Water/Oil Emulsions Prepared by Low Pressure Capillary Homogenization I

Effects of Emulsator and Composition Variables on Mannide Mono-oleate Stabilized Systems

By C. DAVID FOX* and RALPH F. SHANGRAW

An automated reciprocating capillary emulsator is described which duplicates the low-pressure homogenization principle of the interconnected glass syringe method used to prepare repository w/o emulsions. The w/o emulsions produced by this emulsator were evaluated by rotational viscometry and optical microscopy. Rheological flow curves of concentrated emulsions could be correlated with the extent of dispersion and degree of coalescence. Coalescence rates were found to be an inverse function of the surfactant concentration, with 20 per cent mannide mono-oleate furnishing the best relative stability for water-in-mineral oil emulsions. The rate of shear was found to govern the degree of emulsification, with a short capillary of large diameter producing the finest dispersion.

IT HAS been shown that antibody production in animals can be enhanced and prolonged when the aqueous immunologic agent is emulsified in mineral oil and injected intramuscularly to provide a depot of slowly released antigen (1). Since the work of Henle and Henle in 1945 (2), many clinicians have developed their own w/o emulsions of various agents in efforts to achieve a sustained immunologic response in humans (3–6). The best clinical results to date have been obtained by using light mineral oil as the external phase, since it cannot be metabolized by the human body. It is theorized that the mineral oil is removed by a slow phagocytosic process which appears to be the rate-limiting step in the release of antigen from the internal phase (7).

Although numerous reports have confirmed the efficacy of this innovation in hyposensitization therapy for influenza and pollinosis, there is considerable diversity of opinion among clinicians as to which emulsification method and formulation will yield the most stable emulsion with a uniform small particle size. Standardized w/o emulsions of various antigens for annual repository injection are not commercially available. Thus, the physician has had to develop his own individual formulation and emulsification techniques, determine the degree of dispersion, and estimate the appropriate dosage. The most widely used method for preparing these emulsions consists of placing the components to be emulsified in a 10-ml. glass hypodermic syringe and then connecting this syringe to a second 10-ml. syringe by means of a double-hubbed hypodermic needle. The shearing force required for emulsification is achieved by repeatedly forcing the components from one syringe to the other, either manually (4), or by means of an automated device[1] that pneumatically reciprocates the syringe pistons (8).

This low-pressure capillary homogenization project was designed to study the effect of variation in certain operating conditions on emulsification efficiency and to determine how various factors affect the stability of the w/o emulsions prepared. The variables studied were phase volume ratio, concentration of the emulsifying agent, homogenization pressure, capillary hypodermic needle diameter and length, and the number of processing cycles. The definite interrelationship that exists among the numerous variables was found to be reflected in the emulsion flow properties or rheologic behavior. For this reason, the evaluation of the experimental emulsions was based on particle size determination and rheologic flow curves, with an attempt to correlate these 2 parameters.

Mannide mono-oleate[2] was chosen as the emulsifying agent since it has received extensive toxicity evaluation and is generally accepted for use in w/o repository emulsions (9, 10). A purified grade of light mineral oil[3] was selected as the oil

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[1] Brown Emulsor, Andonian Associates, Inc., Waltham, Mass.
[2] Marketed as Arlacel A by Atlas Chemical Industries, Wilmington, Del.
[3] Marketed as Drakeol 6VR by Pennsylvania Refining Co., Butler, Pa.

phase since its suitability and safety is well documented (11).

The maximum volume of 10 ml. of emulsion produced by the glass syringe method is obviously inadequate for extensive evaluation. It was therefore decided to construct an automated capillary homogenizer based on the interconnected glass syringe principle, but capable of producing sufficient emulsion for rheological and stability studies (12).

