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Estimation of Polymorphic Transition Degree of Pharmaceutical Raw Materials[1]

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To investigate kinetically the polymorphism of pharmaceutical raw materials which has been widely recognized as one of the main factors affecting the physical stability or clinical efficacy of pharmaceutics, the quantitative evaluation of the polymorphic transition degree from powder X-ray diffraction measurements was examined. Wakelin's correlation method and the integration method, which were developed to determine the crystallinity index of crystalline materials, and the relative intensity method (I_R method), which was shown in our previous paper to be effective for evaluating the transition degree of semisynthetic fatty suppository bases, were chosen, and the validity of each method was studied both theoretically and experimentally.

Physical mixtures of stable and unstable crystals of tripalmitin, glyceryl monostearate (MGS-B) and Witepsol W-35 were prepared and the applicability of each method were examined. The correlation method gave precise values for all three cases whereas the integration method gave values slightly higher than the expected values. The I_R method is an easy method and gave precise results if suitable angles for the measurement were chosen, but they could not always be found, so its applicability seems to be restricted.

Keywords——polymorphism; transition degree; X-ray diffraction; crystallinity; tripalmitin; Witepsol W-35; glyceryl monostearate; correlation method; integration method; relative intensity method

Some pharmaceutical additives or active ingredients are polymorphic. It is known that the physical properties of pharmaceutics change during storage due to the polymorphism of such materials,[2] and in some cases the bioavailability or the clinical efficacy is affected.[3]

The polymorphism of many substances has often been qualitatively investigated by means of X-ray diffraction, differential thermal analysis, infrared (IR) spectroscopy or other methods. However, to examine the transformation behavior in more detail, in order to identify important factor in the environment or to predict the physical stability of pharmaceutical preparations kinetically, quantitative evaluation of the polymorphic transition seems to be necessary. Little work has been done on this subject by crystallographic methods.[4] In most cases indirect physical indexes such as melting point,[5] consistency,[6] dissolution rate,[7] *etc.* were measured and discussed in connection with the polymorphic transition. One reason for this may be that the evaluation of the crystallographic indexes from X-ray diffraction data is complex and troublesome.

Previously, we tried to evaluate the polymorphic transition rate of semisynthetic fatty suppository bases by using the relative intensity of the specific X-ray diffraction lines of two crystal forms which changed with the progress of transition,[8] but the application of this method seemed to be limited to analogous compounds. Thus, it would be useful to establish a more general method which is applicable to any type of transition. Recent progress in electronics, especially microcomputers, has made complex technical calculation much faster and easier, so we tried to establish a method based on a combination of an X-ray diffractometer, analog-digital converter and microcomputer. In this paper, Wakelin's method[9] which was originally developed for estimation of the crystallinity index, was applied for the determination of the transition degree. The theoretical background of the relative intensity method, the I_R method in our previous papers,[8] is also discussed.

Theoretical

It is known that the process of polymorphic transition varies considerably, depending on (1) the number of polymorphs, (2) the rate of transition, (3) the pathway of transition, that is, whether an unstable crystal form can transform to the most stable crystal form directly or *via* a metastable crystal form or *via* an amorphous state, (4) the temperature reversibility of intereconversion (for example, it is known that some substances such as long chain fatty alcohols show enantiotropism, whereas other substances such as fatty acids and triglycerides show monotropism). However, in any case, the ratio of the weight fraction of the most stable crystal to the crystalline fraction of sample (X_s) can be regarded as an index representing the transition degree. Namely, the transition degree after time $t(T_t)$ can be defined by the equation,

$$T_t=\frac{(X_s)_t-(X_s)_{t=0}}{(X_s)_{t=\infty}-(X_s)_{t=0}} \tag{1}$$

where $(X_s)_t$, $(X_s)_{t=0}$ and $(X_s)_{t=\infty}$ mean X_s at time t, and at the initial and final times, respectively. Another index representing the transition degree may be the ratio of the weight fraction of the most stable crystal to the whole sample including the amorphous portion (Y_s). The transition degree in this case (T'_t) is given by the following equation,

$$T'_t=\frac{(Y_s)_t-(Y_s)_{t=0}}{(Y_s)_{t=\infty}-(Y_s)_{t=0}} \tag{2}$$

Y_s can be expressed as the product of X_s and the crystallinity of sample (C_r), so Eq. 2 can be rewritten in the form,

$$T'_t=\frac{(C_r)_t\cdot(X_s)_t-(C_r)_{t=0}\cdot(X_s)_{t=0}}{(C_r)_{t=\infty}\cdot(X_s)_{t=\infty}-(C_r)_{t=0}\cdot(X_s)_{t=0}} \tag{3}$$

In Eq. 3, C_r can be calculated independently by means of another method such as Ruland's method[10] or Herman's method.[11] If the change of C_r is negligibly small, then Eq. 3 can be approximately by Eq. 1. Thus, whether T_t or T'_t is chosen as the index of transition degree, the estimation of X_s should be necessary. Hereafter, we will concentrate mainly on the method for calculating X_s by powder X-ray diffractometry.

The total scattering intensity of X-ray diffraction for a crystalline material at a given Bragg's angle is given by the sum of the coherent Thomson scattering diffracted by the lattice of crystals (I_c), other Thomson scattering due to the non-crystalline part (I_a), and a diffused background formed by the incoherent Compton scattering and other scattering by air or cosmic rays. For crystallinity measurement, both I_c and I_a are necessary, but for the estimation of X_s, only I_c is necessary because it is defined as the weight fraction of the most stable crystal in the crystalline portion.

According to Alexander and Klug,[12] I_c of each component of the crystalline powder mixture can be expressed by Eq. 4,

$$I_{ci}=\frac{K_i\cdot X_i}{\rho_i\{\mu_i\cdot X_i+\mu_M\cdot(1-X_i)\}} \tag{4}$$

where I_{ci} is the diffracted intensity of component i, K_i is a constant which depends on the properties of component i and the geometry of the apparatus, ρ_i is the density of component i.

