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Study of Crystalline Drugs by Means of Polarizing Microscope. I. Identification of Polymorphic or Solvated Forms in Thiamine Hydrochloride, Bromvalerylurea and Ampicillin[1]

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The microscopic study of crystalline substances using a polarizing microscope and the immersion media was shown to have an advantage of simple and rapid identification of drugs, especially in polymorphs or solvates. Examples of the application of this technique were given for four crystal forms of thiamine hydrochloride, two forms of each bromvalerylurea and ampicillin. The accuracy and reproducibility of the measured refractive indices are discussed with its relation to crystal habit.

Keywords——thiamine hydrochloride; bromvalerylurea; ampicillin, anhydrate and trihydrate; polymorph; solvate; polarizing microscope; refractive indices of crystalline drugs; immersion method

One of the authors, Watanabe, reported about refractive indices of crystalline drugs in the Japanese Pharmacopoeia V determined by the immersion method using a polarizing microscope many years ago.[3] The purpose of the work was to develop the practical method of identification of crystalline drugs, in the presence or absence of some ingredients, using the method which was widely used among mineralogists at that time. The study was further developed by Eisenberg,[4] who microscopically investigated crystallographic characteristics of a number of crystalline pharmaceutical substances. Thus in the "National Formulary," the optical crystallographic data of NF drugs compiled by Eisenberg were tabularly described.[5] Winchell[6] also compiled all the reliable data ever reported about the optical crystallographic properties of organic compounds for the purpose of the identification. Nevertheless such method is not so common in pharmaceutical practices at least in Japan.

Recently the pharmaceutical importance of variations in the physical properties associated with differences in crystalline modifications has been numerously pointed out[7] and the establishment of a rapid and simple method of identifying each crystal form in polymorphs or solvates becomes an urgent problem. In this respect the optical crystallographic microscopy may provide a powerful and economical tool for pharmaceutical investigators as well as hospital pharmacists.

The present report is concerned with experimental proof for the utility of the microscopic method in differentiating various crystal forms of some widely used drugs listed in J. P. IX. Thiamine hydrochloride has recently been found to exist in five forms of poly-

1) A part of this work was presented at the 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1976.
2) Location: *Arise, Ikawadani-cho, Tarumi-ku, Kobe 673, Japan.*
3) A. Watanabe, *Yakugaku Zasshi*, **59**, 2 (1939).
4) W.V. Eisenberg, "Optical-Crystallographic Method of Drug Identification," in "Toxicology: Mechanism and Analytical Methods," Vol. 1, ed. by P. Stewart and A. Stolman, Academic Press, New York, 1961, pp. 660—713.
5) First Supplement of "National Formulary XIII," 1041—1056, American Pharmaceutical Association, Washington, D.C., 1970.
6) A.N. Winchell, "The Optical Properties of Organic Compounds," Academic Press Inc., New York, N. Y., 1954.
7) For example: J.K. Haloblian, *J. Pharm. Sci.*, **64**, 1269 (1975).

morphs or solvates by Watanabe and Nakamachi.[8] Two of them, form I (monohydrate) and form II (anhydrate), which were identified microscopically in the present investigation, are probably on the market, though it is not clearly defined in any of pharmacopoeias presently existed in the world.[9] Two polymorphic forms of bromvalerylurea[10] were also discriminated from each other by observing their refractive indices as well as the related optical properties. Both ampicillin anhydrate and trihydrate have been adopted in the J. P. IX, and it was found that both forms were easily identified under microscope, without relying on the iufrared (IR) spectrometry or X-ray diffraction method. The present study was also undertaken to establish a practical technique of the immersion method useful for pharmacists.

