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Polymorphic Transition Rate of Semisynthetic Fatty Suppository Bases[1,2]

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The degree of polymorphic transition of semisynthetic fatty suppository bases was estimated by means of X-ray diffraction measurements. I_R values, defined as the relative intensities of two characteristic diffraction peaks of unstable A-form and stable B-form were useful for this purpose. Storage experiments using Witepsol H-15 suppositories containing 1% Brilliant Blue were performed. During storage, the changes of melting point, softening time and drug release rate were measured and compared with I_R. Each of these properties showed a correlation with I_R value irrespective of storage temperature or period. With lapse of time, the I_R values commonly fell to a minimum in the earlier period, subsequently increased to a maximum and then remained constant. This change depended markedly on the storage temperature, so we attempted to predict the I_R change using two rate parameters. One was the half-transition time $t_{1/2}$ and the other was the shift factor A_T calculated by the reduced variable method. On plotting these rate parameters against $1/T$, good linear relations were obtained, and the activation energies were 98 kcal and 108 kcal for $t_{1/2}$ and A_T respectively. Using these values, prediction of the physical stability of the suppository seems to be possible. It was also shown that this I_R method is applicable to other commercial fatty vehicles.

Keywords——suppository; stability; polymorphism; semisynthetic fatty suppository base; transition degree; X-ray diffraction; transition rate

Semisynthetic triglycerides have been widely used as oleagenous suppository bases. Many kinds of commercial products are available on the market.[3,4] Most of them are produced from hardened laurine oil such as coconut or palm kernel oil. Their individual physicochemical characteristics depend on the additives or production technique. Therefore, users can select the most suitable vehicle for formulation studies. However, these preparations also have some common properties due to the similarity of their fatty acid composition.

It is now well known that many pharmaceutical properties of fatty suppositories tend to change during storage.[5–9] Previously, the authors showed by X-ray diffraction measurements that the polymorphism of these vehicles showed qualitatively similar behavior.[1] Namely, immediately after preparation, these vehicles were in an unstable crystal form (we denoted this as A-form), but during storage at high room temperature this form gradually changed to a more stable form (we denoted this as B-form). Such a transition is generally accompanied by changes of the pharmaceutical properties. Among them, the decrease of drug release cannot be neglected from the standpoint of clinical therapy efficacy. Therefore, for formulation studies and pharmaceutical evaluation of fatty suppositories, the stability of the vehicle must be carefully examined as well as that of the active ingredient.

From this viewpoint, we studied the relationship between polymorphic transition and pharmaceutical properties, and also attempted to analyze the transition process kinetically in order to predict the physical stability of fatty suppositories.

Experimental

Materials——Witepsol H-15[10a] was mainly used in this study. Its physicochemical properties were as follows: acid value, 0.06; iodine value, 0.8; hydroxyl value, 12.1; saponification value, 237.0; fatty acid composition measured by GLC, 1.2% $C_{10:0}$, 48.1% $C_{12:0}$, 18.5% $C_{14:0}$, 16.9% $C_{16:0}$, 15.3% $C_{18:0}$, and others, none or trace. Witepsol W-35,[10a] Suppocire AM, BM,[10b] S.B-H[10c] and Isocacaobutter MO-5[10d] were

also used for comparison. Brilliant Blue used as a marker for release measurements met the requirements of JSFA IV. The particle size as determined by microscopic observation was about 40 μm.

Preparation of Suppository——Suppositories were prepared by the melt pour method, and weighed about 2.4 g. Freshly prepared samples were placed in a refrigerator overnight, and then storage experiments were carried out in a water bath controlled at the desired temperature within ±0.1°C.

X-Ray Diffraction——Measurement conditions were as follows: apparatus, Geigerflex 2013 (Rigaku Denki); radiation, Ni-filtered Cu-k_α (λ=1.54 Å); voltage/current, 40 kV/35 mA; slits, divergence/receiving/scattering, 0.5°/0.3 mm/0.5°; scanning speed, 2°/min.

