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Estimation of the Degree of Crystallinity and the Disorder Parameter in a Drug by an X-Ray Diffraction Method with a Single Sample

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Crystallinity in a drug has been recognized as an important factor affecting stability, bioavailability and scaling-up of processing operations. In the field of pharmaceutical technology, crystallinity has not yet been studied by Ruland's method, one of the X-ray diffraction methods. The main reason why it is not used may be that extensive and time-consuming calculations are required.

We have developed a modified Ruland's method for determining the degree of crystallinity and the disorder parameter from a powder diffraction pattern of a single sample, suitable for a routine preformulation test. The degrees of crystallinity and the disorder parameters in several samples were measured by Ruland's method and the effects of integral upper limits, integral lower limits, and normalization constants on the results were examined. The integral upper limits seemed to affect the results calculated from the data of a single sample very much, and so did the separation of the crystalline scattering intensity from the total scattering intensity.

We completed a computer program for the automated separation of the noncrystalline scattering intensity and the determination of integral upper limits with a single sample. The results can be obtained from a single sample with satisfactory accuracy for the practical purpose of pharmaceutical technology.

Keywords——crystallinity; disorder parameter; preformulation; Ruland's method; single sample; computer program; automated separation; glutathione; lactose; vitamin B_1

Crystallinity in a drug has been recognized as an important factor affecting chemical stability, physical stability, dissolution rate, bioavailability, and compression characteristics of solid preparations.[1] The crystallinity is, therefore, one of the main subjects of preformulation study. X-ray diffractometry, infrared spectrometry, nuclear magnetic resonance spectrometry, densitometry, and calorimetry have been applied for crystallinity determination.[2] A researcher generally calculates the crystallinity by using various samples of a compound with different crystallinities. At an early stage of drug development, that is, at the preformulation stage, however, the amount of the drug available is usually small. Only a sample from a single small-scale batch may be available for many preformulation tests, and grinding or freeze-drying of the sample to change its crystallinity is often difficult with small amounts of sample. A crystallinity determination with a single sample is, then, a practical requirement in pharmaceutical technology.

Ruland's method[2a] to obtain the degree of crystallinity and disorder parameter from X-ray diffraction data is an academically strict procedure based on paracrystal theory.[3] However, crystallinity in a drug has not yet been studied by Ruland's method in the field of pharmaceutical technology, in spite of the fact that the method can give a well defined crystallinity value for a drug powder. The main reason why it is not used may be that extensive and time-consuming calculations are necessary. The difficulties in Ruland's method were partly overcome by the use of a computer program, and the degree of crystallinity and the disorder parameter in lactose were determined by Nakai *et al.*[4] However, even by Nakai's procedure, crystallinity cannot be studied with one batch of a single sample.

The objective of this study was, therefore, to develop a modified Ruland's method for

determining the degree of crystallinity and the disorder parameter with a powder diffraction pattern of a single sample, suitable for a routine preformulation test. The substances are confined to general organic compounds of low molecular weight for the present investigation.

Experimental

Materials——Crystalline lactose (DMV) was of JP grade. Vitamin B_1 hydrochloride (Kishida Chemical Co., Ltd.) and glutathione (reduced form, crystalline, Sigma Chemical Co.) were of reagent grade. Samples with different crystallinities were prepared by mixing intact crystalline and freeze-dried materials in various ratios. The freeze-dried samples were prepared from 5% aqueous solutions using a Freeze dryer FD-5 (Tokyo Rikakikai Co., Ltd.).

Measurements of X-Ray Diffraction——The X-ray scatterings of these samples were measured at room temperature with a Geiger Flex 2012 diffractometer (Rigaku Denki Co., Ltd.) using a proportional counter. The X-ray source was copper-$K\alpha$ with a nickel filter, voltage 35 kV, current 10 mA, time constant 1 s, receiving slit 0.15°. A symmetrical-reflection goniometer was scanned at 1°/min between $2\theta=151°$ and $2\theta=6°$. Thick samples of about 0.16 cm were used in order to minimize the angular dependence of 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. An analogue-digital converter and a tape puncher were coupled with a recorder. Data were recorded in three digits in 8 bits ISO cord every 0.5 s on paper tape.

