Physical Stability of Semisynthetic Suppository Bases

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Abstract □ Changes in the melting range of several semisynthetic suppository bases were studied over time. X-ray diffraction studies characterized the changes as amorphous to crystalline transitions, with likely polymorphic effects occurring concurrently. Use of a modified Krowczynski apparatus to test pure base and single-ingredient suppositories showed dramatic hardening of the materials over periods as short as 6 weeks. Data were obtained by differential scanning calorimetry on freshly solidified, incrementally aged, and equilibrated samples. These methods may be used as both predictive and ongoing physical stability tests in the evaluation of suppository bases and formulas.

Keyphrases □ Suppositories—physical stability of semisynthetic suppository bases □ Physical stability—semisynthetic suppository bases

Krowczynski (1) noted changes in the deformation time of suppositories during storage using an apparatus of his own design (2). He also noted hardening in polyethylene glycol suppositories (3) but pointed out that plasticizers prevented the phenomenon. Ritschel-Beurlin (4) reported aging in suppository bases by studying the change in "spreading." Torrado-Valeiras (5) studied the stability of suppositories using a hardness tester as well as the Krowczynski device and found that certain excipients could stabilize the "melting point" by a "lubricant action" and stabilization of the metastable form. Mezey *et al.* (6), using a cone flow device, had results similar to those of Torrado-Valeiras. Other studies (7, 8) showed that suppository aging affects the *in vitro* and *in vivo* drug release rates.

Whittam and Rosano (9) studied short-term physical aging in pure monoacid triglycerides using X-ray diffraction analysis, differential thermal analysis, and polarizing microscopy. Lovegren *et al.* (10, 11) utilized differential scanning calorimetry to investigate the polymorphic nature of cocoa butter. Differential scanning calorimetry was employed by Rossell (12) to study cooling phenomena in palm kernel oil products. Correlation to dilatometric means of studying the solids content of fats was shown in a differential scanning calorimetric procedure developed by Miller *et al.* (13).

The aim of this research was to elucidate the aging or hardening phenomena observed in suppositories prepared from semisynthetic suppository bases. The hardening effect, resulting in little or no suppository melting, can cause local irritation, a defecatory reflex, or bowel obstruction. Development of procedures as both predictive and ongoing physical stability tests for both bases and formulated products was undertaken. It was thought that studying the changes through thermal analysis and comparing these results with physical hardening data would allow predictions of shelflife hardening from the initial thermal data. Pure bases and bases with ingredients added were studied.

The combination of the modified Krowczynski procedure, X-ray diffraction analysis, and differential scanning calorimetry gave this study specificity, reproducibility, versatility, and direct application to the dosage form.

EXPERIMENTAL

Materials—Five semisynthetic, commercially available bases were chosen. Table I summarizes their chemical properties and the processes by which they were produced, and Fig. 1 compares their solid fat indexes.

Three excipients were chosen to study the ingredient addition effect. Zinc oxide[1] was selected because of its insolubility, its amorphous nature, and its thickening property in oils. Resorcinol[2] was studied for its crystalline nature and its miscibility with fats. A neutral oil[3], consisting of triglycerides with fatty acid moieties between C-8 and C-12, was used because of its fatty nature and because it was claimed by Mezey *et al.* (6) to inhibit hardening.

Softening Time—The apparatus was a slight modification of Krowczynski's device (2). The original device has a constricted glass tube, with a stoppered bottom, in a glass mantle with an inlet and outlet for thermoregulated water. The suppository sits on the constriction, and a glass rod (29–31 g) is placed on the suppository. When the rod reaches the constriction, the melting time is read. Modifications (Fig. 2) included the use of a constricted U tube to avoid possible back pressure on melting past the constriction, use of a water bath[4] with both refrigeration and heating units (accuracy of ±0.05°), and reduction of the weight of the glass rod from 30 to 14 g.

The first experiment was a screening study of the five bases at 37, 36.5, 36, and 35.5°. Freshly molded suppositories and incrementally aged samples were tested in triplicate. Following Krowczynski's approach, the test was expanded to additional samples if a significant variation was noted. Softening times in excess of 90 min were not continued to the end-point during the screening trials but were noted as such.

Two bases, Bases 1 and 3, were chosen for continuing study because of their stability and instability, respectively, at 37.0°.

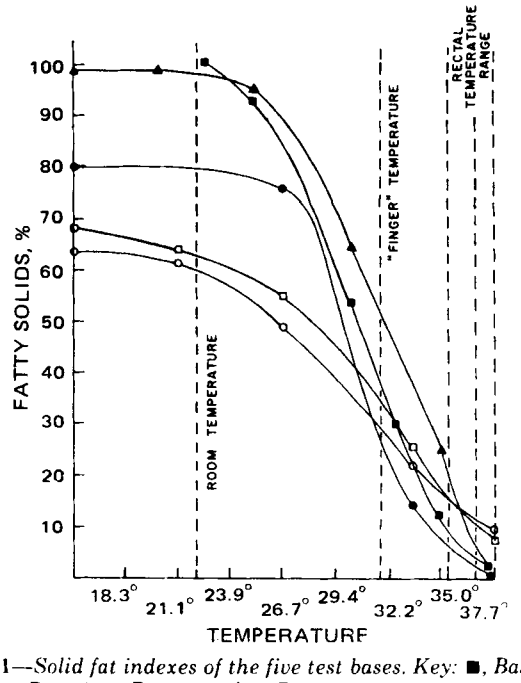

Figure 1—*Solid fat indexes of the five test bases. Key:* ■, *Base 1;* ●, *Base 2;* ▲, *Base 3;* □, *Base 4; and* ○, *Base 5.*