Melting Point——Differential thermal analysis was used. Measurement conditions were as follows: apparatus, Shimadzu DT 20B thermal analyzer; heating speed, 2°C/min.; reference, α-alumina; range. ±100 μV. Accuracy of the temperature was checked by measuring the melting points of methyl stearate (39.2°C) and methyl arrachidate (46.4°C).

Softening Time——Krowczynski's method[11] was used at 37.0±0.1°C.

Release Measurement——The dialysis method was used at 37.0±0.1°C, according to the procedure described in our previous report.[1]

Results and Discussion

Measurement of the Degree of Polymorphic Transition

As mentioned in our previous paper, the X-ray diffraction patterns of semisynthetic vehicles change with time schematically shown in Fig. 1. During storage at room temperature they gradually alter from the A-form having a characteristic diffraction peak at 20.7° (2θ) due to the side spacing spacing of 4.27 Å, to the B-form having a peak at 21.1° due to spacing of 4.23 Å. In the course of the A to B transition process, many intermediary patterns can be observed. It seems reasonable to presume that these intermediates are mixtures of the A and B-forms and that the intermediates rich in B-form change more than ones rich in A-form. This means that the degree of transition of the intermediates can be determined by assay of the concentration of both A- and B-form.

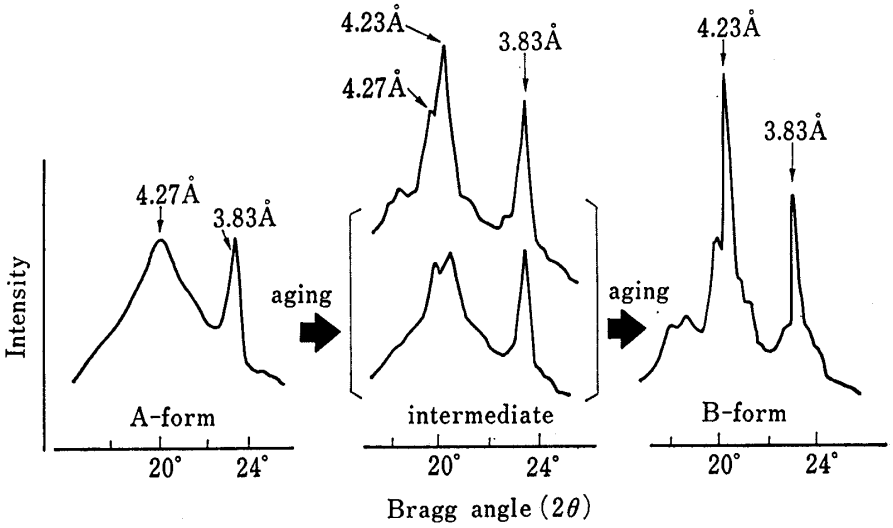

Fig. 1. Schematic Representation of the Changes of X-Ray Diffraction Pattern of Semisynthetic Fatty Suppository Bases

By following the change of both characteristic diffraction peaks, 4.27 Å and 4.23 Å, it can be found that the 4.23 Å peak increases in parallel with the transition. Then, we defined the diffraction intensities of both peaks, I_A and I_B, according to eq. (1) and (2),

$$I_A = I_{20.7°} - I_{30.0°} \quad (1)$$

$$I_B = I_{21.1°} - I_{30.0°} \quad (2)$$

where $I_{20.7°}$ and $I_{21.1°}$ mean the diffraction intensities at Bragg's angle (2θ) values of 20.7° and 21.1°, respectively, and $I_{30.0°}$ is the background intensity. Thus-defined I_A and I_B

should reflect to a certain extent the concentration of A-form and B-form. However, it is generally believed that the X-ray diffraction intensities measured by means of the powder method show poor repoducibility because the area irradiated is apt to differ in every measurement owing to the particle size distribution, orientation of crystals, *etc.* in the measuring cell. In such cases, relative values of two diffraction intensities are often adopted to reduce the experimental errors. For instance, Nakagaki *et al.*[12] used aluminium as an internal standard for evaluation of the crystallinity of zeolite. However, in our case, it is not appropriate to use an internal standard because the physical and pharmaceutical properties of vehicles, such as viscosity, rigidity, melting behavior, drug release and so on, may be changed easily by addition of another material. So, in place of the internal standard method, we defined I_R in eq.(3) and tried to find a correlation between I_R and the degree of A-to-B transition.

$$I_R = I_B/I_A \tag{3}$$

Table I shows I_A, I_B and I_R values obtained in six runs under identical conditions using both crystal forms of Witepsol H-15. The measurement error of I_R assessed in terms of the variation coefficient was distinctly smaller than that of individual I_A and I_B.

