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Effect of Crystallinity on the Percutaneous Absorption of Corticosteroid. I. Determination of the Degree of Crystallinity of Hydrocortisone Acetate in Ground Mixtures with Crystalline Cellulose

MASAMI MORITA,* SADAO HIROTA, KOUJI KINUNO and KATSUO KATAOKA

Pharmaceutical Formulation Research Center, Research Institute, Daiichi Seiyaku Co., Ltd., 16-13, Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan

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The effect of the crystallinity of a topically applied drug on its percutaneous absorption was investigated with hydrocortisone acetate as a model drug. To decrease the degree of crystallinity, the drug was ground with crystalline cellulose. The X-ray diffraction method for determining the degree of crystallinity was shown to be valid in the range of amorphous cellulose ratio of 0 to *ca.* 50% by using simple mixtures of glutathione and amorphous cellulose. Thus, the dissolution rate of hydrocortisone acetate was confirmed to increase quantitatively with decreasing crystallinity.

Keywords——hydrocortisone acetate; crystalline cellulose; glutathione; ground mixture; crystallinity; disorder parameter; Ruland's method; X-ray diffraction; dissolution rate; percutaneous absorption

Percutaneous absorption of drugs has been widely studied in connection with topical dosage forms, and many important factors have been elucidated.[1] However, little basic research has been done on percutaneous absorption in relation to drug crystallinity, though some work has been done on the effects of drug crystallinity on gastro-intestinal absorption.[2]

The purpose of this study was to investigate the relationship of crystallinity to percutaneous absorption by using hydrocortisone acetate as a model drug. The dissolution rates of hydrocortisone and predonisone were reported to increase in the amorphous states (attained by coprecipitation with polyvinylpyrrolidone),[3] but the degree of crystallinity of the corticosteroids was not properly controlled. A method of crystallinity determination based on differential scanning calorimetry may be suitable, but cannot be applied to a drug when it reacts with a coexisting material or when the melting points are close to each other.

In this study, several samples with different crystallinities of hydrocortisone acetate were obtained by grinding the drug with crystalline cellulose in different ratios. It was shown in the previous paper[4] that Ruland's method[5] taking account of lattice imperfections of all kinds including those produced by paracrystalline disorder is a very useful tool for crystallinity determination of a pure drug at the preformulation stage of drug development. The X-ray method has not been applied to the determination of drug crystallinity in a pharmaceutical mixture. The diffracted intensities of the coexisting materials are difficult to eliminate from the observed diffraction pattern. In this paper, Ruland's method is applied to a drug in a mixture when the diffraction pattern of the coexisting material is a halo, and the effect of the coexisting material on the calculated results is examined.

Experimental

Materials——Hydrocortisone acetate and crystalline cellulose were of JP grade. Glutathione (reduced form,

crystalline, Sigma Chemical Co.) was of reagent grade. Samples of glutathione with different crystallinities were prepared by the same method as described in the previous paper.[4] Other chemicals were of reagent grade.

Preparation of a Ground Mixture[6]——A vibrational mill (Heiko Seisakusho model TI-200) made of tungsten carbide was used. The volume of the mill was 140 cm^3, and the length of the rod was 55 mm. The total weight of the materials was 2.0 g, and the grinding time was 5 min.

Preparation of a Physical Mixture——Materials were uniformly mixed in a mortar, avoiding grinding effects.

Measurements of X-Ray Diffraction——A Geiger Flex 2012 diffractometer (Rigaku Denki Co., Ltd.) was used. The measurement conditions were the same as reported in the previous paper.[4]

Calculations——The degree of crystallinity and the disorder parameter of a sample were calculated with a Burroughs 7800 computer by the automated computing procedure reported in the previous paper.[4]

Dissolution Method——The modified JP paddle dissolution apparatus[7] was used. The dissolution medium was 300 ml of a saline solution (JP) at 37 °C. Hydrocortisone acetate (30 mg) was added to the medium, and the amount dissolved was spectrometrically determined (248 nm).