μ_i is the mass absorption coefficient of component i, μ_M is the mass absorption coefficient of components other than component i and X_i is the weight fraction of component i. In the case of polymorphism, the mass absorption coefficient can be regarded as the same in each case, because the atom composition is the same in all cases, and so μ_i can be set equal to μ_M. Conseqeuntly, Eq. 4 can be simplified as follows.

$$I_{ci}=\frac{K_i}{\rho_i\cdot\mu_M}\cdot X_i \tag{5}$$

In this paper, to avoid complexity in the argument, we will make the following assumptions: (1) the transition occurs between two polymorphs; (2) the orientation of crystals in a sample is almost random; (3) the crystalline size does not change much during the transition, and the effect of X-ray diffraction is negligibly small. The last assumption is necessary in the special case where the crystal size is lower than 0.1 μm, because the resolving power of X-ray diffraction decreases and may cause measurement error. However, if the crystal size is not small, then this assumption becomes unnecessary.

When an unstable crystal form (denoted as α-form) transforms to a stable one (denoted as β-form), I_c at a given Bragg's angle can be expressed by the following equation,

$$I_c=I_{c\alpha}+I_{c\beta} \tag{6}$$

From Eq. 5

$$I_{c\alpha}=k_\alpha\cdot X_\alpha=k_\alpha(1-X_\beta) \tag{7}$$

$$I_{c\beta}=k_\beta\cdot X_\beta \tag{8}$$

From Eqs. 6, 7 and 8

$$I_c=(k_\beta-k_\alpha)\cdot X_\beta+k_\alpha \tag{9}$$

where k_α and k_β are constants, and X_α and X_β mean the weight fractions of α- and β-form crystals, respectively. When the diffraction intensity of the standard crystal of α-form ($X_\alpha=1$) is $(I_s)_\alpha$ and that of β-form ($X_\beta=1$) is $(I_s)_\beta$, then $k_\alpha=(I_s)_\alpha$ and $k_\beta=(I_s)_\beta$ from Eqs. 7 and 8. Substituting these values into Eq. 9, the following equation is obtained for X_β.

$$X_\beta=\frac{I_c-(I_s)_\alpha}{(I_s)_\beta-(I_s)_\alpha} \tag{10}$$

Equation 10 indicates that the value of X_β is represented by the ratio of the intensity difference between an unknown sample and α-standard to that between β-standard and α-standard.

The observed diffraction intensity (I_{obs}) obtained by powder X-ray diffractometry can be affected by various factors such as the X-ray intensity, the radiating area, the crystallinity of the sample, *etc*. Therefore, before the application of Eq. 10, I_{obs} must be converted to the normalized value by the following procedure.

a) Primary Normalization——In the case of polymorphs, the theoretical mass X-ray scattering intensity is the same, irrespective of the transition degree or the crystallinity of the sample, because the atom composition is the same. Therefore, the distribution area of X-ray scattering intensity, $\int_{\theta_o}^{\theta_p} I_{obs}(\theta)d\theta$, should be almost equal if the integrated θ range is wide enough. Thus, the primary normalization of I_{obs} is carried out as follows.

$$I^*_{obs}(\theta)=I_{obs}(\theta)\Big/\int_{\theta_o}^{\theta_p} I_{obs}(\theta)d\theta \tag{11}$$

where $I^*_{obs}(\theta)$ means the intensity after normalization and $\int_{\theta_o}^{\theta_p} I^*_{obs}(\theta)d\theta=1$. The range of $\theta_o-\theta_p$ can be determined according to Ruland's equation. Namely, θ is converted first to the magnitude of the reciprocal space vector s ($s=2\sin\theta/\lambda$), and $s^2 I_{\mathrm{obs}}(s)$ is normalized to the electron unit by comparison with the theoretical value. Then, the range of s_o-s_p is determined as Eq. 12 can be satisfied, and the range of $\theta_o-\theta_p$ can be established by converting s to θ again.

$$\int_{s_o}^{s_p} s^2 I_{\mathrm{obs}\alpha}(s)\mathrm{d}s=\int_{s_o}^{s_p} s^2 I_{\mathrm{obs}\beta}(s)\,\mathrm{d}s \tag{12}$$

where $I_{\mathrm{obs}\alpha}$ and $I_{\mathrm{obs}\beta}$ are the normalized I_{obs} values of the α- and β-standard crystals, respectively.

b) Separation of the Diffraction Intensity (I^*_{c}) from Total Scattering Intensity——I^*_{obs} is the sum of many scattering intensities, such as the diffracted Thomson scattering intensity by the crystal lattice (I^*_{c}), the non-diffracted Thomson scattering intensity due to the amorphous portion (I^*_{a}), Compton scattering intensity (I^*_{comp}) and others (I^*_{r}). Among them, I^*_{a}, I^*_{comp} and I^*_{r} form a diffused background intensity (I^*_{b}), that is,

$$I^*_{\mathrm{obs}}=I^*_{\mathrm{c}}+I^*_{\mathrm{a}}+I^*_{\mathrm{comp}}+I^*_{\mathrm{r}}=I^*_{\mathrm{c}}+I^*_{\mathrm{b}} \tag{13}$$

I^*_{b} and I^*_{c} can be separated by drawing a reasonable baseline on the diffraction pattern.

c) Secondary Normalization of I^*_{c}——$\int_{\theta_o}^{\theta_p} I^*_{\mathrm{c}}(\theta)\mathrm{d}\theta$ value reflects the crystalline portion of a sample and is comprised of the distribution area of the diffraction intensity due to α-crystals and β-crystals, as shown in Eq. 14.

