Chem. Pharm. Bull. 26(4)1044—1048(1978)

UDC 615.453.011.3.014.21.073 : 548.33-162

Identification of the Polymorphs of Sulfamethoxydiazine by Optical and Morphological Methods[1]

Teruyoshi Yokoyama, Tsuneo Umeda, Koji Kuroda,[2a] and Atsushi Watanabe[2b]

Pharmacy, Kobe University Hospital[2a] and Faculty of Pharmaceutical Sciences, Kobe-Gakuin University[2b]

(Received July 19, 1977)

Refractive indices and related optical properties of 3 polymorphs and 2 solvates of sulfamethoxydiazine were determined by a polarizing microscope. A morphological measurement was also made for form I and III by a goniometer. All these data were useful for the simple and rapid identification of each form of sulfamethoxydiazine in a hospital pharmacy using a polarizing microscope. Especially the differences in key refractive indices between form II, the most active one, and form I or III are advantageous for discriminating form II from other less active forms in pharmaceutical preparations. Transition behaviors were also examined using a heating stage, from which the stability of 3 polymorphic forms were determined.

Keywords——sulfamethoxydiazine; polymorph; solvate; polarizing microscope; immersion method; refractive indices of crystals; optical properties of crystals; morphological study of crystals; goniometer

It was reported by Mesley and Houghton[3] and Moustafa *et al.*[4] that sulfamethoxydiazine (SMD) existed in three polymorphs (I, II, III) and two solvates (IV, V). The bioavailability of the three polymorphic forms were investigated by Khalil *et al.*[5] and Khalafallah *et al.*,[6] and it was shown that the metastable form II was the most active one.

In the previous paper, Kuroda *et al.*[7] reported on gastrointestinal absorption of polymorphic forms of SMD in rabbits and showed that it was affected by a rate of transition of form II into form I or III in a suspension. The present paper describes the measurement of refractive indices and the determination of optical and morphological properties of various forms of SMD, which are expected to give a simple and rapid method of identification of each polymorphic form in a hospital pharmacy using a polarizing microscope.

Experimental

Materials——SMD was supplied by the courtesy of Robinson Co. Ltd. U.S.A. The preparation of 5 forms of SMD was carried out as follows:

Form I: 0.6 g of SMD crystals was dissolved in 400 ml of boiling water or 60 ml of warm 95% methanol, and then the solution was permitted to stand at a room temperature overnight. The precipitates were filtered, washed with the solvent and dried in vacuum. Needles or long prisms were identified with form I in literatures[4] by infrared (IR) spectra in Nujol mull and X-ray diffraction data. *Anal.* Calcd. for $C_{11}H_{12}N_4O_3S$, mol. wt. 280.3; C, 47.13; H, 4.35; N, 19.99 Found: C, 47.22; H, 4.33; N, 20.10.

1) *a*) Presented at the 26th Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, November 1976; *b*) Study of Crystalline Drugs by Means of Polarizing Microscope. II.; Part I: A. Watanabe, Y. Tanaka, and Y. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2239 (1977).
2) Location: *a*) *Kusunoki-cho, Ikuta-ku, Kobe*; *b*) *Ikawadani-cho, Tarumi-ku, Kobe, 673, Japan.*
3) R.J. Mesley and E.E. Houghton, *J. Pharm. Pharmacol.*, **19**, 295 (1967).
4) M.A. Moustafa, A.R. Ebian, Said A. Khalil, and M.M. Motawi, *J. Pharm. Pharmacol.*, **23**, 868 (1971).
5) Said A. Khalil, M.A. Moustafa, A.R. Ebian, and M.M. Motawi, *J. Pharm. Sci.*, **61**, 1615 (1972).
6) Nawal Khalafallah, Said A. Khalil, and Mamdouh A. Moustafa, *J. Pharm. Sci.*, **63**, 861 (1974).
7) K. Kuroda, T. Yokoyama, and T. Umeda, *Kobe Journal of Medical Science*, **22**, 255 (1976).

Form II: 0.5 g of SMD crystals was dissolved in 80 ml of warm ethanol, which was cooled to crystallize rapidly at about −12°. Small needles or rods were filtered, washed by cold ethanol carefully and dried in vacuum. The results of IR absorption spectra and X-ray diffraction data were identical to those of form II in literatures.[4]

Form III: 1 g of SMD crystals was dissolved in 30 ml of warm acetone, which were permitted to crystallize at a room temperature. Thick tabular or elongated prismatic crystals were filtered, washed by cold acetone and dried in vacuum. The results of IR absorption spectra or X-ray diffraction data were identical to those of form III.