Results and Discussion

Calculation of the Degree of Crystallinity of a Drug in a Mixture

It is assumed that the mixture consists of constituent 1 (a drug) and constituent 2 (an amorphous excipient), and that they do not interact with each other on a molecular level. The diffracted intensities of the mixture, I_3 (cpm-unit) can be normalized to I_3' (electron-unit) in the high-angle region as follows.

$$I_3' = p_1(\overline{f_1^2} + I_{inc1}) + p_2(\overline{f_2^2} + I_{inc2}) = cI_3 \tag{1}$$

p_1: weight fraction of constituent 1
p_2: weight fraction of constituent 2
$\overline{f_1^2}$: mean squared amplitude of atomic scattering factor of constituent 1

$(\overline{f_1^2} = \Sigma N_{i1} f_{i1}^2 / \Sigma N_{i1})$

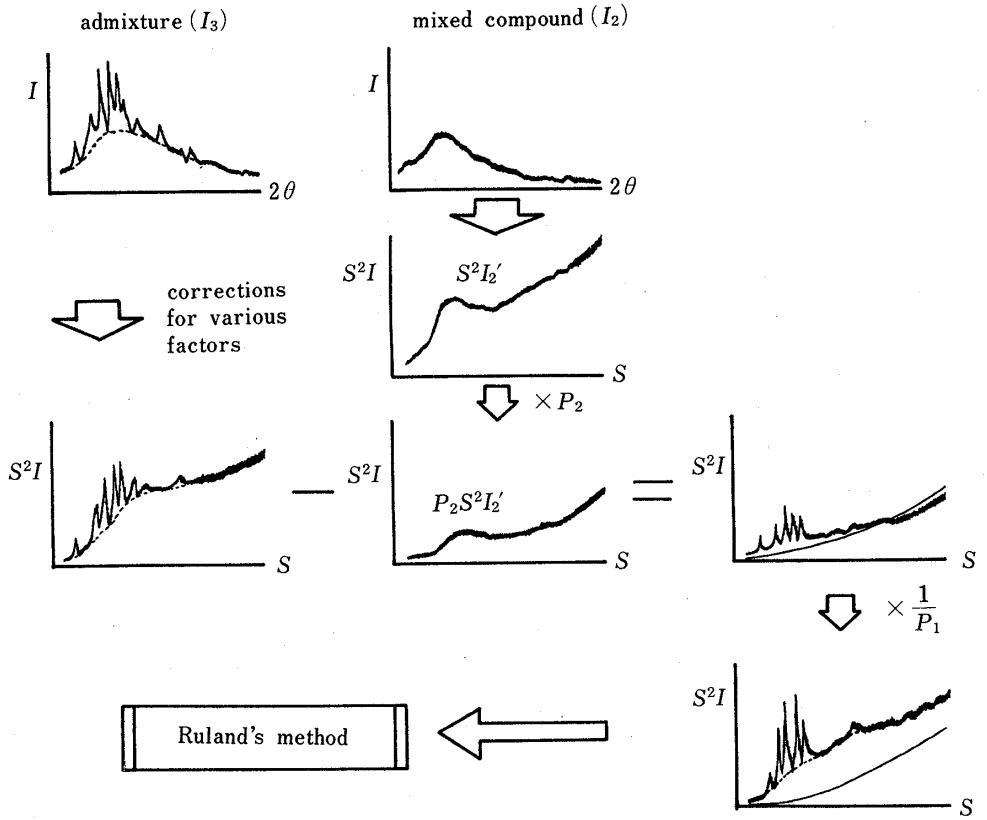

Fig. 1. Determination of the Degree of Crystallinity and the Disorder Parameter of a Drug Mixed with a Noncrystalline Substance

N_{i1}: number of atoms of type i of constituent 1
f_{i1}: atomic scattering factor of an atom of type i of constituent 1
$\overline{f_2^2}$: mean squared amplitude of atomic scattering factor of constituent 2
I_{inc1}: Compton scattering intensities of constituent 1
I_{inc2}: Compton scattering intensities of constituent 2
c: normalization constant

$$p_1+p_2=1 \tag{2}$$

The diffracted intensities of constituent 2, I_2 (cpm-unit) are normalized to I_2' (electron-unit). The intensities of the constituent 1, I_1' can be obtained as follows.

$$I_1'=\frac{1}{p_1}(I_3'-p_2I_2') \tag{3}$$

I_1' is used to calculate the degree of crystallinity, X_{cr}, and the disorder parameter, k, by Ruland's method. This procedure is illustrated in Fig. 1.