$$\int_{\theta_o}^{\theta_p} I^*_{\mathrm{c}}(\theta)\,\mathrm{d}\theta=\int_{\theta_o}^{\theta_p} I^*_{\mathrm{c}\alpha}(\theta)\,\mathrm{d}\theta+\int_{\theta_o}^{\theta_p} I^*_{\mathrm{c}\beta}(\theta)\,\mathrm{d}\theta \tag{14}$$

The value of $\int_{\theta_o}^{\theta_p} I^*_{\mathrm{c}}(\theta)\mathrm{d}\theta$ differs from sample to sample owing to the difference of crystallinity of each sample. As described in the preceding section, X_β ($=\int I^*_{\mathrm{c}\beta}\mathrm{d}\theta/\int I^*_{\mathrm{c}}\mathrm{d}\theta$) is calculated only from the crystalline portion, and the true value is not always necessary. Thus, it is more convenient to correct the value of $I^*_{\mathrm{c}}(\theta)$ by an appropriate conversion so as to be able to compare each sample by an identical measure. Thus, we performed the secondary normalization according to Eq. 15.

$$I^{**}_{\mathrm{c}}(\theta)=I^*_{\mathrm{c}}(\theta)\Big/\int_{\theta_o}^{\theta_p} I^*_{\mathrm{c}}(\theta)\,\mathrm{d}\theta \tag{15}$$

where I^{**}_{c} means the intensity after normalization of I^*_{c}, and $\int_{\theta_o}^{\theta_p} I^{**}_{\mathrm{c}}(\theta)\mathrm{d}\theta=1$.

Thus, by the secondary normalization, a common measure applicable to samples of various crystallinities is established and the application of Eq. 10 become feasible.

Application of Wakelin's Method

Equation 10 indicates that, in theory, X_β can be determined from the intensities at only one Bragg's angle, but nevertheless, the application of Wakelin's methods$^{9)}$ seems to be useful. These methods are statistical ones developed for the derermination of the crystallinity index from many points of intensity data for a sample, and involve two techniques, correlation and integration.

In the case of the correlation method, the difference of the corrected intensity between α- and β-standard samples and that between the α-standard and unknown sample are calculated first over a wide range of angle, as shown in Eqs. 16 and 17, respectively.

$$X(\theta)=I^{**}_{\mathrm{s}\beta}(\theta)-I^{**}_{\mathrm{s}\alpha}(\theta) \tag{16}$$

$$Y(\theta)=I^{**}_{\mathrm{c}}(\theta)-I^{**}_{\mathrm{s}\alpha}(\theta) \tag{17}$$

X_β can be expressed generally as Eq. 18

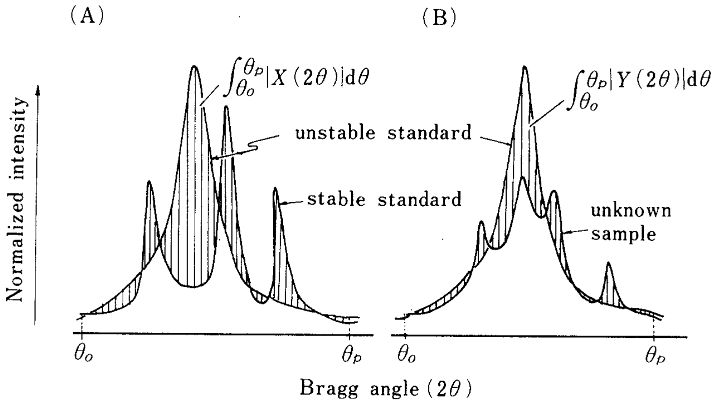

Fig. 1. Schematic Representation of the Integration Method

(A) the area produced by the intensity difference between a stable standard and an unstable standard.

(B) the area produced by the intensity difference between an unknown sample and an unstable standard.

$$X_\beta = Y(\theta)/X(\theta) \tag{18}$$

Thus, when the linear regression of $X(\theta)$ and $Y(\theta)$ is carried out, the slope of the regression line gives X_β of the unknown sample. The correlation coefficient of the regression line can be used to check the reliability of the measurement. The Y intercept should theoretically be zero, so if the deviation from the origin is marked, it indicates that the result is not valid and some systematic errors may be involved in the experimental data.

On the other hand, the integration method considers that X_β corresponds to the ratio of the area enclosed by the curves of $I_c^{**}(\theta)$ and $I_{s\alpha}^{**}(\theta)$ to that enclosed by the curves of $I_{s\alpha}^{**}(\theta)$ and $I_{s\beta}^{**}(\theta)$ over a wide range of Bragg's angle (θ_o—θ_p), as shown in Fig. 1.
Namely, X_β can be calculated by Eq. 19

$$X_\beta = \int_{\theta_o}^{\theta_p} |Y(\theta)|\,d\theta \Big/ \int_{\theta_o}^{\theta_p} |X(\theta)|\,d\theta \tag{19}$$

Relative Intensity Method

Since Eq. 9 should hold at every Bragg's angle, Eqs. 20 and 21 are simultaneously satisfied for the intensities at two different angles, θ_1 and θ_2, in the same X-ray diffraction pattern.

$$I_c(\theta_1) = (k_{\beta 1} - k_{\alpha 1}) \cdot X_\beta + k_{\alpha 1} \tag{20}$$

$$I_c(\theta_2) = (k_{\beta 2} - k_{\alpha 2}) \cdot X_\beta + k_{\alpha 2} \tag{21}$$

where $k_{\alpha 1}$, $k_{\alpha 2}$, $k_{\beta 1}$ and $k_{\beta 2}$ are constants. Then the ratio of diffraction intensities, I_R, is derived as Eq. 22,

$$I_R = \frac{I_c(\theta_1)}{I_c(\theta_2)} = \frac{(k_{\beta 1} - k_{\alpha 1}) \cdot X_\beta + k_{\alpha 1}}{(k_{\beta 2} - k_{\alpha 2}) \cdot X_\beta + k_{\alpha 2}} \tag{22}$$