Calculations——The degree of crystallinity and the disorder parameter in a sample were obtained by the automated computing procedure with a Burroughs B-7800 computer. Several patterns were recorded on an *X-Y* plotter coupled with the computer. The basic program, written by Nakai *et al.*,[4] was modified so that it could be applied to general organic compounds of low molecular weight in routine work. The atomic scattering factors and the Compton scattering intensities[5] were interpolated every 0.01 Å^{-1} of S, the magnitude of the reciprocal space vector, by the spline function (package program by Burroughs). Those which have not appeared in the literature[5] were interpolated from those of the atoms of the greater and lesser atomic numbers by linear regression.

Results and Discussion

Automated Computing Procedure for the Separation of Diffraction Peaks from Background Scattering

The application of this method to routine work requires automated separation of diffraction peaks from continuous scatterings. The diffraction data need to be smoothed for the

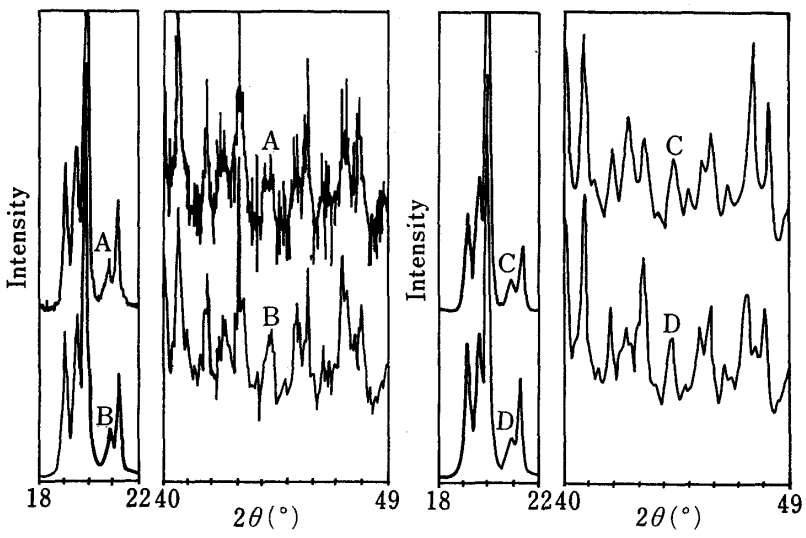


Fig. 1. X-Ray Diffraction Patterns of Lactose by Curve Fitting and Ensemble Mean

A, original data; B, smoothed by spline function; C, smoothed by step-scanning; D, smoothed by ensemble mean.

automated procedure. The use of a large time constant in the smoothing did not give a good resolution of profiles. Numerical filters and other methods for smoothing in a continuous-scanning mode were studied. The results with lactose are shown in Fig. 1. A is the original data plotted every 1/120°. B is smoothed by the use of a spline function[6] with parameters which gave the best results in the literature. C is the reference smoothed by step-scanning. D is smoothed by the ensemble method using twelve data. It is considered that methods B and D can both be used instead of C when step-scanning (known to be suitable for smoothing and automation) is not appropriate. Method D was adopted in this study from the viewpoint of calculation speed.

At what stage the peaks should be separated from the continuous scattering is the next problem. As is clear in Fig. 2b, automated separation on a reciprocal space as in Ruland's method was difficult, for the data interval in the high angle region became small when the θ-scale was converted to the S-scale and the fluctuation of the data was magnified by the factor S^2. The separation seems easier on the original diffraction pattern, as shown in Fig. 2a, as in Hermans' method.[2c] In this case, the crystallinity values depend, of course, on what separation line is adopted. For example, the lower separation line in Fig. 2a gave values of the crystallinity and the disorder parameter of 90% and 2.8 Å^2, and the upper separation line gave 80% and 3.0 Å^2, respectively.