Validation of the Procedure

Amorphous hydrocortisone acetate could not be obtained alone. Therefore, glutathione, the amorphous state of which could be obtained by freeze-drying,[4] was used for the validation of the procedure.

Glutathione with known X_{cr} was mixed with ground crystalline cellulose (the amorphous state) in a ratio of 1:1. In Fig. 2, the value of the Y-function[8] (Eq. 4) calculated for glutathione alone (A) was compared to the value (B) for glutathione in the mixture.

$$Y=\frac{\int_{S_0}^{S_p} S^2I(S)\,dS}{\int_{S_0}^{S_p} S^2\overline{f^2}\,dS} \tag{4}$$

S_0: integral lower limit
S_p: integral upper limit
$I(S)$: coherent scattering intensity at S
$\overline{f^2}$: mean squared amplitude of atomic scattering factor
S: magnitude of reciprocal space vector

$(S=2\sin\theta/\lambda)$

θ: angle between the atomic plane and both the incident and reflected beams
λ: wavelength of the X-rays

These curves show similar patterns to each other on the whole, though the values deviate from each other in the low angle region. Figure 3 shows the curves of Y-functions calculated for glutathione for various X_{cr} in physical mixtures. From these data, 0.3, 0.9 and 1.25 $Å^{-1}$ were taken as S_p at $S_0=0.07$, while the data calculated from glutathione alone gave 0.26, 0.80 and 1.25 $Å^{-1}$ as S_p at $S_0=0.07$.[4]

The S^2I–S curve calculated from glutathione in physical mixtures was compared with that calculated for the drug alone. As shown in Fig. 4, the amplitude of the S^2I-values was observed to be rather large in the high angle region. The amplitude is, however, considered to be eliminated by integration and, therefore, it seems to be possible to calculate X_{cr} and k of glutathione in a physical mixture. These calculated values are shown in Table I. These results agree comparatively well with each other. Therefore, it is considered that such estimation is possible in the range of amorphous cellulose ratio of 0 to *ca.* 50%.

Determination of X_{cr} of Hydrocortisone Acetate in a Ground Mixture with Crystalline Cellulose

The S^2I–S curve of hydrocortisone acetate calculated from a physical mixture with

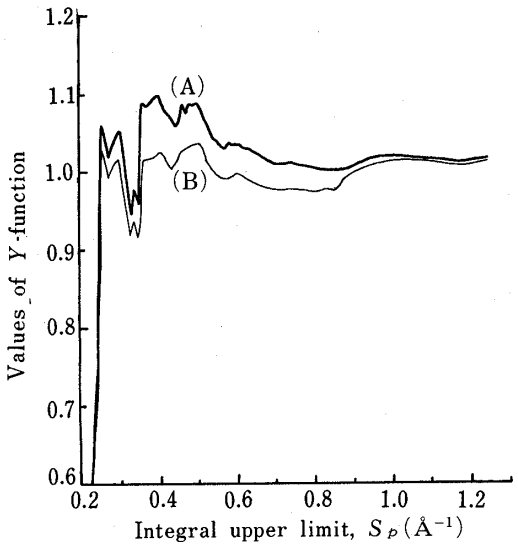

Fig. 2. Comparison between the *Y*-Function Values of Glutathione

(A), Intact glutathione; (B), glutathione numerically separated from a physical mixture with ground crystalline cellulose (1 : 1).

