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Study of Crystalline Drugs by Means of a Polarizing Microscope. VIII.[1] A Simple and Rapid Method to Measure the Thickness of a Crystalline Drug and Its Application to the Quality Testing of Poorly Soluble Drugs[2]

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A practical application of polarizing microscopy has been investigated by using key refractive indices (n_1, n_2) of group A drugs (lamellar, scales or plates). A graphic illustration of the retardation plotted $\log(n_2-n_1)$ as the abscissa and $\log(D)$ as the ordinate, where D denotes the thickness of the sample, gives a simple striped pattern composed of parallel straight lines having various retardations. As group A drugs have unique values of $\log(n_2-n_1)$, the thickness of a crystalline particle can easily be obtained from the graph by observing the interference color of the crystal under crossed polars.

When the thickness of the crystal is obtained, the specific surface area can be calculated by the general microscopic method. This procedure could be useful for quality testing of poorly soluble drugs.

Keywords——polarizing microscopy; key refractive indices; crystal thickness measurement; specific surface area calculation; poorly soluble drug bioavailability; ampicillin anhydride; cephaloridin

The author has studied the practical application of polarizing microscopy and has already reported the key refractive indices[3] as well as related optical characteristics of more than 170 crystalline drugs listed in the Japanese Pharmacopoeia X (JP X) in conjunction with co-workers.[1,4,5] It has been shown that nearly 60% of the investigated drugs are lamellar, scales, thin plates or plates in habit and they can be conveniently characterized and identified by measuring their key refractive indices using an improved immersion method.[6] In the previous paper[1] it was shown that for analytical purposes, a graphic representation of $\log(n_2-n_1)$ against (n_1, n_2) is useful.

In the present paper, the retardation (R) or retardation color is plotted or painted on a graph taking $\log(n_2-n_1)$ as the abscissa and $\log(D)$, the common logarithm of thickness, as the ordinate. This gives a simple striped pattern composed of parallel straight lines or color bands of various retardations or retardation colors as shown in Fig. 2.[7] With this chart, the thickness of a crystalline drug can be easily obtained from its value of $\log(n_2-n_1)$ and the retardation or retardation color estimated from an interference color observed under crossed polars. When the thickness of a crystalline drug is known, the specific surface area can be calculated more accurately than by the general microscopic method, so that the dissolution behavior (and hence bioavailability) can be estimated more reliably, especially in the case of poorly soluble drugs.

Results and Discussion

In Fig. 1 a graphic representation of the key refractive indices (n_1, n_2) of some group A drugs in the JP X is shown taking $\log(n_2-n_1)$ as the abscissa and (n_1, n_2) as the ordinate. The

