

| Time, min      | Contraction, % |
|----------------|----------------|
| Up to 20 ..... | zero           |
| 40 .....       | 0.23           |
| 60 .....       | 0.40           |
| 130 .....      | 0.46           |
| 300 .....      | 0.49           |

After standing overnight at 25C, no contraction was evident and the texture had become sponge-like.

In comparison, samples of cocoa butter solidified after seeding with Form I maintained their good contraction and appearance on aging.

#### Practical Conditions for Maximum Contraction

In commercial operations with cocoa butter the maximum contraction is desired over a reasonably short period of time. With this in mind, the scale reading obtained in the experiments represented in Figures 4 and 5 were examined. The values at the end of 20 min were plotted against solidification temp. So that these values would be comparable, they were corrected to 20C to eliminate the vol change caused by the expansion or contraction of the solid cocoa butter between 20C and the temp of each experiment. The calculated results are plotted in Figure 7.

With the seeded cocoa butter, max volumetric contraction was found at or near 16C. Max volumetric contraction of unseeded cocoa butter was less and occurred at a lower temp.

It should be remembered, of course, that to maintain solidification at 16C in a large block of cocoa butter, an ambient temp below 16C must be used after the surface has solidified.

Good gloss is believed to be due to the presence of minute crystals. This gloss is maintained only if this minute crystal structure remains unchanged. For this reason, solidification procedures which produce minute crystals of the highest melting polymorph will also produce products which have good gloss. The conditions of max linear contraction from the mold are the same as those required for good gloss. This was confirmed by experiment.

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## Effect of Saline Electrolyte on Particle Sizes in Fat Emulsions by Electronic Counting<sup>1</sup>

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### Abstract

The number of particles of specific diams in the fat emulsions SR-151, Intralipid, and Lipofundin, by electronic counting, was critically dependent upon the elapsed time of contact of the emulsions with the diluting saline electrolyte. The most pronounced change in the number of particles as a function of time occurred with the particles of smallest diams, those less than 2 $\mu$ . Increases in the number of particles by approx 100% occurred within 20 min of mixing the emulsions and electrolyte. Apparently coalescence or aggregation of smaller particles caused the increases in numbers. By extrapolation to zero time, accurate counts were obtained.

### Introduction

FAT EMULSIONS which have been developed for intravenous nutrition consist of very fine dispersions of oil particles in aqueous media. Measurement of the diametric sizes of some of the dispersed oil particles in such emulsions by oil immersion light microscopy, while tedious, can be accomplished to provide an approximation of the gross range of particle sizes present. Such observations are very subjective, however, and depend to a great extent on the skill of the observer and the number of representative fields which are examined. If it is desired to determine the number of particles in incremental diametric size ranges as a basis for calculating size distribution approaching statistical significance, the task becomes formidable. Fischer and Harkins (5) made such an analysis of a hydrocarbon emulsion, and observed at least 1,000 particles to determine

the relation between diams of oil spheres and the percentage number of particles of the respective diams. King and Mukherje stopped the Brownian motion in soap-stabilized emulsions of olive oil and kerosene in water and used a projection microscope to observe particle distribution in a like number of particles (6). Pinter and Zilvermit employed a gradient centrifugation method to obtain the distribution of particles below 1 $\mu$  in diam in an "anhydrous" fat emulsion (10). The latter publication presents no information as to the distribution of particles of larger diam, although by oil immersion light microscopy particles of larger sizes are known to be present in similar fat emulsions (13).

An electronic instrument for determining the volume and number of particles in dispersions has recently become available (4), which provides size distribution data in a fraction of the time required by microscopy. There are a number of literature references to the theoretical aspects and reliability of this method (3, 14, 1, 15), and to the use of the instrument for determination of the distribution of particle sizes in a variety of liquid systems (8, 2, 7, 9, 12). As yet there have been no systematic studies of the use of the electronic counting method in determining the particle sizes in fat emulsions, nor of the possible physical effects of this method, particularly the required electrolyte, on the dispersed oil particles of a fat emulsion. This report presents the results of such an investigation.