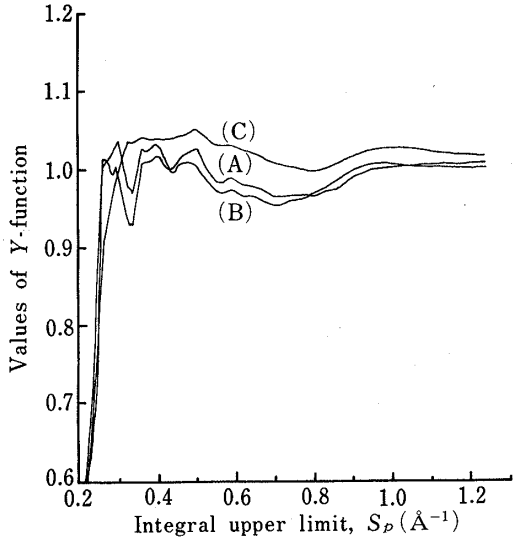

Fig. 3. Determination of Integral Upper Limits for Glutathione in a Physical Mixture with Ground Crystalline Cellulose (1 : 1) by Using $Y(S_p)$-Functions at $S_0 = 0.07$

(A), *Y*-function of sample A in Table I; (B), that of sample B in Table I; (C), that of sample C in Table I.

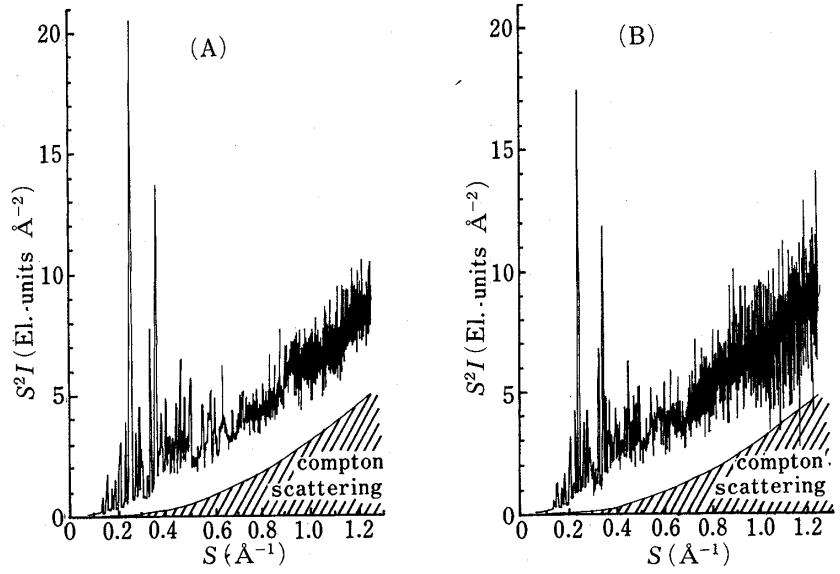

Fig. 4. Comparison between S^2I–S Curve of Intact Glutathione (A) and That of Glutathione Separated from a Physical Mixture with Ground Crystalline Cellulose (1 : 1) (B)

ground crystalline cellulose in a ratio of 1 : 1 was compared with that calculated from hydrocortisone acetate alone. As shown in Fig. 5, similar results were obtained. It was assumed that the diffraction pattern of crystalline cellulose in a ground mixture, a halo pattern, was the same pattern as that of crystalline cellulose ground alone. Figure 6 shows the values of *Y*-functions of hydrocortisone acetate calculated from ground mixtures with crystalline cellulose in mixing ratios of 8 : 2, 6 : 4, and 4 : 6. Values near 0.15 and above 0.6 are taken as S_p at $S_0 = 0.07$. It is considered that the region near 0.15 is not adequate for S_p, because the number of

TABLE I. Evaluation of the Degree of Crystallinity and the Disorder Parameter of Glutathione (GL) in Physical Mixtures with Ground Crystalline Cellulose (GCC)

