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Physicochemical Properties of Crystalline Lactose. I. Estimation of the Degree of Crystallinity and the Disorder Parameter by an X-Ray Diffraction Method

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A computer program for the determination of the degree of crystallinity and the disorder parameter in lactose based on Ruland's method, one of the X-ray diffraction methods, was written. The correctness of the program was confirmed by comparing the computed results with those calculated by the usual Ruland's method and Hermans' method using several samples of lactose with different crystallinities. A series of integral upper limits was determined by rearranging the equation presented by Ruland. The degree of crystallinity and the disorder parameter in crystalline lactose which was used as the 100% crystalline standard in the other methods were estimated as *ca.* 70% and *ca.* 3.5 Å^2, respectively. The effects of the normalization constant on the calculated results were rather small. However, the effects of integral upper limits on those results were large. A series of integral upper limits seems to be a very important factor for crystallinity determination by Ruland's method.

Keywords——lactose; crystallinity; disorder parameter; X-ray diffraction; Ruland's method; Hermans' method; computer program

Crystalline lactose is the most widely used diluent for solid preparations, and many technical studies in which crystalline lactose was used have been reported. However, little basic research on its physicochemical properties related to crystallinity has been done. The crystallization of amorphous lactose powder by moisture up take was reported by Bushill *et al.*[2a] Hüttenrauch *et al.*[2b] indicated that the degree of crystallinity in crystalline lactose dec reased with grinding and that these changes affected the compression characteristics. Otsuka *et al.*[2c,2d] demonstrated that the moisture sorption and volume expansions of amorphous or α-anhydrous lactose tablets occurred even at relatively low humidity, leading to crystallization. Changes of crystallinity in crystalline lactose may cause the frequently observed lot-to-lot variation and affect the macroscopic characteristics relevant to pharmaceutical technology.

The purpose of this work was to develop an accurate method to estimate the degree of crystallinity and the disorder parameter in crystalline lactose. Crystallinity has not often been measured in organic compounds with low molecular weights, though data have been obtained by X-ray diffractometry, infrared spectrometry, nuclear magnetic resonance spectrometry, or calorimetry.[3,4] Many of these methods are based on regression analysis. The values seem to depend considerably on the correct determination of the 100% crystalline and amorphous standards. In this report, Ruland's method,[3b] one of the X-ray methods, was applied. This method is theoretically rigorous, but much time is necessary for rigorous calculations to prepare the nomograms with theoretical intensities, to make corrections for many kinds of factors affecting the intensities, to carry out normalization, and to make many graphical integration measurements (the usual Ruland's method). A computer program based on this method for lactose was written and the values were calculated for crystalline lactose which was used as the 100% crystalline standard.

Theoretical

Ruland's Method

Ruland[3b] and Kilian[3c] presented similar methods of crystallinity determination based on Hosemann's paracrystal theory.[5] According to Ruland's method, the degree of crystallinity, X_{cr}, is given by

$$X_{cr}=\frac{\int_0^\infty S^2 I_{cr}(S)\mathrm{d}S}{\int_0^\infty S^2 I(S)\mathrm{d}S}\times\frac{\int_0^\infty S^2\overline{f^2}\mathrm{d}S}{\int_0^\infty S^2\overline{f^2}D\mathrm{d}S} \qquad \text{(Eq. 1)}$$

where S is the magnitude of the reciprocal space vector ($S=2\sin\theta/\lambda$), θ the angle between the atomic plane and both the incident and reflected beams, λ the wavelength of the X-rays, I_{cr} (S) and I (S) the coherent scattering intensities at S in the crystalline and total regions, respectively, $\overline{f^2}$ the mean squared amplitude of the atomic scattering factor ($\overline{f^2}=\sum N_i f_i^2/\sum N_i$), N_i and f_i the number of atoms of type i and the atomic scattering factor, respectively, and D the disorder function. D is expressed as follows,