### Experimental

*Emulsions.* Three oil/water emulsions were used. One of these, designated SR-151, contained 20 wt % of soybean oil, 1.0 wt % of purified egg lecithin, and an aqueous solution of 2.5% glycerol as the isotonic aqueous phase. This emulsion has been fully

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TABLE I

Effect of Saline Electrolyte on Percent Change in Number of Particles of Constant Diams as a Function of Time in Emulsions SR-151, Intralipid, and Lipofundin

| Time<br>sec | Change in Number of Particles |                 |                 |           |                 |                 |           |                 |                 |           |                 |                 |
|-------------|-------------------------------|-----------------|-----------------|-----------|-----------------|-----------------|-----------|-----------------|-----------------|-----------|-----------------|-----------------|
|             | 1.0 $\mu$                     |                 |                 | 1.2 $\mu$ |                 |                 | 1.6 $\mu$ |                 |                 | 2.0 $\mu$ |                 |                 |
|             | SR-151                        | Intra-<br>lipid | Lipo-<br>fundin | SR-151    | Intra-<br>lipid | Lipo-<br>fundin | SR-151    | Intra-<br>lipid | Lipo-<br>fundin | SR-151    | Intra-<br>lipid | Lipo-<br>fundin |
| 25          | 0                             | -0.1            | 0               | 0         | 0               | 3.5             | 0.1       | 0               | 0               | 0         | 0               | 4               |
| 50          | 0                             | -0.3            | 0.5             | 0         | 0.5             | 7.0             | 1.0       | 0               | 0               | 0.7       | 0               | 9               |
| 100         | 0                             | -1.5            | 3.4             | 0         | 1.3             | 14.0            | 4.4       | 0.8             | 0               | 2.7       | 0               | 16              |
| 200         | -0.3                          | -1.5            | 13.4            | 0.4       | 3.3             | 30.0            | 11.1      | 1.7             | 7.1             | 9.3       | 2.6             | 18              |
| 300         | -1.0                          | +6.2            | 25.9            | 0.7       | 7.4             | 52.5            | 27.8      | 2.9             | 14.2            | 17.3      | 3.1             | 17              |
| 400         | -1.4                          | 13.9            | 39.3            | 0.7       | 17.0            | 84.8            | 38.9      | 5.0             | 21.4            | 26.7      | 3.8             | 17              |
| 500         | -2.1                          | 22.4            | 53.1            | 1.1       | 28.5            | 136.0           | 61.1      | 7.1             | 28.6            | 37.3      | 6.2             | 18              |
| 600         | -2.9                          | 31.7            | 68.7            | 1.1       | 42.5            | 186.0           | 100.0     | 10.0            | 32.1            | 46.7      | 6.9             | 18              |
| 700         | -3.8                          | 41.3            | 83.7            | 1.4       | 55.5            | 239.0           | 188.9     | 12.5            | 60.7            | 61.3      | 11.3            | 19              |
| 800         | -4.7                          | 51.3            | 100.0           | 1.4       | 71.5            | 297.0           | 246.6     | 17.1            | 85.7            | 76.0      | 13.1            | 20              |
| 900         | -5.9                          | 62.2            | 115.0           | 1.8       | 87.0            | 354.8           | 268.8     | 21.7            | 114.3           | 100.0     | 17.5            | 20              |
| 1000        | -7.0                          | 72.5            | 130.3           | 1.8       |                 |                 | 288.9     | 27.5            | 157.1           | 124.0     | 19.4            |                 |
| 1100        | -8.5                          | 83.3            |                 | 2.1       |                 |                 | 304.0     | 33.3            |                 | 150.6     | 23.8            |                 |
| 1200        | -9.7                          |                 |                 | 2.1       |                 |                 | 327.8     | 40.0            |                 | 180.0     | 25.0            |                 |

described in a previous publication (16). Both of the other emulsions were commercial products, Intralipid (11), and Lipofundin (B. Braum, Melsungen). Intralipid contained 20 wt % of soybean oil, 1.2 wt % of egg phosphatide, and isotonic glycerol solution; Lipofundin contained 10 wt % of cottonseed oil, 1.5 wt % of a phosphatide emulsifier, and sorbitol solution as the isotonic aqueous phase.