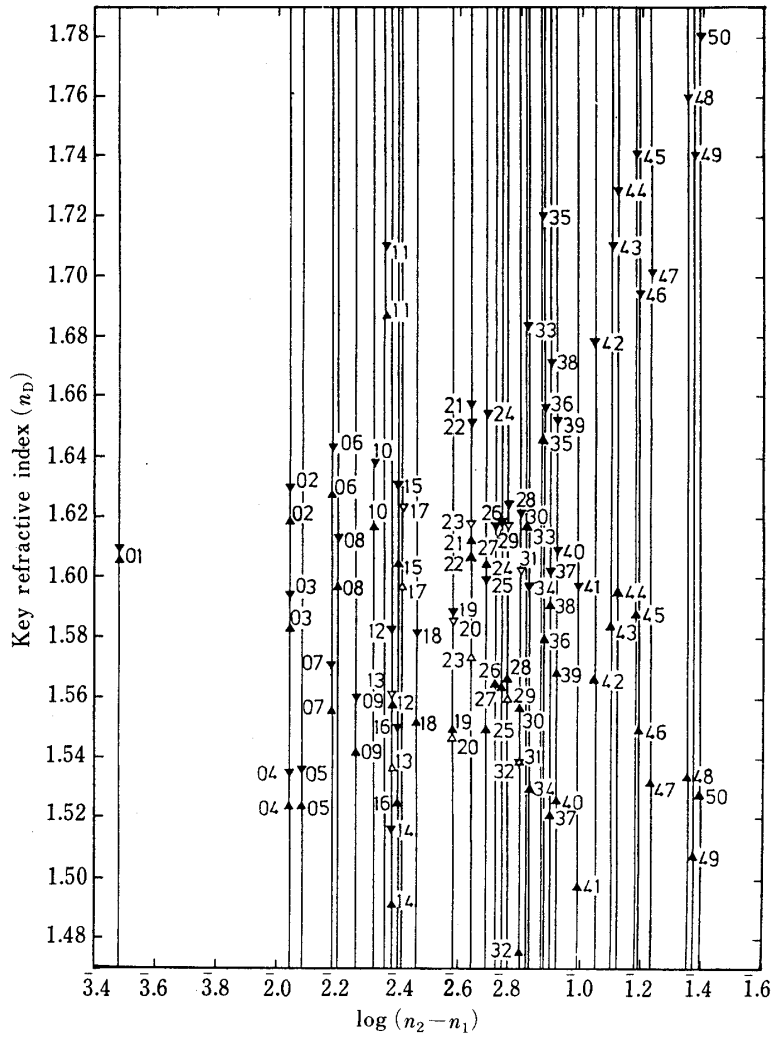


Fig. 1. Correlation between Key Refractive Indices (n_1, n_2) and $\log(n_2-n_1)$ of Some Group A Drugs Listed in JP X

Numbers in Fig. 1 correspond to those in Table I. n_1, ▲ or △; n_2, ▼ or ▽.

names and numbers of the drugs are listed in Table I. As shown in Fig. 1, the straight line drawn along the ordinate from each $\log(n_2-n_1)$ on the abscissa represents a unique character of each drug, and these straight lines are important not only for the purpose of analysis or identification of the drugs but also to measure the thickness of a crystal section with reference to the retardation, as will be described below.

As is known generally among mineralogists, there is a relation between birefringence (n_2-n_1), retardation R and thickness D as shown by the following equation:

$$D=R/(n_2-n_1) \tag{1}$$

where the birefringence (n_2-n_1) is the difference of the two key refractive indices, the retardation R is the value of the wavelength in nm estimated from the main interference color of the crystal section under crossed polars and D is the thickness of the section along the direction of the microscope axis. By taking the common logarithms of both sides of Eq. 1, retardations can be drawn on rectangular coordinates as shown in Fig. 2, in which the value of $\log(n_2-n_1)$ is shown on the abscissa and that of $\log(D)$ on the ordinate. In Fig. 2 the loci of retardations as well as retardation colors of various drugs form a simple striped pattern composed of parallel straight lines or colored bands. When a straight line is drawn from the

TABLE I. Names, $\log(n_2-n_1)$, and Numbers in Fig. 1 and in the Literature of Some Group A Drugs in JP X