Table I. Reproducibility of I_A, I_B and I_R Values for Two Crystal Forms of Witepsol H-15

Run No.	A-Form			B-Form		
	I_A	I_B	I_R	I_A	I_B	I_R
	cps[a] $\times 10^{-3}$			cps $\times 10^{-3}$		
1	3.18	2.80	0.88	2.62	3.82	1.45
2	3.60	2.65	0.85	2.92	4.30	1.47
3	2.85	2.40	0.84	2.77	4.19	1.51
4	3.06	2.58	0.84	2.72	4.10	1.51
5	2.72	2.30	0.85	2.77	4.17	1.51
6	3.01	2.48	0.82	2.95	4.30	1.51
Mean	2.99	2.54	0.84	2.79	4.15	1.49
S.D	0.17	0.18	0.02	0.12	0.18	0.03
C.V (%)	5.7	7.1	2.3	4.4	4.3	2.0

a) Counts per second.

Relationship between I_R and the Fraction Ratio A- and B-Form

Table II shows I_R values of both A- and B-form for three commercial vehicles. As will be described later, I_R values of freshly prepared samples first decrease to a minium value and subsequently increase to a maximum (Fig. 4). Here, A-form corresponds to I_R minimum and B-form corresponds to I_R maximum. To examine the correlation between I_R and the fraction ratio of both forms, the following experiment was performed. Both crystal forms were powdered and blended in a certain ratio, then the mixture was stirred vigorously in cooled 50% aqueous methanol in a homomixer for a few minutes. Next, the solvent was rapidly removed by filtration under reduced pressure. The mixed samples prepared by this

Table II. I_R Values of Both Crystal Forms for Three Commercial Vehicles

Suppository base	A-Form	B-Form
Suppocire BM	0.82±0.02	1.71±0.03
Witepsol H-15	0.85±0.02	1.49±0.02
Isocacaobutter MO-5	0.86±0.04	1.41±0.03

Each value represents the mean±S.D. of five determinations

procedure were immediately subjected to X-ray diffraction measurement, and the I_R values were calculated.

Fig. 2 shows the diffraction patterns of Witepsol H-15 as an example. In this figure, M_1 and M_5 showed almost the same patterns as A- and B-form without treatment, respectively, and their I_R values were also the same. These results indicate that the mixing procedure did not affect the crystal forms of vehicles. In the cases of M_2–M_4, various intermediate patterns were observed. They were very similar to the intermediates observed during storage.

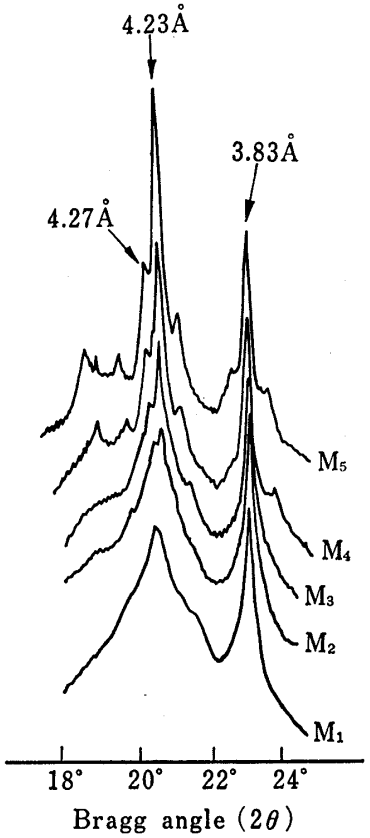

Fig. 2. X-Ray Diffraction Patterns of Witepsol H-15 containing Two Crystal Forms mixed in Various Ratios

M_1; A-form: B-form=100: 0, M_2; 75: 25, M_3; 50: 50, M_4; 25: 75, M_5; 0: 100.