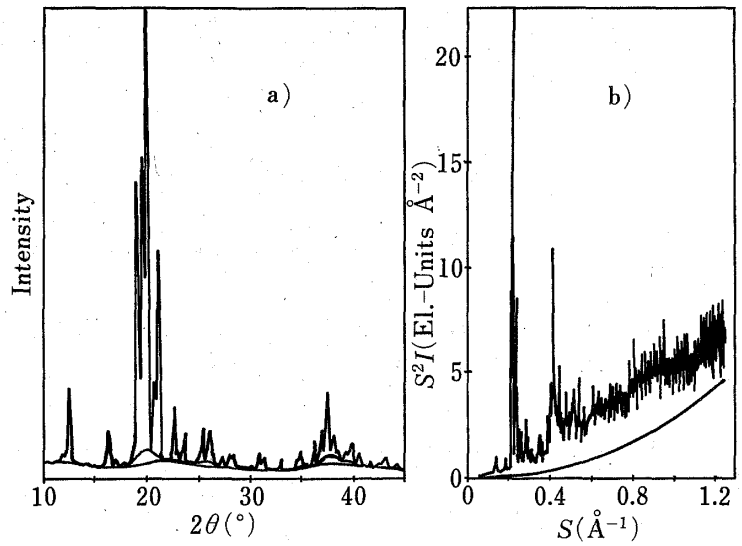


Fig. 2. Separation of Continuous Scattering Intensity from Total Intensity for X-Ray Diffraction Pattern of Lactose (a) and the S–S^2I Plots (b)

A separation method for a standardized noncrystalline scattering line is proposed as follows. First, intensity data are differrentiated with respect to θ and the minimum points are determined. Next, a limiting gradient α of a line between two adjacent minimum points is decided and the skirts of peaks are distinguished from the bottoms between overlapping peaks. Then, the "true skirts" are interpolated from the obtained skirts by means of a spline function (package program by Burroughs). If the gradient between the adjacent minima is greater than α, the bigger minimum is regarded as a bottom of a crevasse between two overlapping peaks and it is not recognized as a "true skirt". In Fig. 3, the separation lines on the diffraction patterns of glutathione samples with different mixing ratios of intact crystals and freeze-dried noncrystalline material are shown. The degree of crystallinity X_{cr} is plotted against the mixing ratio in Fig. 4a. The linearity of the plot together with the small variations of the disorder parameters in Fig. 4b indicated that the proposed method is satisfactory when α

is taken as around 500. The exceptionally high value of X_{cr} at the noncrystalline ratio of 0.75 with $\alpha=300$ in Fig. 4a and the corresponding large k value in Fig. 4b seem to be due to the low value of α as the gradient $\alpha=300$ was smaller than that of the noncrystalline scattering line; thus the regions of crystalline peaks at a low angle were overestimated and so were the degree of crystallinity and the disorder parameter at small values of crystallinity.

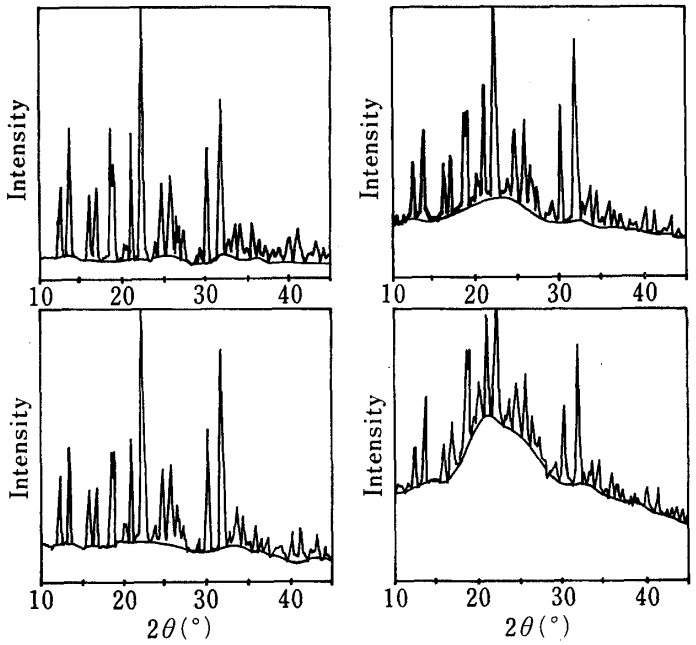


Fig. 3. Separation of Continuous Scattering Intensity from Total Intensity for X-Ray Diffraction Patterns of Glutathione with Various Levels of Crystallinity

