♨ A Pulsed Low Resolution NMR Study on Crystallization and Melting Processes of Cocoa Butter

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ABSTRACT

Pulsed low resolution nuclear magnetic resonance (NMR) was employed to measure the "melting" curves of different series of cocoa butter samples. The samples were prepared from completely liquid phase by cooling and tempering them at different temperatures T_c for varying times Δt. The "melting" curves were measured while keeping the sample at a fixed temperature T_m. The complex shape of each curve was interpreted in terms of cocoa butter polymorphism, and the results were compared with data obtained by other techniques available in the literature. Using just two tempering temperatures (+7 C and -18 C), we were able to distinguish four solid phases and identify them with the phases II, III, IV, and V described in literature. Our data are in full agreement with literature. Several novel results have been also found. These include the kinetic constants of the melting processes of phases II and III, the rate constants of solidification of phase V, the conversion of phase III into phase IV before melting at temperatures $\geqslant$ 30 C, and the growth of phase V out of phase II at -18 C (including the rate constant of this process). We are convinced that NMR may serve as a principal tool in fat polymorphism investigations, especially if it is combined with other techniques such as differential scanning calorimetry. Its advantage, apart from rapidity of measurement, is the fact that the measurement itself does not interfere with the melting or solidification process studied. On the other hand, it does not distinguish in a direct way between different solid phases present in the sample; this can be done only in inference from the behavior upon melting.

INTRODUCTION

Like most fats, solid cocoa butter exhibits a marked polymorphism. The different solid modifications (phases) are quite distinct, and their physical properties such as melting points are well defined. At least six solid phases have been identified by means of X-ray diffraction and by calorimetric techniques (1,2). The growth and stability of the individual solid phases depend upon temperature as well as upon the thermal history of the sample (tempering).

The study of fat polymorphism is complicated by the slow dynamics of the solidification and recrystallization processes, and by the fact that several solid phases are often present simultaneously in the sample. It is therefore advisable to combine as many different experimental techniques as possible in any such investigation.

In this paper we want to show the usefulness of low resolution pulsed nuclear magnetic resonance (NMR) in solid fat polymorphism studies. We have actually employed only one particular NMR technique, namely that of solid/liquid ratio (S/L) determination (3-7). The usefulness of other NMR techniques, such as relaxation time measurements, is yet to be investigated.

The S/L determination by means of NMR is very fast (only a few seconds are needed for a single determination) and thus permits one to follow accurately the kinetics of melting (or solidification) of fats. In this paper we show how this can be used to study the fat polymorphism.

Cocoa butter was chosen because its complex polymorphism has been studied extensively by other techniques. The results we obtained are in full agreement with the published data. We were able to distinguish most of the solid phases described in literature. Moreover, we have found NMR to be well suited for the study of slow kinetics. This enabled us to complement the published data with a set of kinetic constants which would be difficult to obtain otherwise.

EXPERIMENTAL

The technique of solid/liquid ratio determination by NMR has been described in detail elsewhere (3,4). Its principle may be summarized as follows: the sample is placed in a magnetic field and subject to a short (5 μsec) pulse of radiofrequency (RF). The frequency must be matched to the resonance frequency of protons in the magnetic field used. All protons within the sample are excited by the RF pulse in such a way that, once the pulse is terminated, a decaying free induction signal (FID) appears at a receiver coil wound around the sample. The rate of the decay is strikingly different for protons in the liquid phase and those in the solid phase. The solidstate protons give rise to signals decaying in a few tens of microseconds, whereas the liquid-phase proton signal decays in ca. 1 millisec (the latter number depends upon the quality of the magnet). The signal amplitude T immediately after the end of the pulse is proportional to the total number of protons in the sample, while the signal amplitude L at ca. 70 to 150 μsec after the end of the pulse is proportional only to the liquid phase protons. The solid/liquid ratio is therfore easy to determine if both T and L are known. Since for experimental reasons it is impossible to measure T, it is a common practice to measure the signal $\overline{T}$ several microseconds after the end of the pulse and to compensate for the decay of the solid state signal during this period by an empirical extrapolation coefficient f_s. The percentage of the solid (S) is therefore given by the formula (3)

$$S = f_s(\overline{T}\text{-L}) \, / \, [f_s(\overline{T}\text{-L})\text{+L}] \; \cdot 100 \qquad\qquad 1.$$