Sample[a]	A[4]		A		B		B′		C		C′	
Ratio (GL : GCC)	1 : 0		1 : 1		1 : 0		1 : 1		1 : 0		1 : 1	
S_0—S_p[b]	$k=0$	$k=4.2$	$k=0$	$k=4.8$	$k=0$	$k=4.3$	$k=0$	$k=4.7$	$k=0$	$k=4.1$	$k=0$	$k=3.2$
0.07—0.26	0.707	0.833	0.614	0.783	0.521	0.616	0.489	0.620	0.226	0.265	0.213	0.251
0.07—0.80	0.322	0.886	0.221	0.805	0.230	0.647	0.177	0.632	0.103	0.277	0.100	0.261
0.07—1.25	0.150	0.790	0.127	0.756	0.110	0.591	0.103	0.603	0.050	0.255	0.059	0.242
X_{cr}		0.84		0.78		0.62		0.62		0.27		0.25
CV (%)[c]		5.7		3.1		4.6		2.3		4.3		3.8

a) A, Intact glutathione; B, intact 75%+amorphous[4] 25%; C, intact 25%+amorphous[4] 75%; A′, glutathione used is sample A; B′, glutathione used is sample B; C′, glutathione used is sample C.
b) The values of S_p used for samples A′, B′ and C′ were 0.30, 0.90 and 1.25 instead of 0.26, 0.80 and 1.25, respectively.
c) Coefficient of variation of X_{cr} calculated from the data at the above three integral regions.

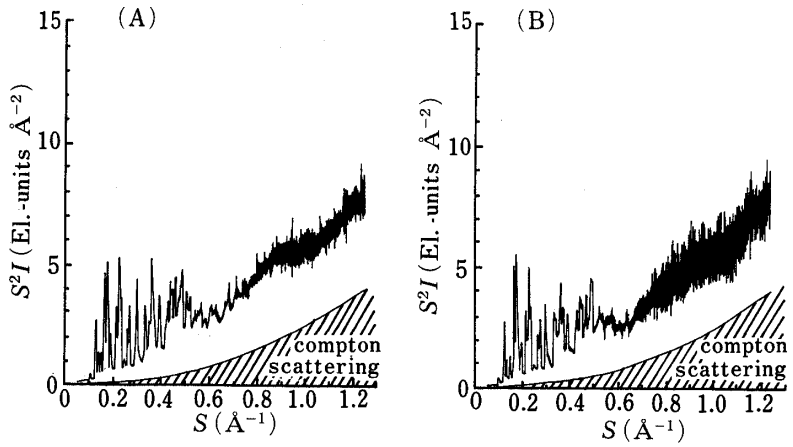

Fig. 5. Comparison between S^2I–S Curve of Intact Hydrocortisone Acetate (A) and That of Hydrocortisone Acetate Separated from a Physical Mixture with Ground Crystalline Cellulose (1 : 1) (B)

peaks is too small in the very low angle region ($S=0.07$—0.15) to examine the decrease effectively. X_{cr}–S_p plots based on Eq. 5 were drawn for these samples.

$$X_{cr}(S_p)=\frac{\int_{S_0}^{S_p} S^2 I_{cr}(S)\,dS}{\int_{S_0}^{S_p} S^2 I(S)\,dS}\times\frac{\int_{S_0}^{S_p} S^2\overline{f^2}\,dS}{\int_{S_0}^{S_p} S^2\overline{f^2}\exp(-kS^2)\,dS} \qquad (5)$$

$I_{cr}(S)$: coherent scattering intensities at S in the crystalline region

Figure 7 shows the X_{cr}–S_p plots for hydrocortisone acetate ground alone. The arrow in the figure shows the point where X_{cr} is constant in the region of $S_p \gtrsim 0.6$. X_{cr} and k of hydrocortisone acetate in ground mixtures with crystalline cellulose in the ratios of 8 : 2, 6 : 4 and 4 : 6 were calculated in a similar manner. From these patterns, X_{cr} of hydrocortisone acetate ground alone is 90%. X_{cr} of hydrocortisone acetate in a ground mixture in the ratio of