Description of the Capillary Emulsator.—The emulsator designed and constructed in this laboratory consists of 2 modified hydraulic cylinders interconnected by a double-hubbed hypodermic needle. The emulsion components are introduced into 1 of the cylinders, and the system is then closed. Under a constant preset pneumatic force, the cylinder pistons alternately force the components repeatedly through the capillary hypodermic needle, producing the shearing action required for emulsification. A front view of the emulsator is shown in Fig. 1. The emulsator constructed has a maximum capacity of 114 ml., but larger volumes of emulsion could be produced with cylinders of increased bore or stroke.

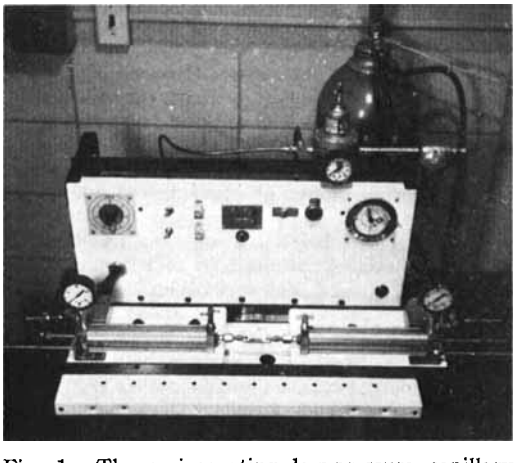

Fig. 1.—The reciprocating low-pressure capillary emulsator.

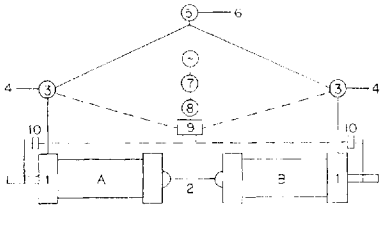

Fig. 2.—The reciprocating low-pressure capillary emulsator. Electrical system (– – –), pneumatic system (——). Key: 1, hydraulic cylinders, 1⅛ in. bore, 7 in. stroke; 2, double-hubbed hypodermic needle; 3, solenoid valves; 4, exhaust lines; 5, air pressure regulator; 6, compressed air source; 7, clock timer; 8, interval timer; 9, impulse relay; 10, microlimit switches.

The reciprocating action of the cylinder pistons is controlled by an impulse switching relay. This relay alternately activates 2 independent circuits—a pneumatic and an electrical circuit to each of two 3-way electromagnetic solenoid valves. A schematic diagram of the pneumatic and electrical system is shown in Fig. 2.

Air pressure on cylinder piston A forces the emulsion in cylinder A through the hypodermic needle into cylinder B. A micro-limit switch is tripped when cylinder piston A has forced all the emulsion into cylinder B. The limit switch directs a pulse of current to the impulse relay which switches current from the cylinder A solenoid to the cylinder B solenoid. This simultaneously exhausts cylinder A, and air pressure is directed into cylinder B. When cylinder piston B has forced the emulsion back into cylinder A, another limit switch is tripped, and the cycle commences again. An electrically operated digital counter is connected to the impulse relay. Each time the relay switches circuits, 1 count is recorded, with 2 counts indicating 1 complete cycle.

The emulsator can be set to commence and cease operation automatically at any desired time within a 12-hr. period, since a clock timer and an interval timer are included in the electrical circuit.

The machine parameters of operation time, capillary diameter, capillary length, and shearing stress can be held constant enabling a valid comparison among emulsions since the dispersions are produced under reproducible and well-defined conditions.

EXPERIMENTAL

Preparation of Emulsions.—In this study, the external phase of all emulsions is composed of light mineral oil and mannide mono-oleate, while the internal phase is distilled water. It should be noted that when all components were placed in 1 cylinder of the emulsator, a stable o/w emulsion is invariably produced when the ratio of oil phase to water phase is 1:1. This was unexpected since mannide mono-oleate is oil-soluble and should tend to produce w/o emulsions. It was therefore necessary to inject the aqueous phase into the oil phase on the first stroke to obtain the desired w/o emulsion. The ease with which mineral oil–mannide mono-oleate systems form o/w emulsions has not been reported in the literature.

