Physical Properties of Lipids Used in Pharmacy II

Use of Heating-Cooling Curves to Study Lipid Materials

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This report describes the use of heating-cooling curves for the study of polymorphism as it occurs in chemically pure or commercial samples of glyceryl mono-, di-, and tristearate, theobroma oil, spermaceti, white petrolatum, and cetyl alcohol. The interpretation of the data obtained by the heating-cooling method has been simplified by using a log temperature difference plot. With this plot, the usual temperature-time curve can be rationalized into a series of interconnecting straight lines. The effects of heating or cooling rates on crystal form and crystal transition temperatures were studied with the aid of the improved heating-cooling curve plots. Distinct polymorphic transitions were observed in the pure glyceryl stearates. On the other hand, in commercial materials there was little evidence that the observed crystal changes were associated with specific polymorphic transformations. The relationship between the transitions observed and the probable storage stability (i.e., the maintenance of film integrity, moisture permeability, etc.) is discussed.

PHARMACISTS, in general, have not taken full advantage of the analytical techniques utilized in other fields for studying the crystal properties of lipids.[1] One purpose of our work was to determine how some of these techniques could be applied to the study of pharmaceutical products containing lipids. Part I of this series (1) presented a photomicrographic method for observing crystal changes and a recent note (2) has shown that polarized light transmission can also be a valuable technique for studying transitions in crystals. This report describes the use of heating-cooling curves for studying polymorphism as it occurs in pure and commercial samples of several lipid materials of pharmaceutical interest. In the heating-cooling curve technique, sample temperature is measured against time as the sample temperature changes in response to a change in environmental temperature.

The pharmaceutical uses of lipids as matrices, additives, coatings, etc., often demand a high degree of physical and chemical stability. To maintain both the acceptability and efficacy of lipid-containing products, such lipid properties as crystal size, refractive index, density, hardness, and film integrity must not change sufficiently during storage to impair the utility of the product. All of these properties are influenced by the crystalline state of the lipid at the time of testing or use.

The heating-cooling curve method is not the newest of the many analytical tools found useful in studies of the crystalline state but, because of its simplicity, it is still worthy of consideration, both as a research and a control procedure. Heating-cooling curves have proven their utility in the studies of chemically pure lipids (3, 4) and, because samples require minimum pretreatment, have also been found useful with commercial lipids for studying the effects of storage history. The studies on commercial lipids are more difficult to interpret, however, because the crystal changes are observed as small discontinuities on a logarithmic curve. The modification proposed in this report (i.e., replotting temperature vs. time data as logarithm temperature gradient vs. time) has been observed to simplify the graphic interpretation of heating and cooling processes (5a, b). With this modification, even minor changes in heat conduction or content appear as deviations from a straight line.

No attempt has been made in this report to document fully the literature of polymorphism, calorimetry, or even heating-cooling theory. A considerable body of recent information is already available in the literature in review form (3, 4).

EXPERIMENTAL

Raw Materials.—The cetyl alcohol was N.F. grade and purchased from M. Michel and Co. The white petrolatum, spermaceti, and theobroma oil were U.S.P. grade and purchased, respectively, from L. Sonneborn and Sons, McKesson and Robbins and Co., and A. N. Stollwerck, Inc. The commercial glyceryl monostearate was obtained from Hercules Chemical Co.; free glycerin 0.8%, monoglyceride content 36%. The commercial glyceryl distearate was obtained from Kessler Chemical Co.; free glycerin 0.6%, monoglyceride content 12%, saponification number 185, acid value 2.

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[1] In this report, the term lipid is used in its broadest sense to include fats, waxes, and other related materials.

The synthetic glyceryl monostearate was prepared by the method of Fischer (6) from 1,2-glyceryl propylidene and octadecanoyl chloride. The saponification number of the purified product was 357.9 (theory 358.5).[2]

The synthetic 1,3-glyceryl distearate was prepared by the method of Jackson (7) from glyceryl monostearate, triphenylchloromethane, and octadecanoyl chloride.[3] The saponification number of the purified product was 311.9 (theory 312.5). The 1,3-structure was confirmed by infrared absorption.

The synthetic glyceryl tristearate was prepared by heating 1,3-distearin in benzene with octadecanoyl chloride and pyridine. The saponification number of the product was 296.1 (theory 297.2). Infrared analysis showed no unesterified hydroxyl groups.

Temperature Recording.—Using crushed ice as a reference bath, sample temperatures were measured with standard, cotton wrapped, 30-gauge iron-constantan thermocouples. The span of a Brown Electronik recorder was set to record temperature directly in °C. For heating curves, a thermoregulator-controlled 5-gal. water bath was used. For cooling curves, either room air or another 5-gal. water bath was used for cooling at or near room temperature, while a 4-L. Dewar flask filled with crushed ice was used for cooling to 0°.

Sample Cell.—Samples were contained in 10 × 75 mm. Pyrex test tubes, which were held in the special Pyrex cell shown in Fig. 1.