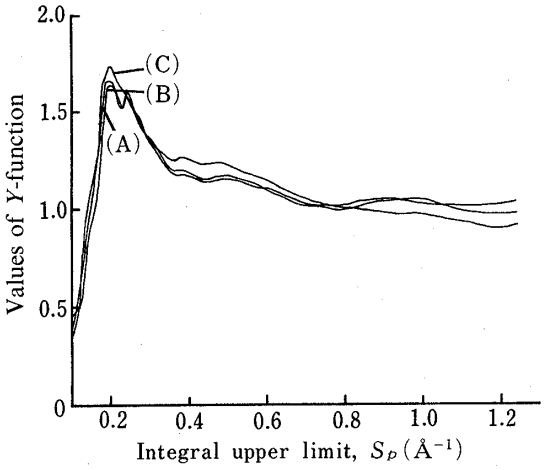

Fig. 6. Determination of Integral Upper Limits for Hydrocortisone Acetate in a Ground Mixture with Crystalline Cellulose (1 : 1) by Using $Y(S_p)$-Functions at $S_0 = 0.07$

(A), Y-function of a ground mixture of hydrocortisone acetate with crystalline cellulose in the ratio of 8 : 2; (B), that in the ratio of 6 : 4; (C), that in the ratio of 4 : 6.

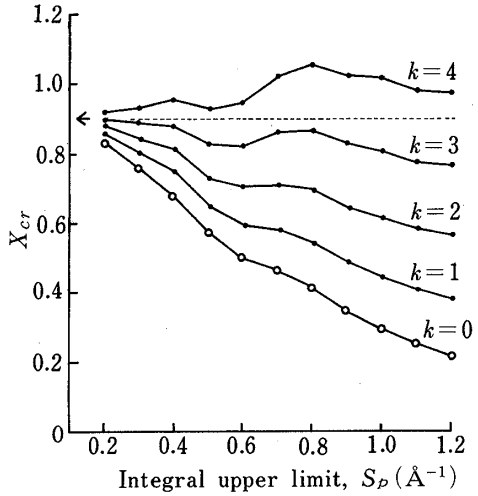

Fig. 7. Determination of the Degree of Crystallinity and the Disorder Parameter of Hydrocortisone Acetate by Using X_{cr}–S_p Plots

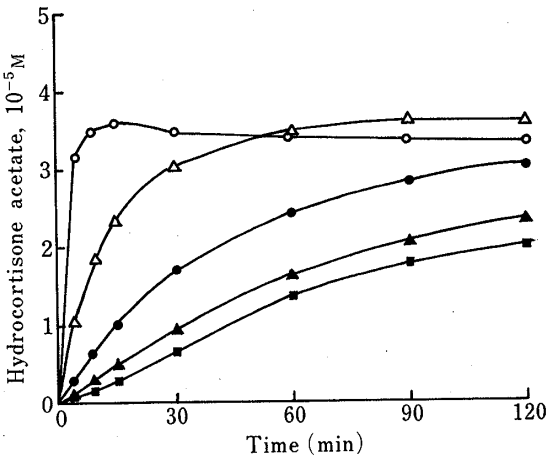

Fig. 8. Dissolution Profiles of Hydrocortisone Acetate in a Ground Mixture with Crystalline Cellulose

○, $X_{cr}=0.0$; △, $X_{cr}=0.45$; ●, $X_{cr}=0.60$; ▲, $X_{cr}=0.65$; ■, $X_{cr}=0.90$.

8 : 2 was 65%, while that in the ratio of 6 : 4 was 60% and that in the ratio of 4 : 6 was 45%. The ground mixtures in the ratios of 2 : 8 and 1 : 9 were apparently amorphous. Thus, X_{cr} of a drug in a ground mixture can be semi-quantitatively determined by this method.

Figure 8 shows the dissolution rates of hydrocortisone acetate with different crystallinities in ground mixtures in saline solution. It was confirmed that the smaller the X_{cr}, the faster the dissolution rate.

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