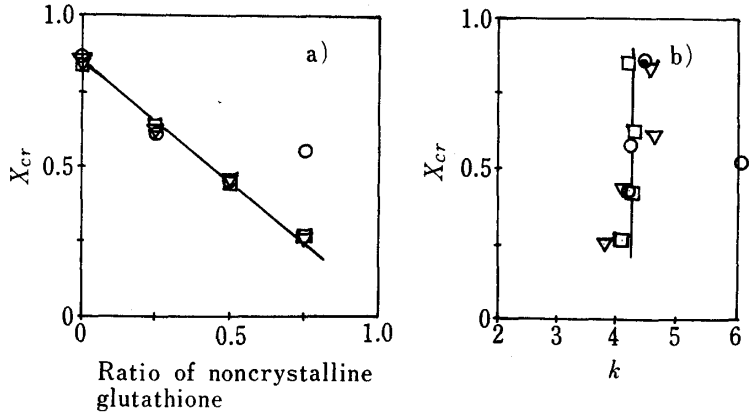


Fig. 4. Effects of Separation Lines between Crystalline Peaks and Continuous Scattering Intensity on Calculated Results for Glutathione at $S_o=0.07$ and $S_p=$ 0.26, 0.80, and 1.25

(○: $\alpha=300$; □: $\alpha=500$; ▽: $\alpha=700$.)

Thus, the crystalline peaks could be separated automatically. The block diagram of the method is shown in Fig. 5.

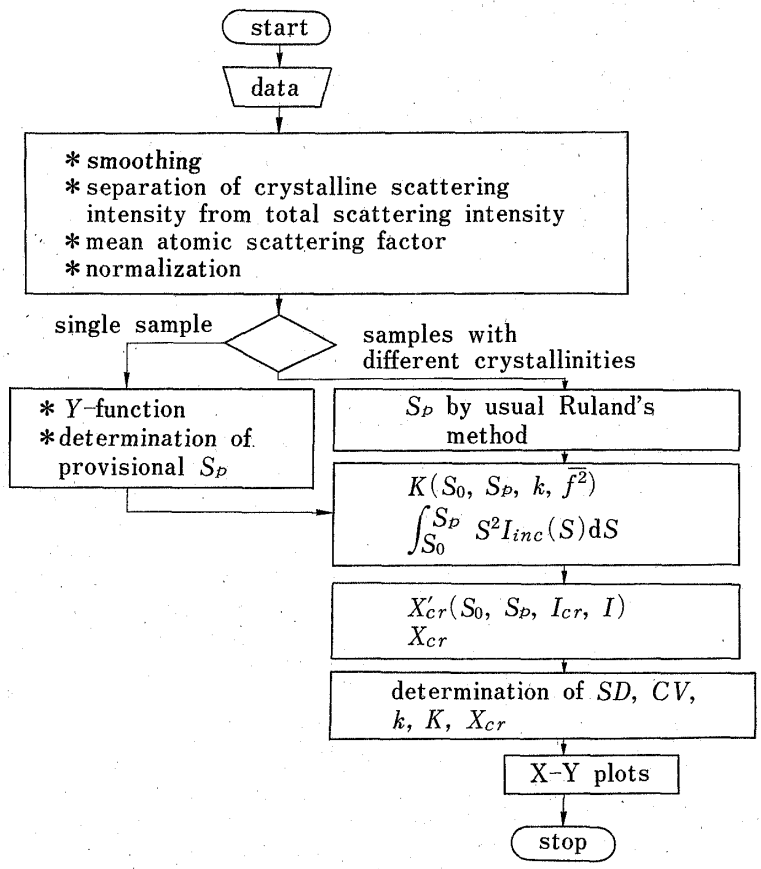


Fig. 5. Block Diagram of Program for Computing Crystallinity and Disorder Parameter

Application of Ruland's Method to Single Samples of Organic Compounds of Low Molecular Weight

Ruland[2a] noted that it should be possible to choose a number of integration intervals (limits S_o and S_p) over larger regions of S such that Eq. 1 becomes independent of the crystallinity of the substance.

$$\int_{S_o}^{S_p} S^2\overline{f^2}\,dS = \int_{S_o}^{S_p} S^2 I(S)\,dS \qquad \text{(Eq. 1)}$$

S : 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
S_p: the integral upper limit
S_o: the integral lower limit
$I(S)$: the coherent scattering intensity at S
f^2 : the mean squared amplitude of atomic scattering factor

($\overline{f^2} = \sum N_i f_i^2 / \sum N_i$)

N_i: the number of atoms of type i
f_i : the atomic scattering factor of the atom of type i

Eq. 1 was rearranged to determine the integral upper limits[4] and the following function Y was calculated in terms of S_p.

$$Y(S_p) = \frac{\int_{S_o}^{S_p} S^2 I(S)dS}{\int_{S_o}^{S_p} S^2\overline{f^2}dS} \qquad \text{(Eq. 2)}$$

Eq. 2 was applied to samples with different crystallinities, and a series of S_p where the Y-function was close to unity irrespective of the crystallinity of samples was determined.