The measurements were performed on a pulsed low resolution NMR spectrometer Minispec P_{20} produced by
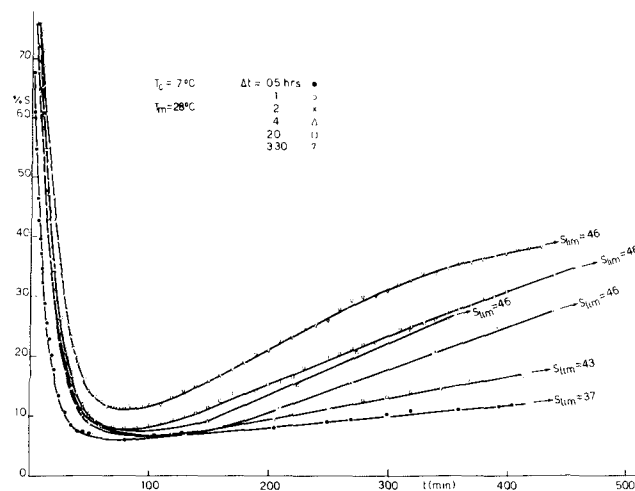

FIG. 1. Melting curves taken at T_m=28 C for samples tempered for different times Δt at T_c=7 C. The solid content S of each sample measured at the time t=1200 min does not change up to the time t=1600 min. This constant value of S was taken as S_{lim}. The lower S_{lim} values observed for samples tempered for Δt = 0.5 and 1 hr may indicate that the equilibrium value is reached at times > 1600 min.

Bruker (Karlsruhe, Germany) and operating at 20 MHz. In combination with the analog computer B-AC5 from the same Company, the instrument permits a rapid and fully automated determination of S. In our case, the signals T and L were measured at 9 μsec and 90 μsec after the end of the pulse, respectively. The extrapolation coefficient f_s was determined from a fully digitized FID; its value was 1.62. The repetition rate between successive pulses was 5 sec.

Each S measurement reported below is the average obtained from ten separate FIDs. The standard deviation of the measurements was ca. 0.5%.

The temperature T_m within the sample compartment of the instrument has been controlled by means of a thermo-stated water-bath to ± 0.1 C. Its absolute value was checked by a mercury thermometer.

The cocoa butter used was an industrial product kindly supplied by the Ferrero Company (Alba, Italy).

The samples were prepared in glass tubes of 10 mm outer diameter. They were first melted and stirred at 70 C for 2 hr and then stored at the desired tempering temperature T_c (7 C or -18 C) for different times Δt. Subsequently, they were placed within the spectrometer and the variation of the solid contents measured as a function of time t. The resulting "melting" curves were evaluated as described in the next section. They will be referred to as melting curves, even though the process is more complicated than a simple melting.

RESULTS AND DISCUSSION

Each melting curve is characterized by the following parameters: (a) the tempering temperature T_c; (b) the time of tempering Δt, and (c) the temperature T_m at which the melting curve was recorded.

In Figure 1 we present the melting curves obtained at T_m = 28 C for samples tempered at T_c = 7 C for different times Δt. In each of these cases, the sample melts within ca. 80 min to a minimum solid content S_{min}. The solid content then starts increasing and reaches a limiting value S_{lim} which is nearly the same for all the samples.

The initial sections of the melting curves, for $2 \leqslant t \leqslant 20$ min, conform to the exponential formula

$$(S-S_{min}) = C \exp(-kt),\qquad 2.$$

where C is a constant, which represents the starting content of the solid phase that melts at the temperature T_m. This has been proved by evaluating the linear regression of ln $(S-S_{min})$ upon time t. The results are reported in Figure 2 and summarized in Table I. The regression coefficients indicate that Eq. 2. is indeed in excellent agreement with the data considered. We interpret this fact by the presence of two phases A and B. The melting temperature of phase B is at least 28 C and its content in the sample is ca. S_{min}. Phase A, the melting point of which is less than 28 C, melts completely during the initial period.

The melting rate constants k reported in Table I vary somewhat with the tempering time Δt, but the variations of kinetics are not very large and tend to zero for large values of Δt. We think that these variations reflect a decreasing concentration of defects within phase A upon increasing tempering times Δt, rather than a varying mixture of two or more phases.