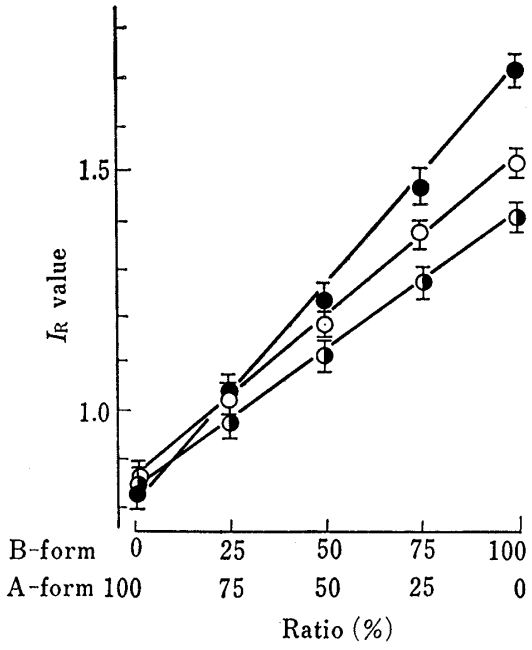

Fig. 3. Relation between I_R Values and Fraction Ratio of A- and B-form

(●) Suppocire BM, (○) Witepsol H-15, (◑) Isocacaobutter MO-5.
Each value represents the mean ± S.D. of five determinations.

Fig. 3 shows the relationship between I_R and the mixing ratio of both crystal forms. Good linear relations were seen for all three commercial vehicles. These results indicate that all intermediates in the course of the transition are mixtures of A and B crystal forms, and that the weight fraction of both forms can be determined by measuring I_R. Thus, the degree of the transition can be estimated from the I_R value for an unknown sample.

Change of I_R during Storage

The change of I_R of Witepsol H-15 placebo suppository is shown in Fig. 4, as a typical example. It decreased to a minimum value ($I_R$min.) in the earlier stage of storage and subsequently increased to the maximum ($I_R$max.) within 4 days. After that, I_R did not change any more. Thus, the polymorphic transition may be completed when I_R reaches $I_R$max..

In the earlier stage of storage, the X-ray diffraction patterns showed apparently A-form, but the intensity of the 3.83 Å diffraction peak increased remarkably (Fig. 5). This peak can be observed in both A- and B-form, and it does not increase any further after I_R reaches its minimum. Thus, it is presumed that another transition which differs from the A-to-B

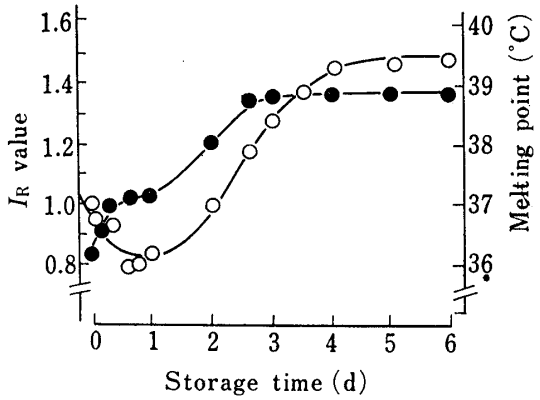

Fig. 4. Changes of I_R Value and Melting Point of Witepsol H-15 during Storage at 30.0°C

—○—; I_R value, —●—; Melting point.

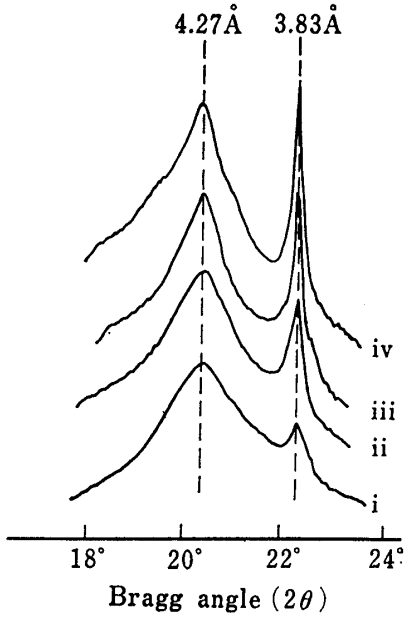

Fig. 5. Changes of X-Ray Diffraction Pattern of Witepsol H-15 during the Induction Stage at Room Temperature

i: Immediately after preparation (I_R=0.92), ii: After 12 h (I_R=0.88), iii: After 24 h (I_R=0.82), iv: After 48 h (I_R=0.79).