[1] Zinc oxide USP, Westwood Chemical Co., Middletown, N.J.
[2] Resorcinol USP, Koppers, Pittsburgh, Pa.
[3] Miglyol 812, Dynamit Nobel, Witten, West Germany.
[4] Model NK-22, Haake, Saddle Brook, N.J.

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Table I—Chemical Components and Method of Manufacture of Suppository Bases

Base	Fatty Acid Moiety Content, %							Di-esters, %	Mono-esters, %	Process
	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18.1}$			
1[a]	0.5	2.0	49.0	19.0	14.0	15.0	0.5	9.0	0.5	Reesterified
2[b]	3.0	3.0	55.0	21.0	9.0	9.0	0.0	0.0	0.0	Solvent fractionation
3[c]	2.1	1.9	38.0	13.0	14.8	30.0	0.2	0.0	0.1	Interesterified
4[d]	0.0	0.0	0.2	0.6	17.5	14.4	67.3	0.0	0.0	Solvent fractionation
5[e]	4.0	4.0	48.0	16.0	8.1	17.9	1.4	3.0	0.3	Interesterified

[a] Witepsol H-175, Dynamit Nobel, Witten, West Germany. [b] Satina III, Glidden-Durkee, Rockville Centre, N.Y. [c] Suppocire BCM, Gattefosse, Saint Priest, France. [d] Kaomel, Glidden-Durkee, Rockville Centre, N.Y. [e] Hydrokote 2-7, Capital City Products, Columbus, Ohio.

The second study tested the effect of several parameters on the hardening characteristics of Base 3 since this base was the least stable at 37°. The parameters studied were storage temperature after molding, cooling rate during molding, and ingredient addition. The storage temperatures used were room temperature (22°), refrigeration (4°), and freezer (−5°). For cooling variation, the suppositories were molded using dry ice, refrigeration, and standing at room temperature, varying the molding time (cooling from 50 to 20°) from 5 to 15 to 30 min, respectively. The ingredient addition studies were carried out using 5, 10, and 20% zinc oxide USP, 5% neutral oil, and 0.2, 0.5, 1, and 2% resorcinol USP. Samples were tested at the beginning of the study and after 1-, 2-, 4-, and 6-week periods.

All suppositories were molded in 50-cavity brass molds. All masses were prepared in a 2-liter beaker nested in a 4-liter beaker containing water on a hot plate. Zinc oxide USP was passed through a comminuting machine[5] at high speed with hammers forward before incorporation. Dispersion was accomplished by a laboratory homogenizing mixer[6].

Differential Scanning Calorimetry—A thermal analyzer[7] was used with the differential scanning calorimeter cell attachment. Samples between 7.0 and 10.0 mg were prepared in tightly covered aluminum cups and were tested against an empty, covered cup blank. Sample weight was determined by subtracting the tare weight of the container. The cell containing the blank and sample cups was chilled to −20° using liquid nitrogen. The test runs then were carried out at a heating rate of 2.0°/min over the range of −10–+45°.

Three samples of each base were tested as individual continuous experiments. The experimental design allowed the same samples to be tested at all incremental age periods (initial, 1, 2, 4, and 6 weeks and 6 months or equilibrium).

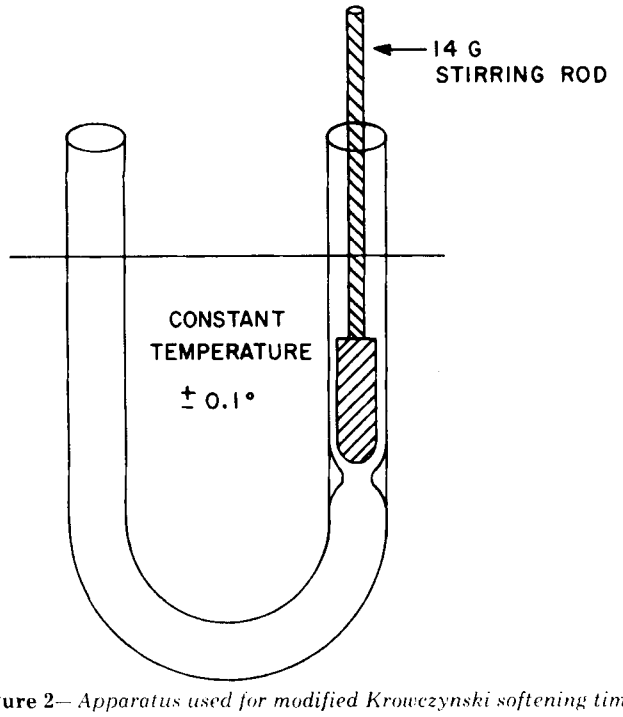

Figure 2— *Apparatus used for modified Krowczynski softening time test.*

[5] Fitzpatrick model M mill with N000 screen.
[6] Arde-Barinco model CJ-3.
[7] Dupont model 900.

