# A comparative study of emulsions prepared by ultrasound and by a conventional method. Droplet size measurements by means of a Coulter Counter and microscopy

Kirsten Eberth and Jill Merry

*Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)*

To inspire confidence in pharmaceutical preparations of emulsions it is important that the droplet size distribution of the dispersed phase is known and alterations with time are predictable. Factors that are known to influence the mean droplet size and size distribution of the droplets include the method of emulsification, the type and concentration of stabilizing agents, the viscosity of the liquid phases in the emulsion and the volume fraction of the dispersed phase.

Pharmaceutical emulsions are normally prepared by mechanical stirrers, emulsifiers, homogenizers or colloid mills. Emulsions can also be prepared by ultrasound, but although the potential of ultrasonics as an emulsifying method was discovered as early as 1927 (Wood and Loomis), its use in today's pharmaceutical production of emulsions is limited. This is perhaps due to the insufficient knowledge of the droplet size distributions obtained, and due to the fact that ultrasonics may cause chemical degradation under certain circumstances.

The effect and mechanism of ultrasonic emulsification have been investigated by many authors with differing results, among the more significant papers are Neduzhii (1965), Li and Fogler (1978a and b) and Higgins and Skauen (1972). It is suggested (e.g. Chendke and Fogler, 1974) that under certain circumstances emulsions prepared by ultrasound are monodisperse systems. Factual information on droplet sizes in ultrasonically prepared emulsions in earlier work is sparse, probably due to insufficient methods of analysis. Emulsions investigated more recently have mostly been of little relevance to pharmaceutical applications; Li and Fogler (1978a and b) have made o/w emulsions of hydrocarbons at very low concentrations at about 75°C and obtained systems with very small ($< 0.3$ $\mu$m) and uniform oil drops. In contrast to these investigations, Weppelmann (1979) has found that o/w emulsions

with low oil concentration, prepared by ultrasound, have rather broad particle size distributions with mean values of about 2 μm. Further research on the effect of ultrasound in preparation of pharmaceutical emulsions is therefore appropriate.

In this work experiments were carried out to compare droplet size distributions produced using different emulsification methods on the same simplified model pharmaceutical emulsion. Emulsions were prepared by ultrasonics (20 kHz) or by a laboratory emulsifying machine, and were characterized by microscopic and Coulter Counter measurements.

The applied model o/w emulsion contained 20 g pristane (2,6,10,14-tetramethyl-pentadecane) (Aldrich-Europe), 2 g Tween 85 (Koch-Light Laboratories) and 178 g distilled water.

The water in the emulsions and the diluents used in analysis were filtered (Millipore filter size 0.22 μm). Electrolyte used in Coulter Counter measurements was 0.9% NaCl aqueous solution.

The emulsion components were premixed by gentle heating (~ 40°C), followed by shaking for 10 min. With this premixing the emulsions obtained a relatively wide distribution of droplet sizes and volume-weighted mean diameters of about 100 μm. The emulsions were then emulsified by either ultrasonics or with a traditional emulsifier.

Ultrasonic treatment (20 kHz) was given by a Branson Sonifier Cell Disrupter model B-30 (Branson Sonic Power, U.S.A.) at room temperature in the same rosette-shaped cell where it was possible to minimize temperature fluctuations to ± 1°C by circulating thermostatted cooling water. The sound probe was immersed at a constant depth and placed centrally in the emulsion, as the position of the probe effects emulsification reproducibility. Ultrasonic treatment was given in pulses lasting 0.5 s in 1 s periods (repeating frequency of 1 cycle/s). Emulsions were prepared at sound intensities recorded as power inputs, of 130 W, 175 W, 190 W and 200 W. The applied time lengths of the ultrasonic treatment were 2 and 10 min, corresponding to 1 and 5 min of constant treatment.

