

razol occurred with and without SKF 525-A pretreatment in all of the barbitalized rats and in some of the normals, using a multiple injection technique. Cook, *et al.* (9), have reported a similar absence of interference with development of tolerance to morphine by SKF 525-A; it did not change its LD₅₀.

On the other hand, pretreatment with SKF 525-A markedly lowered the fatal dose of picrotoxin in barbitalized rats and significantly decreased the convulsive and fatal doses in normal rats. The results with barbitalized rats, pretreated with SKF 525-A, were unusually uniform in that the standard errors were less than 1.8% of the CD₅₀s and LD₅₀s of picrotoxin, compared with 3.5% and 3.1% in normal, pretreated rats.

The major factor of SKF 525-A effect is due to the inhibition of biotransformation of substances susceptible to its influence. The extensive review by Brodie (14) involving, in part, numerous *in vivo* and *in vitro* studies by his own staff, indicates that a similar inhibitory effect by SKF 525-A may be exerted on a variety of metabolic pathways or, as stated by Cooper, *et al.* (7), it inhibits a factor or factors possessed in common by a diversity of drug enzyme systems. Depression of bioacetylation and of acetylcholinesterase activity has since been reported by Kuriaki and Marumo (15). The mechanism involved in the case of the three analeptic drugs tested by us remains to be determined. With

propallylonal, SKF 525-A may prevent the replacement of Br by an OH group and subsequent molecular rearrangement of the ketone form to give an acetonyl group, which leads to formation of a barbiturate that is much weaker and slower in action than the β -bromallyl derivative (16, 17).

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Temperature-Induced Rheological Variability in an Emulsion System

By JAMES C. BOYLAN, H. GEORGE DeKAY, and GILBERT S. BANKER

A study has been made on the effect of emulsification temperature and cooling rate on certain physical properties of a beeswax-mineral oil emulsion. The emulsion, which was prepared at five temperatures and cooled at three rates, was evaluated for plastic viscosity, thixotropy, yield value, water separation rate, and initial average particle size. It was shown that both the emulsification temperature and cooling rate significantly altered these properties.

IN THE PAST few decades, emulsions have been studied from many viewpoints. Despite this, the formulation, preparation, and stability of most emulsion products remains, to a large extent, an empirical art. Investigations to date have failed to pinpoint the rheological characteristics of many emulsions.

It is generally agreed that an increase in temperature facilitates emulsification (1-3). One author (4) contends in rather vague and general terms that an emulsification temperature should

be chosen which will give the optimum dispersion to the materials being emulsified. Spalton (5) specifies that a freshly prepared wax emulsion should be allowed to cool slowly since rapid cooling may result in a granular product. Wellman (6) found that the solubility of the soap, as controlled by temperature, is an important factor in emulsion formation. Several studies have been conducted attempting to relate viscosity of emulsions to temperature (7-9).

In the area of particle size and its effect on viscosity, Sherman (10) states that there is a frequent increase in viscosity when an emulsion is homogenized. He feels that this increase in viscosity is due to a decrease in particle size,

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which leads to increased interfacial area and interaction among the globules.

The objective of this study was to determine the following effects of various emulsification temperatures and rates of cooling on a wax-containing emulsion: (a) the rheological properties (i.e., viscosity, yield value, and thixotropy), (b) the rate of creaming and/or separation of the phases, and (c) the particle size of the dispersed phase.

EXPERIMENTAL

Emulsion Formulation and Preparation.—A beeswax emulsion of the following composition was used throughout this study

	Amount, Gm.
White wax.....	80.0
Liquid petrolatum (heavy).....	250.0
Sodium borate.....	11.0
Purified water.....	659.0
to make.....	1000.0

The emulsion formula was chosen on the basis of its simplicity and the wide cosmetic and pharmaceutical use of its ingredients.

The wax and liquid petrolatum were placed in a 2000-ml. stainless steel beaker (inside height 16.5 cm., inside diameter 12.5 cm.). The sodium borate and the water were placed in a 1000-ml. glass beaker. The aqueous phase was brought to the desired temperature by means of a bunsen burner. The temperature of the oil phase was controlled by means of a heating mantle,¹ regulated by a variac. When the desired temperature of both phases was reached, the aqueous phase was slowly added to the oil phase (which was still encased in the heating mantle). A model V-7 "lightin" mixer² was immediately started at 1875 r.p.m. and allowed to run for 15 seconds to achieve initial emulsification. The emulsion was then removed from the heating mantle and placed in a specially constructed air bath (see Fig. 1) which had been preset to cool the emulsion at one of the prescribed rates. A second "lightin" mixer within the air bath was started and run at 1800 \pm 100 r.p.m. until the emulsion cooled. All emulsions were cooled in the air bath to 30°. With practice, the procedure and time required for enclosing the emulsion in the air bath became nearly constant.