**Electrolyte.** An aqueous solution of sodium chloride at 0.9% concentration was used as the electrolyte for all measurements and counts of particles in the emulsions. The saline solution was filtered through a porous glass funnel (ultrafine), and 0.1% formalin (37% formaldehyde) was added to prevent mold growth. All glassware used was rinsed with this solution. Corrections for background counts of the electrolyte and for "electronic noise" were made in all calculations of particle size and number in the emulsions. These corrections were never more than 1-2%, and usually were less.

The temp of the sample-electrolyte solutions was 24C in all determinations of particle size and count.

**Counter.** The instrument used for determination of particle size and number in the fat emulsions was a Coulter Counter, Industrial Model B. A 30 $\mu$  orifice was used, and the instrument was calibrated with mulberry pollen (average diam 12.6 $\mu$ ), and with standard latex (average diam 1.305 $\mu$ ).

**Procedure for Particle Counting.** The amount of 2 ml of well-mixed emulsion was diluted to 200 ml with distilled water, and designated as stock solution. A portion of the stock solution was then added to

saline electrolyte in an amount inversely proportional to the oil content of the original emulsion, so as to have equivalent volumes of oil in the fully diluted samples of the respective emulsions. Final dilutions were 1:50000 for SR-151 and Intralipid, and 1:25000 for Lipofundin.

As the stock solution of an emulsion was added to the electrolyte, timing was begun with a stopwatch. Elapsed time, from mixing of stock solution and electrolyte to the beginning of the count of particles, was noted. The measured volume of fully diluted emulsion in which particles were counted was 50 $\lambda$  for all samples.

In one set of experiments, the effect of elapsed time of contact of the emulsion with electrolyte on the number of particles of constant volume (i.e., diam) was determined. Repetitive counts of particles of 1, 1.2, 1.6, 2 and 3 $\mu$  diam were made, using a freshly diluted portion of emulsion for each diam size counted. The stated diams represent average values of increments of 0.2 $\mu$ . The initial count was made within 20-30 sec after addition of emulsion to electrolyte, and the elapsed time during which repetitive counts of each diam was made was 15-20 min. In another set, the effect of time of contact on the number of particles in the diametric size range of 1 $\mu$  to 7 $\mu$  was obtained on one portion of each of the emulsions, in a continuous stepwise manner with no overlapping of already-counted particles. Increments of particle size were 0.2 $\mu$  from 1-4 $\mu$ , and 1 $\mu$  for sizes above 4 $\mu$ . Elapsed time to the beginning of counting for each of the sizes was noted; counts then were cor-

TABLE II

Observed and Corrected Distribution of Particles from 1-7 $\mu$  in SR-151, Intralipid and Lipofundin