Emulsions prepared by traditional emulsification were made with a Diaf Emulsifying machine, type ET, Diaf A/S, Copenhagen. The mixture is passed through a set of rotating cogwheels and then forced through a needle valve that was adjusted to

TABLE I

THE VOLUME (IN PERCENT) OF THE DISPERSE PHASE (PRISTANE) REGISTERED BY THE COULTER COUNTER OF EMULSIONS (10% w/w PRISTANE, 1% w/w TWEEN 85 AND 89% w/w WATER) PREPARED WITH ULTRASONICS (20 kHz), WHERE EMULSIFICATION TIME AND SOUND INTENSITY HAVE BEEN VARIED *

| Emulsifica-tion time | Sound intensity | | | |
|---|---|---|---|---|
| | 130 W | 175 W | 190 W | 200 W |
| 1 min | – | 64 ± 10 | 41 ± 8 | 26 ± 2 |
| 5 min | 60 ± 4 | 16 ± 2 | 9 ± 1 | 7 ± 2 |

* The indicated error is the standard deviation of the mean.

give the smallest droplets possible. Each emulsion was treated 5 times.

Pre-investigations of the disperse phase were made by measurements with a projection-microscope (Visopan, Reichert, Wien, Austria). The emulsions were diluted 1 : 200 and measured in a haemocytometer (cell depth 0.1 mm).

A Coulter Counter model TA II (Coulter Electronics, U.K.) was used for the further analysis. An aperture size of 30 $\mu$m was chosen after pre-examination of the droplet sizes with the microscope. Samples were diluted in two steps, using water as diluent in the first step and electrolyte in the second. A computer program calculated the average droplet count per sample, corrected data for background contamination and calculated the volume of the dispersed phase per ml emulsion in each size class, the total volume of measured droplets per ml emulsion, the volume-weighted mean diameter and plotted histograms of the measured droplet size distributions. The algorithms used in the calculations are given in the Appendix. Emulsification with the Diaf emulsifying machine produced emulsion systems with volume-weighted droplet size distributions where Coulter Counter measurements indicated that close to 100% of the total volume of the disperse phase consisted of droplets between 0.6 and 13 $\mu$m.
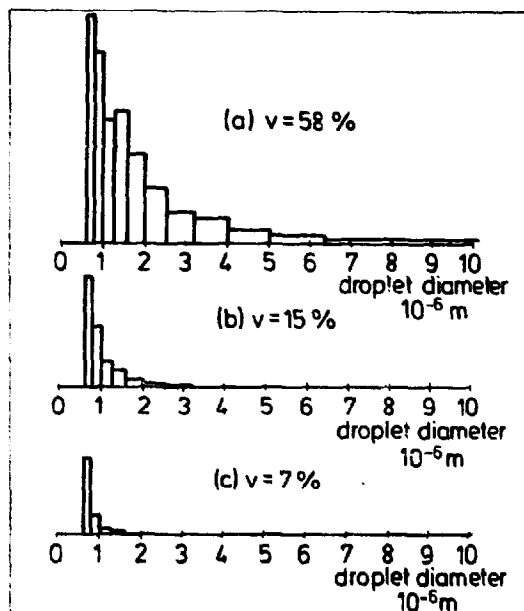
Fig. 2.

Fig. 1.

Fig. 1. The relationship between the droplet size distribution (as measured by the Coulter Counter) and the applied ultrasonic intensity during emulsification. The areas of each column are proportional to the volume of the disperse phase in each column. Ultrasonic treatment time 5 min. Applied sound intensities: (a) 130 W; (b) 175 W; and (c) 200 W. The volume fractions of pristane registered by the Coulter Counter are about 58%, 15% and 7%, respectively.
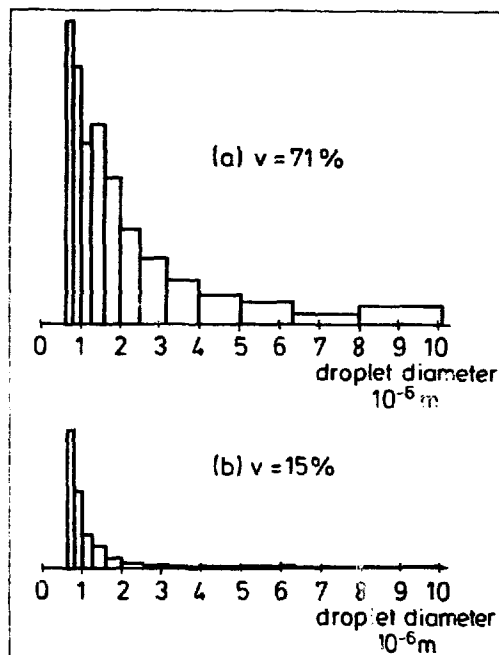
Fig. 2. The relationship between the droplet size distribution (as measured by the Coulter Counter) and ultrasonic emulsification time. The areas of each column are proportional to the volume of the disperse phase in each column. Applied ultrasonic intensity 175 W. Treatment time: (a) 1 min; and (b) 5 min. The volume fractions of pristane registered by the Coulter Counter are about 71% and 15%, respectively.