This equation has the shape of a hyperbola. However, if the angle where $k_{\beta 2} = k_{\alpha 2}$ is chosen as θ_2, Eq. 22 becomes a simple linear equation as follows,

$$I_R = p \cdot X_\beta + q \tag{23}$$

$$p = (k_{\beta 1} - k_{\alpha 1})/k_{\alpha 2}$$

$$q = k_{\alpha 1}/k_{\alpha 2}$$

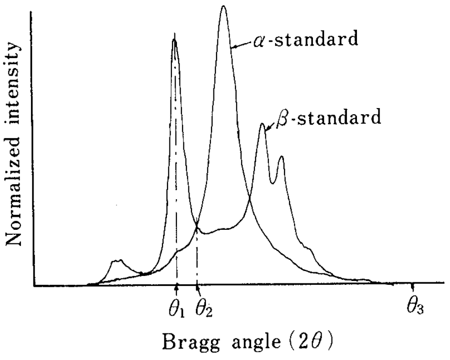

Fig. 2. The Normalized Diffraction Patterns of α- and β-Form of TP and the Position of the Bragg Angle for X_s Calculation by the I_R Method

$\theta_1 = 19.2°$; $\theta_2 = 19.8°$; $\theta_3 = 32.9°$.

The values of the constants p and q can be obtained from the I_R values of α- and β-crystal standards (denoted as $I_{R\alpha}$ and $I_{R\beta}$, respectively), that is, $p = I_{R\beta} - I_{R\alpha}$ and $q = I_{R\alpha}$, and therefore Eq. 23 can be rewritten as Eq. 24,

$$X_\beta = \frac{I_R - I_{R\alpha}}{I_{R\beta} - I_{R\alpha}} \tag{24}$$

This equation seems to resemble Eq. 10, but the normalization of diffraction intensity is not necessary in this case, because the I_R value is the ratio between two intensities in the same X-ray diffraction pattern, so that the common correction factor for normalization such as $1/\int I_{obs}(\theta)d\theta$, can be canceled out. In short, the I_R value itself can be regarded as the normalized value. Thus, to apply Eq. 24, the choice of θ_2 where $k_{\alpha 2} = k_{\beta 2}$ is necessary first. At the ideal angle of θ_2, Eq. 25 should be satisfied, from Eq. 21,

$$I^{**}_{s\alpha}(\theta_2) = I^{**}_{s\beta}(\theta_2) \tag{25}$$

Thus, θ_2 corresponds to the angle where both standard diffraction curves, $I^{**}_{s\alpha}(\theta)$ and $I^{**}_{s\beta}(\theta)$, cross each other, as shown in Fig. 2. At the same time, all the diffraction curves of various X_β should also cross this point. Therefore, it can be said that the intensity at θ_2 acts as a sort of inner standard. On the other hand, as regards θ_1, an angle such that the differerence of intensities of both standards is sufficiently large should be chosen in order to increase the precision. In addition to these angles, in order to eliminate the background intensity from the observed intensity, one more angle, θ_3, where only background intensity is seen, is to be chosen. The background intensity may differ between θ_1 and θ_2, but it can be regarded as approximately equal to that at θ_3 if this is chosen so as to be as near to θ_1 and θ_2 as possible and if it contains no diffraction line. Then the I_R value can be obtained by Eq. 26,

$$I_R = \frac{I_{obs}(\theta_1) - I_{obs}(\theta_3)}{I_{obs}(\theta_2) - I_{obs}(\theta_3)} \tag{26}$$

where $I_{obs}(\theta_1)$, $I_{obs}(\theta_2)$ and $I_{obs}(\theta_3)$ mean the observed intensities directly obtained from the chart of the X-ray diffraction pattern at the angles θ_1, θ_2 and θ_3, respectively. In Fig. 2, the selected angles, θ_1, θ_2 and θ_3, in the normalized X-ray diffraction patterns of tripalmitin(TP) standard crystals are shown as an example. As is clear in Fig. 2, there were two clear cross-points and the calculated results were almost the same. However, it seems reasonable to choose 19.8° for θ_2, because it is the nearest point to θ_1.

This method seems to be very convenient because X_β can be estimated from only three points on the same diffraction chart and without any complex calculations. We experimentally confirmed the applicability of this method to semisynthetic fatty materials and used it for the examination of the polymorphic behavior of these materials.

Experimental

Materials——Tripalmitin (Nakarai Chemical Co.) was of reagent grade. MGS-B (monoglyceryl stearate, Nikko Chemical Co.) and Witepsol W-35 (semisynthetic fatty suppository base, Dynamit Nobel Co.) were used as received.

Preparation of Standards of Polymorphic Form——The α-form of TP was prepared as a fine powder by adding molten TP dropwise to cooled 50% aqueous methanol under vigorous stirring in a homogenizer, while the β-form of TP was prepared by allowing the α-form to stand for two weeks at 40 °C. The α-form of MGS-B (MGS) and the A-form of Witepsol W-35 (W-35) were prepared by rapidly cooling the melt at room temperature, and the solid masses were powdered in a mortar in a cool place. The β-form of MGS and the B-form of W-35 were prepared by allowing the α-form to stand for two weeks at 40 °C and the A-form to stand for ten days at 30 °C, respectively. Each of the crysral forms was checked by measuring the X-ray diffraction. The crystalline sizes of standard crystals measured by microscopic observation were as follows: α-form of TP, 50—100 μm; β-form of TP, 20—50 μm; α-form and β-form of MGS, 20—50 μm, respectively; A-form and B-form of W-35, 20—50 μm, respectively.