After the equilibrium testing of a sample (*e.g.*, 6 months old), the melted sample was held at 45° for 5 min and then cooled back to room temperature using compressed air or nitrogen through the cell over ~20 min. The test then was repeated immediately, with freezing to −20° and running as outlined to establish the initial endotherm. After holding and cooling to room temperature a second time, the sample was stored at the desired temperature for 1, 2, 4, or 6 weeks. Retesting and continued storage were repeated, resolidifying the sample each time, so that full testing of all time periods took 13 weeks. If any test periods were missed, a longer period was measured, and the desired period was repeated that many weeks later.

Bases 1 and 3 then were retested in triplicate at equilibrium, at the initial point, and at 6 weeks, but they were stored at 4, 20, and 30°. Base 3 then was tested with 5% neutral oil (duplicate), 10% zinc oxide USP (duplicate), and 2% resorcinol USP (duplicate as well as single samples with 1, 0.5, and 0.2% resorcinol USP). As before, these samples were tested only at equilibrium, at the initial point, and at 6 weeks.

The recorded endotherms were examined for the position and height of the major peak. This height was measured vertically from the peak tip to a horizontal line drawn between the two equilibrium points at the beginning and end of the run. The height in centimeters was converted to degrees, divided by the sample weight, and recorded as degrees per gram. Heats of fusion, ΔH_f, for the samples were determined by cutting and weighing sample endotherms *versus* a gallium standard.

X-Ray Diffraction—An X-ray diffractometer[8] was used. Samples of each base were prepared by melting the base and molding it in a 0.2-mm thick aluminum frame backed by masking tape. The frame opening created a sample surface measuring 2.0 × 1.0 cm. All five bases were studied initially upon molding and after 1, 2, 4, and 6 weeks. Since this test is nondestructive to the sample, a study could be run in 6 weeks. A second study was done on Bases 1 and 3 stored at 4, 20, and 30° *versus* a polypropylene control since variability was seen in the amplifier from week to week.

RESULTS AND DISCUSSION

Softening Time Testing—Table II summarizes the results of the base screening study performed with the modified Krowczynski apparatus. Observed softening times were noted as a function of temperature for suppositories stored for up to 54 days. Bases 2 and 5 showed no increases in softening time throughout the range of rectal temperature. While Bases 1 (Fig. 3) and 4 showed only slight hardening at 37°, the softening times were increased significantly at lower body temperatures. Base 3 (Fig. 4) showed drastic hardening at all temperatures studied.

Table III summarizes the results of the effects of cooling time, storage temperature, and adjuvant addition on the rise in softening time of Bases 1 and 3 at 37°. Cooling time had no effect on the degree of hardening noted at the test periods, whereas storage temperature had an initial separating effect on the samples but not a lasting one. This finding suggested a discrete, point-to-point process that occurred more slowly as the temperature was lowered as opposed to an ongoing, continuous process. As a control, Base 1 showed little change.

Ingredient addition studies showed the effect on the hardening rate of the three additives: neutral oil, resorcinol USP, and zinc oxide USP. The two oil-soluble materials yielded more dramatic results than the insoluble zinc oxide due to the viscosity-imparting property of this dispersed powder in the melted base. Therefore, the smallest addition of zinc oxide was the most effective in reducing hardening.

Conversely, reducing the concentration of soluble resorcinol reduced its effect on prevention of hardening.

X-Ray Diffraction Studies—In the initial study, all five bases showed similar results both upon molding and after storage.

[8] North American Phillips Co. (Norelco).

Table II—Base Screening Study: Softening Time Stability after Storage at 22°

Age of Base, days	Time to Soften at Test Temperature, min			
	37.0°	36.5°	36.0°	35.5°
Base 1				
1	8.5	10	12	14.5
11	14.5	21	31	60
38	16	22	33	72
53	16	20	28	100
Base 2				
12	6.5	8	10	12
28	7	9	11	12
54	8	9.5	11.5	14
Base 3				
1	14.5	17	21.5	35
9	38	75	>100	>100
18	49	>100	>100	>100
Base 4				
1	8.5	12	18	32
10	11	17	30	44
28	11	21	32	85
43	14	25	35	>100
Base 5				
7	9	11	13.5	30
15	9	11.5	15	22
33	9	11	13.5	30
48	9	11.5	15	22

All bases, upon molding, exhibited a broad peak (~2.2° wide at mid-height) at a 2θ value centering at 20.3–20.6°. A sharper peak (~0.6° wide at half-height) also was seen at a 2θ value of 22.7–23.0°. In the initial samples, the low point between the two peaks was at ~37% of the height of the peak at 20.5°.