Measurement of Flow Properties.—The instrument used in this study was the Haake Rotovisko rotational viscometer (Gebrüder Haake K.G., Berlin, Germany), with a rotor radius of 2.004 cm. and a cup radius of 2.10 cm., which gave a range of shear

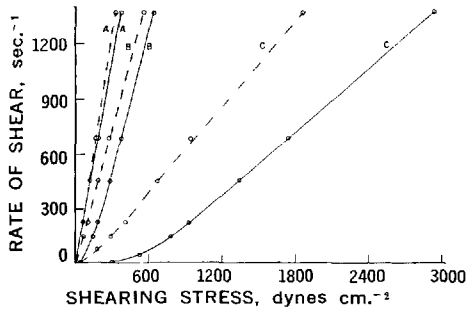

Fig. 3.—Effect of phase volume variation on w/o emulsion flow curves. Key: ———, initial; – – – –, 30 days. (Distilled water internal phase) A, 5%; B, 20%; C, 50%.

rate from 7×10^{-2} to 1.14×10^{3} sec.$^{-1}$ and a range of shear stress from 15 to 3×10^{3} dynes/cm.^{2} All determinations were made immediately after homogenization and after a 30-day storage period, at a temperature of 30°.

Particle Size Determination.—Dark-field microscopy was chosen as the method for particle size analysis. Although this method has inherent limitations in measuring submicron particles, it was still preferable to the problems of coping with sedimentation or light-scattering techniques when applied to w/o systems. A size–frequency analysis was performed on each emulsion immediately after homogenization and again after 30 days, with a Leitz Ortholux dark-field microscope (E. Leitz, G. m. b. H., Wetzlar, Germany) equipped with a 95/1.32 N.A. fluorite oil immersion objective, immersed substage condenser, and 25× oculars for a total magnification of 2375×. A small aliquot of each emulsion was diluted 1:400 with light mineral oil and a drop placed in a Petroff-Hauser bacteria counting chamber (Arthur H. Thomas Co.) of 0.02-mm. cell depth and covered with a cover glass of 0.25-mm. thickness, thus permitting critical focusing. Fields from 2 slides were examined with the diameters of 400 particles being measured with a screw micrometer eyepiece.

Storage of Emulsions.—The portion of each emulsion used for the initial rheological determination was discarded and the remainder stored in 4-oz. clear glass bottles for 30 days at room temperature.

Effect of Variation in Phase Volume.—Representative flow curves for w/o emulsions containing 5, 20, and 50 vol. % of distilled water are shown in Fig. 3, with size–frequency data presented in Table I. These emulsions all contain 20% mannide mono-oleate. Emulsions containing smaller amounts of emulsifying agent exhibited lower apparent viscosities, but their flow curves had the same general shape. Emulsator constants were: homogenizing pressure, 50 psig; capillary diameter, 18 gauge; capillary length, 1.25 cm.; and cycles, 25.

It can be seen that emulsions containing 5% internal phase possess Newtonian flow properties, and despite the high degree of coalescence that occurred in 30 days, little change is reflected in the flow curve. Emulsions containing 50% water in the internal phase, however, exhibit flow curves which show a high degree of correlation with the extent of droplet coalescence. Furthermore, this concentration is the one most commonly utilized by clinicians for their repository emulsions.

Effect of Variation in Mannide Mono-oleate Concentration.—The effect of 4 different concentrations of mannide mono-oleate on simple 50:50 w/o systems is shown by the size–frequency distribution data presented in Table II. Concentrations of 2, 5, 10, and 20 vol.% were used in the light mineral oil external phase replacing the oil, and each emulsion contained 50% distilled water internal phase. Emulsator constants were: homogenizing pressure, 50 psig; capillary diameter, 18 gauge; capillary length, 1.25 cm.; and cycles, 25. Varying each of the constants in turn for emulsions of identical composition yielded similar data. It is evident that a high percentage of mannide mono-oleate is required to furnish the best relative stability; hence, a concentration of 20% was used for the emulsions in the remainder of this study.