Both mixers utilized a three-blade marine-type propeller, the length of each blade being 1.6 cm. The propeller was mounted on a 26.9-cm. shaft. In each case, the propeller was placed off center in the same quadrant and one-third of the distance from the bottom of the beaker relative to the height of the liquid, with the mixer shaft at an angle of about 25° from the perpendicular. A Stobatac³ was used to standardize the mixer speeds. A small oscillating fan inside the air bath maintained air circulation and a 12-inch evaporating dish filled with distilled water minimized fluctuations in relative humidity during the experiment.

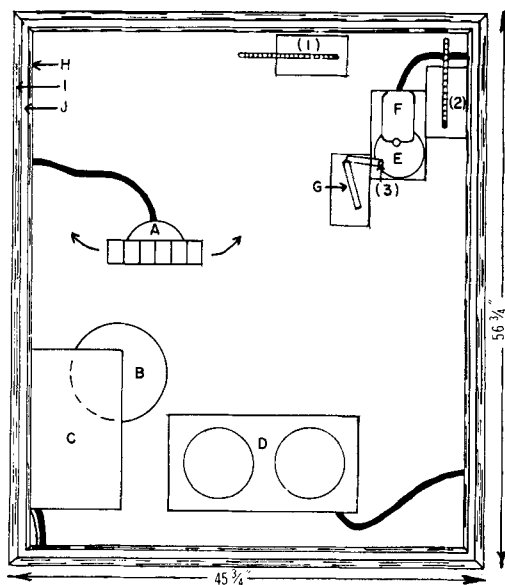


Fig. 1.—Top view of the air bath. The materials are: $\frac{3}{4}$ and $\frac{3}{8}$ inch plywood, glass wool (insulator), and double glass window above the mixer area. The inside of the roof and the sides are lined with aluminum foil. Thermometers, supported on ring-stands, are at points 1, 2, and 3. A, rotating fan; B, evaporating dish; C, refrigeration unit; D, hot plate; E, emulsion; F, mixer; G, magnifying mirror; H, $\frac{3}{8}$ inch plywood; I, $\frac{3}{4}$ inch plywood; J, $\frac{3}{4}$ inch glass wool.

The emulsions were prepared at five temperatures and cooled at three rates (see Table I). When a sample was to be cooled slowly, the air bath was preset at $25 \pm 0.5^\circ$. Since the heat lost by the emulsion upon cooling remained in the hermetically sealed air bath, the temperature inside the bath rose to $29\text{--}30^\circ$. If the emulsion was to be cooled at a moderate rate, the air bath was preset at $11 \pm 1^\circ$. The temperature within the bath then rose to $14\text{--}16^\circ$. In the case of rapidly cooled products, the stainless steel beaker containing the emulsion was placed in a 10-inch water bath containing crushed ice. This was then introduced into the precooled air bath ($11 \pm 1^\circ$).

TABLE I.—SUMMARY OF EMULSION MANUFACTURE

Emulsion Notation ^a	Emulsification Temperatures, °C.		Rate of Cooling to 30°, min.	Temperature when Placed in Air Bath, °C.
	Aqueous Phase	Wax Phase		
1-S	70	70	127	68
1-M	70	70	66	68
1-F	70	70	21.5	67
2-S	80	80	127.5	78
2-M	80	80	75	78
2-F	80	80	13.5	72
3-S	90	90	134	85
3-M	90	90	78	85
3-F	90	90	15.5	80
4-S	70	90	127	74
4-M	70	90	72.5	73
4-F	70	90	16	70
5-S	90	70	128	80
5-M	90	70	76	80
5-F	90	70	15.3	75

^a S, slow cooled; M, medium cooled; F, fast cooled.

¹ Glas-col Apparatus Co., Terre Haute, Ind.

² Mixing Equipment Co., Inc., Rochester, N. Y.

³ General Radio Corp., Chicago, Ill.