transition proceeds in this period. This suggestion is supported by the change of melting point curve shown in Fig. 4. It rose in the earlier period and showed a shoulder when I_R became minimum; then, it rose again in parallel with I_R. However, the melting point reached an equillibrium state earlier than I_R. It became constant when I_R reached 1.3, which corresponds to about 70% B-form fraction ratio as estimated from the calibration curve shown in Fig. 3. Comparing both curves, it seems that the melting point does not always reflect the overall polymorphism and that I_R values give more information about the transition.

The polymorphism of pure triglycerides was investigated in detail by Lutton and others.[13,14] According to their classification, our A-form somewhat resembles the β'-form having two diffraction peaks near 4.7 Å and 3.8 Å. However, it cannot be strictly assigned to any of their types. Generally, fatty vehicles are not pure but are mixtures of various kinds of triglyceride, and may be composed of various unit crystal cells which differ from each other in fatty acid composition and crystal form. In such a case, the X-ray diffraction pattern is considered to the sum of individual diffractions of various kinds of unit cells.[15] This situation may hold for the A- and B-form, so it may be difficult to define these forms in terms of micro levels of unit cell as if they were pure triglyceride.

It is also well known that a pure triglyceride solidifies as the α-form having a broad single diffraction peak near 4.15 Å when the melt is cooled rapidly. For the suppository base, however, we could not obtain the α-form in spite of many trials; there were always two peaks. However, from the behavior of the 3.83 Å diffraction peak shown in Fig. 5, it is reasonable to consider that the vehicles are relatively rich in α-like form in the earlier stage of storage. The α-like form is unstable and is easily transformed to another form, possibly β'-like form. The decrease of I_R value may reflect the loss of unstable α-like form.

Hitherto, we classified both A- and B-form only qualitatively from the X-ray diffraction patterns, but to simplify the discussion, hereafter we designate the crystal form at I_R min. as A-form, that at $I_R$max. as B-form, the I_R decreasing period till $I_R$min. as the induction stage and the I_R increasing period from $I_R$min. to $I_R$max. as the A-to-B transition stage.

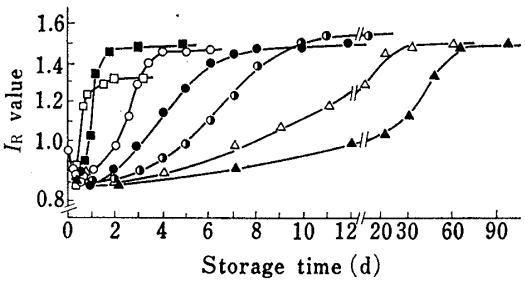

Fig. 6. Changes of I_R Values of Witepsol H-15 during Storage at Various Temperatures

□; 34.0°C, ■; 32.0°C, ○; 30.0°C, ●; 29.0°C, ◑; 28.0°C, △; 27.0°C, ▲; 26.0°C.

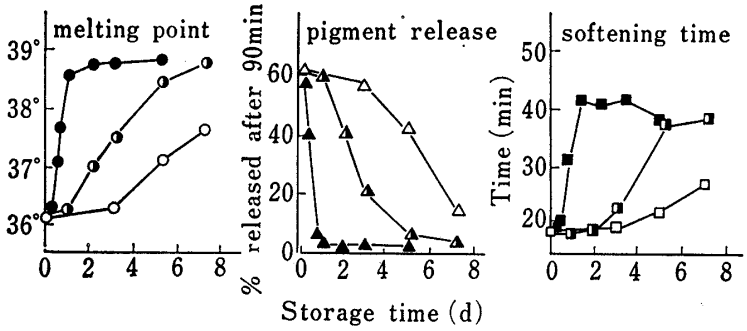

Fig. 7. Changes of Pharmaceutical Properties of Witepsol H-15 Suppositories containing 1% Brilliant Blue during Storage at 26.0, 28.0 and 30.0°C

○△□; 26.0°C, ◑▲◨; 28.0°C, ●▲■; 30.0°C.