$$D=\exp(-kS^2) \qquad \text{(Eq. 2)}$$

$$k=k_{\mathrm{T}}+k_{\mathrm{I}}+k_{\mathrm{II}} \qquad \text{(Eq. 3)}$$

where k expresses the disorder parameter, k_T the thermal motion, and k_{I} and k_{II} the lattice imperfections of the first and second kinds. Eq. 1 is applicable to a number of integration intervals over larger regions, so that X_{cr} can be written as

$$X_{cr}=X'_{cr}\times K \qquad \text{(Eq. 4)}$$

$$X'_{cr}=\frac{\int_{S_0}^{S_p} S^2 I_{cr}(S)\mathrm{d}S}{\int_{S_0}^{S_p} S^2 I(S)\mathrm{d}S} \qquad \text{(Eq. 5)}$$

$$K=\frac{\int_{S_0}^{S_p} S^2\overline{f^2}\mathrm{d}S}{\int_{S_0}^{S_p} S^2\overline{f^2}D\mathrm{d}S} \qquad \text{(Eq. 6)}$$

where the integral upper limit S_p and the integral lower limit S_0 satisfy Eq. 7 and are independent of the crystallinity.

$$\int_{S_0}^{S_p} S^2\overline{f^2}\mathrm{d}S=\int_{S_0}^{S_p} S^2 I(S)\mathrm{d}S \qquad \text{(Eq. 7)}$$

These integral regions can be found by examining several samples with different crystallinities, and then a series of X'_{cr} is calculated by using the series of determined S_p from Eq. 7. Since X_{cr} does not depend on these upper limits, a series of K which keeps X_{cr} constant can be determined. Thus, the degree of crystallinity and the disorder parameter can be simultaneously obtained.

Hermans' Method[3a)]

The relationships between the mass and the integrated intensity of the reflection are given by

$$M_c=k_c\int I_c(\theta)\mathrm{d}\theta \qquad \text{(Eq. 8)}$$

$$M_a=k_a\int I_a(\theta)\mathrm{d}\theta \qquad \text{(Eq. 9)}$$

$$M=M_c+M_a \qquad \text{(Eq. 10)}$$

where M_c, M_a, and M denote the mass of crystalline, amorphous and total regions, respectively, I_c (θ) and I_a (θ) the coherent scattering intensities in the crystalline and the amorphous regions, and k_c and k_a are constants. From Eqs. 8, 9, and 10 the following relationships are obtained:

$$\int I_a(\theta)\mathrm{d}\theta=-(k_c/k_a)\int I_c(\theta)\mathrm{d}\theta+M/k_a \qquad \text{(Eq. 11)}$$

$$X_{cr}=\frac{M_c}{M}=\frac{\int I_c(\theta)\mathrm{d}\theta}{\int I_c(\theta)\mathrm{d}\theta+(k_a/k_c)\int I_a(\theta)\mathrm{d}\theta}. \qquad \text{(Eq. 12)}$$

Eq. 11 indicates a linear relationship between $\int I_a(\theta)d\theta$ and $\int I_c(\theta)d\theta$, and the gradient gives the value of k_c/k_a. Then, the degree of crystallinity is given by substituting the k_a/k_c value into Eq. 12.

Experimental

Materials——Crystalline lactose (Wako Pure Chem. Ind., Ltd.) was of reagent grade. The samples with different crystallinities were prepared by grinding with a shaker ball mill (Yanagimoto Co., Ltd.). Grinding conditions were as follows. The volume of the stainless steel mill was about 38 cm^3, the number of balls 17, the diameter of each ball 11 mm, and the sample weight 1 g. Grinding time was changed from 1 h to 10 h.

Measurements of X-Ray Diffraction——The X-ray diffraction scattering was measured at room temperature with a Geiger Flex D-3F diffractometer (Rigaku Denki Co., Ltd.) using a Geiger–Müller counter. The X-ray source was copper-Kα with a nickel filter, voltage 30 kV, current 10 mA, time constant 1 s, receiving slit 0.15°. A symmetrical–reflection goniometer was scanned at 2° a minute between $2\theta=150°$ and $2\theta=5°$. Thick samples were used in order to minimize the absorption in the samples. The count range was changed in several regions for one sample and the X-ray scattering was measured in the most appropriate count range. The angular calibration was performed using silicon reflections.

