Method for Evaluating the Emulsibility of Insecticide Concentrates

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An apparatus designed for testing the spontaneity of emulsification of heavier-than-water emulsible concentrates proved to be satisfactory for measuring the stability, making it possible to study both characteristics in one operation. The results with four toxaphene concentrates in four types of water and one lindane concentrate in five types of water are reported. A commercial toxaphene concentrate showed satisfactory spontaneity and stability of emulsion in distilled, softened, and three types of hard water. One concentrate showed satisfactory spontaneity and emulsibility even in the hardest water and was readily redispersed after 24 hours. Two concentrates were unsatisfactory. The lindane concentrate showed satisfactory spontaneity and emulsibility only in the standard Army and Navy hard waters.

MOST FIELD SITUATIONS require that emulsible insecticide concentrates exhibit spontaneity of emulsion and subsequent emulsion stability. Concentrates with these qualities are especially desirable for use in sprayers not equipped with agitators.

The methods in common use for measuring emulsion stability and evaluating spontaneity of emulsification were recently reviewed by Selz (*4*). At this laboratory, the spontaneity of emulsification of heavier-than-water lindane concentrates has been measured for the time required for the sedimentation of 0.1 ml. of cream from 350 ml. of a 1% emulsion. A glass tube, 4 feet long and attached to a 15-ml. measuring tube, has been used as the spontaneity tube, and the tests have been made with Army standard hard water (342 p.p.m. in terms of calcium carbonate). Any lindane concentrate (*2*) that requires 5 minutes or more for the sedimentation of 0.1 ml. of cream is considered satisfactory.

The apparatus used for spontaneity tests is satisfactory for simultaneously measuring the spontaneity and stability of emulsion of heavier-than-water concentrates. Descriptions of the apparatus and its use, as well as the results of tests with four toxaphene concentrates and one lindane concentrate in several types of water, are reported in this paper. The toxaphene concentrates were included in this study because these concentrates must show especially good spontaneity and emulsion stability to be suitable for general use in insect-control work. These qualities are also necessary in lindane concentrates and are more easily attained with lindane than with toxaphene.

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Apparatus

The emulsibility tube, formerly called the spontaneity tube, in which the tests were conducted consisted of a borosilicate glass tube [(Corning No. 234220), 4 feet long, 22 mm. in outside diameter] joined to a 15-ml. conical centrifuge tube (Corning No. 8080). Although a ground-glass connection has been used, a 1-inch length of Tygon tubing with $^1/_2$-inch inside diameter and $^3/_{32}$-inch wall thickness makes a satisfactory connection. The mouth of the centrifuge tube is fitted flush with the end of the 4-foot tube. Centrifuge tubes graduated in 0.1-ml. divisions are used for making the sedimentation readings.

A stemless funnel, prepared by cutting off the stem of a 2-inch 60° funnel and grinding the base to a smooth flat surface, is used to introduce the concentrate into the emulsibility tube.

A 25-ml. graduated cylinder is used to measure the lindane concentrate.

A 5-ml. tipless measuring pipet was prepared by cutting off the tapered tip below the 5-ml. mark and grinding the end to a smooth, flat surface. This pipet is used for introducing the small samples of the toxaphene concentrate to the emulsibility tube.

A buret stand and clamp assembly or a suitable tube rack is used to hold the emulsibility tubes during the test.

Test Materials

Emulsible Concentrates. The composition of the concentrates used in these tests are given in Table I. Concentrate *A*, a commercial product, was said to contain 14% of inert ingredients, including the emulsifier. Emulsifier CTX-54 and Emulsol C83-27A are emulsifier blends of nonionic and anionic surfactants.

Test Waters. Distilled water and four synthetic waters of varying hardness were used. The type and composition of the waters are given in Table II.

The naturally softened water was prepared to simulate the type *E* water (*7*) that occurs in the Atlantic and Gulf Coastal Plains, and the half Army hard water was prepared by diluting Army hard water to half strength with distilled water.