In order to better distinguish the melting points of the two phases, we have determined the initial section of the melting curves of samples tempered for times longer than 200 hr at 7 C, varying T_m from 25 C to 33 C (Fig. 3). Obviously, phase B melts completely at 30 C. On the other hand, phase A melts even at T_m = 25 C, and the rate of this
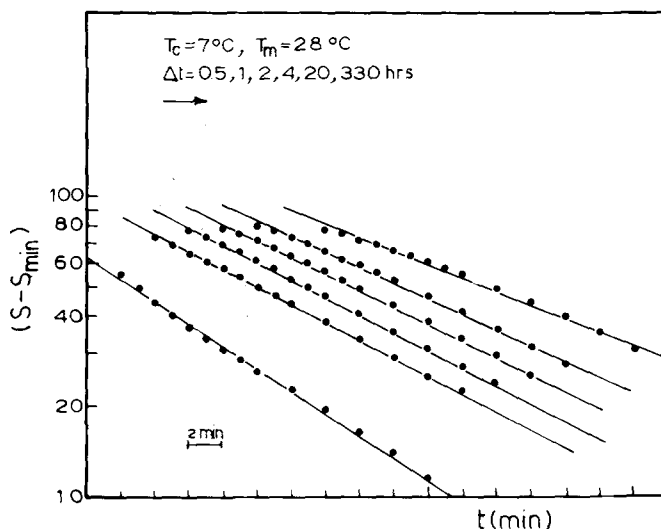

FIG. 2. Exponential regressions of the melting curves reported in Figures 1 in the time range 2-20 min according to the formula $S-S_{min}=C \exp (-kt)$. In order to avoid overlaps, the reported curves were shifted in the time axis in such a way that the first experimental point of each curve corresponds to the time t = 2 min.

TABLE I

Melting Rates k and Regression Correlation Coefficients r^2 of Cocoa Butter Samples for T_c = 7 C, T_m = 28 C

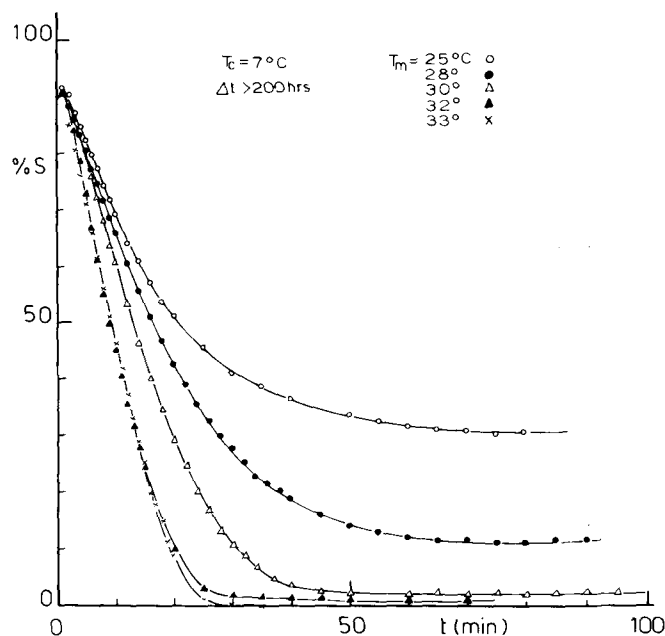| Δt(hr)	k(min^{-1})	r^2
0.5	.084	.998
1	.067	.999
2	.066	.999
4	.063	.998
20	.059	.997
330	.050	.995

FIG. 3. Melting curves of samples tempered more than 200 hr at T_c = 7 C taken at different temperatures T_m.

melting process is nearly equal to the rate of melting at T_m = 28 C (Fig. 4). The difference between the S_{min} values for the curves obtained at T_m = 25 C and T_m = 28 C, respectively, can be explained either by incomplete melting of phase A at 25 C or by incomplete melting of phase B at 28 C.

The above results are in good agreement with data reported in literature (1,2) provided that phase A and B are identified with the phases denoted conventionally as III and IV, respectively. It has been shown, in fact, that upon tempering cocoa butter samples at temperature T_c between 5 and 10 C, the liquid phase solidifies into phase III which then slowly recrystallizes into phase IV. Phase IV may also grow directly from liquid but only at rather high temperatures ($\geqslant$ 16 C). The melting temperatures of the two phases are reported to be ~25.5 C for phase III and ~27.5 C for phase IV (1). Given the generally low reproducibility of the melting points of fats, it is quite possible that phases III and IV indeed melt partially at T_m=25 C and T_m=28 C, respectively.