Effect of Variation in Homogenizing Pressure.—The first emulsator parameter to be investigated was that of homogenizing pressure, and the influence of pressures varying from 20 to 75 psig is shown in the flow curves of Fig. 4. Each emulsion is composed of mannide mono-oleate, light mineral oil, and distilled water in the volume ratio of 2:3:5, respectively. Emulsator constants were: capillary diameter, 18 gauge; capillary length, 1.25 cm.; and cycles, 25.

A microscopical examination of the freshly prepared emulsions revealed that droplet diameters in each emulsion were essentially all below 1 μ. Since a decreased particle size is the only factor which could have caused the increased apparent viscosities, it is assumed that the increase in pressure resulted in a small but significant decrease in droplet diameters. Previous work in this laboratory had shown that droplet diameters below 1 μ have a profound influence on flow curves of concentrated emulsions. However, in this study, significant differences in particle diameters below 1 μ could not be discerned due to the inherent limitations of the microscope technique employed. It was decided to keep the homogenizing pressure constant at 50 psig for all subsequent emulsions since this is the maximum pressure that 10-ml. glass syringes will withstand without leakage past the plungers.

Effect of Variation in Capillary Diameter.—Emulsions were prepared using hypodermic needles of 20, 18, and 15 gauge (nominal inside diameters of 0.58, 0.84, and 1.37 mm., respectively). An analysis of the rheological and micromeritical data for these emulsions showed that, among the gauges used, an

Table I.—Size–Frequency Distributions for Fig. 3 Emulsions,[a] Phase Volume Variation

Diam., μ	Emulsions A[b] Time, Days 0	A[b] Time, Days 30	B[c] Time, Days 0	B[c] Time, Days 30	C[d] Time, Days 0	C[d] Time, Days 30
<0.5	388	25	392	5	389	20
0.5–1	12	75	8	15	10	60
1–3	...	160	...	75	1	80
3–5	...	80	...	100	...	60
5–8	...	40	...	100	...	100
8–15	...	15	...	100	...	70
>15	...	5	...	5	...	10

[a] Each determination based on 400 measured droplets. [b] 5% aqueous internal phase. [c] 20% aqueous internal phase. [d] 50% aqueous internal phase.

TABLE II.—SIZE FREQUENCY DISTRIBUTIONS FOR 50:50 W/O EMULSIONS STABILIZED WITH MANNIDE MONO-OLEATE[a]

	Percentage (v/v) Mannide Mono-oleate in Light Mineral Oil External Phase							
	2%		5%		10%		20%	
	Time, Days		Time, Days		Time, Days		Time, Days	
Diam., μ	0	30	0	30	0	30	0	30
<0.5	235	...	229	...	256	20	389	30
0.5–1	100	...	117	...	109	40	10	80
1–3	65	...	54	40	35	80	1	80
3–5	...	10	...	32	...	53	...	60
5–8	...	32	...	48	...	67	...	70
8–15	...	78	...	100	...	60	...	70
>15	...	280	...	180	...	80	...	10

[a] Each determination based on 400 measured droplets.

increase in capillary diameter resulted in emulsions with slightly higher apparent viscosities and a more uniform particle size distribution.

Effect of Variation in Capillary Length.—The effect of variation in the capillary length on flow curves for w/o emulsions is shown in the flow curves of Fig. 5. Each emulsion is composed of mannide mono-oleate, light mineral oil, and distilled water in the volume ratio of 2:3:5, respectively. Emulsator constants were: homogenizing pressure, 50 psig; capillary diameter, 18 gauge; and cycles, 25. It is readily evident that as the needle length is decreased, apparent viscosities increase, again undoubtedly due to a decrease in droplet size and increased uniformity. The same effect is also noted in using either 20- or 15-gauge capillaries of various lengths. Thus, all subsequent emulsions were prepared with the largest diameter and shortest length double-hubbed capillary hypodermic needle, *i.e.*, 15 gauge and 0.625 cm., respectively.