After storage, no new major peaks were seen. However, the peak at 20.3° grew sharper and more distinct from the peak at 22.7°, eventually having a smaller width similar to the other peak. The low point between the peaks after 6 weeks approached the baseline (Fig. 5). In Bases 3 and 4, peaks that appeared initially as shoulders on the major peak at 20.5° became more distinct, but in no case did any shift occur to a new position nor did any peak evolve that was not observed initially.

These observations suggest an amorphous to crystalline shift in the materials studied rather than polymorphic changes. Figure 5 shows a typical initial and aged sample scan made on Base 3.

In interpreting these data, it must be kept in mind that all commercial fat products, including semisynthetic suppository bases, are mixtures of many fatty molecules. Thus, these data may not be examined in the same depth as the data for a pure compound or a single crystal. Never-

Table III—Effect of Cooling Time, Storage Temperatures, and Adjuvants on the Softening Time Stability of Bases 1 and 3 at 37°

Base	Excipient	Cooling Time, min	Storage Temperature	Softening Time, min			
				Initial	2 Weeks	4 Weeks	6 Weeks
3[a]	None	15	22°	37	68	103	205
		30	22°	22	70	112	208
		5	22°	24	77	95	200
		15	4°	37	34	90	203
		15	−5°	37	28	27.5	190
	Neutral oil	15	22°	25	34	40	39
	Resorcinol, 0.2%	15	22°	15	35	38	42
	Resorcinol, 0.5%	15	22°	17	33	37	39
	Resorcinol, 1.0%	15	22°	22	31	33	35
	Resorcinol, 2.0%	15	22°	17	26	27	27
	Zinc oxide, 5%	15	22°	35	105	106	102
	Zinc oxide, 10%	15	22°	35	78	95	130
	Zinc oxide, 20%	15	22°	40	80	108	150
1[b]	None	15	22°	7.5	12	20	20
		30	22°	8	10	12	16
		15	4°	8	8	10.5	10

[a] Suppocire BCM. [b] Witepsol H-175.

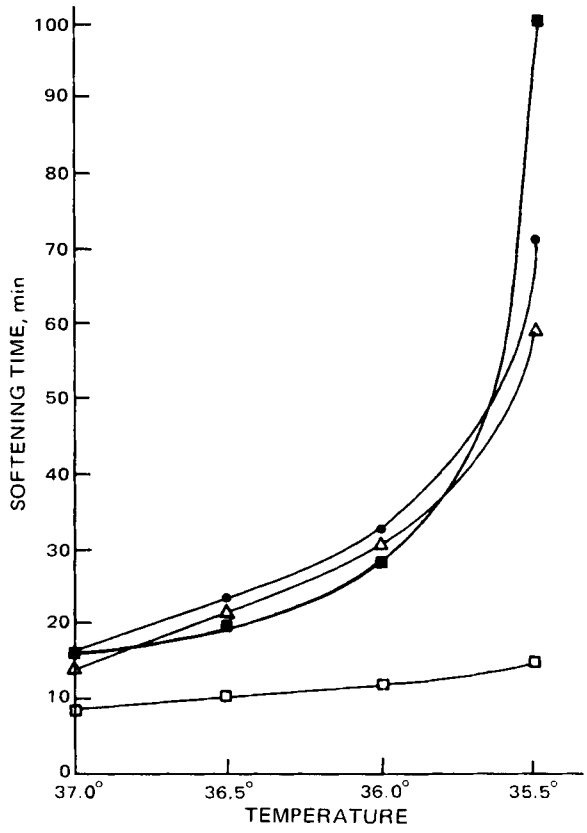

Figure 3—*Softening time stability for Base 1 stored at 22°. Key: □, 1 day; △, 11 days; ●, 38 days; and ■, 53 days.*

theless, the graphs are definitely characteristic. Therefore, while it would be presumptuous to claim that no polymorphism contributes to (or detracts from) the phenomenon under study, it still is obvious from the scans generated that the mechanism responsible for the effect being

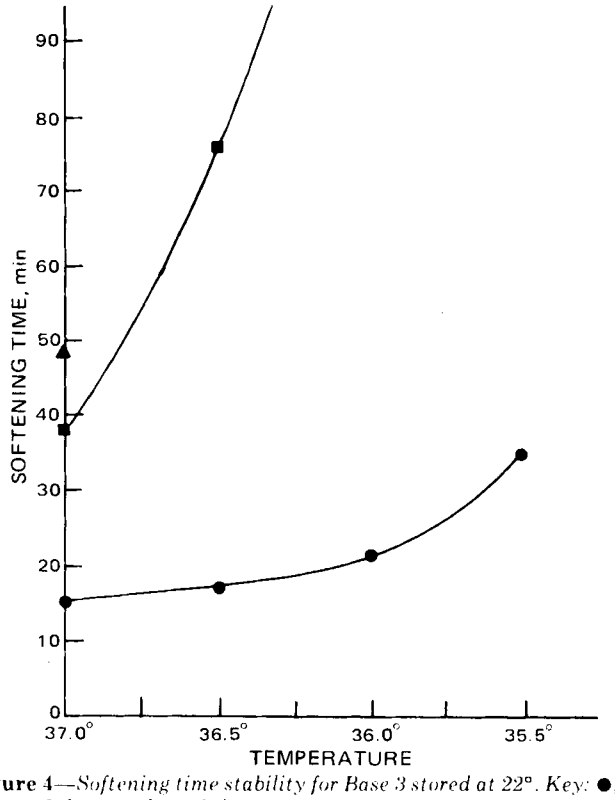

Figure 4—*Softening time stability for Base 3 stored at 22°. Key: ●, 1 day; ■, 9 days; and ▲, 18 days.*