At temperatures higher than 28 C (and therefore above the melting point of phase (B), the initial parts of the melting curves no longer conform to formula 1. The curvature of the semilogarithmic plots of $(S-S_{min})$ with respect to t (Fig. 4) cannot be explained by a simple combination of two concurrent melting processes with different melting rates for the two phases. The formula describing such a process would be

$$S = S_A \exp(-k_A t) + S_B \exp(-k_B t), \qquad 3.$$

where S_A and S_B are the initial concentrations, and k_A and k_B are the melting rates of the phases A and B, respectively. Unfortunately, semilogarithmic plots corresponding to Eq. 3 give concave curves for *any* set of the parameters S_A, S_B, k_A, and k_B, while the experimental curves are
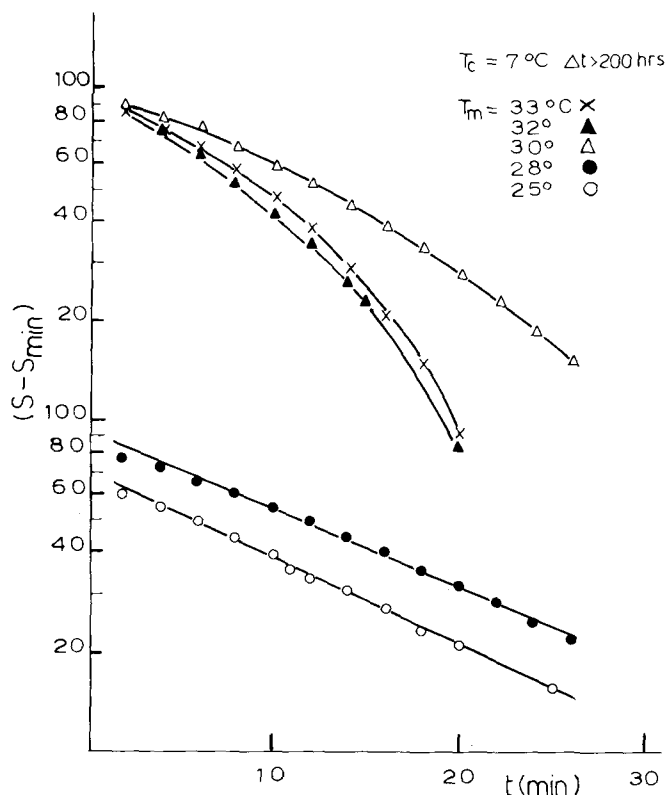
TABLE II

Solidification Rates k and Regression Correlation Coefficient r^2 of Cocoa Butter Samples for T_c = 7 C, T_m = 28 C.

Δt(hr)	$k \times 10^3$ (min^{-1})	r^2
0.5	0.6	.989
1	1.2	.982
2	2.8	.996
4	3.6	.992
20	2.7	.992
330	5.0	.994

convex.

A possible explanation of the high temperature melting curves is based on a melting scheme in which phases A and B both melt to liquid but, at the same time, phase A converts to phase B. If phase B melts faster than phase A and if, as is the case, phase A is much more abundant at the beginning, then it can be shown that such a reaction scheme indeed leads to convex semilogarithmic plots.

We must now explain the resolidification process taking place at T_m=28 C after the initial melting period of ca. 80 min. From the literature it is known (1,2) that at temperatures $\geqslant$ 26 C phases III and IV both convert to phase V which melts at ~33.8 C (1). This phase is also known to grow directly from liquid at temperatures $\geqslant$ 25 C. We therefore interpret the resolidification observed in Figure 1 as a growth of phase V from liquid with little interference from other processes.

That the solidification is relatively simple can be shown by a linear regression fit of ln $(S_{lim}-S)$ as a function of time for t $>$ 130 min. The results of such a fit are reported in Table II. The correlation coefficients demonstrate an excellent agreement with the simple formula

$$(S_{lim}-S) = C \exp(-kt), \qquad 4.$$

where C is a constant. The solidification rates k vary with the time of tempering Δt. This fact is probably related to a varying concentration and/or size of nucleation centres of phase V in the tempered samples.