| Diam<br>$\mu$ | Observed and Corrected Distribution of Particles in Equivalent Vols of Emulsions |                     |         |            |                     |        |            |                     |        |
|---------------|--|---------------------|---------|------------|---------------------|--------|------------|---------------------|--------|
|               | SR-151   |                     |         | Intralipid |                     |        | Lipofundin |                     |        |
|               | Obs.   | Cor.<br>for<br>Time | Cor.    | Obs.       | Cor.<br>for<br>Time | Cor.   | Obs.       | Cor.<br>for<br>Time | Cor.   |
|               | n  | %                   | n       | n          | %                   | n      | n          | %                   | n      |
| 1.0           | 62,083   | -0.5                | 62,394  | 28,602     | -2.0                | 29,181 | 30,963     | 16.0                | 26,692 |
| 1.2           | 29,468   | 0.7                 | 29,263  | 2,606      | 4.9                 | 2,484  | 2,278      | 55                  | 1,470  |
| 1.4           | 11,065   | 35.0                | 8,196   | 260        | 12.0                | 232    | 141        | 59                  | 89     |
| 1.6           | 3,374  | 100                 | 1,687   | 167        | 9.0                 | 153    | 29         | 32                  | 22     |
| 1.8           | 1,163  | 141                 | 483     | 32         | 19.0                | 27     | 9          | 57                  | 6      |
| 2.0           | 287  | 110                 | 137     | 27         | 17.5                | 24     | 8          | 25.1                | 6      |
| 2.2           | 89   | 116                 | 41      | 10         | 8.0                 | 9      | 9          | 10.2                | 8      |
| 2.4           | 37   | 150                 | 15      | 7          | 5.0                 | 7      | 9          | 2.2                 | 9      |
| 2.6           | 21   | .....               | .....   | 3          | .....               | .....  | 2          | .....               | .....  |
| 2.8           | 7  | .....               | .....   | 2          | .....               | .....  | 1          | .....               | .....  |
| 3.0           | 5  | .....               | .....   | 3          | .....               | .....  | 3          | .....               | .....  |
| 3.2           | 5  | .....               | .....   | 2          | .....               | .....  | 2          | .....               | .....  |
| 3.4           | 3  | .....               | .....   | 1          | .....               | .....  | 5          | .....               | .....  |
| 3.6           | 1  | .....               | .....   | 1          | .....               | .....  | 2          | .....               | .....  |
| 3.8           | 1  | .....               | .....   | 1          | .....               | .....  | 1          | .....               | .....  |
| 4.0           | 1  | .....               | .....   | 4          | .....               | .....  | 2          | .....               | .....  |
| 4.3           | 1  | .....               | .....   | 2          | .....               | .....  | 3          | .....               | .....  |
| 5.0           | 3  | .....               | .....   | 1          | .....               | .....  | 5          | .....               | .....  |
| 6.0           | 2  | .....               | .....   | 2          | .....               | .....  | 1          | .....               | .....  |
| 7.0           | 0  | .....               | .....   | 1          | .....               | .....  | 0          | .....               | .....  |
| $\Sigma$      | 107,616  |                     | 102,266 | 31,734     |                     | 32,140 | 33,473     |                     | 28,329 |

rected for the time effect by extrapolation to zero time, using the data obtained with the first set of experiments.

**Microscopy.** The emulsions were examined with a light microscope, oil immersion lens, mag 950 $\times$ , equipped with a calibrated eyepiece scale.

### Results and Discussion

The percent change in the number of particles at constant diam were plotted as functions of the elapsed time during which the emulsions were in contact with electrolyte. From the curves, the change in number of particles in each emulsion at even multiples of time were obtained, and these values are given in Table I.

The number of 1 $\mu$  particles began to decrease within about 3 min of mixing SR-151 and electrolyte, and steadily decreased during the time period observed. Similarly, the number of 1 $\mu$  particles in Intralipid initially decreased for about 5 min after mixing, and thereafter the number increased. There was no decrease observed in the number of 1 $\mu$  particles in Lipofundin, nor in the number of particles of any of the other diametric sizes of the emulsions, except as already noted. Rather, the number of particles increased with increasing time.

With respect to SR-151 and Intralipid, apparently the 1 $\mu$  particles began to coalesce shortly after mixing the emulsions with electrolyte, and formed particles of larger diam, or aggregates. The coalescence or aggregation of smaller particles seems to offer a reasonable explanation for the increases which were observed in the number of particles greater than 1 $\mu$  in all of the emulsions. Since there was no decrease in the number of particles at any of the observed diam in Lipofundin, the obvious explanation is that Lipofundin contained particles which were much smaller in size than those in SR-151 and Intralipid, and these smaller particles, less than 1 $\mu$ , coalesced to form particles 1 $\mu$  and larger in diam.