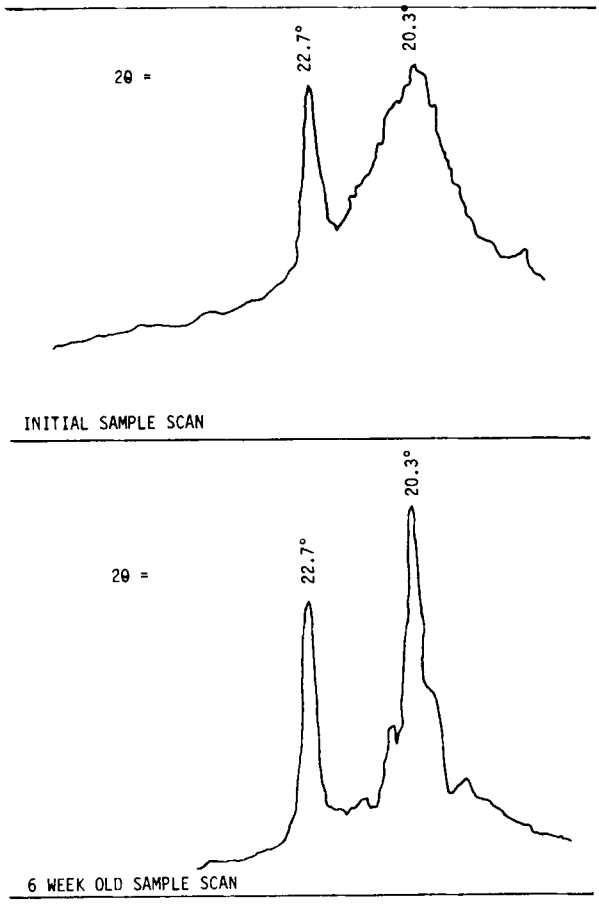

Figure 5—*Comparison of X-ray diffractometer scans for initial and 6-week-old samples of Base 3 stored at 22°.*

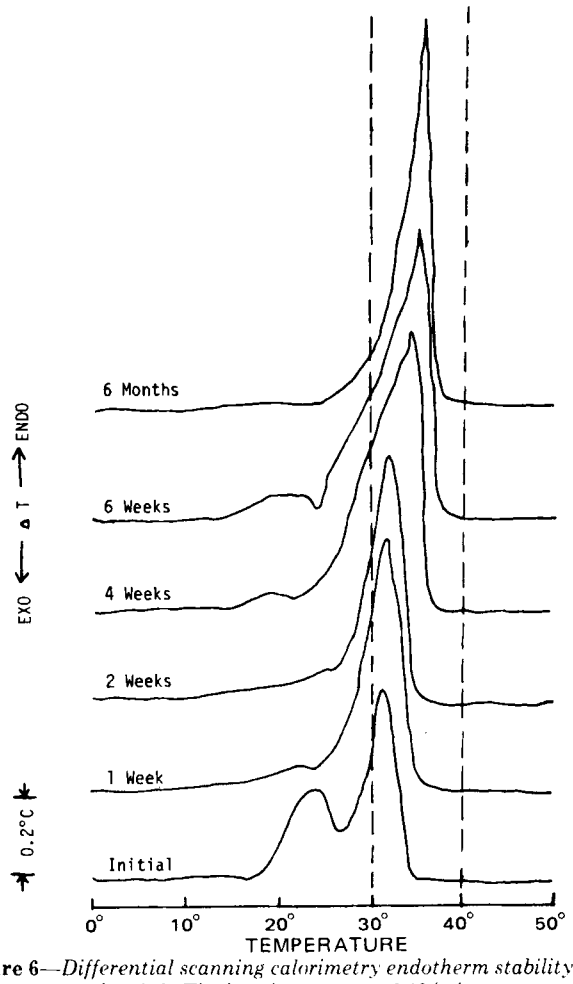

Figure 6—*Differential scanning calorimetry endotherm stability scan of Base 1 stored at 22°. The heating rate was 2.0°/min.*

measured is a crystallization phenomenon. This result reinforces the observation made on comparisons of softening times that a discrete process characterized by a temperature-dependent rate of hardening was taking place.

The hardening rate dependence on temperature was reinforced by the second X-ray study, which showed more rapid peak sharpening at higher storage temperatures in samples aged for 6 weeks at 4, 20, and 30°.