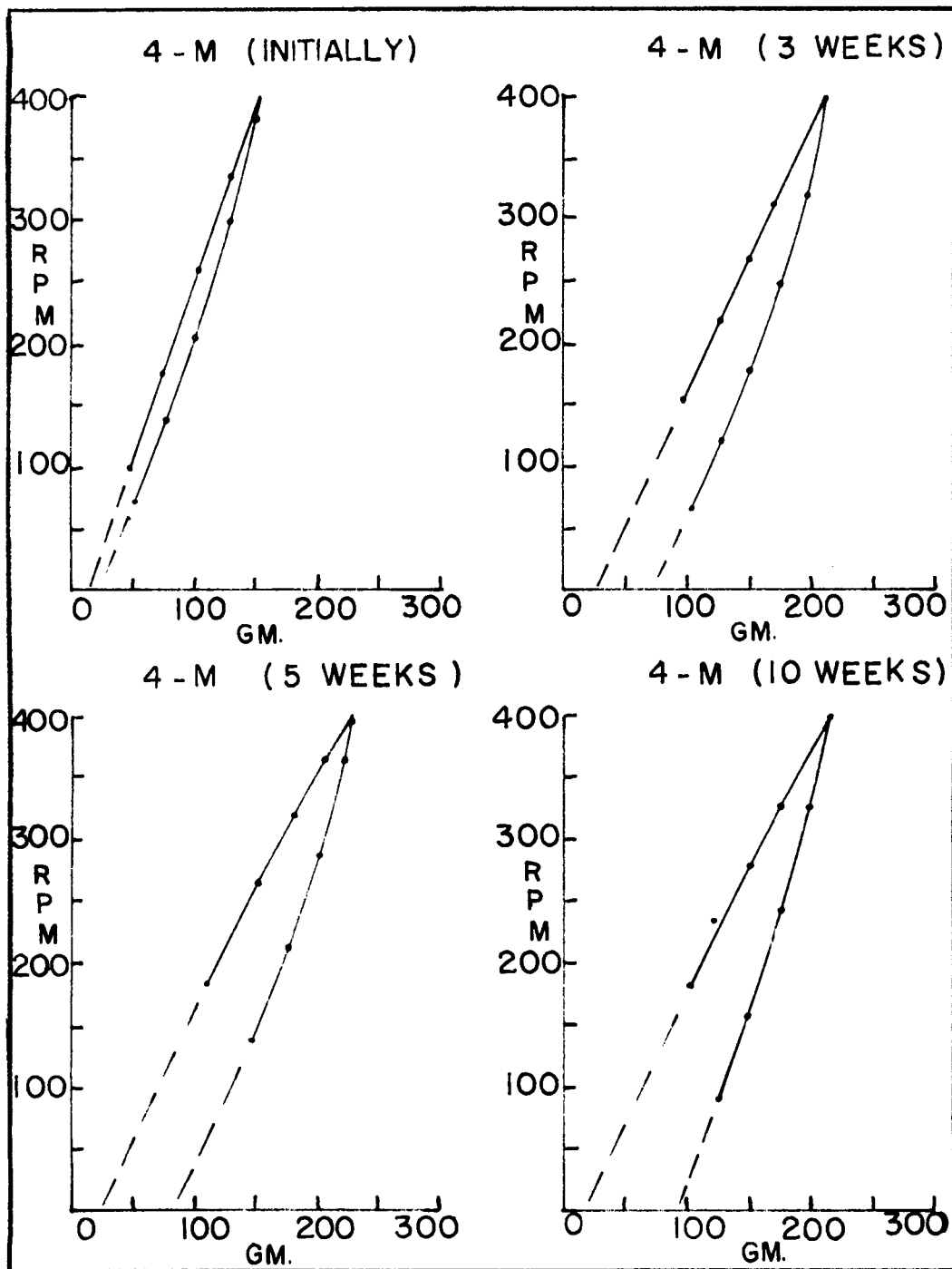


Fig. 2.—Emulsion 4-M rheograms showing the effect of aging.