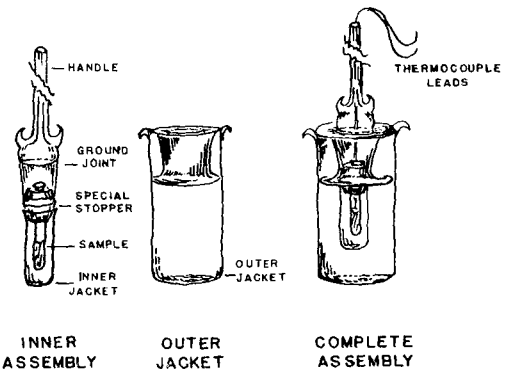

Fig. 1.—Pyrex cell for holding lipid samples during heating-cooling determinations.

Sample Preparation.—Solid samples are normally melted and cast directly into the sample tube. If prior history is being studied, however, the sample is cut into plugs which are then placed in the sample tube. Before measurement, a 2 × 100 mm. melting point capillary is set in the sample to serve as a thermocouple well and a small cork stopper is used to hold this capillary in the center of the test tube. Good contact between the sample and thermocouple capillary is important. Powdered material may be used if it is packed firmly around the capillary, but very coarse material was found to give poor results. Samples should be about 2 Gm. in size, but exact weight is not critical.

[2] The authors gratefully acknowledge the assistance of Dr. Robert J. Meyer of Morton Chemical Co., Woodstock, Ill., for the synthesis of the synthetic mono-, di-, and tristearins.

[3] The octadecanoyl chloride used was prepared from 97% stearic acid by fractional distillation of its methyl ester. By freezing point, its purity was estimated to be 99.9%.

Measurement Procedure.—The prepared test tube containing the sample is fitted into the inner assembly by using a double tapered rubber stopper (Fig. 1). The thermocouple is then inserted and the completed inner assembly is cooled to the starting temperature. (No heat transfer medium was used; the improvement so afforded did not warrant its use.) When the inner assembly has properly cooled, the outer jacket is attached, the complete cell immersed into a hot bath held about 5° above the melting point of the sample, and the temperature rise recorded. When the sample has melted, the cell is placed in the ice-filled Dewar flask until the cooling curve record indicates that the sample temperature is less than 20°, whereupon the sample is again placed in the hot bath and remelted. If desired, the effects of a smaller cooling gradient (i.e., slower cooling) may be studied by recooling the sample in a bath which is at either room temperature or 5° below the sample's freezing point.

Plotting Procedure.—The recorded curve (representing temperature in °C. vs. time) must be replotted as log temperature difference (bath temperature minus sample temperature) vs. time, or simply as log ΔT vs. time. By using 2-cycle semi-log paper and the procedure of Ball (5a), this replotting can be simplified. For heating curves, label the bottom ordinate as "bath temperature − 1°" then continue to label each ordinate upward in order,

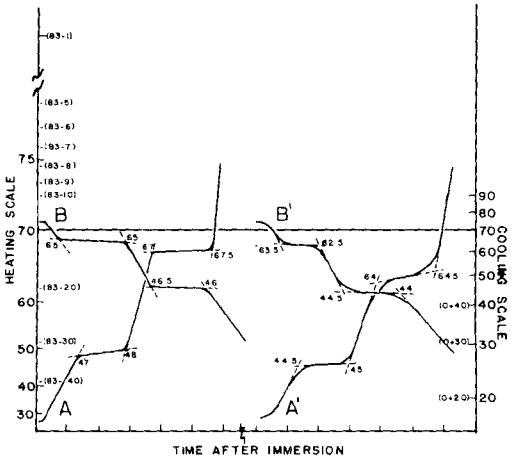

Fig. 2.—Log ΔT plots for a hypothetical substance. Curves A and B depict plausible heating and cooling curves, respectively, for a chemically pure substance. The addition of an impurity to this pure substance might alter the heating and cooling curves to those labeled A′ and B′, respectively. The labeling of the ordinates as described in the text is shown. In this labeling, the single number in the margin is the sample temperature. The first number within the parentheses is the bath temperature, while the second number is the difference between the bath temperature and the sample temperature. It is this latter (difference) number which controls the ordinate positions of the curves. The sample and bath temperatures are added only for convenience in plotting and reading the curves. Each curve is labeled as to (*a*) heating or cooling, (*b*) order of running (e.g., heating-1-, run before cooling-2-), and (*c*) the equilibrium bath temperature. Figures 3 to 13 were plotted in a manner similar to Fig. 2, but only the sample temperatures are designated on the ordinates.

bath temperature − 2°, *bt* − 3°, . . ., *bt* − 10°, *bt* − 20°, etc., until *bt* − *x*° reaches zero. Temperature vs. time is then plotted directly from the recorder curve using this temperature scale as the ordinate. For cooling curves, the bottom ordinate should be labeled "bath temperature + 1°" and the next *bt* + 2°, . . ., *bt* + 10°, *bt* + 20°, etc., and data plotted as before. The procedure described is shown in more detail in Fig. 2.

In the figures shown, all heating plots have been inverted to permit them to be studied in the same perspective as the cooling plots.