Form IV: 1 g of SMD crystals were dissolved in warm 25 ml of dioxane, which was stayed at a room temperature overnight. Long plates were filtered, washed with cold dioxane and dried carefully. *Anal.* Calcd. for $C_{11}H_{12}N_4O_3S \cdot C_4H_8O_2$, mol. wt. 368.4: C, 48.90; H, 5.40; N 15.20. Found: C, 48.60; H, 5.23; N, 15.76.

Form V: 1.0 g of SMD crystals were dissolved in warm 550 ml of chloroform, which were permitted to crystallize at a room temperature. Long prisms or long plates were filtered, washed with cold chloroform and dried carefully. *Anal.* Calcd. for $C_{11}H_{12}N_4O_3S \cdot CHCl_3$, mol. wt. 399.7: C, 36.06; H, 3.28; N, 14.02. Found: C, 36.12; H, 3.24; N, 14.07.

Instruments——IR absorption spectra in Nujol mull were taken with a Shimazu mod. 400 spectrophotometer. The X-ray diffraction patterns were obtained with the Rigakudenki mod. RV-3 X-ray diffractometer with: target, Cu-Kα; filter, Ni; voltage, 40 kV; current, 75 mA. Optical properties of crystals were determined by the Nikon mod. PHO polarizing microscope. In addition, the Fedrow's universal stage as well as the microscopic heating stage were used to determine the optical orientation and to observed transition behavior of polymorphic forms. Refractive indices of crystals were measured by the immersion method using the chart of the immersion media, which was described in the previous paper by one of the authors.[8]

Crystal morphological investigation was carried out for form I and III measuring the facial angles by uniaxial goniometer of R. Fuss, which was allowed to use by the courtesy of Takeda Chemical Industries Co., Ltd.

Results and Discussion

Morphological and Optical Properties of Form I and III

Among the 5 forms of SMD, the crystals of form I and III were obtained in large size that were able to perform goniometric investigation. The optical properties were determined by the polarizing microscope and the universal stage. The results are shown in Table I, Fig. 1 and Fig. 2.

Table I. Morphological and Optical Properties of Form I and Form III of Sulfamethoxydiazine

	Form I		Form III	
Crystal system	Monoclinic, C_{2h}		Monoclinic, C_{2h}	
Axial angle	$\beta=111°\ 19'$		$\beta=97°\ 56'$	
Axial ratio	a:b:c=0.447:1:0.626		a:b:c=1.140:1:0.681	
	obsd.	calcd.	obsd.	calcd.
Facial angles	110:010=67° 22′	—	100:001=82° 04′	—
	110:1$\bar{1}$0=45° 19′	—	100:110=48° 28′	—
	001:110=69° 58′	—	001:111=39° 28′	—
	$\bar{1}$10:$\bar{1}$12=65° 55′	—	010:111=61° 24′	—
	$\bar{1}$01:$\bar{1}$12=32° 15′	32° 16′	110:111=45° 21′	45° 06′
	$\bar{1}$10:$\bar{1}$01=46° 40′	46° 40′	001:$\bar{1}$11=44° 25′	44° 21′
	010:$\bar{1}$12=73° 51′	72° 55′	$\bar{1}$11:$\bar{1}$10=50° 56′	50° 53′
	112:$\bar{1}\bar{1}$2=32° 37′	32° 48′	010:$\bar{1}$11=58° 19′	58° 17′
	$\bar{1}$12:001=43° 51′	43° 44′	111:1$\bar{1}$1=53° 00′	52° 31′
Optical properties	Optic axial plane: parallel to (010); Z ∧ c=*ca.* 20° (in obtuse angle of β) Optic axial angle: *ca.* 83° Optic sign: positive		Optic axial plane: parallel to (010); Z ∧ c=*ca.* 8° (in acute angle of β) Optic axial angle: *ca.* 41° Optic sign: negative	

8) A. Watanabe, Y. Tanaka, and Y. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2239 (1977).