Rheological Evaluation.—The viscometer chosen for this study was a Stormer viscometer provided with the modified cup and bob as suggested by Fischer (11). A weight hanger with various slotted weights provided the shearing stress. A constant temperature water bath maintained the sample temperature at $25 \pm 0.1^\circ$. A calibration curve for the cup and bob was prepared using seven N.B.S.

standard viscosity oils. The emulsion sample was placed in the cup and deaerated by gentle suction. The cup was carefully raised into position, taking care not to entrap air under the bob. The system was then conditioned for 2 hours before beginning a run. The rheogram was obtained by adding successively increasing weights to the hanger. A point on the curve was obtained by noting the

TABLE II.—EFFECT OF EMULSIFICATION TEMPERATURE, RATE OF COOLING, AND SAMPLE AGE ON THE RHEOLOGY OF A WAX-CONTAINING EMULSION

Emulsion Notation ^a	Rheological Parameter	0					
		Days ^b	1 wk.	3 wk.	5 wk.	6 wk.	10 wk.
1-S	V ^c	59.25	62.87	82.69	78.00	85.72	92.10
	T ^d	2.86	1.86	6.63	5.75	9.14	9.02
	f ^e	22.74	25.63	39.00	29.62	35.34	37.26
1-M	V	68.52	66.96	82.69	96.76	98.17	122.1
	T	3.67	6.20	8.95	14.85	14.71	20.15
	f	32.18	27.57	34.35	43.87	41.10	25.43
1-F ^g	V	70.93	67.95	72.58	92.73	93.80	95.01
	T	7.36	8.00	8.21	17.04	17.50	22.00
	f	34.08	33.10	32.29	57.78	58.16	59.50
2-S	V	46.48	41.77	69.55	76.58	80.01	71.96
	T	0.89	0.95	4.86	8.16	8.09	8.22
	f	17.86	15.38	34.98	42.53	37.06	36.88
2-M	V	61.75	77.58	71.54	79.63	89.64	85.72
	T	3.72	7.00	5.98	7.98	10.68	11.47
	f	25.02	22.20	23.96	27.80	21.39	30.69
3-S	V	40.10	45.45	81.64	80.63	80.63	88.09
	T	0.77	1.31	7.05	5.75	5.48	10.14
	f	13.20	12.48	32.46	38.01	38.01	30.69
3-M	V	64.30	78.00	89.64	94.19	93.80	96.76
	T	3.68	6.97	14.09	15.54	14.50	18.03
	f	25.68	27.77	39.99	37.32	38.25	27.07
4-S	V	41.01	76.58	77.00	74.96	70.93	84.67
	T	1.00	6.04	6.41	6.41	5.92	6.84
	f	15.02	40.68	30.53	34.17	35.00	46.45
4-M	V	62.83	92.10	90.07	85.10	90.65	87.61
	T	3.68	12.84	11.77	15.37	14.70	15.48
	f	28.37	18.63	42.80	52.98	43.74	35.34
5-S	V	41.01	65.44	83.08	86.11	81.64	89.06
	T	1.33	4.76	8.18	8.27	9.00	9.30
	f	17.67	34.85	46.43	41.85	40.81	39.06
5-M	V	66.96	91.62	103.8	105.9	95.16	101.8
	T	3.92	13.19	18.51	18.26	16.35	15.07
	f	25.73	40.94	29.95	60.91	42.92	47.69

^a See Table I for the relationship between the emulsion notation and the method of manufacture. ^b The first rheograms were prepared immediately after manufacture and cooling of the emulsion samples. ^c Plastic viscosity in centipoises. ^d Thixotropic area in square inches. ^e Yield value in dynes/cm.². ^f Rheological data for emulsion samples 2-F to 5-F could not be obtained due to the wax aggregates present.

time required for a given weight to drive the bob through 100 revolutions. This value was then converted to r.p.m. When a maximum of 380 to 410 r.p.m. was reached, the weight on the hanger was reduced in increments. An interval of 60 seconds was observed between each point on the curve. The calculations necessary to obtain the instrumental constants, plastic viscosity, and yield value were identical to those of Fischer (11). The thixotropic area was measured directly in square inches with a compensating planimeter.⁴ At least two samples of each emulsion were run immediately after manufacture, and after 1, 3, 5, 6, and 10 weeks, the samples being stored at room temperatures which ranged from 22 to 29°.

Separation Rate Evaluation.—Preliminary work showed that water separation was the only visible change to occur in the emulsion. Consequently, a 100.0-ml. fraction of each emulsion sample was placed in a rubber stoppered Pyrex glass tube as outlined by Peck (12). Water separation was measured at frequent intervals for 180 days.