RESULTS AND DISCUSSION

Log Temperature Difference Plot.—The heating-cooling curves for chemically pure compounds are characterized by sharp inflections at the transition points. On the other hand, the curves of impure compounds, such as the usual commercial materials, have only minor inflections which are difficult to observe. To make the data on commercial materials easier to interpret, all heating and cooling curves were plotted as log (bath temperature minus sample temperature) vs. time. Called the log temperature difference plot (log ΔT plot), this temperature-time relationship has been found to be useful where samples are heated in constant temperature baths or ovens (5). In theory, as long as any crystalline form does not undergo change during heating or cooling, a log ΔT plot over this region will be a straight line whose slope is a function of the quantity of heat transferred and the specific heat of the crystalline form or forms present. Since phase transitions are accompanied by heat effects, however, the beginning or end of these will be indicated by inflections in the curves (3). Again, in theory, at the melting or transition point a straight line of different slope will be obtained as long as the coefficient of heat input remains fairly constant. These ideas suggest that a melting or solidification curve for a polymorphic material can be represented by a series of intersecting straight lines having different slopes as a result of the phase changes.

The degree of linearity of log ΔT plots is shown in Fig. 3 which depicts the heating and cooling of a substance (water) whose specific heat and phase

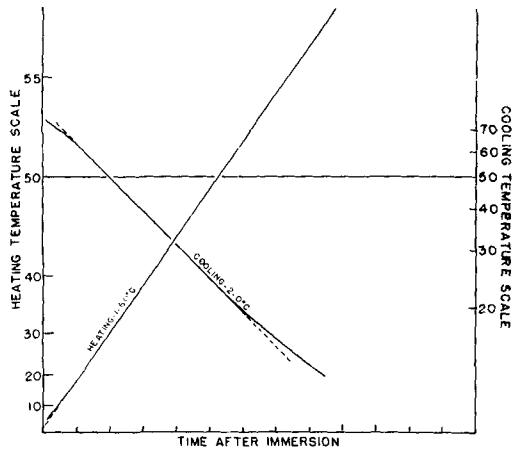

Fig. 3.—Log ΔT plots for the heating and cooling of distilled water.

structure remain the same throughout the temperature range studied. After an initial (10 minutes) equilibration period, in which a steady state heat input is being attained, the heating curve shows good linearity. The cooling curve shows a similar initial equilibration period with subsequent linearity until the sample temperature reaches 10–15°, where it deviates, probably because the ice bath was not stirred. Ball (5a) has observed that log ΔT plots are linear only until log ΔT = *ca.* 5° (i.e., until the sample temperature approaches to within 5° of the bath temperature). For this reason, the recording of heating-cooling curves is generally stopped when the sample temperature is within 2 or 3° of the bath temperature. This procedure limits the recording time in our studies to approximately 30 minutes for samples melting under 60°.

Heating and Cooling Studies of Nine Lipid Materials.—The log ΔT plots of heating-cooling curves for the nine lipid materials studied are shown in Figs. 4–13. The heating ordinates on these graphs are on the left, while the cooling ordinates are on the right. Because the scales cannot be made the same (the heating scale is an inverted log function, while the cooling scale is a normal log ordinate), some compromise had to be made in order to plot heating and cooling on the same graph. One temperature near the center of both curves was used as a point of correlation and the rest of the values were graphed from it (see 50° line, Fig. 3). Only at this temperature are both scales exactly the

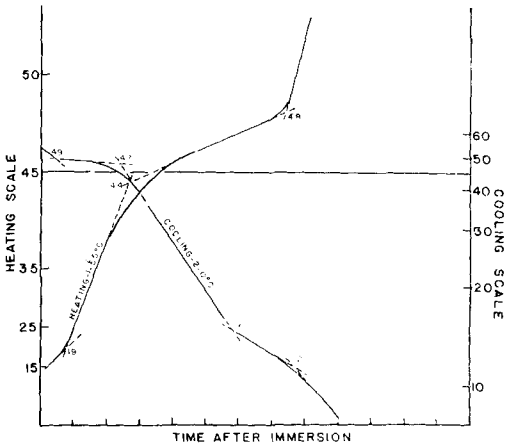

Fig. 4.—Log ΔT plots for the heating and cooling of cetyl alcohol.

same. For clarity, the temperatures of all inflection points have been noted directly on the graphs (e.g., 47 and 48° on curve A of Fig. 2). Each curve is labeled as to (*a*) heating or cooling, (*b*) order of running (e.g., heating-1-, run before cooling-2-, run before heating-3-, etc.), and (*c*) the equilibrium bath temperature.