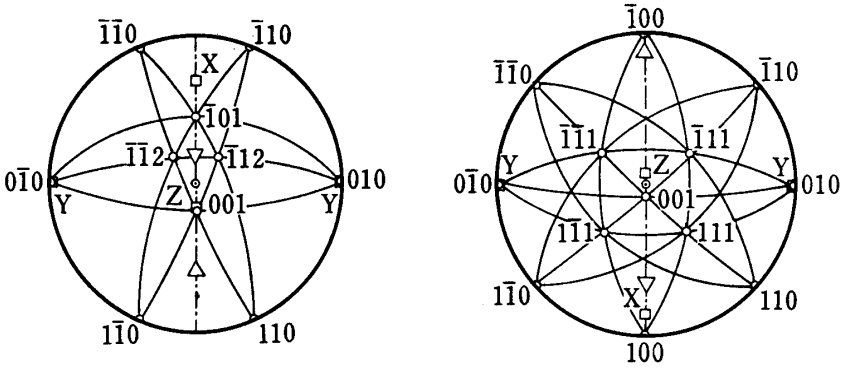
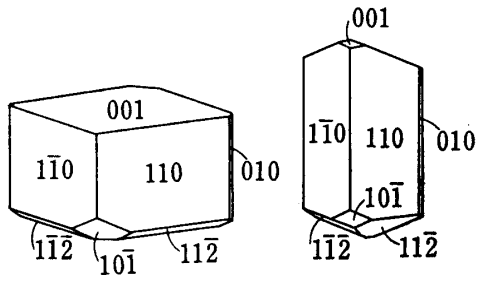


Fig. 1. Stereographic Projections of the Crystals of Sulfamethoxydiazine, Form I and III, where —▷———◁— denotes Optic Axis and Optic axial Plane



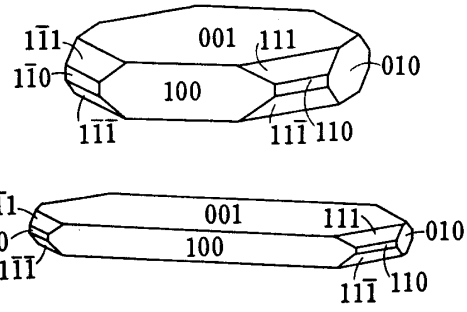


Fig. 2. Orthographic Projections of the Crystals of Sulfamethoxydiazine, Form I and III

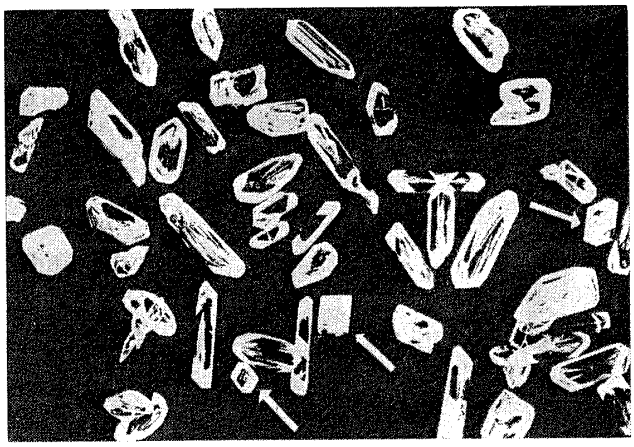


Fig. 3. Microscopic Photograph of the Crystals of Sulfamethoxydiazine, Form III including Form I (→ mark)

As shown in Fig. 2, form I and III appeared in a few different habits. In form I (110) predominant long prisms were mostly common, through (001) predominant thick lozenge shaped tables sometimes appeared. Form III appeared in slightly elongated prisms or thick tabular crystals as shown in Fig. 2.

Now, it was revealed by the morphological observation that isolated form I crystals exsist in many samples of form III. This evidence was described also by Bettinetti *et al.*,[9] though they could not detect it by IR analysis. Fig. 3, the microscopic photograph of form III, showed the existence of form I crystals (pointed by arrow mark).

Measurement of the Refractive Indices and the Related Optical Properties of 5 Forms of SMD

Three polymorphs, form I, II and III, and two solvates, form IV and V, were investigated by polarizing microscope using both orthoscopic and conoscopic methods. Thick crystals such as form III and sometimes form I were to be crushed into pieces of crystals in an agate

9) G.P. Bettinetti, F. Giordano, and A. La Manna, *Il Farmaco. Ed. Pr.*, **29**, 493 (1974).

mortar before the microscopic observation. Crystals of form III were apt to cleave parallel to (001), therefore the refractive indices of these crystals could be measured conveniently. The results were tabulated in Table II. In Fig. 4 the experimental method of determining

Table II. Refractive indices and Related Properties of Various Forms of Sulfamethoxydiazine