Influence of the Storage Temperature on the Transition Rate

Fig. 6 shows the changes of I_R for Witepsol H-15 placebo suppository stored at various temperatures between 26.0°C and 34.0°C. All the curves show a similar pattern, that is, decreasing to $I_R$min., subsequently increasing to $I_R$max. and afterwards being almost constant. It is also clear from this figure that the transition rate depends markedly on the storage temperature. Namely, the higher the temperature, the faster the transition proceeds. At storage temparatures below 32.0°C, the values of $I_R$max. were almost constant (1.46—1.50), but I_R was considerably lower at 34.0°C (1.34). This phenomenon can be explained as follows. At temperatures close to the melting point, such as 34.0°C, both solid and liquid phase coexist in the vehicle. The solid phase may transit rapidly to B-form, but when the suppository is taken out and cooled for X-ray measurement, the liquid phase recrystallizes as A-form. Consequently, the sample is relatively rich in A-form and shows a lower I_R value.

Relation between I_R and Pharmaceutical Properties

In the previous paper, we showed that pharmaceutical properties of suppositories tend to change during storage at high room temperature. Fig. 7 shows the changes of melting point, drug release properties and softening time of Witepsol H-15 suppository containing 1% Brilliant Blue stored at 26.0, 28.0 and 30.0°C. It is clear from this figure that the higher the storage temperature, the faster these properties change. The temperature dependencies

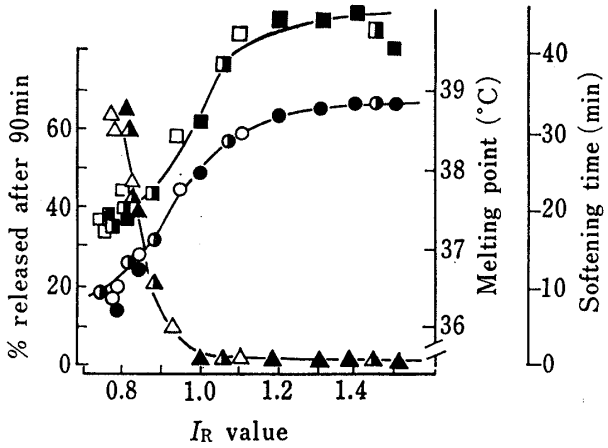

Fig. 8. Relation between Pharmaceutical Properties and I_R Values for Witepsol H-15 Suppositories

Symbols: see caption of Fig. 7.

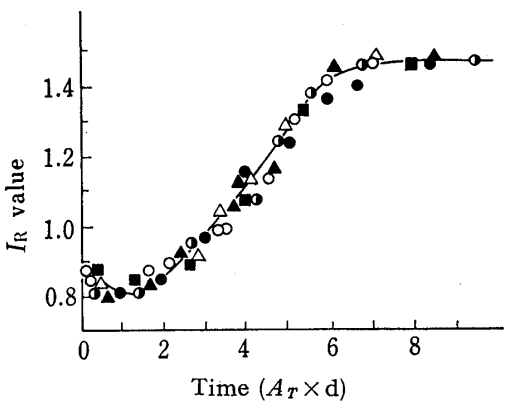

Fig. 9. I_R Curves Superposed by the Use of a Reducing Time Factor

Symbols: see caption of Fig. 6.

seem to have some analogy to the I_R curve, so we plotted them against the I_R values (Fig. 8). It can be seen that they depend upon the I_R values irrespective of storage temperature or period. This result means that these pharmaceutical properties can be determined to some extent by measuring I_R.