Differential Scanning Calorimetry—Accuracy in melting-range determinations was gained by utilizing a heating rate of 2°/min. The poor heat transfer characteristics of fats (14) prevented the use of faster speeds due to the lag time in the return to a ΔT equilibrium. Speeds of 5°/min and higher totally washed out and shifted the sharp peaks observed at 2°/min. In an experiment where changes in the peak positions of 1° were being sought and metastable transformations were not, this choice was mandatory since slower heating rates yielded sharper endo- or exotherms and located an equilibrium transformation temperature more accurately (10).

Figures 6 and 7 show the general trend observed for Bases 1 and 3 in the initial study conducted with samples stored at room temperature. In all bases, regardless of the initial peak position, a 2.5–3.0° shift upward in the peak positon, a sharpening of the peak, and an increase in peak height were observed over the test period. Tables IV and V summarize the data collected to quantify the measurable parameters of the endotherms in the initial study. Table IV lists the changes in temperature positions of the major peak. Table V lists the ratio of the major peak

Table IV—Base Screening Study: Temperature Position of Major Endotherm Peak [a]

		Storage Time at Room Temperature					
Base	Sample	Initial	1 Week	2 Weeks	4 Weeks	6 Weeks	6 Months
1	A	33.0	34.0	34.5	35.0	34.0	36.0
	B	32.0	34.0	35.0	34.0	34.0	36.0
	C	32.0	32.0	32.0	32.0	32.0	36.5
2	A	31.5	31.5	31.5	32.0	31.0	33.0
	B	31.5	31.5	32.0	31.5	32.0	34.0
	C	31.0	33.5	34.0	34.0	34.0	32.0
3	A	33.0	34.5	36.0	37.5	37.5	37.5
	B	33.5	37.0	37.0	36.5	36.5	37.5
	C	33.0	35.0	37.5	37.0	37.0	37.5
4	A	33.0	33.5	34.0	36.0	34.5	36.0
	B	32.0	33.5	34.0	34.0	34.0	37.0
	C	32.0	34.0	34.0	34.0	34.0	37.0
5	A	33.0	33.0	35.0	35.5	36.0	36.5
	B	32.5	33.0	34.0	35.5	35.5	36.5
	C	32.5	33.5	35.0	35.0	34.0	36.5

[a] Values are in degrees.

Table V—Base Screening Study: Ratio of Height of Major Peak to Sample Weight [a]

		Storage Time at Room Temperature					
Base	Sample	Initial	1 Week	2 Weeks	4 Weeks	6 Weeks	6 Months
1	A	65.6	79.2	70.1	90.9	66.7	109.5
	B	77.5	85.2	59.4	56.8	63.3	148.4
	C	63.6	82.0	79.8	68.5	65.8	88.2
2	A	77.9	75.7	106.6	71.2	112.1	92.9
	B	79.7	80.7	80.2	81.7	72.3	87.2
	C	85.7	48.6	48.1	45.2	48.6	67.2
3	A	51.7	53.3	58.0	73.5	81.1	79.6
	B	47.2	73.7	66.1	66.4	68.1	86.2
	C	42.1	42.1	73.2	71.4	68.1	79.3
4	A	40.6	51.8	63.9	44.3	49.5	50.6
	B	35.3	48.2	47.6	42.9	40.5	58.8
	C	40.5	64.8	67.8	78.7	72.8	65.4
5	A	32.7	40.7	70.9	60.6	54.2	58.6
	B	34.4	33.9	30.4	42.4	38.4	54.8
	C	33.7	35.4	42.2	43.4	47.4	51.4

[a] Values are in degrees per gram.

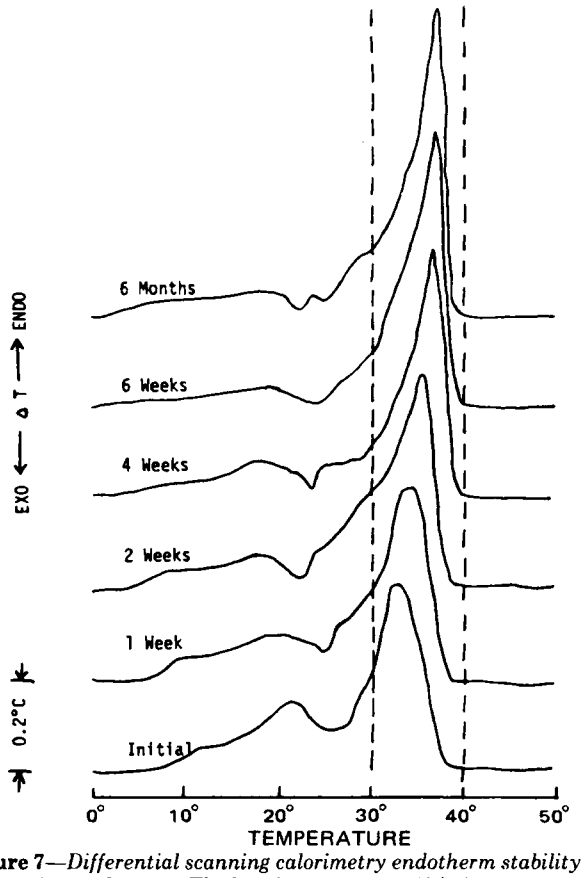

Figure 7—*Differential scanning calorimetry endotherm stability scan of Base 3 stored at 22°. The heating rate was 2.0°/min.*

height to the sample weight and the changes in this parameter over time.