Form	Molecular weight	Crystal system	Refractive indices[a] n_1	n_2	Remarks
Form I	280.3	Monoclinic	1.677 ($n_{\beta'}$)	1.709*($n_{\gamma'}$)	Inclined extinction, *ca.* 8.8°. (110) predominant.
Form II	280.3	Monoclinic	1.635*(n_{β})	1.659 ($n_{\gamma'}$)	Parallel extinction, (001) predominant. Elongation −, Optic sign −.
Form III	280.3	Monoclinic	1.671 ($n_{\alpha'}$)	1.707*($n_{\beta'}$)	Shape irregular, refractive indices were measured in the crashed crystals. (001) predominant.
Form IV	368.4	Monoclinic	1.613 ($n_{\alpha'}$)	1.646*($n_{\gamma'}$)	Long prisms. Parallel or slightly inclined extinction. Elongation +.
Form V	399.7	Orthorhombic	1.629 (n_{α})	1.661*(n_{γ})	Long plates. Parallel extinction. Elongation +.

a) The symbol * denotes the key refractive index. See detail in the text.

the refractive indices using the brightness of Becke-line is shown, in which the estimated brightness are given in abscissa so that the refractive indices are obtained by interpolation at the center line.

From the results of the refractive indices as well as the optical properties of SMD shown in Table II or in Fig. 4, it is supposed that each form of SMD will be identified under the polarizing microscope. Especially form II, the most active one, will be discriminated easily from form I or III by measuring their refractive indices, because there is an enough difference between the key index of form II and that of form I or III as shown in Table II or Fig. 4. However, when a sample contains both form I and III, it is rather difficult to discriminate from each other, because the key refractive indices of both forms are quite similar. In such cases the observation of the related optical and crystallographic properties will be necessary. For instance, when crystals of form I exist in (110) predominant elongated habit, they are discriminated from form III by determining extinction angle.

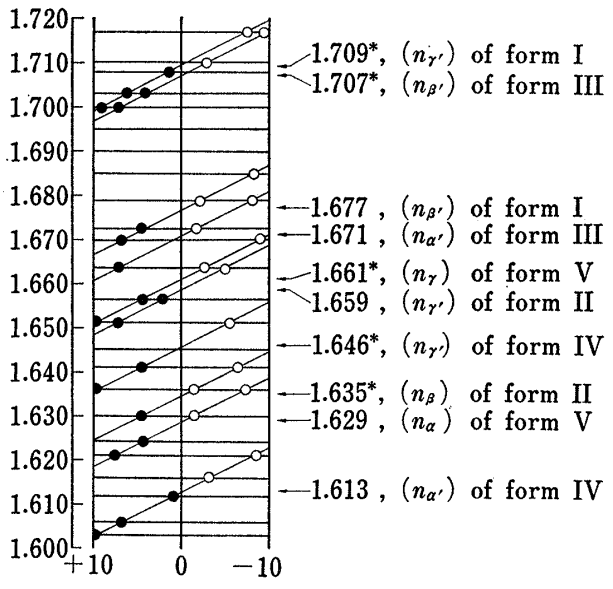


Fig. 4. The Chart for the Determination of Refractive Indices of 5 Forms of Sulfamethoxydiazine, given in Abscissa by a Grade Number of the Brightness of Becke-Line when a Refractive Index of a Crystal is Greater (●) or Smaller (○) than the Immersion Medium

Transition Behavior and Stability of 5 Forms of SMD

On heating the crystals of form I, II and III by the heating stage under a microscope, form I melted simply at 212—214°, while form II transformed into form I at 150—156° showing an immediate change of a birefringence and an extinction angle. On further heating the changed crystals of form I melted at about 212°. On heating the crystals of form III, the transition into form I was observed at about 195° showing the slight change of a birefringence in most cases, and in a few cases the crystals of form III melted at about 190°, then crystallized again on further heating occuring the transition into form I and finally melted at about 212°.

From the above mentioned transition and melting behavior of 3 polymorphic forms of SMD we are able to conclude as followings: Form I, mp 212°, is the stable form at higher temperatures and metastable at the normal temperature. Form II, the biological most active one, is the stable form at lower temperatures metastable at the normal temperature, and unstable at temperatures higher than 150°. Form III is then an only stable form in the normal temperature. Form IV (solvate of dioxane) and form V (solvate of chloroform) are unstable in the air and apt to change opaque crystals releasing their solvents.