In Hermans' method, the goniometer was scanned between $2\theta=52°$ and $2\theta=5°$. The well-ordered crystal of sucrose was used to make corrections for the diffuse scattering due to the thtrmal agitation of the atoms and the Compton scattering. The background intensity was subtracted from that of lactose over the range. Crystalline peaks were separated from the diffuse scattering due to the noncrystalline scattering and the lattice imperfections by the usual freehand method.

Calculation——The degree of crystallinity and the disorder parameter were calculated automatically using a MELCOM 9100 computer (Mitsubishi Electric Co., Ltd.). The atomic scattering factors6) and the Compton scattering intensities6) of atoms which constituted a lactose molecule were obtained by interpolation every 0.01 Å^{-1} of S from 0.10 Å^{-1} to 1.25 Å^{-1} before these values were fed into the computer. The diffraction data for total and diffuse scattering intensities were also entered at intervals of 0.1° of 2θ from $2\theta=150°$ to $2\theta=8.1°$. In this automated procedure the diffuse scattering intensity was at first separated from total scattering intensity on the observed diffraction chart, although in the usual Ruland's method's it was separated in a converted reciprocal space.

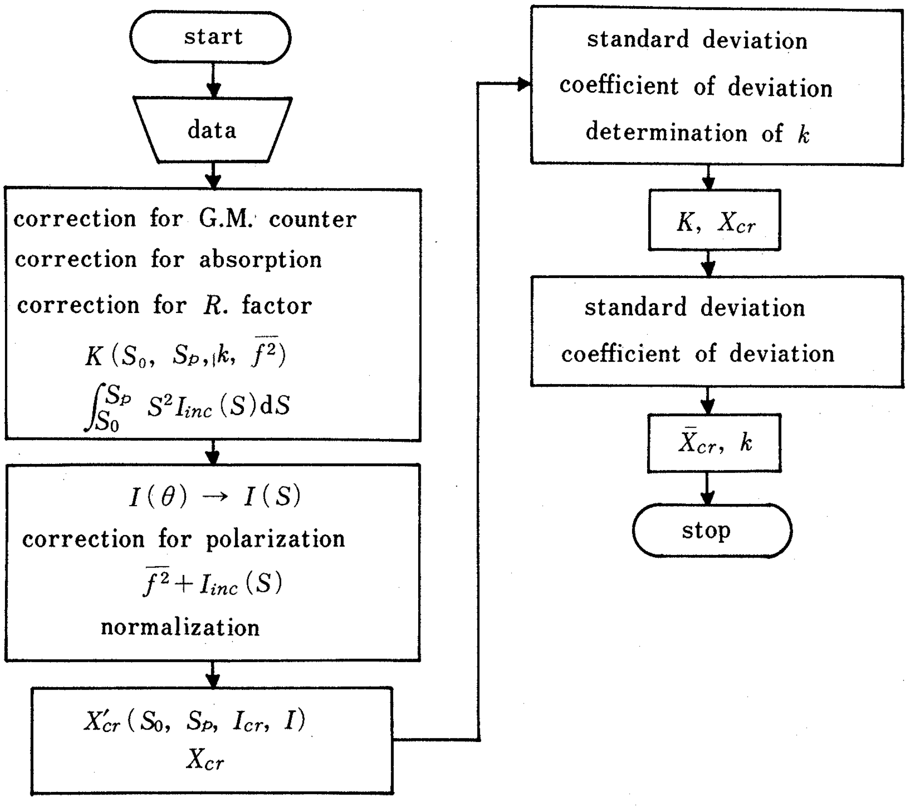

Chart 1

Results and Discussion

Automated Computing Procedure based on Ruland's Method

Ruland's method is theoretically rigorous as described above. However, much time is required for rigorous calculations, so we sought to prepare a computer program based on this method. The obtained flow-chart is shown in Chart 1. This program consists of the following corrections and calculations.