The changes in sample endotherms over the 6-week period correlated perfectly with the observations of Krowczynski softening times and X-ray diffraction patterns. In all cases, a gradual change was seen. As seen in the X-ray diffraction data, changes were observed in all five bases, regardless of the production method or chemical composition.

The differential scanning calorimetry data explain the base to base variation observed in Krowczynski softening times through a correlation to the solid fat index. The solid fat index is determined dilatometrically[9] on samples immediately after solidification and may be considered only as an initial collection of data subject to change upon aging. A correlation between initial differential scanning calorimetry data and the solid fat index was shown by Miller *et al.* (13). Therefore, it is a reasonable assumption that an upward shift in the differential scanning calorimetry endotherm represents a similar upward shift in the solid fat index, *i.e.*, a higher solids content at any measured temperature. A higher solids content at any given temperature would raise the viscosity of the suppository melt at that point and, in turn, increase its softening time in the Krowczynski test.

A correlation between the degree of hardening of the five bases and their solid fat indexes at ~33° was seen (Fig. 1). Base 3, which hardened the most at 37°, had the highest solid fat index at 33° (42%). Bases 1 and 4 were next in hardening and next in decreasing solid fat index values at this temperature (37 and 35%). Base 5 at 33% and Base 2 at 17% hardened the least and were the lowest in the solid fat index at 33°.

While this correlation assumes a total shift of the curves by 4°, this shift is not necessarily the case since the shape of the curve could be changing. Until a method to measure the solid fat index of an aged sample is readily available, the procedure used for this study provides adequate correlation for prediction of hardening effects, rationalized through an upward shift in the solid fat index. In comparison, initial chemical and process information provides only the most general clues as to the melting behavior of the finished base at any age after resolidification. No useful conclusions could be drawn from the heat of fusion data collected or the peak height to sample weight ratios measured.

[9] American Oil Chemists' Society Test Method 113a.

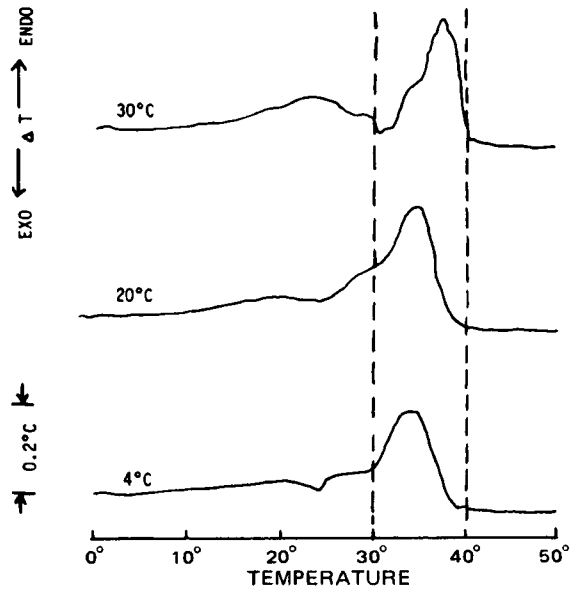

Figure 8—*Effect of storage temperature on differential scanning calorimetry endotherm stability for 6-week-old samples of Base 3. The heating rate was 2.0°/min.*

The effect of the storage temperature on the hardening rate was confirmed by measurements of the endotherms of Bases 1 and 3 stored for 6 weeks at 4, 20, and 30°. The comparative endotherms for Base 3 are shown in Fig. 8. The samples stored at temperatures closer to, but below, their melting ranges reached equilibrium crystallinity more rapidly. This phenomenon is similar to the annealing or tempering process used in metal alloy and glass preparation.

The ingredient addition trial endotherms of Base 3 showed no variation from the endotherms of Base 3 tested without additives either initially or after storage. Thus, it can be concluded that not only is there no significant freezing-point depression but that the mechanism involved in slowing the hardening rate, as evidenced by changes in softening time, is not involved with the phenomenon itself. Since the endotherm still is changing, the amorphous to crystalline shift is still occurring and, likewise, so is the shift in the solid fat index. The explanation lies in the fact that the overall phenomenon being prevented is an increase in the viscosity of the melting suppository since that is what the softening time is measuring. Addition of ingredients provides numerous pockets of discontinuity in the gross crystalline structure of the fatty solids present, lowering the gross viscosity of the semisolid suppository under testing.

This explains the effect seen from different concentrations of resorcinol. As less resorcinol was employed, there was less of a decrease of hardening. With zinc oxide, which is not soluble in the base, diminished hardening was noted at smaller concentrations, but this trend began to reverse when the powders began to add to the viscosity of the melt at higher concentrations.