Table I. Composition of Emulsible Concentrates

Components	Concentrate and % Composition				
	A	B	C	D	E
Toxaphene	61	50	50	62	..
Lindane	..	..	..	..	20
Kerosine	..	..	40	25.5	..
Isophorone	..	..	..	..	40
Petroleum distillate	25	..	..	..	..
Mineral spirits	..	40	..	..	..
Velsicol AR-60	..	..	..	..	32.5
Emulsifier CTX-54	..	10	10	12.5	..
Emulsol C83-27A	..	..	..	..	7.5

Table II. Composition of Test Waters

Type of Water	Reference	Hardness, P.P.M. of $CaCO_3$	Composition, Gram per Liter			
			$CaCl_2.2H_2O$	$CaCl_2$ (anhydrous)	$MgCl_2.6H_2O$	$NaHCO_3$
Distilled	..	..	..	..	..	..
Naturally softened	(1)	20	0.0094	..	0.0103	0.8000
Half Army hard		171	..	0.1519	0.0694	..
Army hard	(5)	342	..	0.3037	0.1388	..
Navy hard	(3)	500	0.2345	..	0.2680	..

The tests were initiated at 70°–77° F., and temperatures ranged from 65° to 77° F. during the 24-hour test period. These were the narrowest ranges obtainable under existing conditions, but, in practice, a much wider temperature range would be expected.

Experimental Procedure

Toxaphene Emulsible Concentrates The emulsibility tube was held vertically in the buret clamp or special rack and filled with test water to a mark $2\frac{1}{2}$ inches below its mouth. About 350 ml. were required. Sufficient concentrate to give a 0.5% toxaphene emulsion (2.3 ml. for the 61 and 62% and 3.5 ml. for the 50% concentrates) was pipetted into the tube with the tipless pipet. The pipet was held vertically so that the delivery tip was in the same plane and in the center of the mouth of the emulsibility tube. The rapidity and extent of disintegration of the concentrate were noted, and observations for bottom creaming or breaking of the emulsion were made after 0.5 and 24 hours.

Lindane Emulsible Concentrate The emulsibility tube was set up as described and filled with the test water to a mark of $4\frac{1}{2}$ inches below its mouth. About 323 ml. were required. The stemless funnel was placed over the mouth of the tube, and 17.5 ml. of concentrate poured rapidly through the funnel from the graduated cylinder, in order to obtain an emulsion containing about 1% of lindane. The initial disintegration was noted and the amount of bottom creaming after 0.5 and 24 hours. If the emulsion was not broken in 24 hours, the tube was inverted through 10 complete cycles, and another observation was made after 0.5 hour.

Results and Discussion

The results of spontaneity and stability tests with the four toxaphene concentrates and one lindane concentrate in five types of water are shown in Table III.

Toxaphene concentrate *A* gave the best over-all performance in these tests, showing satisfactory spontaneity and stability in all types of waters. This concentrate has been reported by Sparr *et al.* (6) to remain stable in livestock dipping vats for six months. Concentrate *C* showed satisfactory spontaneity and emulsibility in all waters after 0.5 hour, but slightly excessive creaming was evident after 24 hours. However, it was readily redispersed. Concentrates *B* and *D* were unsatisfactory in hard waters, and their emulsions were not completely satisfactory in distilled or softened water after standing for 24 hours.

Lindane concentrate *E* did not disperse properly, and oil separation was observed in distilled, softened, and half-strength Army waters after 0.5 hour. The material dispersed satisfactorily in standard Army and Navy hard waters, but excessive bottom creaming was evident after 24 hours. However, the cream was readily redispersed by 10

Table III. Emulsibility of Emulsible Concentrates in Several Types of Water

Concentrate	Type of Water	Initial Disintegration	Bottom Creaming, Ml.	
			After 0.5 hour	After 24 hours
Toxaphene A	Distilled	Complete	<0.05	0.15
	Softened	Nearly complete	0.05–0.10[a]	0.12[b]
	Half Army hard	Complete	<0.05	<0.05
	Navy hard	Complete	<0.05	<0.05
Toxaphene B	Distilled	Complete	<0.05	Partially broken
	Softened	Complete	<0.05	1.0
	Half Army hard	Complete, coarse particles discernible	1.2	3.7
	Navy hard	Complete	1.4	Completely broken
Toxaphene C	Distilled	Complete	0.3	2.1
	Softened	Complete	<0.05	<0.05[a]
	Half Army hard	Complete	<0.05	Partially broken
	Navy hard	Complete	0.5	2.2
Toxaphene D	Distilled	Complete	<0.05	Partially broken
	Softened	Complete	<0.05	0.7
	Half Army hard	Complete, coarse particles discernible	0.8[a]	Broken
	Navy hard	Complete	0.9	Broken
Lindane E	Distilled	Negligible	11.7 oil	....
	Softened	Complete	12.5 oil	....
	Half Army hard	Complete, slight particularity	4.5 oil	10.7 oil
	Army hard	Complete	<0.05	24.0[c]
	Navy hard	Complete	<0.05	20.0[c]