How to determine a series of S_p, therefore, becomes the probelm for the application of Ruland's method to a single sample. The relationship between $I(S)$ and $\overline{f^2}$ is given by

$$I(S) = \overline{f^2} + \frac{1}{\sum N_i} \sum_{m}^{m \neq n} \sum_{n} f_m f_n \frac{\sin(2\pi S r_{mn})}{2\pi S r_{mn}} \qquad \text{(Eq. 3)}$$

where i, m, and n denote the kinds of atoms constituting a molecule, f_m and f_n the respective atomic scattering factors of mth and nth atoms, and r_{mn} the distance between these atoms. As Ruland noted, the oscillations of $I(S)$ around $\overline{f^2}$ (related to the oscillations of Y-function) were a function of r_{mn}, *i.e.*, the molecular structure and its molecular packing structure. In general, the maximum part of noncrystalline scattering, which corresponds to the most frequently appearing interatomic distance, appears in a low angle region, where the crystalline diffraction peaks also appears. Therefore, it fluctuates very much along a line of $Y=1$ the low angle region and the fluctuations diminish as the value of S increases. Ruland chose this low angle region ($S_{p1}=0.3$ for polypropylene) since there appeared to be little practical error.[2a] One of S_p can be determined near here where the value of Y-function is close to unity, independent of the crystallinity of the substance. However, it is difficult to determine the value of other S_p (provisional S_p) from a single sample.

In organic compounds of low molecular weight, a crystal lattice seems to contain only the first kind of lattice imperfection. According to Ruland,[7] the effects of the first kind of a lattice imperfection on the intensity can be expressed as follows,

$$\exp(-k_I S^2) = \exp(-4\pi^2 \overline{u^2} S^2) \qquad \text{(Eq. 4)}$$

$$k_I = 4\pi^2 \overline{u^2} \qquad \text{(Eq. 5)}$$

where $\overline{u^2}$ is the average of the mean square deviation of the atoms in any direction relative to their ideal position, and we can see that S is related to the order of reflections.[3,8] We used the other S_p as follows,

$$S_{pM} = M \times S_{p1} \qquad (M=2,\ 3,\ 4,\cdots) \qquad \text{(Eq. 6)}$$

where S_{p1} is the point of the smallest value of a series of S_p.

Thus, as the series of "provisional" S_p can be determined, it becomes possible to obtain the degree of "provisional" crystallinity with a single sample. The effects of these "provisional" S_p on the results need to be clarified.

(i) **Characteristics of the *Y*-Function**——The profiles of the Y-functions of glutathione and vitamin B_1 hydrochloride with various crystallinities are shown in Fig. 6, where the S_p data was taken at 0.01 Å^{-1} intervals. As the crystallinity increased, so did the fluctuations. The fluctuations subsided as the S value increased with all samples, as was theoretically expected. A very steep rise of the Y-function was observed in the low angle region regardless of the crystallinity of the sample. Although the curve shifts vertically with possible changes of normalization constants, the coefficient for the scaling of experimental intensity (cpm) to electron-units (Fig. 7a), and of integral lower limits (Fig. 7b), the intercept of the curve to $Y=1$ line near $S_p=0.25$ scarcely changes because of the steepness of the curve. The normalization constants were obtained by comparing the smoothed experimental intensity *versus* S curve with the calculated curve at thirteen points between $S=1.00$ Å^{-1} and $S=1.24$ Å^{-1} with an interval of 0.02 Å^{-1}. The max., mean, and min. described in Fig. 7a are of the thirteen normalizing constants.