Correction for Geiger-Müller (G.M.) Counter——The resolving-time losses of the G.M. counter were corrected for, since their large resolving time resulted in significant count losses. Nonlinearity losses were determined by the following multi-foil method.[7] The scattering intensity of the (200) plane of sodium chloride was reduced by a series of aluminum foils of uniform thickness t set just before the receiving slit. The counting rate for n foils is given by

$$N = N_0 \exp(-n\mu t) \qquad \text{(Eq. 13)}$$

where N denotes the true couunting rate, N_0 theobserved counting rate, and μ the linear absorption coefficient. The plot of 1n N_0 *versus* n is shown in Fig. 1. The theorertical straight line was obtained by extrapolating the observed straight line from the low counting rate region. The plots of $(N-N_0)/N_0$ *versus* N_0 provided two linear relationships for nonlinear response correction as shown in Fig. 2.

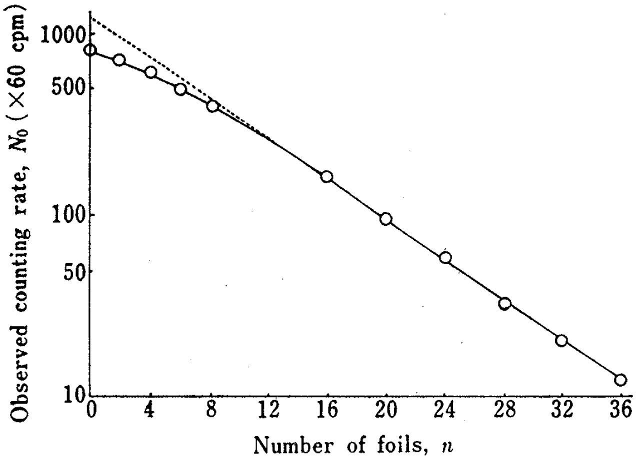

Fig. 1. Plot of Theoretical and Experimental Counting Rates as a Function of Number of Absorbing Foils in the Multi-Foil Method

——: experimental curve. -----: theoretical curve.

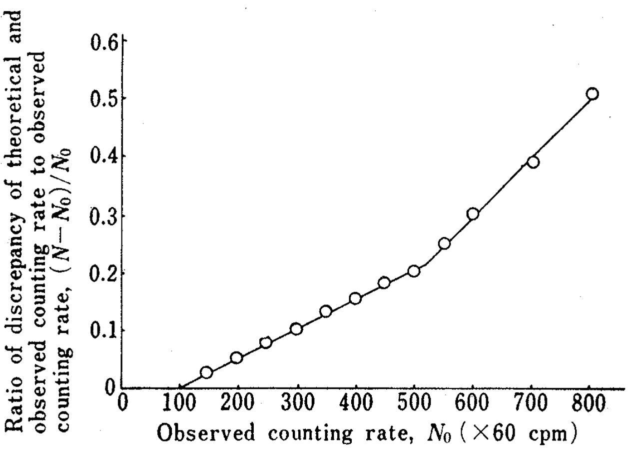

Fig. 2. Determination of Experimental Equation for Nonlinear Response Corrections by Plotting $(N-N_0)/N_0$ *versus* N_0

Correction for the Absorption and the Recoil Factor——The Compton scattering intensity was corrected for both the absorption within the sample and the Breit-Dirac recoil factor. The correction function for the former was taken from the literature.[3b] For the latter, in the case of counters, the correction function is given by[7]

$$R = (\lambda'/\lambda)^2 = (1+2h/mc \times \sin^2\theta/\lambda)^2 \qquad \text{(Eq. 14)}$$

where λ' and λ are the wavelengths of the incoherent and the coherent scattering, h Plank's constant, m electronic rest mass, and c velocity of light. Absorption in the air path was not corrected for.