[a] Cream oily brown.
[b] Tube inverted through three complete cycles after introduction of concentrate.
[c] Easily redispersed.

inversion cycles, and after 0.5 hour, creaming was negligible (<0.05 ml.). It also passed the emulsion-stability test as set forth in the military specification (*2*).

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MICROBIOLOGICAL FAT PRODUCTION

Effect of Fermentation Variables on Rate of Fat Formation by *Rhodotorula gracilis*

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A study was made of the effect of experimental variables on the rate of fat formation as distinguished from cell growth. Shake flasks and a fermentor assembly which included an automatic pH recorder-controller were employed. The fat content was expressed as a ratio of fat to nonfat yeast; this ratio increased linearly with time of fattening. The rate of fat formation varied linearly with pH between pH 3.0 and 8.5 and increased from 2.1 to 3.1 grams of fat per 100 grams of nonfat yeast per hour. Decreasing the temperature from 28° to 22° C. lowered the fat rate to less than half the original value. Under the experimental conditions employed, the addition of accessory growth factors, or of the cations calcium, sodium, and iron, to the growth medium appeared to be unnecessary for fattening of the yeast. Neither sugar concentrations up to 16% by weight of medium nor type of hexose had a significant effect on fattening. Acetate was inhibitory at pH 5 and was of no value as an adjunct at pH 8. Ethyl alcohol and glycerol did not give increased fat formation.

THE RATE OF FAT FORMATION of a variety of microorganisms has been investigated (*9*). Enebo *et al.* (*2, 9*) studied fat production by various *Rhodotorula*. They chose *Rh. gracilis* for detailed study because this yeast grew readily in submerged culture, it formed fat rapidly and abundantly, and the cells were easily removed from the culture medium. They demonstrated that when a medium containing soluble nitrogen and a relatively high concentration of sugar is inoculated, two cultural phases can be observed. During the first or "protein" phase the cells proliferate at a normal logarithmic rate and have a low fat content. In the second or "fattening" phase, which begins when the medium has been depleted of nitrogen, the cell population remains nearly constant but the fat content increases. These findings were confirmed by Pan *et al.* (*10*).

In these, as in most other investigations on fat formation by microorganisms, multiplication and fattening of the cells were carried out successively under the same set of conditions. Thus the investigator allowed the culture to grow under a given set of experimental conditions and then, without change in conditions, to form fat. No logical basis could be found for assuming that optimum conditions for multiplication would likewise be the optimum for fat formation.

If the nonfat portion of the yeast cell is considered as a factory for fat production, then, under a given set of conditions, fat should be produced at a constant rate. The specific rate of fattening for a given weight of yeast would depend on experimental conditions. This concept is supported by the data presented in this paper. The objective of this investigation was to determine the effect of various experimental conditions on the rate of fat formation by *Rh. gracilis* when the fattening phase was separated from that of growth or multiplication. Should fat production by this organism become commercially desirable, the rate of fat formation would be an important consideration.

Materials and Methods

Equipment A fermentor was used in the studies on pH and temperature; a shake flask technique was employed in the case of all other variables. The fermentor assembly, shown in Figure 1, included an automatic pH recorder-controller in addition to the usual air sparger, constant temperature water bath, and stirrer. The unbaffled stainless steel fermentation tank was 28.5 cm. in both diameter and depth.

The pH control equipment consisted of an immersion-type stainless steel electrode holder carrying Beckman industrial size electrodes (shown removed from the fermentation vessel), an amplifier, a controller, and a diaphragm-type pump. When the recorded pH deviated from the set point, an external electric circuit was closed, causing the pump to admit neutralizing solution from a reservoir. Because in these experiments the deviation was always toward the acid side, 1*N* sodium hydroxide was the only solution required. The total variation in pH