Measurement of X-Ray Diffraction——The X-ray scattering of samples was measured at room temperature with a Geigerflex 2013 diffractometer (Rigaku Denki Co., Ltd.) using a proportional counter. The X-ray radiation was nickel-filtered copper K_α radiation ($\lambda = 1.54$ Å). Other measurement conditions were follows; voltage 40 kV; current 35 mA; time constant 1 s; slits, divergency/receiving/scattering, 0.5 °/0.3 mm/0.5 °. A symmetrical-reflection goniometer was scanned at 4 °/min between 3 and 50 ° in the case of transition degree measurement, and between 3 and 150 ° in the case of Ruland's crystallinity measurement. An analog-digital converter (3456 A digital voltmeter, Hewlett-Packard Co.) and a microcomputer (HP-85, Hewlett-Packard Co.) were coupled with a recorder. The scattering data analog output was digitized at every 0.1 ° of Bragg's angle 2θ interval in the case of transition degree measurement and at various intervals from 0.1 to 1 °, changing with increasing Bragg's angle in the case of Ruland's crystallinity measurement. Data obtained were recorded on tapes.

Calculation——The weight fraction of stable crystal (X_s) according to Wakelin's method was calculated by the correlation and integration method using an HP-85 microcomputer. In the case of the I_R method, X_s was manually calculated by using the intensity data read directly from the X-ray diffraction chart. The calculation of Ruland's crystallinity was also performed by referring to Morita's singel sample method.[10c)]

Results and Discussion

The Polymorphs and Crystallinity of Samples

Figure 3 shows the change of X-ray diffraction patterns of TP, MGS and W-35 during storage at 30 or 40 °C. TP crystallized as the hexagonal α-form when the melt was rapidly cooled, showing a single diffraction line of 4.15 Å in the side spacing region of the X-ray

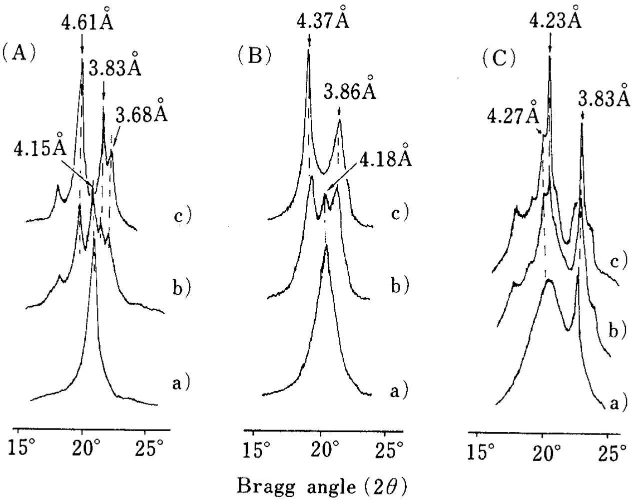

Fig. 3. Change of X-Ray Diffraction Patterns of TP, MGS and W-35 during Storage

(A) TP, at 40 °C; a) α-form, immediately after solidification, b) intermediate, after 16 h, c) β-form, after 2 weeks.

(B) MGS at 40 °C; a) α-form, immediately after solidification, b) intermediate, after one day, c) β-form, after 2 weeks.

(C) W-35 at 30 °C; a) A-form, immediately after solidification, b) intermediate, after 2 d, c) B-form, after 2 weeks.

Table I. The Crystallinity and Disorder Parameter (k) of the Standard Crystals Obtained by Ruland's Method

Standard crystal		Crystallinity (%)	k value
TP	α-form	83.3	0.5
	β-form	84.4	0.9
MGS	α-form	59.1	1.6
	β-form	57.3	1.4
W-35	A-form	49.2	1.5
	B-form	53.8	1.1

Calculations were performed using the following integral limits, $s_o=0.1$, $s_p=0.35$, 0.60, 0.85, 1.1.

diffraction pattern. This form transformed to the most stable triclinic β-form, having three main diffraction lines of 4.61, 3.83 and 3.68 Å under appropriate temperature conditions. Many analogous substances are known to take the metastable monoclinic β′-form besides these forms, and α-to-β′-to-β transition occurs,[13] but in the case of pure TP, β′-form did not appear in the transition process. Thus, this transition was regarded as a simple α-to-β transition.

MGS was also obtained as α-form immediately after solidification of the melt, having a single peak of 4.18 Å. It transformed to the most stable β-form having two main peaks of 4.37 and 3.86 Å during storage at near 40 °C. In the cases of some pure monoglycerides, it is known that four polymorphs, sub α, α, β′ and β, are present and the interconversion is very complex,[14] but in the case of MGS, only α- and β-form could be detected and the α-to-β transition occurred.

W-35 is a mixture of many kinds of glycerides and is obtained as the unstable A-form immediately after solidification of the melt. It transforms to the more stable B-form at higher room temperature. The change of X-ray diffraction pattern accompanying this transition may be the sum of each change of unit crystals constituting W-35, but it can be macroscopically regarded as an A-to-B transition.

These substances seemed to be suitable for this investigation, because when the melt was cooled, they crystallized as spherulites or sheaf-like crystallites which grew radially from a primary crystal core and therefore showed a statistically random orientation.

The crystallinities of the standard crystals used in this study were determined by Ruland's method and the results are listed in Table I. The crystallinity hardly varied for a given substance, though it was different among substances. Thus, it was presumed that the crystallinity scarcely changed in the process of each transition. Therefore, T_t calculated from Eq. 1 is regarded as being almost the same as T_t' in Eq. 2. Hereafter, T_t value is used as the index of transition degree in this paper.