Experimental

Materials——Thiamine hydrochloride was purchased from Wako Pure Chemical Ind., Ltd. These crystals (Catalog No. 205-00855) were revealed as form I (monohydrate) by IR spectrometry.[8] Form II (anhydrate) and form IV (methanolate) were prepared by recrystallization of form I.[8] Form III (anhydrate, stable form at higher temperature) was prepared by heating form I at about 150° for an hour or so before the microscopic study.[8] Bromvalerylurea was purchased from Nippon Shinyaku Co., Ltd. These crystals were form I (triclinic). Form II (orthorhombic) was prepared by recrystallization of form I from water.[10] Both ampicillin anhydrate and trihydrate were purchased from the market, and both forms were confirmed by IR spectrometry.[11]

A Polarizing Microscope and Its Accessories——Polarizing Microscope Nikon-POH (Nikon-Kogaku Ind., Ltd.), Na-lamp with spectral starter (Toshiba), microscopic camera Nikon AFM, heating stage for microscope MHS (Union Optical Co., Ltd.) with slide regulator (Matsunaga MFG Co., Ltd.) and Fedrow's universal stage (E. Leitz) were used.

Preparation of the Set of Immersion Media——Abbe refractometer (Atago-Kogaku Ind., Ltd.) was used. A permanent set of immersion media covering the range between 1.42 and 1.70 at intervals of 0.003—0.006 was prepared according to the method mainly originated by Watanabe.[3] The following media and their mixtures were used: *n*-decane (1.420), olive oil (1.470), ceder wood oil (1.522), α-bromonaphthalene (1.658) and methylene iodide (1.703). The refractive indices of immersion media were corrected by the temperature coefficients.[3]

Measurement of Refractive Indices of Crystals——Refractive indices of crystals were determined by immersing the crystals in a suitable immersion medium and observing the movement of Becke line as the microscope objective was moved up and down through the focus. In the present study the brightness of the Becke line was evaluated by the number between +10 and −10. When the refractive index of the crystal was greater than that of the liquid, the brightness was described by a positive number, +10 to 0, while in the opposite case a negative number, 0 to −10, was assigned. The observed brightness expressed in this way was then plotted on the chart of immersion media and drew a straight line through the plots. The refractive index of the crystal was then determined from the point of intersection with the center line as shown in Fig. 1 for the case of thiamine hydrochloride.

Results and discussion

Thiamine Hydrochloride

Form I, form II and form IV were stable for the microscopic observation at room temperature. The measured refractive indices as well as the optical properties are shown in Fig. 1 and Table I.

Form I, II and IV can be identified from each other by their refractive indices. Especially form II was easily distinguished from form I or IV by observing their interference color using the gypsum plate since form I and IV showed far stronger birefringence than form II.

8) A. Watanabe and H. Nakamachi, *Yakugaku Zasshi*, **96**, 1236 (1976).
9) For example: U.S. Pharm. XIX, Europ. Pharm. I, Brit. Pharm. (1973), Pharm. Franc. IX, Deutsch. Arzneib. 7 Auf. and J.P. IX adopted anhydrous formula, while Internat. Pharm. WHO, Pharm. Helv. VI, Nedel. Farm., Pharm. Hung. VI and Osterreich. Arzneib. 9 Auf. adopted monohydrous formula without any description about their modification.
10) A. Watanabe, *Yakugaku Zasshi*, **58**, 41 (1938).
11) K. Kamiya and M. Nishikawa, *Takeda Kenkyusho Nempo*, **29**, 617 (1970).

It was also noticed that on heating the stage a part of form II was converted into form III[8] accompanied with apparent change of the interference color.

Form III obtained from form I by heating at about 150° on the heating stage showed nearly the same microscopic appearance as that of form I, though the result of the measurement of refractive indices revealed the slight difference between them. Form V[8] obtained from form IV by releasing methanol was a opaque congregation of fine crystals and it was difficult to determine any optical crystallographic data from these crystals.