So far, only the behavior of samples tempered at 7 C has been discussed. We have prepared a different set of samples
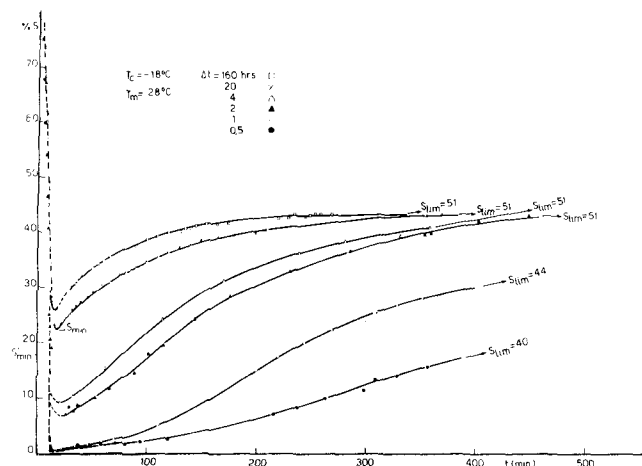

FIG. 4. Semilogarithmic plot of $(S-S_{min})$ vs. time, in the time range 2-20 min, for the melting curves reported in Figure 3.

FIG. 5. Melting curves taken at T_m = 28 C for samples tempered for different times Δt at T_c = -18 C. The solid content S of each sample measured at the time t=1200 min does not change up to the time t=1600. This constant value of S was taken as S_{lim}. The lower S_{lim} values observed for samples tempered for Δt = 0.5 and 1 hr may indicate that the equilibrium value is reached at times > 1600 min.

TABLE III

Melting Rates k and Regression Correlation Coefficients r^2 of Cocoa Butter Samples for T_c = -18 C, T_m = 28 C

Δt(hr)	$k_{(min^{-1})}$	r^2
0.5	.18	.993
1	.19	.997
2	.22	.998
4	.27	.990
20	.23	.995
160	.23	.991

which were tempered at T_c = -18 C for different time Δt. Their melting curves measured at T_m = 28 C are reported in Figure 5. Like in the preceeding case an initial melting period is followed by a resolidification process. The initial melting is substantially faster for this set of samples and the minimum solid content S_{min} is reached within only 20 min.

The initial sections (from 2 to 14 min) of the melting curves exhibit a linear dependence of $\ln (S-S'_{min})$ on time t where S'_{min} is put equal to the extrapolated value of the growing resolidification branch (e.g., Fig. 5). The melting rate coefficients k and the regression correlation coefficients are reported in Table III. The melting process may therefore be described by a formula similar to Eq. 2. (except for the modified interpretation of S_{min}). The melting rates are substantially higher than for samples tempered at 7 C. This indicates that the phase which melts at T_m = 28 C (denoted as C) is not identical with phase A(III). From the literature (1,2) it is known that at temperatures $T_c <$ 0 C cocoa butter solidifies in phase I (melting point ~17.3 C) which subsequently recrystallizes into phase II (melting point ~23.3 C); the recrystallization process takes 15 sec to 15 min at 0 C and ca. 4 hr at -30 C (1). It is therefore likely that our phase C coincides with phase II. Any portion of phase I that might be present in the sample at the end of tempering for short times Δt is likely to be converted into phase II before the melting point of phase I is reached.

From Figure 5 it is apparent that the samples tempered at -18 C contain a varying amount S'_{min} of a solid phase (denoted as D) which does not melt at 28 C. The content of this phase depends strongly upon the tempering time Δt which means that phase D is a recrystallization product of phase C (II). In order to better determine melting point of phase D, we have taken a number of samples tempered at -18 C for more than 120 hr and measured their melting curves at different temperatures T_m (Fig. 6). It appears that while a partial melting of phase D starts at 32 C, it melts completely only somewhere between 33 and 35 C. The only phase described in literature with a melting point lying in this region is phase V (melting point ~ 33.8 C). We may therefore safely identify our phase D with phase V.

The formation of phase V from phase II at low temperatures (-18 C) has never been reported in the literature. It was therefore interesting to determine the rate constant of this recrystallization process. In order to do so we have prepared a series of samples tempered at -18 C for different times Δt and determined their melting curves at T_m = 30 C at which temperature the solid content after phase II has melted grows extremely slowly so that S_{min} and S'_{min} coincide almost exactly (Fig. 7). The values of S_{min} may then be interpreted as the starting content S_V of phase V in the sample. The maximum content S_V^∞ of phase V was found to be ca. 24% reached after 22 hr of tempering. A check sample tempered for 400 hr did not differ at all from that tempered for 22 hr.
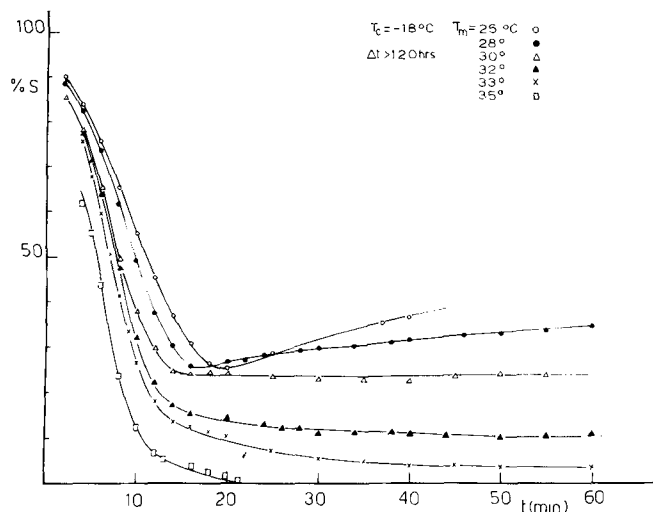