Particle Size Determination.—Immediately after manufacture, each sample was viewed with a standard optical microscope equipped with a 10× eyepiece and 43× objective. Emulsion samples were "mixed" prior to sampling by gently tumbling them in the sample container by hand. The sample was

prepared by diluting one drop of emulsion as delivered by a standard U.S.P. medicine dropper with 5.0 ml. of distilled water. One drop of this dilution was then placed on a glass slide, a cover slip carefully put in place, and the sample viewed. The longest regular dimension of each particle was measured using a micrometer in the eyepiece.

The method of Harris, Horvitz, and Moon (13) was applied to preliminary particle size data obtained from two different emulsion samples to determine the number of particles required for a statistically valid sampling. With the test a maximum confidence interval of 0.5 μ , 99% confidence coefficient, 95% probability, with 99 degrees of freedom, a sample size of 300 to 400 particles was indicated. Subsequently, 400 particles were counted from each emulsion sample in the determination of particle size parameters.

RESULTS AND DISCUSSION

Rheology.—The temperature of emulsification and rate of cooling altered specific rheological properties of this emulsion system. All samples demonstrated plastic viscosity and thixotropy (Table II and Figs. 2-5). As shown by Table II, thixotropy increased greatly with age in all samples. The rate of cooling determined, to a large extent, the amount of thixotropy of the emulsion both initially and on aging.

⁴ Keuffel and Essel Co., Model #4242.

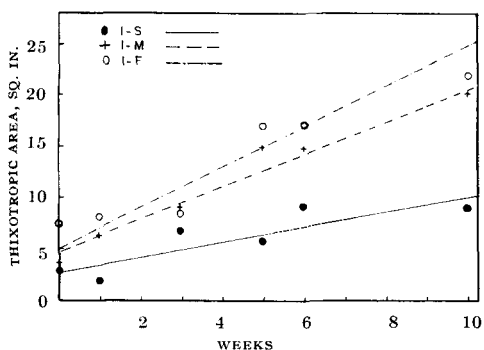


Fig. 3.—The effect of cooling rate and sample age on the thixotropy of a wax emulsion.

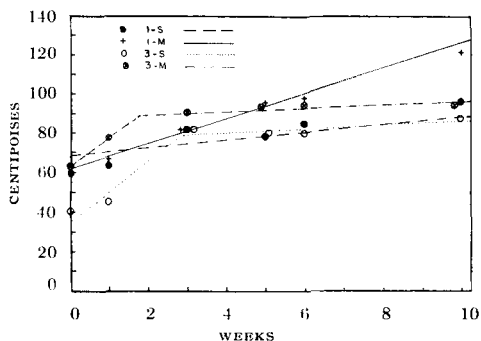


Fig. 4.—The effect of emulsification temperature, cooling rate, and sample age on the plastic viscosity of a wax emulsion.

The more rapid the cooling rate, the greater the thixotropy of the emulsion samples.

Emulsions made at different temperatures and cooled slowly showed, without exception, that the lower the temperature of emulsification, the greater the plastic viscosity, thixotropic area, and yield value of the freshly prepared samples (Tables I and II). After 10 weeks this was still true for the yield values. Compared with emulsions cooled at the fast and medium rates, the slowly cooled emulsions demonstrated the lowest values for plastic viscosity and thixotropic area (Table II).

As illustrated by Table II, emulsions prepared at different temperatures and cooled at a medium rate initially had only about a 10% difference in plastic viscosity. After 10 weeks there was a variance of about 40%. Similarly, the products cooled at a medium rate initially possessed nearly equal thixotropic areas; however, after 70 days there was a great percentage variance in the areas (Table II). Initially, the yield value of emulsion 1-M was greater than emulsions 2-M and 3-M (2-M and 3-M being nearly equal to each other). After 10 weeks there were only small differences in the yield values in emulsions 1-M, 2-M, and 3-M.

The results indicate that the rapidly cooled products possessed the greatest thixotropy and yield value of all emulsions.

Despite the frequently published statement that the separate emulsion phases should not be prepared at widely differing temperatures, with this emulsion it seemed to make little difference in any property measured whether the phase temperatures varied by as much as 20° or not.

TABLE III.—PARTICLE SIZE DATA

Emulsion	Diameter, μ (Arithmetic Mean)	Emulsion	Diameter, μ (Arithmetic Mean)
1-S	6.1	3-F	4.9 ^a
1-M	5.3	4-S	5.7
1-F	5.0	4-M	5.0
2-S	7.0	4-F	4.5 ^a
2-M	6.2	5-S	5.9
2-F	5.9 ^a	5-M	4.6
3-S	6.3	5-F	3.9 ^a
3-M	5.9		

^a These emulsions contained, in addition to the single crystals, many 35 to 50 μ aggregates.