Determination of the Range of Bragg's Angle for Analysis

For Wakelin's technique, it is necessary to determine the angle region required in the calculation. Thus, the P value, defined by Eq. 27, was calculated in the appropriate range of magnitude of the reciprocal space vector s.

$$P(s_p)=\frac{\int_{s_o}^{s_p} s^2 I_{s\alpha}(s)\,\mathrm{d}s}{\int_{s_o}^{s_p} s^2 I_{s\beta}(s)\,\mathrm{d}s} \qquad (27)$$

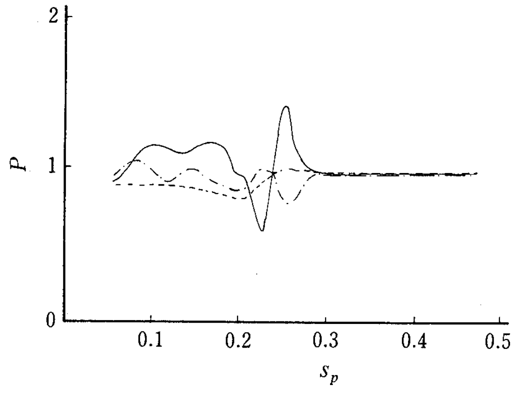

Fig. 4. Influence of the Integral Upper Limit s_p on the Calculated P Values

——, TP; — - —, MGS; - - - -, W-35. Lower limit $s_o = 0.034$ ($2\theta = 3.0°$).

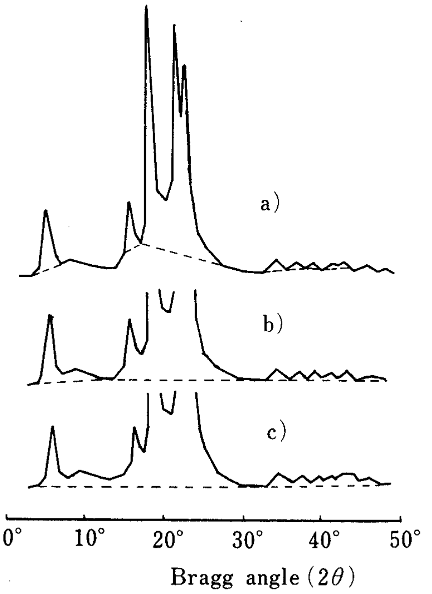

Fig. 5. Examples of Separation Lines Determined by the Automated Computing Procedure

a) $F = 0.0012$; b) $F = 0.0006$; c) $F = 0.0002$.

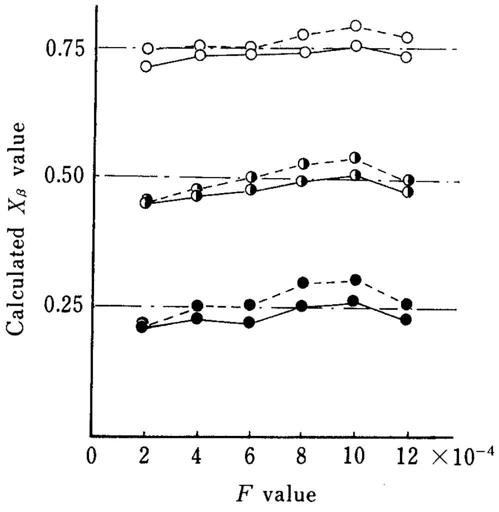

Fig. 6. Influence of the Value of Limiting Gradient F on the Calculated X_β of Various Physical Mixtures of TP

○, $\alpha/\beta = 1/3$ phys. mixt.; ◑, $\alpha/\beta = 1/1$ phys. mixt.; ●, $\alpha/\beta = 3/1$ phys. mixt.

——, correlation method; - - - -, integration method.

Figure 4 shows the change of P value against the upper limit of s, s_p, when the lower limit s_o is 0.034 (Bragg angle $2\theta = 3°$). It was shown that the P values of TP, MGS and W-35 were almost equal to 1 above $s_p = 0.3$ ($2\theta = 27°$). Thus, it is considered that the region of 3—50° is enough for P values to become equal to 1 and we adopted this region for all the calculations.

Automated Separation of the Diffracted Intensity

The separation of diffraction peaks due to crystals from the diffused background scattering was carried out by an automated computing procedure. The separation line was determined by following procedure; (1) the observed intensity (I_{obs}) was normalized; (2) the normalized intensity (I^*_{obs}) was differentiated with respect to 2θ and the minimum points were determined; (3) the limiting gradient F of a line between two adjacent minimum points was decided and if the gradient between the adjacent minima was greater than F, the bigger minimum was regarded as the bottom of a trough between two overlapping peaks and excluded; (4) the selected minima were combined linearly, and consequently a separation line was obtained; (5) if the obtained separation line was judged to be unreasonable, then the value of the limiting gradient F was changed.

The background scattering intensity of each angle (I_b^*) was calculated by means of interpolation from this line. Thus, the crystallline diffraction intensity (I_c^*) was obtained from Eq. 13 at each angle.

Figure 5 shows as an example of how the separation lines change with F value (from 0.0002 to $0.0012\theta^{-1}$) in the case of TP β-form standard. The effect of F values on the calculated X_β was examined by using 25, 50 and 75% physical mixtures of TP standards (Fig. 6). As is clear in this figure, the results were near the expected values (the deviation from the expected value was within ±0.03 or ±0.04 in each case), so that the F value does not seem to affect the result much. Thus, this calculation method can be regarded as valid for our purposes.

Calibration for X_s Measurement Using Physical Mixtures

Various physical mixrures of TP, MGS and W-35 were prepared by thorough mixing of the unstalbe and stable standards in ratios of 4:1, 3:2, 2:3 and 1:4. X_s of every sample was determined by the correlation method and integration method. The results are shown in Table II. The values calculated by the correlation method were close to the expected values and showed good linearity in all cases, as is clear from their correlation coefficient values. The values obtained by the integration method were generally higher than expected and the linearity of the regression line was a little poorer than that in the case of the correlation method, especially for W-35. The reason may be that, in the integration method, both positive and negative deviations of the intensity occurred, and such noise signals are evaluated as positive values since the absolute values are used, as shown in Eq. 19. On the other hand, in the correlation method, positive and negative noise signals should be produced with the same probability and should cancel each other out. Thus, the correlation method seemed superior to the integration method for practical use.