Effect of Variation in Processing Cycles.—The final emulsator variable to be studied was the number of homogenization cycles to which the emulsion is subjected. The number of cycles was varied from 13 to 100. For any given set of conditions, essentially constant rheological values were obtained after 25 cycles, and no discernible improvement in particle size reduction could be observed by continuing the homogenization process for more than 25 cycles.

DISCUSSION

Since 1945, the formulation and production of w/o repository emulsions for human use has undergone extensive improvement. These improvements have, for the most part, resulted from subjective observations and appear to have been empirically derived. At present, most reported formulations are composed of mannide mono-oleate, light mineral oil, and aqueous antigenic extract in the volume ratio of 2:3:5, respectively, and it is generally recognized that a droplet diameter of less than 1 μ is an essential requirement for satisfactory *in vivo* sustained antibody production (13).

Previous workers have probably been misled by the limitations of their optical equipment. It is quite likely they have reported lower particle size limits above those which actually existed. No attempt is made in this work to claim accurate particle size distributions in the submicron range, but only to make comparisons of those particles which can be accurately determined, since, for the purpose of this research, it was of greater importance to know the size–frequency distribution of droplets greater than 1 μ. The measurement of the droplets in the experimental emulsions was facilitated by the fact that Brownian motion was negligible, an observation directly opposed to that reported by Silverman (14), who stated that Brownian motion was especially significant in his series of w/o emulsions, which are similar in composition to the ones used in this study.

In the area of particle size and its effect on viscosity, this investigation has confirmed the opinion of Sherman (15, 16), who reported that the observed increase in apparent viscosity of homogenized emulsions is due to a decrease in droplet size. This leads to increased interfacial area and interaction among droplets in concentrated emulsions, especially when the droplet diameters are below 2 μ.

The representative flow curves furnish additional evidence to establish the fact that particle size can be correlated with rheological data, *i.e.*, the relative extent of flow curve deviation after storage when

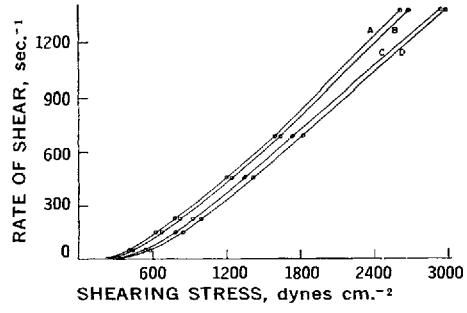

Fig. 4.—Effect of homogenizing pressure on flow curves of 50:50 w/o emulsions. Key: A, 20 psig; B, 25 psig; C, 50 psig; D, 75 psig.

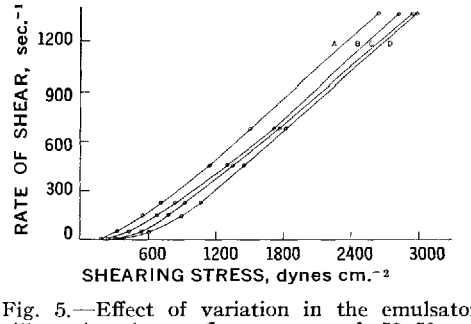

Fig. 5.—Effect of variation in the emulsator's capillary length on flow curves of 50:50 w/o emulsions. Key: A, 5.0 cm.; B, 2.50 cm.; C, 1.25 cm.; D, 0.625 cm.