To clarify interpretation of the data, heating-cooling curves of a hypothetical sample are presented in Fig. 2, preceding a discussion of the data of the lipid samples. In Fig. 2, curves A and B might be the heating and cooling curves, respectively, of a pure substance, while A′ and B′ might be the corresponding curves for the same substance in a less pure state. For simplicity, the same transition is

shown in all four curves, i.e., a reversible (enantiotropic) transition at approximately 47° in the pure substance (curve A). Transition temperatures are often lower on cooling curves because of a tendency to supercool. All four curves exhibit straight line segments, but the slope changes are more abrupt with the pure substance. At the beginning of each curve, there is a time lag before steady state heat transfer occurs across the cell. Most transitions in the lipid substances occur over a temperature range, e.g., in curve A, the transition starts at 47° and ends at 48°. To get exact transition temperatures, it is usually necessary to extrapolate straight line segments until they intersect, e.g., 44.5° on curve A

The terminology used in the following discussion is that found in the literature (3, 4). In general, the forms are named gamma (γ), alpha (α), beta prime (β'), and beta (β), in order of increasing melting points. The β form is the stable form in the materials reported, but in rare cases it is not known to exist and the β' is the stable form.

Figures 4, 5, and 6 show the heating and cooling curves of commercial samples of cetyl alcohol, white petrolatum, and spermaceti. These same N.F. and U.S.P. materials have been studied dilatometrically by Ravin and Higuchi (8). Cetyl alcohol shows a smooth heating curve with a break at 19° and a melting range (44–48°) which ends sharply at 48°.[4] The inflection at 19° probably represents the melting of a component of the commercial material which is normally liquid at room temperature. On the cooling curve, this

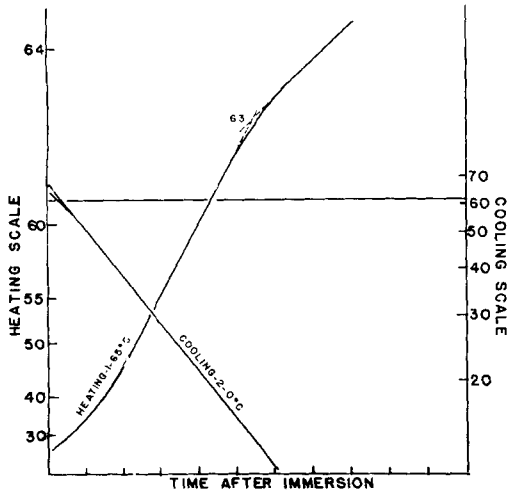

Fig. 5.—Log ΔT plots for the heating and cooling of white petrolatum.

transition occurs between 15 and 12° rather than at 19°. An inflection at 40–45°, observed by Ravin and Higuchi, could not be observed in our studies. In contrast, since they did not study cetyl alcohol below room temperature, they did not report the transition at 19°. Both white petrolatum (Fig. 5) and spermaceti (Fig. 6) show smooth, uninterrupted approaches to the melting point, i.e., there are no detectable transition points. The results are

[4] It is generally a misnomer to speak of melting points in lipids. Any sensitive method will detect a melting range.

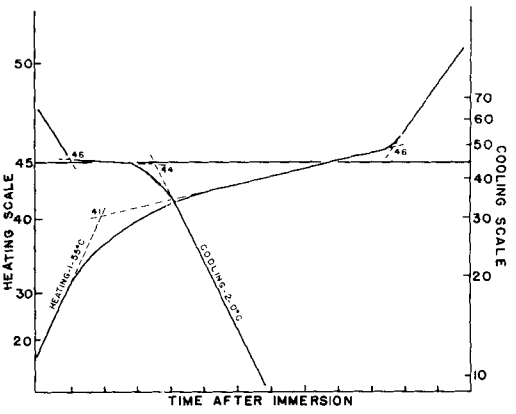

Fig. 6.—Log ΔT plots for the heating and cooling of spermaceti.

essentially the same as those obtained dilatometrically.

Heating and cooling curves run on synthetic glyceryl monostearate (monostearin) are shown in Fig. 7. This material is reported to exist in four forms; namely sub-alpha, alpha, beta prime, and beta (4). The curve labeled heating-1- is the heating curve of material stabilized by prolonged storage at room temperature. Two distinct inflections (65–68° and 74–76°) can be observed and, although they are 5° less than those reported by Malkin (9a), they probably represent the α (along with the subsequent $\alpha \rightarrow \beta$ transition), and the β melting points. The β' form is difficult to observe by these methods (9b). A heating curve, heating-3-, run after rapid cooling (i.e., there was a large cooling gradient), shows only melting of α form crystals, no β form being present or formed during the heating. There is an indication on curve heating-3- of an arrest at 39°, but it is too indefinite to be conclusive. Slow cooling (i.e., using a small cooling gradient) yields essentially the same results except for a definite arrest at 47°, which probably represents alpha → sub-alpha transition (see curve cooling-4-). Commercial

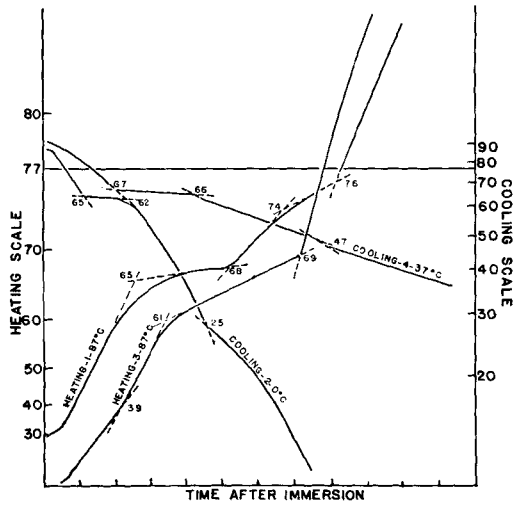

Fig. 7.—Log ΔT plots for a heating-cooling study of synthetic glyceryl monostearate.

monostearin shows little, if any, evidence of polymorphic change when it is remelted after being rapidly cooled (see curves cooling-2- and heating-3- on Fig. 8). While polymorphic transitions probably do occur in commercial monostearin, the presence of large amounts of other glycerides (palmitates, oleates, etc.) and/or other substitutions (di- and triester) apparently obscures the heat effects of these changes.

