An Arrhenius plot of the log of the first-order rate constants determined at various temperatures *versus* the reciprocal of the absolute temperature is shown in Fig. 8. Kinetic data including rate constants, activation energy, and half-life for the

transformation of Form I to Form II in aqueous suspension, as well as extrapolated values at 25°, are shown in Table III. The half-life value obtained by extrapolation (≃14 hr) was in fair agreement with the experimental value (≃16 hr) determined for suspensions stored at room temperature.

According to the results of the present study, it might be concluded that the preparation of physically stable aqueous suspensions of succinylsulfathiazole could best be obtained by using the water-stable Form II. If Form I is available commercially, as is frequently the case, adequate measures have to be taken to inhibit the transformation of the crystal form and its accompanying crystal growth, caking, and difficult resuspendability. A study of the effect of potential transformation retardants will be the subject of future reports.

REFERENCES

(1) T. Higuchi, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 657(1958).

(2) K. J. Frederick, *J. Pharm. Sci.*, **50**, 531(1961).

(3) W. I. Higuchi, P. K. Lau, T. Higuchi, and J. W. Shell, *ibid.*, **52**, 150(1963).

(4) E. Shefter and T. Higuchi, *ibid.*, **52**, 781(1963).

(5) A. J. Aguiar, J. Krc, Jr., A. W. Kinkel, and J. C. Samyn, *ibid.*, **56**, 847(1967).

(6) J. Haleblian and W. McCrone, *ibid.*, **58**, 911(1969).

(7) Armour Research Foundation of Illinois Institute of Technology, *Anal. Chem.*, **21**, 1293(1949); through *Chem. Abstr.*, **44**, 13003(1950).

(8) R. J. Mesley and E. E. Houghton, *J. Pharm. Pharmacol.*, **19**, 295(1967).

(9) A. E. Steel, *Nature (London)*, **168**, 877(1951); through R. I. Block, E. L. Durrum, and G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic, New York, N.Y., 1955, p. 241.

(10) M. A. Moustafa, S. A. Khalil, A. R. Ebian, and M. M. Motawi, *J. Pharm. Pharmacol.*, **24**, 921(1972).

(11) M. Maruyama, N. Hayashi, and M. Kishi, *Takamine Kenkyusho Nempo*, **13**, 176(1961); through *Chem. Abstr.*, **56**, 11708d(1962).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 22, 1973, from the *Department of Pharmaceutics, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.*

Accepted for publication February 20, 1974.

† Deceased.

ˣ To whom inquiries should be directed.

Inhibitory Effects of Central Hypertensive Activity of Angiotensin I and II by 1-Sar-8-ala-angiotensin II (Saralasin Acetate)

THOMAS A. SOLOMON* and JOSEPH P. BUCKLEYˣ

Abstract □ The cerebroventricular administration of 0.25–2.00 μg/kg of angiotensin I or II into α-chloralose-anesthetized cats produced significant increases in mean systemic pressure. These central hypertensive effects were inhibited by the intraventricular injection of 0.5 or 1.0 μg/kg of 1-sar-8-ala-angiotensin II (saralasin acetate). The response to angiotensin I was attenuated to a lesser extent than that to angiotensin II with this preparation. However, when both the agonists and antagonists were administered intravenously, there was equal inhibition of the effects of both angiotensin I and II. Saralasin acetate alone had little or no effect on mean blood pressure or heart rate when administered by either route; when injected intravenously, it did not significantly alter the bradycardia induced by vagal stimulation, the pressor responses to bilateral carotid occlusion or intravenously administered epinephrine, and the depressor effect of intravenous acetylcholine. The difference in the levels of antagonism at central and peripheral sites suggests that the receptors for the angiotensins are not identical in these two areas.

Keyphrases □ Angiotensin I and II—inhibition of central hypertensive activity by saralasin acetate, intravenous and intraventricular administration, cats □ Saralasin acetate (1-sar-8-ala-angiotensin II)—inhibitor of central hypertensive activity of angiotensin I and II, intravenous and intraventricular administration, cats □ 1-Sar-8-ala-angiotensin II (saralasin acetate)—inhibitor of central hypertensive activity of angiotensin I and II □ Hypertensive activity, central—inhibition of effects of angiotensin I and II by saralasin acetate

Bickerton and Buckley (1) presented evidence that angiotensin II (an octapeptide) could exert an effect on the central nervous system (CNS). Utilizing the dog cross-circulation technique, they showed that administration of the peptide into the vascularly isolated head of the recipient produced a pressor effect due to peripheral vascular constriction. Since these initial observations, several investigators (2–11), utilizing similar as well as other preparations, have confirmed this effect of angiotensin. This action appears to be mediated *via* an increase in sympathetic outflow from the CNS, since it may be blocked by the intravenous administration of α-adrenergic blockers (1) into the periphery and consists mainly of an increase in peripheral resistance, with cardiac activity only slightly altered (12).

This effect of angiotensin II has been postulated to play an important role in the central control of the cardiovascular system (13), and the possibility exists that the interaction of angiotensin II with central receptor sites contributes to the development of cardiovascular hypertensive disease (14). A strong cor-