In Table III, the results calculated by the I_R method for the same samples are listed. On the whole, the deviations between the expected value and the X_s value obtained by the I_R method tended to be greater than those in the correlation method, and the extent of deviation differed from substance to substance. It was found that the deviations of MGS were large, whereas those of W-35 were comparatively small. This might be because, near the angle θ_2

TABLE II. Comparisons of the X_s Values Calculated by the Correlation Method and the Integration Method with the Weight Fractions of Stable Crystal Form in Various Physical Mixtures of TP, MGS and W-35

Weight fraction of stable crystal form	Calculated X_s value[a]					
	TP		MGS		W-35	
	Cor.[b]	Int.[c]	Cor.	Int.	Cor.	Int.
0.20	0.216±0.017	0.250±0.021	0.200±0.010	0.230±0.021	0.215±0.003	0.367±0.033
0.40	0.408±0.009	0.431±0.012	0.394±0.009	0.469±0.053	0.406±0.033	0.436±0.020
0.60	0.605±0.005	0.624±0.016	0.577±0.007	0.627±0.028	0.614±0.007	0.675±0.075
0.80	0.799±0.007	0.824±0.019	0.798±0.019	0.835±0.030	0.815±0.042	0.891±0.014
(Regression line)						
Slope	0.973	0.958	0.989	0.987	1.004	0.906
y-Intercept	0.021	0.054	−0.002	0.047	0.011	0.135
r	0.9999	0.9998	0.9992	0.9972	0.9998	0.9785

a) Each value represents the mean±S.D. for four determinations.
b) Correlation method.
c) Integration method.

Table III. Comparisons of the X_s Values Calculated by I_R Method with the Weight Fractions of Stable Crystal Form in Various Physical Mixtures of TP, MGS and W-35

Weight fraction of stable crystal form	Calculated X_s value[a,b]		
	TP	MGS	W-35
0.20	0.208 ± 0.018	0.168 ± 0.020	0.145 ± 0.011
0.40	0.344 ± 0.021	0.286 ± 0.051	0.403 ± 0.007
0.60	0.559 ± 0.040	0.519 ± 0.072	0.543 ± 0.046
0.80	0.834 ± 0.021	0.865 ± 0.026	0.798 ± 0.073
(Regression line)			
Slope	1.047	1.162	1.049
y-Intercept	-0.037	-0.122	-0.053
r	0.9891	0.9761	0.9939

a) Each value represents the mean ± S.D. for four determinations.
b) Angles where the diffraction intensities were measured were as follows: TP ($\theta_1 = 19.2°$, $\theta_2 = 19.8°$, $\theta_3 = 32.9°$), MGS ($\theta_1 = 19.5°$, $\theta_2 = 20.0°$, $\theta_3 = 35.1°$), W-35 ($\theta_1 = 21.1°$, $\theta_2 = 20.7°$, $\theta_3 = 30.0°$).

where the intensity curves cross, the slope of the curve of MGS was so steep that a slight difference in reading the angle would produce a large error. On the other hand, θ_2 of W-35 was located at the shoulder of the curve where the change of the intensity produced by a reading error in the angle would be small. Thus, the applicability of the I_R method is rather limited compared with that of the correlation method.

Determination of the Change of Transition Degree during Storage

Based on the above examination, the changes of transition degree of TP, MGS and W-35 were practically determined during storage at 30 or 40 °C. The change of the normalized X-ray diffraction patterns of TP is shown in Fig. 7 as an example. A typical α-to-β transition can be seen, and the α-form transformed to the β-form within only one or two days at 40 °C. The mode of the change of the patterns was similar to that of physical mixtures (the β-form crystal content gradually increased). This suggests that both forms coexist and only the weight fraction of them changes with the lapse of time. From the intensity data of these patterns, the transition degree was calculated at each time of storage. In this case, the initial sample was conveniently regarded as α-standard, and the sample after storage for two weeks at 40 °C was regarded as β-standard.

An examples of analysis by the correlation method is shown in Fig. 8. A good linear relation was found with each sample, so the calculated X_β values seem to be valid.

The changes of transition degree for TP obtained by the correlation method, the integration method and I_R method are plotted together in Fig. 9. In all cases, regarding $(X_\beta)_{t=\infty}$ as $(X_\beta)_{t=2\text{ weeks}}$, the transition degree at each time was represented by $T_t\{=(X_\beta)_t/(X_\beta)_{t=2\text{ weeks}}\}$. The calculated values were nearly the same in all three methods, but the integration method tended to give a slightly larger value than the others. This tendency was the same as in the case where the physical mixtures were analyzed. The correlation method seemed to be reliable because the correlation coefficients gave satisfactory values of from 0.93 to 0.99 and the y intercept of the regression line was almost zero.

The analytical results for MGS obtained by the correlation method and the integration method are plottd in Fig. 10. The I_R method was excluded because the calibration line was not good, as shown in Table III. In this case, the initial sample was regarded as the α-standard, and the sample after storage for two weeks at 40 °C was regarded as β-standard.

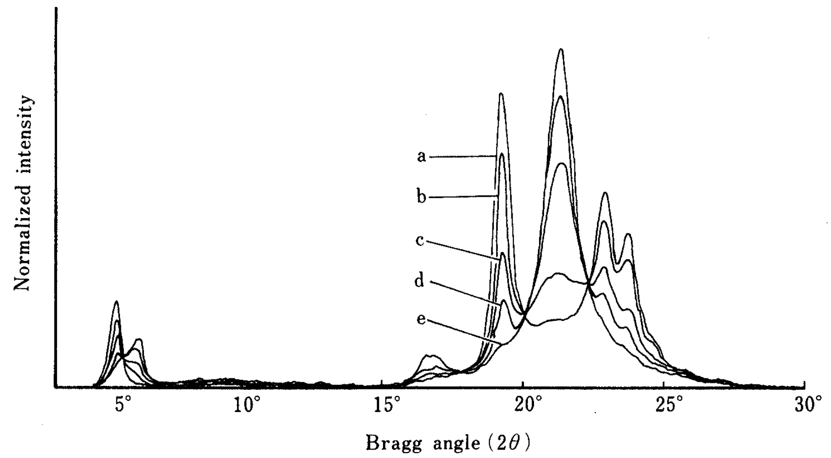

Fig. 7. Changes of the Normalized Diffraction Pattern of TP during Storage at 40 °C

a) after 24 h; b) after 16 h; c) after 8 h; d) after 4 h; e) initial.