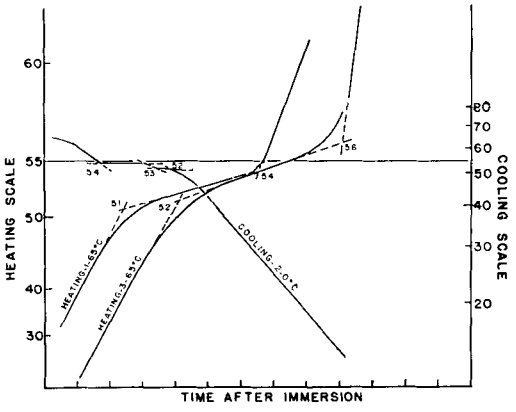

Fig. 8.—Log ΔT plots for a heating-cooling study of commercial glyceryl monostearate.

The heating and cooling behavior of the synthetic glyceryl distearate (distearin) is shown in Figure 9. Forms reported in the literature are α, β', and β (4). Clearcut evidence of two crystal forms appears in the heating curve run on a sample cooled rapidly from a melt (see heating-3-) with the transition occurring at 57–60°. These two forms correspond roughly to the α and β' forms reported by Baur, *et al.* (10a), and Malkin and Shurbagy (10b). The β' melting observed on reheating (heating-3-) could have resulted either from rapid transition of α to β' as the heating curve was being run or from β' crystals formed during the cooling. The curve obtained during rapid cooling (cooling-2-) suggests that very little β' form crystallizes out with the α form. At slower cooling rates, however, both forms definitely appear (cooling-4-).

Note that cooling curves normally produce freezing points well below the melting points of the same form. Smit (11) has theoretically explained this as due to the kinetic nature of heating and cooling determinations, and suggests that only heating curves be used for exact work.

For the same reasons as indicated above with commercial monostearin, the heating-cooling curves of commercial distearin do not show the polymorphic forms found in the pure material (Fig. 10). As expected, the melting and freezing points of this commercial distearin are considerably below those of the synthetic material. The melting point of the commercial mixture is lowered by rapidly cooling the melt (compare heating-3- with heating-1-), but none of the three curves indicates a characteristic polymorphic transition. Lutton (12) has suggested that the higher melting points of aged samples (also observed in synthetic distearin) are due to crystal aging or stabilization. This point is discussed further under theobroma oil.

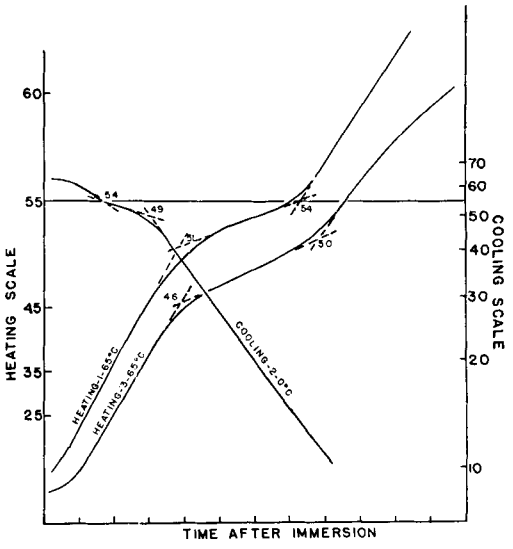

Fig. 10.—Log ΔT plots for a heating-cooling study of commercial glyceryl distearate.

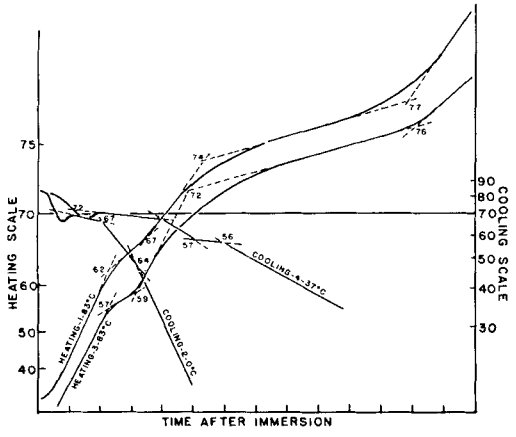

Fig. 9.—Log ΔT plots for a heating-cooling study of synthetic glyceryl distearate.