Prediction of Transition Rate

It seems to be practically desirable to predict the changes of pharmaceutical properties of fatty suppositories by means of kinetic analysis of polymorphic transition. However, only a few studies on the kinetic analysis of the polymorphism of triglycerides have been carried out. Goto *et al.*,[16] for example, analyzed the transition from unstable α-form to stable β-form for some saturated monoacid triglycerides. They used the following equation on the assumption that the transition follows a first-order reaction:

$$\ln(1-X) = -kt \qquad (4)$$
$$X = I_\beta/(I_\alpha+I_\beta)$$

where I_α and I_β represent the diffraction intensities of characteristic peaks of the α-form and β-form, respectively, X is the degree of transition, t is the time of storage and k is the first-order rate constant. On the other hand, Dafler used eq. (5) for the transition of tristearin.[17] This equation is based on Pearlstein's theory[18] regarding the transition as an atom-jump process from an unstable α-lattice location to a stable β-lattice location,

$$C_\beta/C_\alpha = -m \ln t + C_0 \qquad (5)$$

where C_β and C_α are the concentrations of the β- and α-form. They considered that C_β/C_α should be proportional to I_β/I_α; m and C_0 are constants.

We tried to apply these equations to I_R values but the results were not satisfactory. This may be attributable to the presence of more than one transition process, as mentioned in the preceding paragraph, or to other unknown factors involved in the transition mechanisms. However, from the practical viewpoint, it may be possible to predict the transition rate by empirical techniques. Thus, we attempted to apply the reduced variable method as follows.

The I_R curves in Fig. 6 display rather similar patterns irrespective of storage temperature, with the exception of 34.0°C, and it may be possible to superpose them on a single curve by conversion of the time-scale. Namely, if the I_R curve at T°C is represented as a function of time, $F_T(t)$, the following equation can be set up by the choice of the most suitable shift factor A_T.

$$F_{T_1}(A_{T_1}t) = F_{T_2}(A_{T_2}t) = \cdots\cdots = F_{T_n}(A_{T_n}t) \qquad (6)$$

Then, we used the I_R curve at 29.0°C as a standard and calculated A_T values by means of the least-squares method. In making the calculation, we regarded the time of $I_R$min. (t_{min}) as the starting point ($t=0$), because the reproducibility of I_R values of freshly prepared samples was not good, but they all pass $I_R$min. during storage. Such poor reproducibility at the initial stage may be due to minor differences of preparation or storage conditions before X-ray measurement. A_T values obtained are listed in Table III. The I_R values are plotted against reduced time ($A_T \times t$) in Fig. 9. All the I_R curves superpose well on the standard curve $F_{29}(t)$. These results indicated that the transition mechanism is the same under all these storage conditions, and only the transition rates are different. The shift factor A_T is a parameter of transition rate and a function of temperature, though the details of the mechanism are not clear.

Fig. 10 shows a plot of log A_T *vs.* reciprocal absolute temperature $1/T$ according to Arrhenius's equation. A good linear relationship was obtained, and the activation energy calculated from the slope was about 108 kcal. Goto *et al.* obtained values of 46, 62 and 83 kcal for the α-to-β transition of trilaurin, tripalmitin and tristearin, respectively.[16] Compared with

Table III. A_T and $t_{1/2}$ Values for the Transition of Witepsol H-15 during Storage at Various Temperatures

Storage temp. (°C)	A_T	$t_{1/2}$ (h)
26.0	0.135	624
27.0	0.347	226
28.0	0.683	130
29.0	1.000	86
30.0	1.845	48
32.0	3.996	22
34.0	—	8

these values, our value seems to be a little large, but they also stated that the activation energies of impure triglycerides are somewhat larger than those of pure ones, so 108 kcal may be reasonable.

It is also possible to regard the time required for transfer from a certain state to another state as a rate parameter. In this case, the half-changing time $t_1/_2$ is often used. Thus, we defined $t_{1/2}$ as the time required for the I_R value to reach $(I_R\text{max.}+I_R\text{min.})/2$ from $I_R$min. The found $t_{1/2}$ values are listed in Table III, and $-\log t_1/_2$ is also plotted in Fig. 10 as a dashed line. It also showed a good linearity and the activation energy was 98 kcal, close to the value obtained from A_T. Using this parameter, we can estimate the physical stability of suppositories. For examples, we predicted the $t_{1/2}$ values of Witepsol H-15 at 20°C and at 15°C to be about 1.4 years and about 26.4 years, respectively.