Table I. Refractive Indices and Related Properties of Thiamine Hydrochloride

Modification	Molecular weight	Crystal system	Refractive indices n_1	Refractive indices n_2	Remarks
Form I (Monohydrate)	355.3	Monoclinic	1.605 (n_α)	1.689 (n_γ)	Long plates, lamellar. Extinction inclined (17.6°).
Form II (Anhydrate)	337.3	Monoclinic	1.617	1.638	Irregular plates. Extinction inclined.
Form III (Anhydrate)	337.3	Monoclinic	1.607 (n_α)	1.695 (n_γ)	Shape and extinction are the same as those of form I.
Form IV (Methanolate)	369.3	Triclinic	1.590 ($n_{\alpha'}$)	1.675 ($n_{\gamma'}$)	Tabular or triclinic pinacoid. Extinction inclined (18.6°).

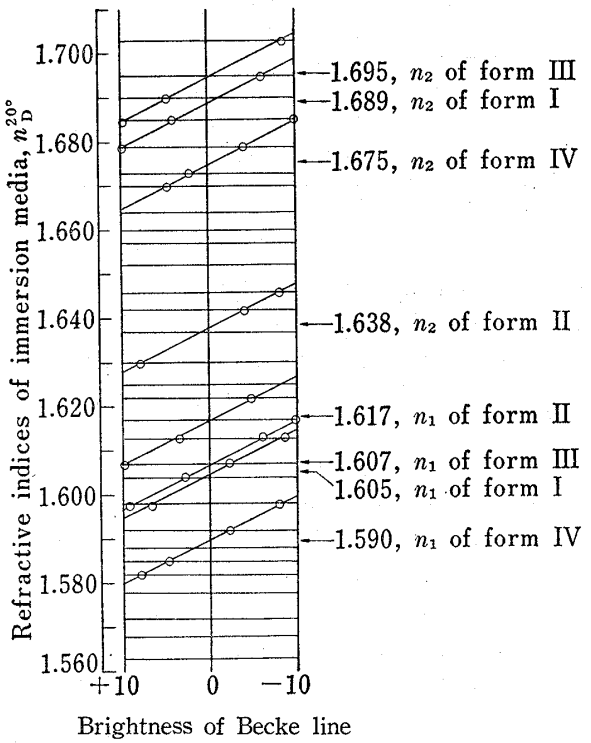

Fig. 1. Determination of Refractive Indices of Thiamine Hydrochloride

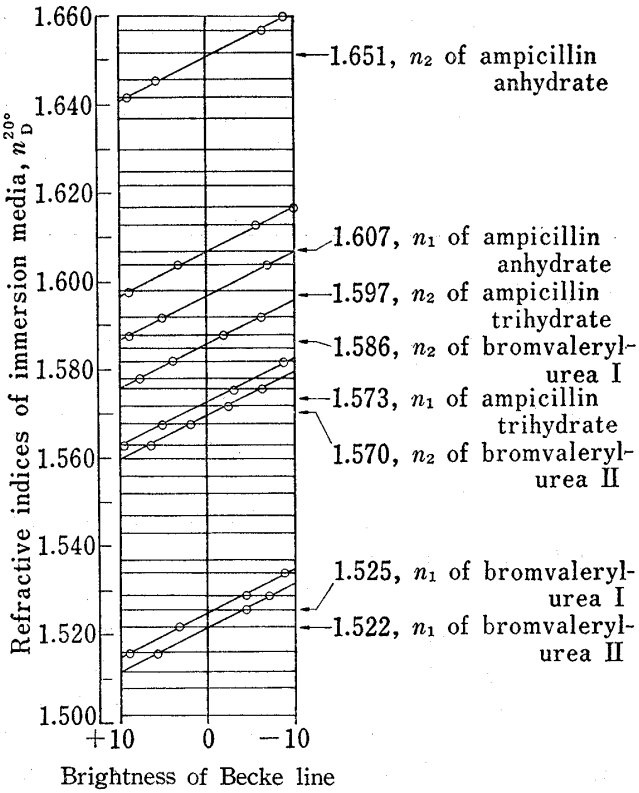

Fig. 2. Determination of Refractive Indices of Bromvalerylurea and Ampicillin

Bromvalerylurea

This substance was known to exist in two polymorphic forms.[10] Form I crystallized in triclinic trapezoidal thin plates, and form II was obtained in orthorhombic prisms or rods, sometimes in needles, elongated along the c-axis. As the thin plates of form I were grown