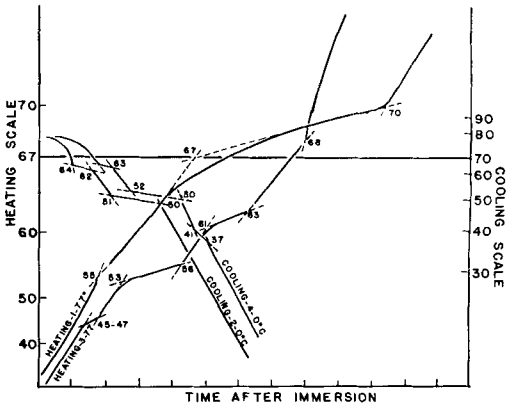

Fig. 11.—Log ΔT plots for a heating-cooling study of synthetic glyceryl tristearate.

Rapidly cooled synthetic glyceryl tristearate shows four distinct melting or transition points, while storage stabilized samples show one or perhaps two (Fig. 11, curves heating-3- and heating-1-, respectively). Three of these points correspond well to the α, β', and β melting points reported by Clarkson and Malkin (13) (melting points found 53–56°, 61–63°, 67–70°; reported in the literature 55°, 65°, 72°). The lowest form observed in this work, melting at 45–47°, is not identifiable from the literature.

Theobroma oil (cocoa butter), although a mixture of several glycerides, has a heating-cooling curve similar to that of a pure compound. This has been explained (14–16) as being due to the high percentage (more than 50%) of one glyceride, 2-oleopalmitostearin. The forms γ, α, β', and β melting at 16–18°, 21–24°, 27–29°, and 34–35° have been reported (17, 18). The heating-cooling characteristics of a commercial sample of theobroma oil are shown in Figure 12. The storage stabilized sample (heating-1-) shows essentially one melting point (33°), that of the stable β form. Although this curve shows no indication of β' crystals (m.p. 28°), some are probably present because both β' and β forms are quite stable at room temperature (17a). Cooling molten theobroma oil to 0°

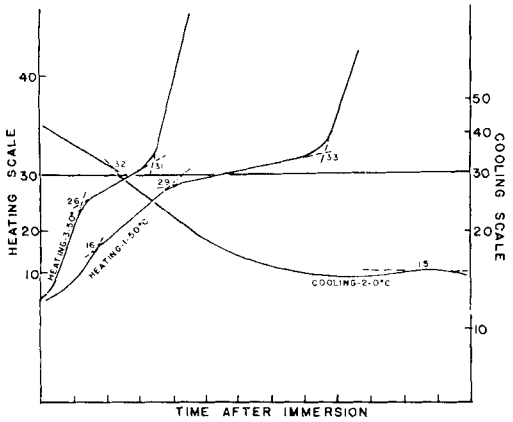

Fig. 12.—Log ΔT plots for a heating-cooling study of a storage stabilized sample of theobroma oil (cocoa butter). Curve cooling-2- was run within 2 hours of curve heating-1-. Curve heating-3- was obtained after the sample used for curve cooling-2- had stood at room temperature for 24 hours.

results in a congealing point of 15–16°. From its translucent appearance, this is probably a glassy form, although Vaeck describes it as the γ form (17c). If this glassy form is immediately reheated, it melts at 16–25° (see, for example, heating-1-, Fig. 13). On the other hand, if the glassy form is allowed to stand for 24 hours at room temperature, complete transformation occurs, primarily to the α form, but at least in part to the β form. If the sample is now reheated (see heating-3-, Figs. 12 and 13) the influence of the β nuclei can be observed; complete melting of the sample occurs above the β' melting point (i.e., >28°).

If theobroma oil (cocoa butter) suppositories are prepared by standard suppository procedures (melt at 40°, pour into 0–2° mold), the suppositories, when immediately reheated, exhibit the behavior shown in heating-1- of Fig. 13. Only $\gamma \rightarrow \alpha$ transition is observed, plus α melting at 25°. When the suppositories are allowed to stand at room temperature for 24 hours after casting, the heating curve (heating-3-) shows that complete transition to the α form and some $\alpha \rightarrow \beta$ transition has occurred. It is unlikely that $\alpha \rightarrow \beta'$ transition occurs in studies conducted under the described conditions because this transition is quite slow when compared to the $\alpha \rightarrow \beta$ transition (17b).

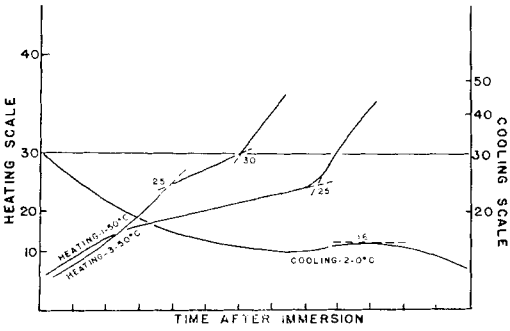

Fig. 13.—Log ΔT plots for a heating-cooling study of theobroma oil (cocoa butter) suppositories (cast at 0 to 2° from a 40° melt). Curves heating-1- and cooling-2- were obtained within 2 hours of casting. Curve heating-3- was obtained after the suppository had stood at room temperature for 24 hours.