No. in Fig. 1	Name	$\log(n_2-n_1)$	No. in lit.
1	Methylbenactizium bromide	$\bar{3}.48$	231[5]
2	Cephalexin	$\bar{2}.04$	A15[4]
3	Cycloserine	$\bar{2}.04$	A12[4]
4	Diethylcarbamazine citrate	$\bar{2}.04$	643[1]
5	Ethambutol hydrochloride	$\bar{2}.08$	224[5]
6	Sulfamethizole	$\bar{2}.18$	656[1]
7	Kainic acid	$\bar{2}.18$	642[1]
8	Cocaine hydrochloride	$\bar{2}.20$	812[6]
9	Procaine hydrochloride	$\bar{2}.26$	144[1]
10	Thiamine hydrochloride II	$\bar{2}.32$	302′[1]
11	Tetracycline hydrochloride	$\bar{2}.36$	A-5[4]
12	Aspirin aluminium	$\bar{2}.38$	102[5]
13	L-Isoleucine	$\bar{2}.38$	321[1]
14	Erythromycin ethylsuccinate	$\bar{2}.38$	A-3[4]
15	Phenytoin	$\bar{2}.40$	116[5]
16	Nicotinamide	$\bar{2}.40$	306[1]
17	Diphenhydramine hydrochloride	$\bar{2}.42$	162[1]
18	L-Valine	$\bar{2}.46$	324[1]
19	Methycilline sodium	$\bar{2}.58$	A28[4]
20	Cortisone acetate	$\bar{2}.58$	151[1]
21	Quinine hydrochloride	$\bar{2}.64$	641[1]
22	Ampicillin anhydride	$\bar{2}.64$	A-2[4]
23	Dextromethorphan hydrobromide	$\bar{2}.64$	227[5]
24	Imipramine hydrochloride	$\bar{2}.69$	106[5]
25	Tolubutamide I	$\bar{2}.69$	354[1]
26	Cephalotin sodium	$\bar{2}.72$	A16[4]
27	Oxyphenbutazone	$\bar{2}.74$	108[5]
28	Norethisterone	$\bar{2}.76$	245[5]
29	Naphazoline nitrate	$\bar{2}.76$	147[1]
30	Phenobarbital	$\bar{2}.80$	118[5]
31	Epinephrin	$\bar{2}.80$	223[5]
32	Trimethadione	$\bar{2}.80$	112[5]
33	Tetracycline	$\bar{2}.82$	A18[4]
34	L-Threonine	$\bar{2}.83$	323[1]
35	Sulfisoxazole	$\bar{2}.87$	657[1]
36	Prednisolone acetate	$\bar{2}.88$	152[1]
37	Sulpyrine	$\bar{2}.90$	111[5]
38	Quinidine sulfate	$\bar{2}.90$	212[5]
39	Aspirin	$\bar{2}.92$	101[5]
40	Chloramphenicol	$\bar{2}.92$	A-9[4]
41	Chloramphenicol sodium succinate	$\bar{2}.99$	A10[4]
42	Propranolol hydrochloride	$\bar{1}.05$	204[5]
43	Haloperidol	$\bar{1}.10$	114[5]
44	Cephaloridin	$\bar{1}.12$	A17[4]
45	Sulfisomidine	$\bar{1}.18$	658[1]
46	Noscapin hydrochloride	$\bar{1}.19$	228[5]
47	Acetazolamide	$\bar{1}.23$	201[5]
48	Trichlormethiazide	$\bar{1}.35$	208[5]
49	Isoniazid	$\bar{1}.37$	651[1]
50	Hydralazine hydrochloride	$\bar{1}.39$	202[5]