TABLE IV.—SUMMARY OF ANALYSIS OF VARIANCE

Source	d.f.	SS.	M.S.	$F_{\text{calcd.}}$	$F_{0.05}$	Significant
Among columns, (cooling rate)	2	4.6	2.3	30.7	4.46	Yes
Among rows, (emulsion temp.)	4	4.3	1.08	14.3	3.84	Yes
Error	8	0.6	0.075	
Total	14	9.5				

Figure 5 shows that rheological replication was possible with this emulsion system. Emulsions 4-S and 4-S' were made, on separate days, at the same temperature and cooled at the same rate.

Separation Rate.—All samples showed water separation. Fast cooled emulsions, with the exception of emulsion 1-F which was prepared at a temperature just above the melting point of white wax (70° both phases), showed rapid pronounced water separation (Fig. 6). After 180 days, 27 to 32 ml. of water had separated from the fast cooled emulsions; however, only 13 ml. of water had separated from emulsion 1-F during this period. Water separation in the samples cooled at the slow and moderate rates varied from 10 to 14 ml. after 180 days. This difference was apparently due to the fact that in the fast cooled emulsions the phases had considerably less time for complete emulsification and mixing (Table I). Emulsion samples cooled at a medium rate consistently showed the least water separation over the test period.

Particle Size.—The study of the particles and their relative sizes under the microscope was quite revealing (Table III). The average particle size was definitely related to the rate of cooling and temperature of emulsification. In the emulsion samples, the average particle size varied from 3.9 μ in emulsion 5-F to 7.0 μ in emulsion 2-S. Without exception, smaller particles resulted as the rate of cooling was increased. However, fast cooled samples also contained large (35 to 50 μ) aggregates in addition to the small individual particles. This was probably due to incomplete emulsification of the sample. It is important to point out that only a few isolated oil globules were seen under the microscope. The mineral oil and the white wax apparently merged, forming needle-like crystals. De Navarre (14) states that when reacting sodium