In most of the materials where an aged sample was compared with a recently crystallized sample (Figs. 7–13), the melting point of the recently crystallized sample was found to be lower. This has been discussed by Steiner (18) who suggests that these low melting points are due to "mutually soluble binary mixtures." In light of the observation that rapid cooling also produces low melting points, the simpler explanation suggested by Lutton (12), that all samples which are not completely "aged" will give low melting points (lower by as much as 3°), seems a more acceptable one.

Stability Interpretations of Heating-Cooling Data.—Polymorphism is defined as the ability of a substance to exist in more than one crystal form. These crystal forms have different physical properties, e.g., melting points and different energy contents. They are of importance because transitions from one crystal form to another, with simultaneous changes in physical properties, can cause stability problems in pharmaceutical products even under room temperature storage conditions.

Essentially two large classes of polymorphic transitions have been identified in lipids. In the more common type, called monotropism, one crystal form is stable up to the melting temperature. Under these conditions, metastable crystals will tend to transform, with time, to the stable form, irrespective of storage temperature; however, the storage temperature may influence the rate of monotropic transitions. In the less common type of polymorphism, called enantiotropism, each crystal form has a temperature range in which it is stable, and reversible transformation occurs between two forms at the transition temperature.

Because the main concern in lipid stability is the presence and rate of polymorphic transitions rather than their direction, both types of polymorphism may be discussed together.[5] The transition from one polymorphic form to another, with its consequent liberation of heat (heat of transition), probably occurs as both a $solid_1 \rightarrow solid_2$ and a $solid_1 \rightarrow liquid \rightarrow solid_2$ phenomenon (11, 13). The extreme slowness of known $solid_1 \rightarrow solid_2$ transitions (e.g., methyl stearate and palmitate) (19), along with the sharpness and reproducibility of lipid transition temperatures, suggest that $solid_1 \rightarrow liquid \rightarrow solid_2$ transition is the predominant mechanism for transitions occurring at temperatures near the maximum transition temperature. At temperatures below this temperature, some much slower mechanism may be responsible for the crystal transitions (e.g., $solid_1 \rightarrow solid_2$). Since solid phase transitions would logically be functions of viscosity, transformations which have high transition temperatures will probably proceed at negligible rates at room temperature in solids which have a low liquid content. Complete transition, therefore, may require minutes, days, or years, depending on the transition rate at the temperatures of storage.

Estimation of a lipid material's potential for polymorphic change, or in other words its tendency to be unstable, can be made by comparing the heating curve of a sample stored for prolonged periods at room temperature (i. e., stabilized) with one of a sample which has recently been rapidly frozen from a melt. Freezing from a melt usually produces the least stable (γ and/or α) forms of lipids, and reheating the sample will then show the presence of these forms. If a stabilized sample of material is not available, the observation of multiple melting points in a rapidly-frozen sample may be used as criteria of polymorphic potential.

All of the synthetic stearates studied show great polymorphic potential. Synthetic glyceryl monostearate (Fig. 7), glyceryl distearate (Fig. 9), and glyceryl tristearate (Fig. 11), all show multiple melting points even after prolonged storage at room temperature. The degree of transition to the stable polymorphic form was so great in the di- and tristearates, however, that considerable information would have been lost if the rapidly-cooled samples had not been reheated. The commercial stearates (Figs. 8 and 10) show no such polymorphic potential. The difference in melting points observed on the two heating curves of commercial distearin is related to the difference in the "ages" of the two samples, as was discussed in a previous section. It was also mentioned previously that the absence of distinct transition points in commercial materials should not be interpreted to mean that polymorphism is absent. Chemical heterogeneity of a given sample tends to obscure its polymorphic transitions. Careful studies, including the use of purified components of the sample, may be necessary to prove their existence.

The real value of heating-cooling curves, from a stability point of view, is in estimation of the relative rate of a polymorphic transition at room or storage temperature. With lipid-containing pharmaceuticals, we are concerned only with a rather narrow range of transition rates and can adjust the parameters of the heating-cooling measurement procedure so that these rates will be observed. On the one hand, if the transition rates are so high that low melting forms are not observable by heating-cooling methods, the crystal changes in the lipid will probably be completed during the manufacturing process and cause no stability problems in the product. On the other hand, if the crystal changes are extremely slow at the transition temperature or if the transition temperatures are much higher than the storage or use conditions, the rate of transition at room temperature may be negligible and, again, there probably will be no stability problems. Therefore, it seems likely that stability problems occur in pharmaceutical products only when the transitions proceed at rates between these extremes under the temperature conditions to which the product is exposed.