Calculation of K and Determination of S_p——The next procedure is the calculation of K in Eq. 6. The determination of integral regions was necessary before this calculation. Ruland stated that these regions could be found experimentally by studying samples with different crystallinities. A series of integral upper limits, S_p, was determined as follows. Eq. 7 can be rewritten as follows

$$Y=\frac{\int_{S_0}^{S_p} S^2 I(S)\mathrm{d}S}{\int_{S_0}^{S_p} S^2\overline{f^2}\mathrm{d}S}. \qquad \text{(Eq. 15)}$$

Eq. 15 was applied to several samples with different crystallinities. The points at which Y values are close to unity independently of the crystallinity can be selected as a series of S_p. The plots of Eq. 15 as a function of S_p are shown in Fig. 3. The Y values in the low angle region do not agree with each other, so that the point at which the mean Y value of these samples was close to unity was selected as the first S_p. On the other hand, the Y values in the high angle region agreed with each other, but they were not close to unity. Considering the fluctuation of the Y values, 1.20 was selected as S_p in this region. Therefore, 0.32, 0.86, and 1.20 Å^{-1} were taken as S_p at $S_0=0.10$ Å^{-1}. A series of K values in Eq. 6 was calculated with Simpson's numerical integration formula by varying k from 1 to 10 using the determine dintegral regions. In this step, the values of $\int_{S_0}^{S_p} S^2 I_{inc}(S)\mathrm{d}S$ were calculated simultaneously, where $I_{inc}(S)$ denotes the Compton scattering intensities.

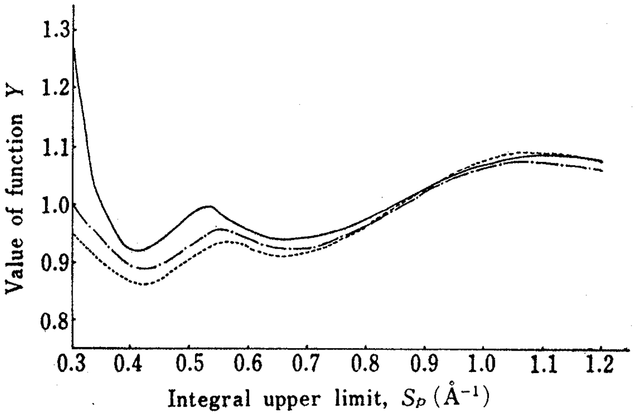

Fig. 3. Determination of Integral Upper Limits Using Function Y at $S_0=0.10$ Å^{-1}

Crystallinity of lactose: —— 75%; —— 35%; ---- 15%.

Table I. Relationship between Normalized Points in the High Angular Region and Corresponding 2θ Regions

Normalized point (Å^{-1})	2θ (°)
1.00	99.5—102.2
1.02	102.3—105.0
1.04	105.1—108.0
1.06	108.1—111.0
1.08	111.1—114.2
1.10	114.3—117.6
1.12	117.7—121.1
1.14	121.2—124.8
1.16	124.9—128.7
1.18	128.8—133.0
1.20	133.1—137.6
1.22	137.7—142.7
1.24	142.8—148.8

Normalization——The θ-scale was converted to the S-scale after the correction of the polarization, and the normalization constant was selected so as to fit the observed curve $I_{co}+I_{inc}$ to the theoretical curve $\overline{f^2}+I_{inc}$ at large values of S where I_{co} denotes the coherent scattering intensity. The theoretical curve is accurately given by

$$I(S)=\overline{f^2}+\frac{1}{\sum N_i}\sum_{j\neq k}\sum f_j f_k\frac{\sin(2\pi r_{jk}S)}{2\pi r_{jk}S}+I_{inc}(S) \qquad \text{(Eq. 16)}$$

where f_j and f_k are the respective atomic scattering factors of the jth and kth atoms, and r_{jk} is the distance between these atoms. The second term in Eq. 16 was neglected since the effect was small at large values of S. The points where the normalization constant was determined are given in Table I The obtained constants in Table I are the means of calculated values at these points.

Calculation of X'_{cr}——X'_{cr} was calculated from Eq. 5 by use of the trapezoidal rule. The matrixes of X_{cr} were obtained as a function of k and S_p from Eq. 4. The coefficient of variation, CV, in a series of X_{cr} was calculated and the value of k which minimized CV was selected.

Validity of the Program and Crystallinity Determination in Crystalline Lactose

The results calculated by our program were compared with those calculated by the usual Ruland's methods using nomograms. The typical S^2I–S curve of crystalline lactose isshown in Fig. 4. Nomograms of K as a function of k and S_p obtained by the usual Ruland's method and those obtained with the computer program are shown in Fig. 5. As shown in Table II, the final results for X_{cr} and k in crystalline lactose obtained by the two methods agreed, reasonably well.