Transition Rate Analysis of Other Commercial Vehicles

Thus far, we have dealt only with the transition of Witepsol H-15, but the method described above is not restricted to this vehicle; it should be widely applicable to other semisynthetic vehicles.

Fig. 11 shows the changes of I_R values for six commercial fatty vehicles during storage at 30.0°C. All the curves exhibit qualitatively similar patterns. In Table IV, values of $I_R$max., $I_R$min. and $t_{1/2}$ are summarized. It is clear from the table that $I_R$min. takes almost the same value but that $I_R$max. and $t_1/_2$ differ remarkably. This may reflect the differences

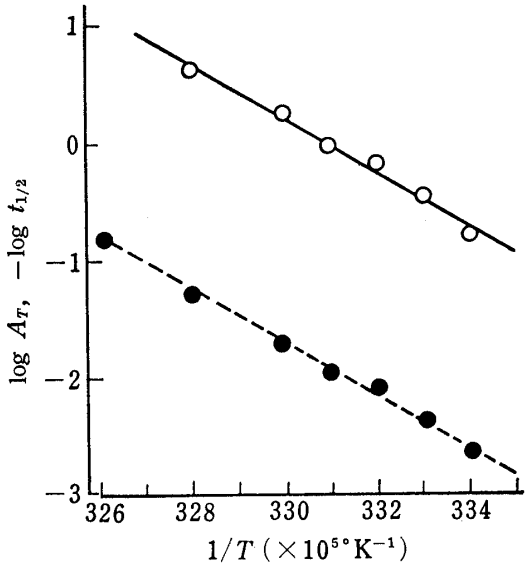

Fig. 10. Arrhenius Plots of A_T and $t_{1/2}$
—○—; A_T, --●--; $t_{1/2}$.

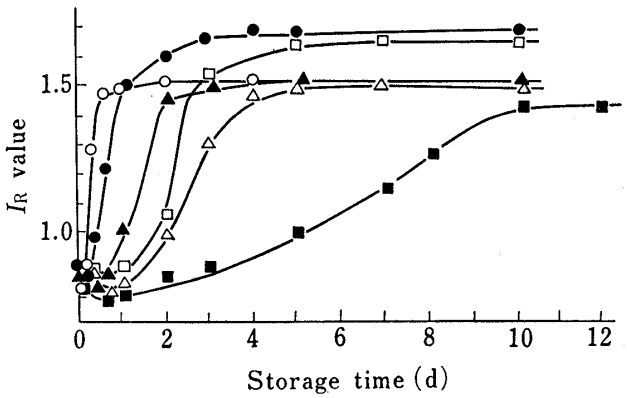

Fig. 11. Changes of I_R Value for Various Oleaginous Suppository Bases during Storage at 30.0°C

○; Suppocire AM, ●; Suppocire BM,
△; Witepsol H-15, ▲; S.B-H,
□; Witepsol W-35, ■; Isocacaobutter MO-5.

Table IV. Comparison of Transition Rates for Various Synthetic Oleagenous Suppository Bases during Storage at 30°C

Suppository Base	I_R max.	I_R min.	$t_{1/2}$ (h)
Witepsol H-15	1.49	0.78	48.0
Witepsol W-35	1.68	0.82	44.4
Suppocire AM	1.51	0.82	7.2
Suppocire BM	1.71	0.84	15.6
Isocaobutter MO-5	1.41	0.76	135.6
S.B-H	1.47	0.77	24.6

of physical properties of the vehicle due to differences of fatty acid composition, additives and so on. At present, it is not clear how these factors affect the polymorphic transition rate. However, this information would be very interesting and important for formulation studies, and we intend to investigate these subjects in detail.

References and Notes

1) This paper forms Part II of "Studies on the Stability of Suppositories." Part I; H. Yoshino, M. Kobayashi, and M. Samejima, *Yakuzaigaku*, **41**, 102 (1981).
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