If one considers the synthetic stearin series (Figs. 7, 9, and 11) and in particular the transitions in the range 58–68° (monostearin, $\alpha \rightarrow \beta$; distearin, $\alpha \rightarrow \beta'$; tristearin $\beta' \rightarrow \beta$) the relative rates of these transformations can be estimated. At the transition temperature (61–69°) monostearin showed no transformation to the β form (no 76° m.p.), tristearin (at 61–63°) transformed very slightly (slight melting at 68°), and distearin rather completely. One could expect that the polymorphic stability of these pure lipids would decrease in a similar order (i.e., stability of monostearin > tristearin > distearin). At room temperature, the absolute rate of these transitions would also be influenced by the transition temperature itself. The higher transition temperatures of mono- and tristearin would further suggest that their room temperature transition rates would be less than that for distearin. Another example of this type is shown in theobroma oil, which has the same slow $\alpha \rightarrow \beta$ transition as was shown by rapidly cooled mono- or tristearin (e.g., see Fig. 13, heating-1-). The room temperature transition rate of theobroma oil is fairly rapid (heating-3-) because the transition temperature is near room temperature.

Another possible use for studies like those illustrated here concerns the effects of heat treatment (tempering) on the crystalline state of the lipid. Crystal transformation could be monitored easily by storing the material at the desired temperature and observing the effect of storage on the heating curve of the stored material. Tempering near the transition temperature would then continue until the undesired form had disappeared from the curve.

In general, our experience suggests that the smoother and straighter the entire log ΔT vs. time plot of a heating-cooling curve, the greater the crystal stability of a lipid. Secondarily, the higher the transition temperature, the greater the crystal stability at room temperature.

Effects of Polymorphic Forms on Lipid Properties. —A basic characteristic of polymorphic transitions is the decrease in specific volume which normally occurs as the crystalline form changes from $\gamma \rightarrow \alpha \rightarrow \beta' \rightarrow \beta$. This decrease in specific volume, if it occurs after a container has been filled or a lipid film has been applied, can cause shrinkage away from the container walls or loss in integrity of the film. Changes in the crystal form can also cause more subtle changes, such as in the vapor and

[5] If the storage conditions of stability testing include temperatures above and below the enantiotropic transition temperature, this assumption is not necessarily true.

liquid permeability of a film. It has been observed, for instance, that rapidly cooled wax films transmit water vapor much faster than stabilized films (20, 21), which suggests that crystal size, shape, form, and orientation may have a pronounced effect on vapor and liquid transmission.

Pharmaceutical Utility of Heating-Cooling Curve Techniques.—It has been shown that most fats and waxes are polymorphic and on aging may undergo crystalline changes (3). Since these changes sometimes occur over a significant time span they can cause physical changes in pharmaceutical products during storage, e.g., changes in ointment consistency, changes in the appearance of suppositories, or changes in the integrity of tablets, pellets, or other dosage forms containing lipids. The development pharmacist needs good methods for determining what lipids are likely to undergo such changes, the temperatures at which the changes are accelerated, the rates at which they occur, and methods for eliminating or minimizing these changes during or after the manufacture of dosage forms which contain lipids.

Heating-cooling curves are useful for detecting the propensity of these pharmaceutical agents to undergo polymorphic change, for determining the temperatures of rapid polymorphic transitions, for evaluating the effects of time-temperature history on the polymorphic form which a lipid may assume, and for establishing what manufacturing and handling procedures result in the unstable or the relatively stable crystalline forms.

If, for example, heating-cooling curve data indicate that a substance undergoes polymorphic change in a pharmaceutical product at the usual temperatures of storage and transit, the pharmacist must be alert to the fact that a stability problem may result. Specific manufacturing conditions may be necessary to assure that the integrity of the product remains unchanged from lot to lot. Products containing cocoa butter, for example, fall into this category and may show different degrees of polymorphic change and different physical stabilities, depending upon their methods of preparation, despite the fact that their chemical compositions may be identical.

In other instances, polymorphic changes may be completely inimical to the purpose for which the product is intended. In such instances a stable form must be attained when the product is initially prepared. This can either be accomplished through the use of tempering or special manufacturing techniques. More simply, the lipids may be chosen on the basis of their inabilities to undergo significant polymorphic changes in the product formulas selected and under the anticipated storage conditions. In all product development problems involving lipids, the heating-cooling curve technique can be of considerable value in evaluating the raw lipid materials and as a control method for establishing the materials and manufacturing procedures necessary for attaining the required physical integrity.

The heating-cooling curve measurements described in this study require only relatively simple apparatus, are inexpensive to perform, and can be performed by unskilled personnel with a minimum of training. The quantity of sample required is only about 1 or 2 Gm.; however, in some instances this might be considered inordinately large, as for example when evaluation of a pellet or tablet film is involved. In such an instance, the light transmission technique (2), which utilizes samples weighing only a few mg., might be a preferred evaluation technique.

Because the heating-cooling curve technique of this study is a nonequilibrium method, it is much more rapid than the dilatometric technique (8). A number of samples may be run in a relatively short period of time. On the other hand, it gives no direct indication of the volume changes involved when polymorphic transitions occur. When volume change data are important, the dilatometric technique is valuable, and the heating-cooling approach of this study is of little use unless a direct correlation can be found between the two methods with the particular system under study.

Within the limitations discussed, the heating-cooling curve technique has value in studies of polymorphism. It represents an approach which could see greater use in the solution of pharmaceutical problems relating particularly to the use of lipids in dosage forms.

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