The results calculated by Hermans' method were compared with those calculated by

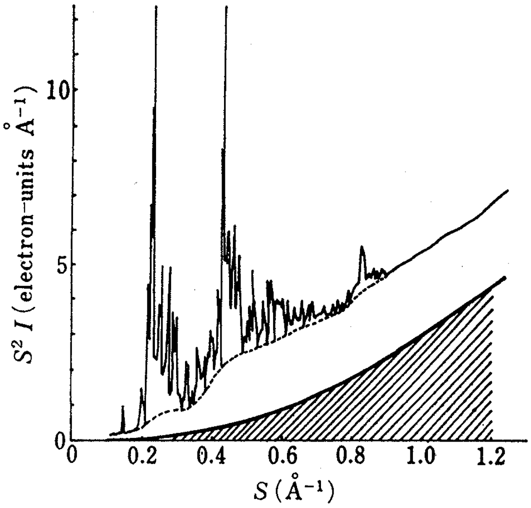

Fig. 4. S–S^2I Curve of Crystalline Lactose

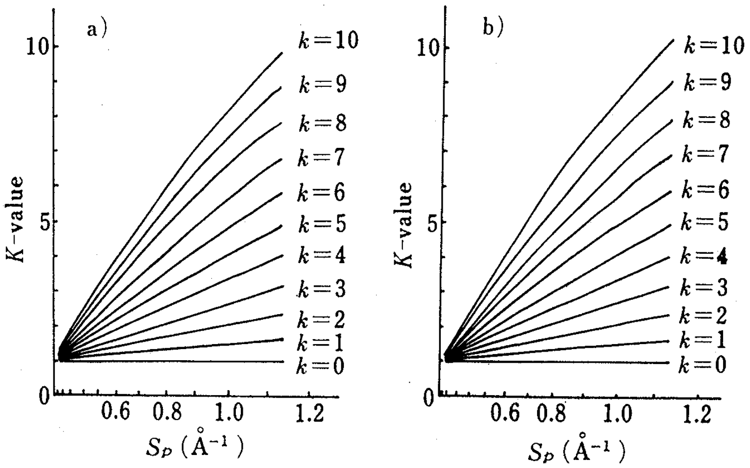

Fig. 5. Nomograms of K Values as a Function of the Disorder Parameter and Integral Upper Limit at S_0=0.10 Å^{-1}

a) With our computer program.
b) By the usual Ruland's method.

Table II. Comparison of the Degree of Crystallinity and Disorder Parameter of Crystalline Lactose calculated by Means of Our Computer Program based on Ruland's Method with Those calculated by the Usual Ruland's Method

	$k^{a)}$	$X_{cr}{}^{b)}$
Usual Ruland's method	3.3	0.67
Computed result	3.5	0.70

a) Degree of disorder parameter, mean value of 5 samples.
b) Degree of crystallinity, mean value of 5 samples.

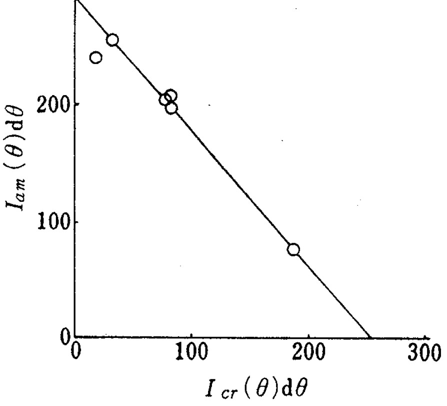

Fig. 6. Regression Line for Lactose with Different Crystallinities obtained by Hermans' Method

Table III. Comparison of the Degree of Crystallinity calculated by Means of Our Computer Program based on Ruland's Method with Those calculated by Hermans' Method

Sample No.	Hermans' method	Computed result
4	0.76	0.68
6	0.32	0.39
7	0.32	0.33
8	0.30	0.36
9	0.12	0.19
10	0.07	0.15