The first integral upper limit S_{p1} can thus be determined even when the normalization is not correct and the integral lower limit is not adequate. The second and the larger integral upper limits, S_{p2}, S_{p3} $\cdots$ are not determined easily from intercepts of the Y-function on the

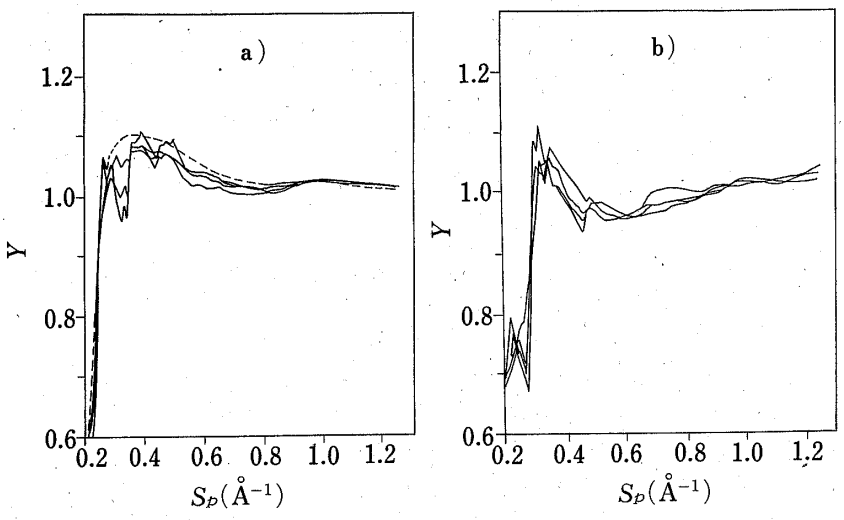


Fig. 6. Determination of Integral Upper Limits for Glutathione (a) and Vitamin B_1 Hydrochloride (b) by "S_p *versus* Y" Plots at $S_o=0.07$

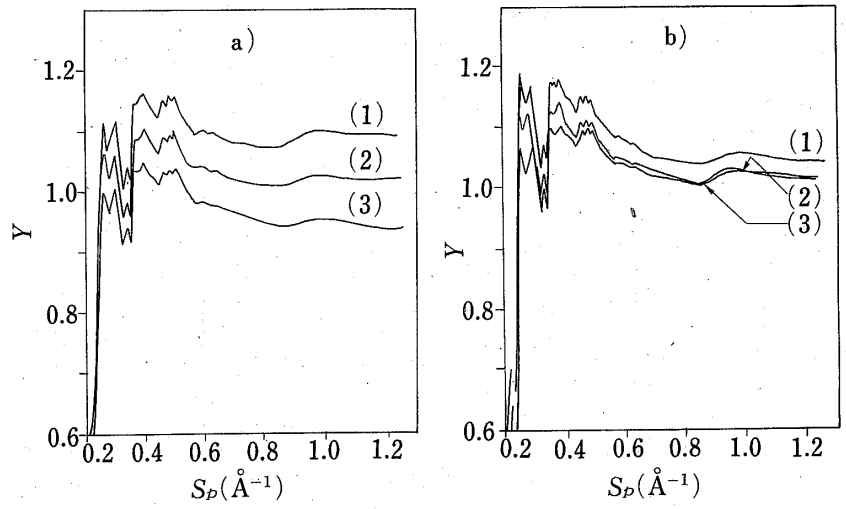


Fig. 7. Effects of Normalization Constants and Integral Lower Limits on Determination of Integral Upper Limits for Glutathione from an X-Ray Diffraction Pattern of a Single Sample

(a) normalization constant=(1) max.; (2) mean; (3) min. at $S_o=0.07$; (b) integral lower limit=(1) 0.08; (2) 0.12; (3) 0.07 at normalization constant=mean.

$Y=1$ line, because the structure factor or the crystalline scattering affects the Y-function. They are better determined provisionally by means of Eq. 6.