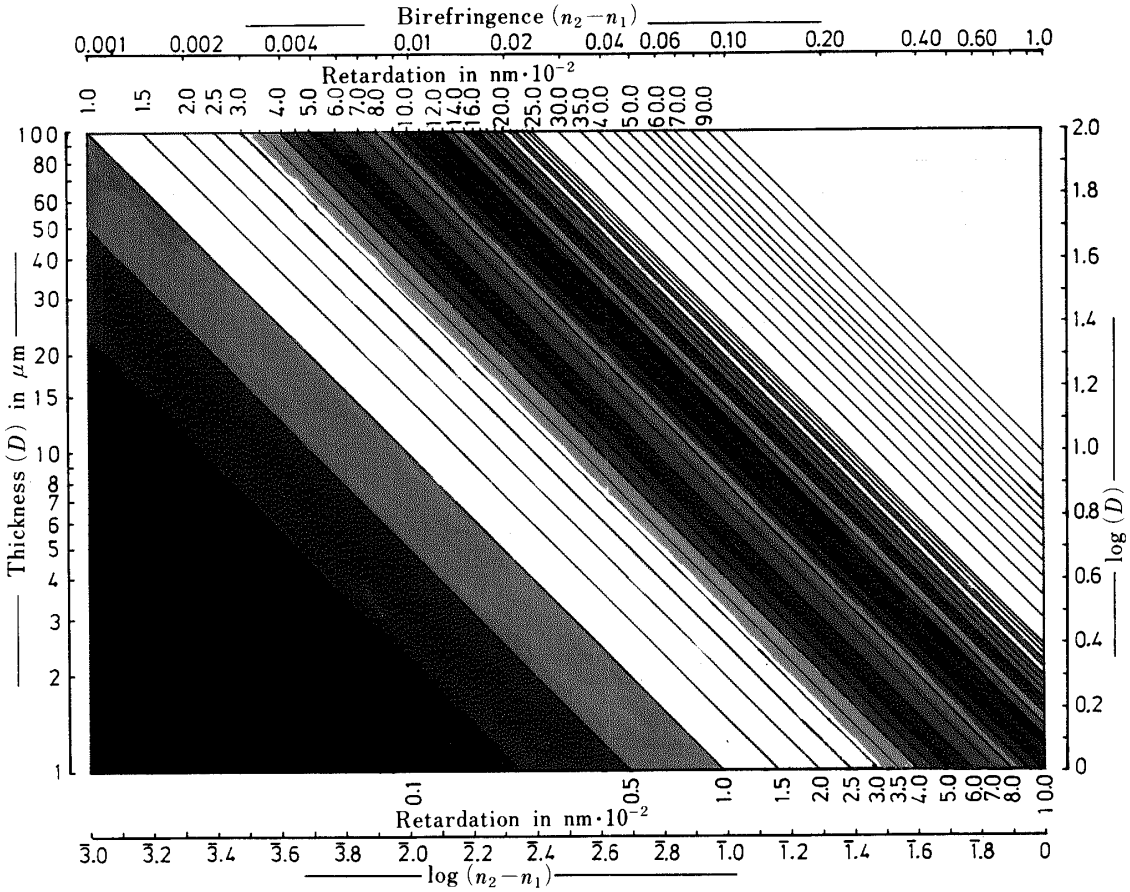


Fig. 2. Chart for Evaluating the Thickness of a Crystal Section from the Retardation or Retardation Color and $\log(n_2-n_1)$

point of the measured value of $\log(n_2-n_1)$ along the ordinate (as in Fig. 1) in the case of Fig. 2, the line intersects at various points with the oblique lines of retardations or cuts various colored bands. Each intersecting or cutting position corresponds to the thickness of the crystalline particle. In this way, Fig. 2 can be used to estimate the thickness of drugs if the key refractive indices are known; it is straight forward to find a color with similar tone to the interference color of the crystalline particle. However, the colored region is restricted to between 50 to 2100 nm at most, and the colors between 50 to 250 nm are white or whitish grey, so that it is difficult to evaluate the retardation accurately. In such cases, the use of a gypsum plate is recommended.[8] The names of retardation colors and their abbreviations, and the changes of the colors seen upon inserting a gypsum plate (R: 530 nm) are shown in Table II.[9]

In general, when the thickness is obtained, it is easy to calculate a specific surface area as reported in the case of aspirin crystals.[10] In the case of crystals belonging to group A, three dimensional parameters a, b, and c (thickness) can be approximately evaluated by usual microscopic observation as in the case of aspirin, and by using those parameters the specific surface area can be calculated as follows:

$$\text{surface area of an average-sized crystal} = 2(ab+bc+ca)$$

$$\text{volume of an average-sized crystal} = abc$$

$$\text{specific surface area} = 2(ab+bc+ca)/abc\rho, \ (\rho\text{: density}) \qquad (2)$$

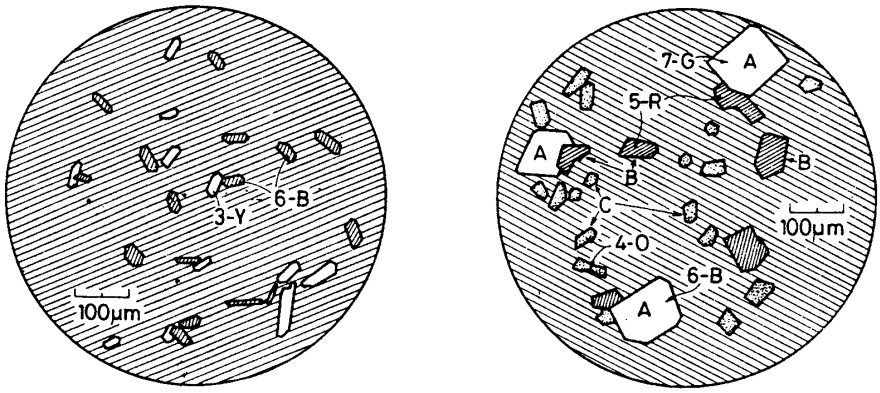
When the specific surface area is obtained, the dissolution behavior can be estimated by the Noyes–Whitney equation:

TABLE II. Retardations, Interference Colors (Abbreviations), and Their Changes upon Inserting a Gypsum Plate (R: 530 nm)

Retardation in nm	Color (Abbreviation)	Changes of colors inserting a gypsum plate	
		Vib. direction coincident	Vib. direction opposed
0	Black (0-Bl)	5-R	5-R
50	Iron grey (0-IGr)	5-V	4-O
100	Lavender grey (1-LGr)	6-I	3-Y
150	Grey (1-Gr)	6-B	3-Y
200	White (2-W)	7-G	2-LY
250	Light yellow (2-LY)	7-YG	2-W
300	Yellow (3-Y)	8-Y	1-Gr
350	Yellow (3-Y)	8-Y	1-LGr
400	Orange (4-O)	9-O	0-IGr
450	Orange (4-O)	9-OR	0-Bl
500	Red (5-R)	10-OR	1-LGr
550	Violet (5-V)	10-VR	1-Gr
600	Indigo (6-I)	11-I	2-W
650	Blue (6-B)	11-I	2-LY
700	Green (7-G)	12-GB	3-Y
750	Yellow green (7-YG)	12-G	3-Y
800	Yellow (8-Y)	13-GY	4-O
850	Yellow (8-Y)	13-GY	4-O
900	Orange (9-O)	14-C	5-R
950	Orange red (9-OR)	14-C	5-R
1000	Orange red (10-OR)	15-DP	5-V
1050	Dark violet red (10-VR)	15-DP	6-I
1100	Indigo (11-I)	16-GB	6-B
1150	Indigo (11-I)	16-GB	7-G
1200	Greenish blue (12-GB)	17-G	7-YG
1250	Green (12-G)	17-G	7-YG
1300	Greenish yellow (13-GY)	18-LG	8-Y
1400	Carmine (14-C)	19-GGr	9-O
1500	Dull purple (15-DP)	20-WGr	10-VR
1600	Grey blue (16-GB)	21-FR	11-I
1700	Bluish green (17-BG)	22-W	12-GB
1800	Light green (18-LG)	23-W	13-GY
1900	Greenish grey (19-GGr)	24-W	14-C
2000	Whitish grey (20-WGr)	25-W	15-DP
2100	Fresh red (21-FR)	26-W	16-GB

$$dm/dt = KS(C_s - C) \tag{3}$$

where dm/dt is the dissolution velocity, K is the velocity constant, C_s and C are the concentrations of the substance in saturated and initial solutions and S is the specific surface area calculated by using Eq. 2. In pharmaceutical preparations of crystalline drugs, the particle size of a drug often differs depending on the manufacturer, or even in different lots from a single manufacturer, and particles of various sizes may coexist in a pack of a preparation. In such cases, by measuring different sets of parameters, various specific surface areas can be calculated, and the dissolution behavior can be discussed for samples having different values of S in Eq. 3. This is important in relation to the bioavailability of powdered crystalline drugs, especially poorly soluble drugs, and the procedure should therefore be applicable to quality testing.



(a) ampicillin anhydride under crossed polars with a gypsum plate
▨, vib. direct. coincid. 6-B;
▱, vib. direct. opposed 3-Y.

(b) cephaloridin under crossed polars
□, size A, 6-B or 7-G;
▨, size B, 5-R;
▩, size C, 4-C.

Fig. 3. Tracings of Photomicrographs of Ampicillin Anhydride and Cephaloridin

Experimental

Instrument——A polarizing microscope, Olympus POS, was used.

Material——JP X grade drugs were used.