FIG. 6. Melting curves of samples tempered more than 200 hr at T_c = -18 C taken at different temperatures T_m.

We have found that the recrystallization process may be described accurately by the formula

$$(S_V^\infty - S_V) = S_V^\infty \exp (-kt) \qquad 5.$$

where t is the time of tempering Δt.

The linear regression of $\ln (S_V^\infty - S_V)$ upon t gives k = 0.30 hr^{-1} and a correlation coefficient r^2 = 0.998.

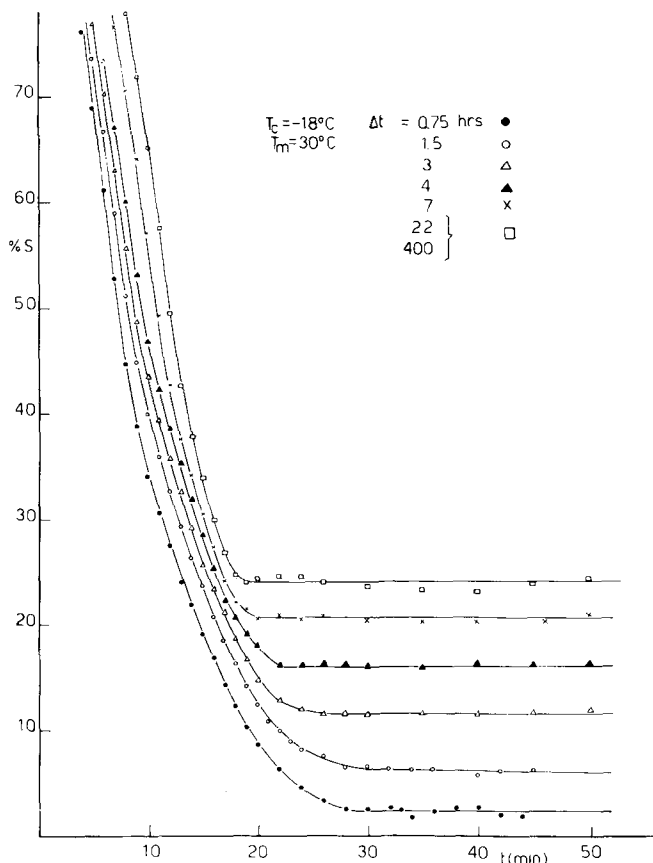Returning to Figure 5, we may now interpret the growth of the resolidification of the sample for t > 20 min (i.e.,

FIG. 7. Melting curves taken at T_m = 30 C for samples tempered at T_c = -18 C for different times Δt.

TABLE IV

Solidification Rates k and Regression Correlation Coefficients r^2 of Cocoa Butter Samples for T_c = -18 C, T_m = 28 C

Δt(hr)	$k_{(min^{-1})} \times 10^3$	r^2
0.5	2.2	.995
1	3.7	.996
2	4.6	.995
4	4.8	.993
20	5.6	.998
160	6.1	.990

after phase II has completely melted) as growth of phase V from the liquid phase. The equilibrium solid/liquid ratio for phase V at 28 C, denoted as S_{lim}, would appear to be ca. 51%, which is somewhat higher than the 46% found for the samples tempered at 7 C. The difference may be caused, however, by a small difference in the temperature T_m between the two series of experiments; else, one might suspect a partial conversion of phase V into phase VI occurring only in the samples which were tempered at -18 C. Notice that according to the literature (1), phase VI which melts at 36.3 C cannot grow directly from liquid.

As for the samples tempered at 7 C, the solidification process apparent from Figure 5 conforms to Eq. 4. The results of a linear regression fit of ln (S_{lim}-S) upon time are listed in Table IV. As expected, the solidification rates are similar to those found in the former case (see Table II).

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