(ii) **Examination of Provisional S_p**——The effects of the integral upper limits on the calculated results for glutathione are shown in Table I. The values on the first line were obtained from the values of S_p by the usual Ruland's method using samples with different crystallinities, and those on the second to the fourth lines were obtained from arbitary values of S_p. Below the fifth line the S_{pi} values were determined by the method proposed in this paper. The S_{p1} value at the third line is 0.6, while the values at the other lines are 0.25—0.29. In the third line, the value with the minimum coefficient of variation was determined as X_{cr}, though the value was influenced little by the S_p value when k was large, as shown in Table II. The X_{cr} values in the third line seem to be too large, while the values at the other lines are

Table I. Effects of Integral Upper Limits on Calculated Results for Glutathione

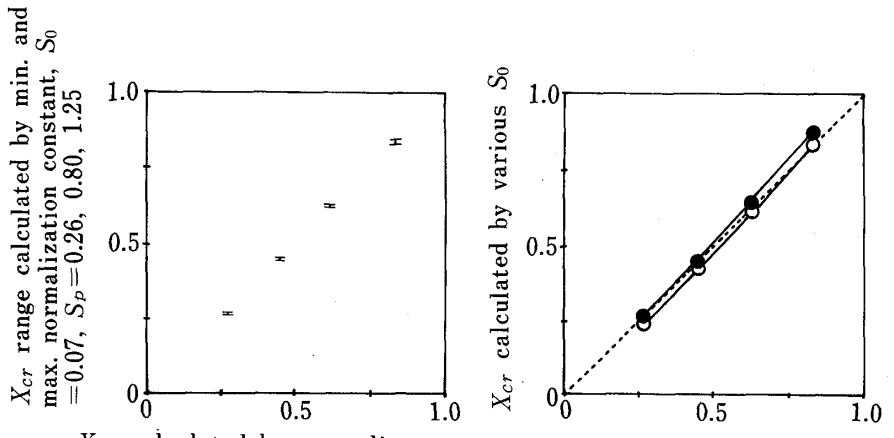
Sample S_p	A		B		C		D	
	$\widehat{k}$	$\widehat{X_{cr}}$	$\widehat{k}$	$\widehat{X_{cr}}$	$\widehat{k}$	$\widehat{X_{cr}}$	$\widehat{k}$	$\widehat{X_{cr}}$
0.26, 0.80, 1.25 (obtained by the usual Ruland's method)	4.2	0.836	4.3	0.618	4.3	0.446	4.1	0.266
0.25, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2	4.3	0.868	4.0	0.587	4.1	0.433	3.9	0.258
0.6, 0.8, 1.0	6.1	1.199	6.3	0.884	5.7	0.581	5.5	0.349
0.25, 0.6, 0.8, 1.0	3.9	0.825	3.6	0.558	3.7	0.410	3.5	0.244
0.25, 0.50, 0.75, 1.00	4.0	0.840	—	—	—	—	—	—
0.27, 0.54, 0.81, 1.08	—	—	4.2	0.614	—	—	—	—
0.29, 0.58, 0.87, 1.16	—	—	—	—	4.1	0.430	3.7	0.243

Table II. Effects of k Value on a Series of X_{cr} calculated by using only High Angle Regions for "A" Sample of Glutathione

S_o—S_p	$k=0$	$k=4$	$k=6$	$k=8$
0.07—0.60	0.470	0.899	1.179	1.499
0.07—0.80	0.322	0.852	1.203	1.594
0.07—1.00	0.220	0.796	1.160	1.552
$\bar{X}_{cr}$		0.85	1.18	1.55
CV		6.09	1.86	3.09

nearly identical and smaller than those at the third line. The X_{cr} values for S_{p1} of 0.25—0.30 are considered to be adequate, as Ruland and Kilian used 0.3—0.4 as the S_{p1} values.[2a,b]

The effects of normalization constants and integral lower limits on the calculated results for glutathione are shown in Fig. 8. Changes of normalization constants did not affect the results, and the effects of changes of S_0 on the results were also rather small. However, it was thought that the S_0 should be decided as the place where as many low order reflections entered the integration region as possible, and where the direct beams were not mixed in the diffracted beams.



X_{cr} caluclated by normalization constant=mean, S_0=0.07, S_p=0.26, 0.80, 1.25

X_{cr} calculated by usual Ruland's method at S_0=0.07

Fig. 8. Effects of Normalization Constants and Integral Lower Limits on Calculated Results for Glutathione

○: S_o=0.08, S_p=values obtained by the proposed method; ●: S_o=0.12, S_p= values obtained by the proposed method.

Conclusion

From the results described above, it is concluded that the degree of crystallinity and the disorder parameter of a general organic compound of low molecular weight can be obtained from the X-ray powder diffraction pattern of a single sample with satisfactory accuracy for practical purposes of pharmaceutical technology.

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