Measurement of the Thickness, and Calculation of the Specific Surface Area——Example 1. Ampicillin Anhydride: A tracing of a photomicrograph of ampicillin anhydride crystals under crossed polars through a gypsum plate is shown in Fig. 3(a),[11] in which the interference colors of the crystals are described by the abbreviations listed in Table II. They are either blue or yellow depending on the vibration direction of normal and abnormal light against the inserted gypsum plate, and the value of retardation was estimated to be about 150 nm from Table II. Ampicillin anhydride crystals are lamellar and the key refractive indices are as follows[4]: $n_1 = 1.602$, $n_2 = 1.651$, $\log(n_2 - n_1) = \bar{2}.64$. A straight line is drawn parallel to the ordinate from the point of $\bar{2}.64$ on the abscissa and the point of intersection with the oblique line of retardation of 150 nm was obtained. A straight line was then drawn along the abscissa from this point to the ordinate. Thus the thickness was estimated to be 3.5 μm, and the habit parameters of the ampicillin anhydride crystals were obtained as follows: $a = 0.02$ mm, $b = 0.05$ mm and c(thickness) $= 0.0035$ mm. Results of the calculations: V(volume) $= abc = 0.0000035\,\text{mm}^3$, S(surface area) $= 0.00245\,\text{mm}^2$, SS(specific surface area) $= S/V = 700\,\text{mm}^{-1}/\rho$.

Example 2. Cephaloridin: A tracing of a photomicrograph of cephaloridin crystals under crossed polars is shown in Fig. 3(b),[11] in which the interference colors are indicated by the abbreviations listed in Table II. As cephaloridin crystals showed different particle sizes, they were divided into 3 groups A, B and C. These groups showed different interference colors but had the same refractive indices. Using the same straight line drawn from the point of $\log(n_2 - n_1) = \bar{1}.12$ on the abscissa, 3 intersecting positions were obtained for 3 kinds of retardation or retardation color in Fig. 2. By the same method as described in example 1, habit parameters were obtained microscopically, and the 3 values of specific surface area were calculated. The results are as follows: Habit parameters (mm), A: a 0.09, b 0.11, c 0.005; B: a 0.06, b 0.08, c 0.004; C: a 0.02, b 0.04, c 0.003. Surface area (mm^2), A: 0.0218; B: 0.0407; C: 0.00196. Specific surface area (mm^{-1}), A: 440; B: 558.3; C: 816.7. Content (%), A: 48.4; B: 37.5; C: 14.0. The mean value of specific surface area (mm^{-1}) was 530.8.

References and Notes

1) Part VII: A. Watanabe, Y. Yamaoka, K. Kuroda, T. Yokoyama, and T. Umeda, *Yakugaku Zasshi*, **105**, 481 (1985).
2) This work was presented at the 35th General Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Kyoto, Nov. 1985.
3) Key refractive indices are those measured from the natural section lying parallel to the microscope stage by the immersion method. They may coincide with the principal refractive indices or not, depending upon the crystal system and the habit. A. Watanabe, Y. Yamaoka, and K. Kuroda, *Chem. Pharm. Bull.*, **28**, 372 (1980).
4) A. Watanabe, Y. Yamaoka, K. Kuroda, and T. Yokoyama, *Yakugaku Zasshi*, **104**, 896 (1984).
5) A. Watanabe, Y. Yamaoka, E. Akaho, K. Kuroda, T. Yokoyama, and T. Umeda, *Chem. Pharm. Bull.*, **33**, 1599

(1985).
6) A. Watanabe, Yu. Tanaka, and Yo. Tanaka, *Chem. Pharm. Bull.*, **25**, 2239 (1977).
7) As an example of a graph correlating retardation color, thickness and (n_2-n_1), there is Michel-Levy's interference color chart. S. Tsuboi, "Henkokenbikyo," Iwanami Shoten, Tokyo, 1975, p. 161.
8) N. H. Hartshone and A. Stuart, "Crystals and the Polarizing Microscope," E. Arnold Ltd., London, 1970.
9) This table corresponds to the colored figure in the previous paper. A. Watanabe, Y. Yamaoka, E. Akaho, K. Kuroda, T. Yokoyama, and T. Umeda, *Chem. Pharm. Bull.*, **33**, 1601 (1985).
10) A. Watanabe, Y. Yamaoka, and K. Takada, *Chem. Pharm. Bull.*, **30**, 2958 (1982).
11) Shown by the colored figure in A. Watanabe, Y. Yamaoka, K. Kuroda, and T. Yokoyama, *Yakugaku Zasshi*, **104**, 900 (1984).