approximately on the X-Z plane, the refractive indices, $n_{\alpha'}$ and $n_{\gamma'}$ were determined as shown in Fig. 2. In the case of form II, Z was parallel with the *c*-axis (elongation +) and n_γ was accurately determined, while the measurement of the accurate value of n_α or n_β was rather difficult owing to the crystal habit, which probably caused the discrepancies between the data in the 'NF' and ours. The transition of form I into form II was observed at about 96° on heating the stage. In Table II the measured refractive indices as well as the optical microscopical data are shown.

Table II. Refractive Indices and Related Properties of Bromvalerylurea and Ampicillin

Substance and Form	Molecular weight	Crystal system	Refractive indices n_1	Refractive indices n_2	Remarks
Bromvalerylurea					
Form I	223.1	Triclinic	1.525 ($n_{\alpha'}$)	1.586 ($n_{\gamma'}$)	Trapezoidal plates. Extinction inclined. NF: 1.526(n_α), 1.57(n_β), 1.586(n_γ).
Form II	223.1	Orthorhombic	1.522 ($n_{\alpha'}$)	1.570 (n_γ)	Rods or needles. Extinction parallel. Elongation positive. NF: 1.519(n_α), 1.583(n_β), 1.599(n_γ).
Ampicillin					
Anhydrate	349.4	Monoclinic	1.607 (n_β)	1.651 ($n_{\gamma'}$)	Lamellar. Extinction parallel. Elongation negative.
Trihydrate	403.4	Orthorhombic	1.573 (n_α)	1.597 (n_β)	Needles. Extinction parallel. Elongation positive or negative (rare).

Ampicillin

X-ray crystallographic data of two forms of ampicillin were reported by James, *et al.*[12], who described the crystal system of ampicillin anhydrate and trihydrate as monoclinic and orthorhombic, respectively. The refractive indices as well as other optical crystallographic characteristics of these two forms observed microscopically in the present study are shown in Fig. 2 and Table II.

Crystals of ampicillin anhydrate were thin small plates, which had parallel extinction, negatively elongated along Y, and were nearly perpendicular with an acute bisectrix X. Therefore n_β and $n_{\gamma'}$ were determined under microscope. While ampicillin trihydrate appeared in needles having parallel extinction and positive elongation along Y. In most of the cases, Z was normal to the narrow plane of needle, but in rare cases when the needle rotated around the needle axis in the immersion medium, X became normal to the needle (that is, X was parallel along the microscope axis). In such cases elongation was observed negative and an approximate value of n_γ was determined.

The above results suggest that we can identify a certain form in polymorphs or solvates using a polarizing microscope simply and rapidly. The refractive indices of each form measured by the immersion method furnish an important key for the identification of each form. Therefore wide acceptability of this method in future concerns mostly with the accuracy and reproducibility of this technique. In this respects we tried to establish a routine

12) M.N.G. James, D. Hall, and D.C. Hodgkin, *Nature*, **220**, 168 (1968).

but accurate technique of measuring two refractive indices, n_1 (lower) and n_2 (higher), from crystals in their natural crystal habit using the chart of the immersion media as described in the experimental section. From the results investigated in this article we found that the error of the data is usually about ± 0.001 though it depends upon the crystal habit of substance. From platy or lamellar crystals refractive indices, n_1 and n_2, are accurately measurable, while from acicular or prismatic crystals usually only one of these indices can be measured accurately when the crystals have parallel extinction. When the crystals have parallel extinction and positive elongation, n_2 is accurately measured, and when the crystals have parallel extinction and negative elongation, n_1 is accurately measured. In these cases the measurement of another refractive index (that is n_1 and n_2, respectively) is usually difficult, because crystals are apt to rotate around the axis of the elongated direction in the immersion medium, as occurred in ampicillin trihydrate crystals.

A further investigation on this line is currently under way in our laboratory.

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