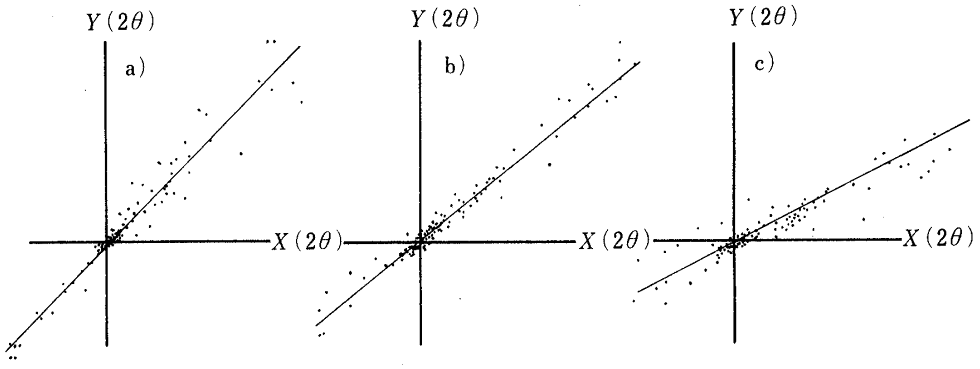

Fig. 8. Correlations between the Intensity Differences of α-Standard–β-Standard and Those of α-Standard–Stored Sample of TP

a) after 24 h at 40 °C, $T_t = 0.953$, $r = 0.9808$; b) after 16 h, $T_t = 0.787$, $r = 0.9930$; c) after 8 h, $T_t = 0.412$, $r = 0.9636$.

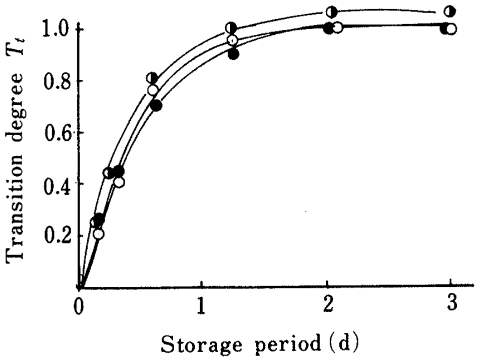

Fig. 9. Changes of the Transition Degree of TP during Storage at 40 °C Calculated by the Three Methods

○, correlation method; ●, integration method; ◑, I_R method.

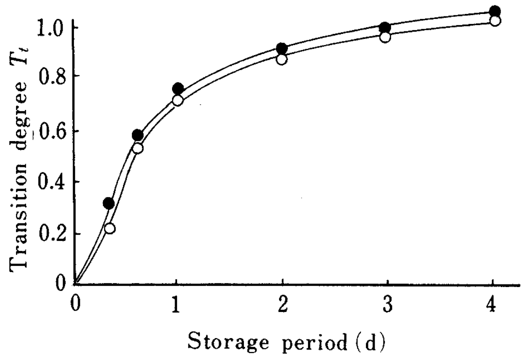

Fig. 10. Change of the Transition Degree of MGS during Storage at 40 °C Calculated by the Correlation Method and Integration Method

○, correlation method; ●, integration method.

The two methods gave a comparatively good agreement but the integration method produced a slightly larger value.

In the case of W-35, the X-ray diffraction pattern changed from A- to B-form and the

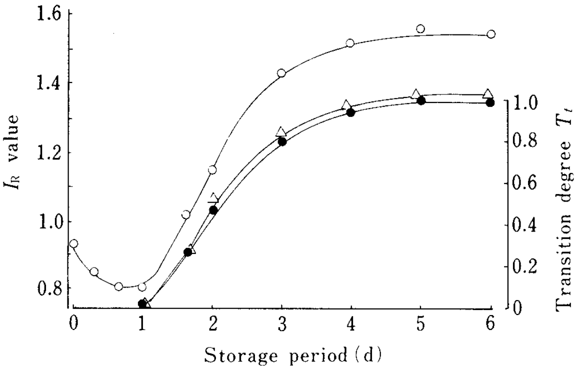

Fig. 11. Change of I_R Value and the Transition Degree of W-35 during Storage at 30 °C

○, I_R value; ●, T_t value by I_R method; △, T_t value by correlation method.

change was almost completed within a week at 30 °C. At first, the sample immediately after preparation and that after ten days at 30 °C were used as the standards of A-form and B-form, respectively, but in this case, the reliability of the results calculated by the correlation method was not high. These seem to be some other changes at the initial stage of storage, as we previously reported.[8] The changes of I_R of W-35 are shown in Fig. 11. The value first decreased and reached the minimum after one day, then increased to the maximum value within a few days. This I_R change suggests that some initial change occurs, and then the A-to-B transition follows. Therefore, the sample stored for one day (minimum I_R value) was chosen as the A-form and the correlation method was applied again. The result is plotted in Fig. 11. The transition degree was also calculated by the I_R method using Eq. 24 and the results are plotted in Fig. 11, and compared with those of the correlation method. As shown in Fig. 11, these results agreed well with each other, and the correlation coefficient of the correlation method was high, so these methods are applicable to this substance.

From these results, it is considered that the correlation method may be the most precise and universally applicable method for the determination of transition degree provided that the transition behavior is not too complex. This method should be useful to analyze the transition process kinetically and to examine in detail the relation between polymorphism and the change of physical properties of many pharmaceutical raw materials. The I_R method is an easy and convenient method, but its practical use seems to be more restricted, because suitable angles for measurement cannot always be found for every substance.

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