compared with the initial flow curve is a direct indication of the extent of droplet coalescence. As shown in Fig. 3, the validity of the above statement increases as the volume per cent internal phase increases. When emulsions contain a low percentage of internal phase, the extent of particle–particle interaction is only a minor contributing factor to the over-all rheologic behavior of the system, and the flow curve is influenced primarily by the viscosity of the external phase. The anomalous flow behavior and high viscosity of concentrated homogenized emulsions is due primarily to the high resistance to flow offered by the tightly packed droplets and the increased adsorption of emulsifying agent at the greatly extended interface. As the droplets coalesce, the emulsion becomes polydisperse and cubic-packing decreases with a concomitant reduction in interfacial area and contact among droplets. The net result of these reductions will be reflected in the flow curve by a significant decrease in both apparent viscosities and shear dependence. This work confirms the rotational viscometric work of Saunders (17), who observed that when the internal phase volume becomes greater than 5%, shear dependence increases due to hydrodynamic interference. Sherman (18), however, has reported observing Newtonian flow in w/o emulsions at internal phase volumes of up to 50%, as determined by capillary viscometry, a method which possesses definite inherent limitations when used to obtain rheological data on shear dependent systems.

While o/w emulsions can generally be stabilized by low concentrations of surfactants, w/o repository emulsions require extremely high concentrations of mannide mono-oleate, with 1 report stating that 35% was required (19). This research has confirmed the fact that high percentages of mannide mono-oleate are required, but as repository w/o emulsions prepared by the interconnected glass syringe method are subjected to very low shear conditions, this would not be unexpected. The majority of emulsions in this study were homogenized at 50 psig to correlate the data with that reported by workers in the repository emulsion field.

The development of automatic devices for the production of small quantities of repository w/o emulsions has enabled the physician to achieve some degree of process standardization, but a review of the literature reveals that tremendous variations exist in methods of preparing essentially identical emulsions in interconnected glass syringes with pneumatically operated syringe plungers. The double-hubbed hypodermic needle length, in almost all cases where reported, is given as 5 cm., but the needle gauge and process time vary over an extremely wide range, as illustrated by 1 report which states emulsions were homogenized for 1 hr. through an 18-gauge needle and then for 3 hr. through a 22-gauge needle (20). Another author reports processing 50 min. through an 18-gauge needle, followed by 20 min. through a 22-gauge needle, and finally, 20 min. through a 25-gauge needle (6).

In reviewing the data, it was noted that an increase in capillary diameter, an increase in homogenizing pressure, and a decrease in capillary length were each associated with an increased emulsion velocity through the capillary. This is in agreement with Poiseuille's law for liquid flow through capillaries, which states that the volumetric flow rate is a direct function of the the capillary radius and pressure differential, and an inverse function of capillary length. The fact that an increased emulsion velocity through the capillary resulted in increased apparent viscosities with concomitant decreases in the mean diameter of the dispersed droplets would indicate that the degree and uniformity of dispersion is influenced by the rate of shear to which the emulsion is subjected. While commercial homogenizers operate in the range of 1000 to 5000 psig and can subject dispersions to shear rates of several hundred thousand reciprocal seconds, the highest approximate rates of shear encountered in this study, as calculated by the method of Henderson *et al.* (21), were 17,800, 20,00, and 20,900 sec.$^{-1}$ for 3 emulsions of identical composition prepared using 20, 18, and 15-gauge capillaries, respectively, of 0.625 cm. length at 100 psig. The majority of emulsions, however, were prepared under approximate shear rate conditions of 10,000 sec.$^{-1}$. In attempting to extend the calculations of rheologic parameters based directly and rigorously on capillary tube viscometry and Newtonian liquids to the highly viscoelastic non-Newtonian systems encountered in this study, it is realized that considerable error is introduced by the assumptions made and by failure to include additional parameters to account for the effects of viscoelasticity. However, approximate calculations of the Reynolds number for representative emulsions in this study yielded values less than 100, indicating that under the conditions employed, velocity profiles could be assumed laminar, thus permitting reasonable approximations of the mean shear rates.