Table IV. Variation of Calculated Normalization Constant[a] on Various Normalized points

Sample No.	Normalized point (Å^{-1})													
	1.00	1.02	1.04	1.06	1.08	1.10	1.12	1.14	1.16	1.18	1.20	1.22	1.24	Mean[b]
1	3.35	3.40	3.47	3.55	3.60	3.67	3.73	3.73	3.75	3.78	3.85	3.93	4.00	3.68
2	4.37	4.23	4.18	4.42	4.47	4.35	4.45	4.57	4.58	4.80	4.95	4.98	4.93	4.56
5	2.52	2.38	2.50	2.48	2.70	2.67	2.67	2.72	2.82	2.88	2.98	3.00	3.18	2.73
7	3.08	2.95	3.23	3.10	3.25	3.28	3.13	3.37	3.37	3.42	3.37	3.45	3.50	3.27
9	3.10	2.88	2.90	3.12	3.15	3.20	3.30	3.28	3.52	3.38	3.38	3.55	3.57	3.26

a) $\times 10^{-3}$ Electron-units/cpm.
b) Mean value of 13 normalized points.

Table V. Effects of Normalization Constants on the Calculated Results

Sample No.	Normalization[a] constant	k[b]	X_{cr}[c]
3	4.77	3.6	0.714
3	4.32	3.3	0.697
3	3.70	2.8	0.672
7	3.50	5.3	0.342
7	3.27	5.0	0.335
7	2.95	4.6	0.330
9	3.57	8.3	0.196
9	3.26	7.8	0.191
9	2.88	7.1	0.185

a) Maximum, mean, and minimum values of 13 normalization constants ($\times 10^{-3}$ Electron-units/cpm).
b) Degree of disorder parameter (Å^2).
c) Degree of crystallinity.

means of the program. Hermans' method required several samples with different crystallinities. Therefore, the samples were ground to obtain suitable specimens. The regression line obtained by Hermans' method is shown in Fig. 6. The X_{cr} values calculated by Hermans' method as shown in Table III agreed with those computed using our program.

Effects of Normalization Constant and Integral Upper Limits on Calculated Results

In general, normalization is performed so as to fit the observed curve to the theoretical one at large values of S. However, Table IV indicates that the normalization constants were not the same. This may be due to our neglect of the second term in Eq. 16. Such variation could not be eliminated, so the effects of normalization constants on the calculated results were investigated. The X_{cr} and k values were calculated for the same sample using the mean,

TABLE VI. Effects of Integral Upper Limits on the Calculated Results

S_0[a]—S_p[b]	Sample No. 3 X_{cr}[c] (k[d]$=3.3$)	Sample No. 7 X_{cr} ($k=4.6$)	Sample No. 9 X_{cr} ($k=7.1$)
0.1—0.3	0.708	0.311	0.164
0.1—0.4	0.644	0.305	0.186
0.1—0.5	0.694	0.332	0.209
0.1—0.6	0.730	0.387	0.207
0.1—0.7	0.738	0.376	0.200
0.1—0.8	0.720	0.357	0.190
0.1—0.9	0.702	0.348	0.179
0.1—1.0	0.682	0.312	0.172
0.1—1.1	0.678	0.311	0.173
0.1—1.2	0.691	0.318	0.179
0.1—1.3	0.684	0.311	0.177
$\bar{X}_{cr}$[e]	0.697	0.330	0.185

a) Integral lower limit (Å^{-1}).
b) Integral upper limit (Å^{-1}).
c) Degree of crystallinity.
d) Degree of disorder parameter (Å^2).
e) Mean value of 11 points.

maximum, and minimum values of obtained normalization constants. As shown in Table V, nearly the same values of K and X_{cr} were obtained for given samples, since Eq. 5 was obtained by multiplying both fractions of observed intensities by the same normalization constant. The errors involved in selecting the normalization constant were confirmed to be small. The effects of integral upper limits on the results are shown in Table VI. The discrepancies indicate that the selection of these limits is very important for crystallinity determination according to Ruland's method.

References and Notes

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