The number of particles larger than 3 $\mu$  in diam remained constant for at least 20 min in the 3 emulsions.

The distribution of particle sizes in the range of 1 $\mu$  to 7 $\mu$  were determined for the 3 emulsions by the stepwise method described. Approximately 20 min were required to determine this distribution, and the number of particles counted at each of the several diams were then corrected to zero time by the percent change as a function of time noted in Table I. The diams given are the average values of the increments between successive diams. These results are given in Table II.

The results of Table II indicate that the observed cumulative count of particles in SR-151 and in Lipofundin were about 5% and 18%, respectively, greater than were actually present, whereas the observed cumulative count was 1% low for Intralipid.

The distribution of particles in the 3 emulsions as given in Table II was determined as rapidly as possible after addition of the emulsion to electrolyte. In another determination of distribution, the emulsions were added to electrolyte and allowed to stand for 30 min before the beginning of particle count. The distribution of particles in the range 1-2 $\mu$  was obtained, and from the number of particles at known diam, the volume of oil present in the form of particles at the stated diam was calculated. These results, for both corrected and noncorrected volumes, are given in Table III.

TABLE III

Volume of Oil as Particles of Stated Diams in Emulsions Diluted with Electrolyte and Standing 30 Min Before Counting

| Diam     | Vol of Oil as Particles in Equivalent Vols of Emulsions |          |            |          |            |          |
|----------|---|----------|------------|----------|------------|----------|
|          | SR-151  |          | Intralipid |          | Lipofundin |          |
|          | Vol   | Cor. Vol | Vol        | Cor. Vol | Vol        | Cor. Vol |
| $\mu$    | $\mu^3$   |          | $\mu^3$    |          | $\mu^3$    |          |
| 1.0      | 27,728  | 33,463   | 11,846     | 15,729   | 28,131     | 14,387   |
| 1.2      | 25,598  | 27,258   | 2,621      | 2,298    | 2,355      | 1,360    |
| 1.4      | 18,922  | 16,155   | 438        | 339      | 639        | 130      |
| 1.6      | 10,655  | 7,322    | 597        | 332      | 308        | 48       |
| 1.8      | 3,835   | 3,582    | 123        | 86       | 31         | 18       |
| 2.0      | 2,916   | 1,209    | 15         | 101      | 67         | 34       |
| $\Sigma$ | 89,654  | 88,989   | 15,640     | 18,885   | 31,531     | 15,977   |

From the results of Table III, it is evident that the largest volume of oil in the 3 emulsions was present in the form of particles of small diam. Since the effect of electrolyte is most evident with respect to the numbers of these smaller particles, failure to correct for the time involved may result in significant error in calculating the volume distribution of particles in fat emulsions.

To establish that the dilution of emulsions with distilled water, in preparing the stock solution, had no measurable effect on the number of oil particle sizes, SR-151 was added directly to electrolyte without dilution with water, and counts made as functions of time. The percent changes in the number of particles with increasing time were the same as those observed previously.

Each emulsion was examined by microscopy after diluting 1:5 with distilled water. The largest number of particles were 1 $\mu$  or less in diam, and no coalescence or aggregation was evident. Addition of saline resulted in greatly increased Brownian movement and, with time, agglomeration and some coalescence of the oil particles. Because of the rapid movement of the oil particles, no attempt was made to obtain a count of particles, nor accurate measurement of diametric sizes. However, by visual observation, there was no apparent gross change in particle size within several seconds following the addition of saline to the emulsion. These visual results substantiate the results obtained by the electronic method.

### Summary

The effect of saline electrolyte on the number of particles of specific diam in the emulsions SR-151, Intralipid, and Lipofundin was a variable, nonlinear function of time. To obtain correct electronic counts of the particles, it is necessary to know the elapsed time from dilution of the emulsion with electrolyte to the beginning of each of successive counting cycles. Extrapolation of observed count to zero time can then be used to obtain the correct count of particles, from which size distribution can be calculated in emulsions of the type proposed for intravenous nutrition.

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