On the basis of the data presented, and as noted by other authors (22, 23), the use of capillary needles 5 cm. in length and of gauges larger than 18, *i.e.*, smaller diameter, actually will hinder effective emulsification by reducing the rate of shear which consequently reduces the work done on the dispersion. However, the lengthy process time reported by the majority of clinicians undoubtedly offsets the decreased dispersion efficiency resulting from lower rates of shear. Thus, where repository emulsions are prepared by means of 2 interconnected glass syringes, the use of a very short, large-diameter doubled-hubbed capillary (0.625 cm., 15 gauge) for a short interval will result in a greater work input and subsequent particle size reduction than can be achieved by the use of a long and smaller diameter capillary for extended periods of time.

Although the interconnected glass syringe method of emulsification is extensively employed for the extemporaneous preparation of small quantities of repository w/o emulsions, the recently developed Mulsi-Churn unit (Mulsi-Jet Inc., Elmhurst, Ill.) (24) for the preparation of these emulsions would appear to be more suitable due to the extremely high shear rate produced by jet velocities of 300 to 800 in./sec. which would tend to insure a maximum dispersion of the aqueous antigenic extract in a minimum of time. However, this device was not evaluated in this study.

SUMMARY AND CONCLUSIONS

1. An automated reciprocating capillary emulsator has been developed which duplicates the low-pressure capillary homogenization principle of the 2 interconnected glass syringe method for preparing repository w/o emulsions.

2. The evaluation of w/o emulsions by the use of flow curves obtained with a concentric cylinder viscometer is demonstrated. Rheological flow curves were found to be an effective method for indicating the extent of dispersion and degree of droplet coalescence in concentrated emulsions, which behave as general plastic solids.

3. Under low shear conditions, concentrations of mannide mono-oleate in excess of 10% are required to prepare and furnish adequate relative stability to water-in-mineral oil emulsions, although absolute stability is poor.

4. The rate of shear governs the degree of emulsification where the emulsion components are repeatedly forced through a double-hubbed hypodermic needle, and a short capillary of large diameter produced the finest dispersion.

5. For any given set of preparative conditions, a maximum degree of particle size reduction is achieved quite rapidly, and emulsator operation for extended time periods appears unnecessary.

6. For the preparation of w/o repository emulsions, high shear rate emulsators would appear to offer substantial advantages over the low shear rate interconnected glass syringe method.

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Water/Oil Emulsions Prepared by Low Pressure Capillary Homogenization II

Stabilizing Influence of Inorganic Electrolytes, Secondary Emulsifiers, and Temperature

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Aqueous dispersions in light mineral oil stabilized with mannide mono-oleate were prepared under a set of standard conditions and evaluated by rotational viscometry in conjunction with optical microscopy. Inorganic electrolytes in the aqueous internal phase, at concentrations as low as 0.01 *M*, increased apparent viscosity, retarded sedimentation, and had a marked stabilizing influence. The addition of small amounts of water-soluble surfactants to the internal phase yielded extremely fine dispersions, but these agents decreased stability and tended to cause inversion. Storage of w/o emulsions at 5° had a definite stabilizing influence when compared to room temperature storage.

IN AN earlier report (1), a reciprocating capillary emulsator was described which is similar in principle to an emulsator[1] that is in general use by physicians for the extemporaneous preparation of small quantities of repository antigenic w/o emulsions. However, the new emulsator produces a quantity of emulsion sufficient for experimental purposes and allows a much greater flexibility in controlling shearing stress. It was also shown that rheological flow curves furnish an adequate means for evaluating w/o emulsion stability, thus obviating the necessity for tedious size-frequency analyses. Fine dispersions of water-in-mineral oil were found to require high concentrations of mannide mono-oleate,[2] but the

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Previous paper: Fox, C. D., and Shangraw, R. F., *J. Pharm. Sci.*, **55**, 318(1966).
[1] Brown Emulsor, Andonian Associates, Inc., Waltham, Mass.
[2] Marketed as Arlacel A by Atlas Chemical Industries, Wilmington, Del.