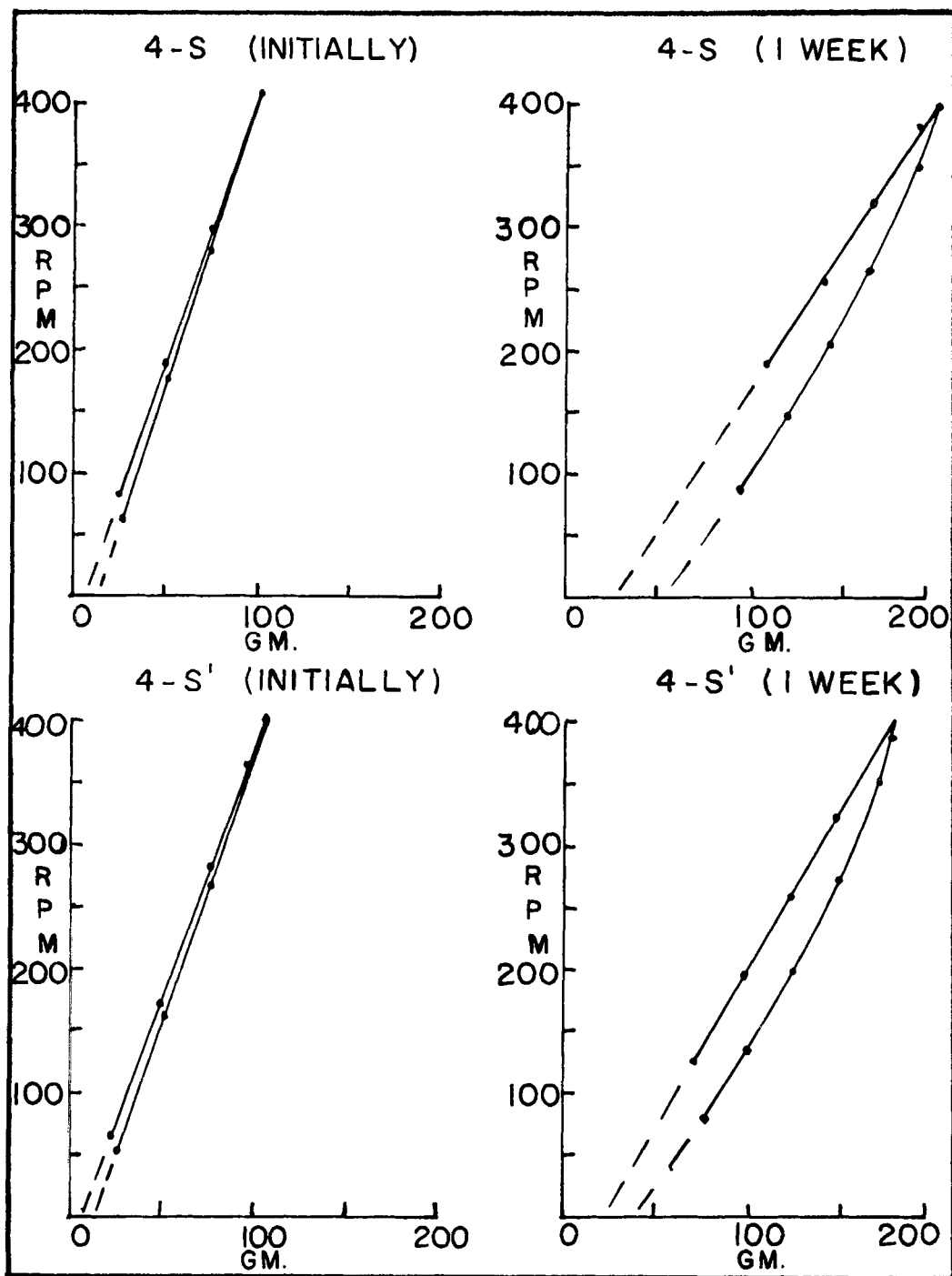


Fig. 5.—Rheological replication of emulsion 4-S.

borate with white wax to form a cold cream, sodium cerotate and several other minor soaps are formed which emulsify the oil and the waxes in the water. To determine if the oil and the wax would combine without the aid of an emulsifier, 80 Gm. of white wax and 250 Gm. of mineral oil were heated together, with mixing, to 80° and maintained at that tem-

perature until all the wax melted. The mixture was then slowly cooled to room temperature. A uniform, semisolid, one-phase product resulted.

A two-way analysis of variance (15) revealed that both the rate of cooling and the temperature of emulsification produced significant changes in the average particle size (Table IV).

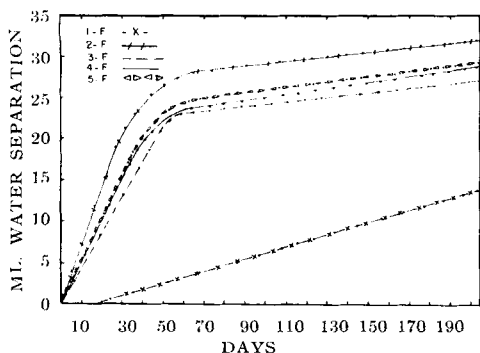


Fig. 6.—Water separation from fast cooled emulsions.

SUMMARY AND CONCLUSIONS

All the samples studied demonstrated plastic viscosity and thixotropy and possessed a yield value. However, the temperature of emulsification and rate of cooling definitely altered these and other physical properties of the emulsion. The rate of cooling was the main variable factor which determined the degree of thixotropy in the sample; the faster the cooling rate, the greater the thixotropy. As a general rule, plastic viscosity and thixotropy increased with age. Emulsions cooled at a medium rate showed more variance in their rheological values and trends than did the slowly cooled products.

Upon cooling, the wax and the mineral oil in the emulsion combined, forming needle-like crystals. Both the cooling rate and the emulsification temperature significantly changed the size of the emulsion particles; without exception, smaller particles resulted as the rate of cooling was increased. However, fast cooled emulsions

formed wax-oil aggregates as well as individual particles.

All fast cooled emulsions, with the exception of the one prepared at 70° (both phases), showed rapid water separation over a 180-day interval. All other emulsions showed only slight water separation in the 180 day test period. Fast cooling is not recommended when preparing this emulsion.

Despite the frequently published statement that the separate emulsion phases should not be prepared at widely differing temperatures, with this emulsion it appeared to make little difference visually, microscopically, or rheologically whether the phase temperatures were the same or not.

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