CONCLUSIONS

The hardening phenomenon has been characterized by multiple test methods to be a finite amorphous to crystalline transition. The rate of this transition is storage temperature dependent but is independent of cooling time during solidification within the bounds of suppository molding rates. This transition is manifested in an upward shift in the solid fat index in the range of body temperatures. This rise in the percent solids in the melt at the temperature under examination results in a rise in viscosity at that temperature, which is perceived as hardening. In other words, there are less meltable solids at body temperature in a suppository that has hardened. Ingredients added to the base do not hinder the transition but may have one of two effects. Base-soluble ingredients disturb the gross crystalline structure of the fatty solids, lowering the achieved viscosity upon melting. Base-insoluble ingredients, in higher concentrations, may raise the viscosity of the melt simply by providing a higher solids content.

Suppository base selection may be simplified in two ways. First, examination of the solid fat index for potential problems upon shifting is an easy investigative early-warning device to narrow the field of choice. Second, differential scanning calorimetric examination of an aged base

sample (as received from a potential supplier) and an instantaneous rerun yield initial *versus* aged data on melting-range shifting after an investment in testing time of <1 hr. Even though the formula ingredients may retard the rate of transition, this test produces a best and worst case melting range to evaluate.

The results of this study in no way reflect on the quality of any base tested or on the ability of any of the manufacturers to supply a range of bases suitable to individual needs.

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Kinetic Study of USP Blue Tetrazolium Assay with Methylprednisolone, Hydrocortisone, and Their Hemisuccinate Esters by High-Pressure Liquid Chromatography

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Abstract □ The reactions of blue tetrazolium (I) with methylprednisolone and hydrocortisone and their hemisuccinate esters at ambient temperature under USP assay conditions in alcohol USP and in absolute alcohol were followed by high-pressure liquid chromatography (HPLC) and spectrophotometry. The disappearance of the ester and the increase and then decrease in the alcohol, as well as the formation of several reaction products with time, were observed by HPLC analysis. The reduction of I was observed spectrophotometrically. A sequential kinetic model was used to describe the overall reaction. The rate constants for the hydrolysis of the hemiester (k_1), the reaction with I (k_2), and the degradation of the parent steroid (k_3) were determined by discrete kinetic experiments using HPLC. The following observations were made: (*a*) k_1 is proportional to $[H_2O]$ and is the rate-limiting step, (*b*) k_2 is about 100 times the value of k_3, and (*c*) k_2 for methylprednisolone is about the same as for hydrocortisone and appears to be independent of the concentration of water. With these rate constants, simulated time–concentration profiles for the reaction of the esters with I favorably compared with experimental data in alcohol USP and absolute alcohol. This study shows that the USP blue tetrazolium assay with these esters has potential for variability and is not stability indicating.

Keyphrases □ Methylprednisolone—and hemisuccinate ester, high-pressure liquid chromatographic and spectrophotometric monitoring of USP assay with blue tetrazolium □ Hydrocortisone—and hemisuccinate ester, high-pressure liquid chromatographic and spectrophotometric monitoring of USP assay with blue tetrazolium □ High-pressure liquid chromatography—kinetic study of USP blue tetrazolium assay with methylprednisolone and hydrocortisone and their hemisuccinate esters

The official USP assay for the corticosteroids, including their esters, is the blue tetrazolium (I) reaction (1). The base-catalyzed reduction of I by the C-17 side chain of corticosteroids is a classical reaction in steroid analysis (2). Meyer and Lindberg (3) established that the α-ketol moiety in corticosteroids is responsible for the reduction of I. However, the reaction mechanism for the C-21 esters of corticosteroids is unknown.

BACKGROUND

Johnson *et al.* (4) reported that color development was unusually slow with hydrocortisone hemisuccinate compared to other steroids. In a study of the reaction of I with cortisone acetate, a C-21 ester, Guttman (5) showed that as the concentration of base catalyst was decreased, the lag time increased and the sigmoid shape of the curve was emphasized. He concluded that hydrolysis of the ester was a prerequisite to a reaction resulting in the generation of formazan. Graham *et al.* (6) also suggested that ester hydrolysis occurs prior to and during formazan development. Their conclusions were derived from graphical kinetic treatment of the spectrophotometric data.

The hydrolysis of the hemiester of corticosteroids in aqueous media has been studied by several investigators. Mauger *et al.* (7) reported that the degradation of its hemisuccinate ester to hydrocortisone was first order. Further degradation to a species devoid of the 17-dihydroxyacetone also was observed. This degradation was followed by separation of the alcohol and ester and subsequent reaction with I. Garrett (8) studied the alkaline hydrolysis of hydrocortisone hemisuccinate by constant pH titration.

High-pressure liquid chromatography (HPLC) has been used recently to study the degradation of hydrocortisone (9). An HPLC method was described (10) that determined simultaneously hydrocortisone (II*a*) and its hemisuccinate ester (III*a*) or methylprednisolone (II*b*) and its hemisuccinate ester (III*b*).

This paper reports a kinetic study of the reaction of I with II and III using this HPLC method. The hydrolysis rates of III to II under compendial blue tetrazolium assay conditions also are reported.

The effect of water in the final reaction mixture of I has been studied. The percentage of water in the official USP method [due to the use of alcohol USP and 10% aqueous tetramethylammonium hydroxide (IV) in the preparation of reagents and samples] is 5.8 (11). Rechnagel and

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