Emulsification by means of ultrasonics produced emulsions with volume-weighted size distributions wh re Coulter Counter measurements and microscope investigations indicated that only between about 7 and 70% of the volume of the disperse phase consisted of droplets with diameters between 0.6 and 13 $\mu$m (Table 1), with few or no oil droplets larger than 13 $\mu$m.

The Coulter Counter measurements are presented in Figs. 1 and 2. The histograms indicate that the non-registered fractions of the size distributions consist of droplets smaller than 0.6 $\mu$m. The Coulter Counter measurements show that an increase in the applied ultrasonic intensity (Fig. 1) and an increase in the applied ultrasonic treatment time (Fig. 2) both result in a decrease in the amount of the disperse phase registered by the Coulter Counter, probably due to an increase in the fraction of droplets smaller than 0.6 $\mu$m.

The Coulter Counter measurements of the ultrasonically prepared emulsions are complicated by the unknown and probably very large quantity of droplets with diameters smaller than 0.6 $\mu$m. This has in preliminary investigations shown to give rise to secondary coincidence phenomena, unless droplet counts per sample are kept at an extremely low level (for some systems below 10% of the maximum count that according to the manual corresponds to a coincidence error of 5%).

The measurements show that ultrasonic treatment with the applied experimental conditions did not produce monodisperse emulsion systems. Monodispersity is possibly obtained by ultrasound in other circumstances — studies are in progress to investigate this in further detail.

The work described in this paper is being supplemented by quasielastic light-scattering measurements to obtain further information of the droplet size distribution in the region below the Coulter Counter size range.

### Appendix

In this work droplet dispersities are compared by the volume-fractions of the disperse phase with droplet sizes within the applied size range. These values cannot be calculated from the volumes of the disperse phase in each size class presented by the Coulter Counter TA II as these, although exact, are volumes relative to an arbitrary chosen total volume.

Instead the volumes are calculated from the measured number of droplets in each size class per sample, an estimated mean droplet volume for each class, the dilution ratio and sample size.

In the Coulter Counter manual the arithmetic mean is used as an estimated mean droplet volume:

$$\bar{v}_j = \tfrac{1}{2}(v_j + v_{j+1})$$

$v_j$, $v_{j+1}$ and $\bar{v}_j$ denoting the lower and upper threshold droplet volumes and the mean droplet volume in the $j^{th}$ class, respectively.

In these experiments a simplified density function, f(v), was applied to obtain a better approximation than the arithmetic mean. f(v) is calculated by normalizing a linear function from $(v_j, n_j)$ to $(v_{j+1}, n_{j+1})$, $n_j$ denoting the number of particles in the $j^{th}$ size class; this calculation results in

$$f(v) = \frac{\left(n_{j+1} - n_j\right)\dfrac{v}{v_j} + 2n_j - n_{j+1}}{\dfrac{v_j}{2}\left(n_{j+1} + n_j\right)}$$

which by integration becomes

$$\bar{v}_j = \int_{v_j}^{2v_j} v\, f(v)dv = \frac{v_j\left(5n_{j+1} + 4n_j\right)}{3\left(n_j + n_{j+1}\right)}$$

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