International Journal of Pharmaceutics, 46 (1988) 177–178
Elsevier

IJP 01588

A transmission electron microscope method for determination of droplet size in parenteral fat emulsions using negative staining

J. du Plessis [1], L.R. Tiedt [2], A.F. Kotze [1], C.J. van Wyk [1] and C. Ackermann [1]

Departments of [1] Pharmaceutics and [2] Electronmicroscopy Unit, Potchefstroom University for CHE, Potchefstroom (South Africa)

(Received 29 March 1988)
(Accepted 12 April 1988)

Key words: Parenteral fat emulsion; Droplet size; Transmission electron microscope

Summary

Du Plessis et al. (1986) reported a microcapsule technique which was suitable for the determination of droplet size in parenteral fat emulsions (PFE). This technique involved the enclosure of the emulsion in small agar capsules which were used to prepare ultra thin sections of the PFE. Although well-defined droplets were obtained, this was a tedious and expensive method which took about 30 h before results could be obtained. The objective of this study was to find a more rapid and less expensive method by which particle size analysis could be performed.

This new method involved dilution (1 : 8) of the emulsion with distilled water. One milliliter of the diluted emulsion was added to 1 ml of 2% osmium tetroxide solution. This mixture was left standing for 15 minutes for fixation. A thin layer of the fixed emulsion was placed on a Formvar-coated 200-mesh copper grid. The excess fluid was removed with filter paper and left to air-dry. Three samples of a parenteral fat emulsion were prepared. The grids were examined with a Philips CM10 TEM and 3 micrographs of each sample were taken. Well-defined droplets were obtained.

A particle size analysis was done with a Zeiss particle size analyzer. The mean volume diameter for a parenteral fat emulsion was 250 nm. This

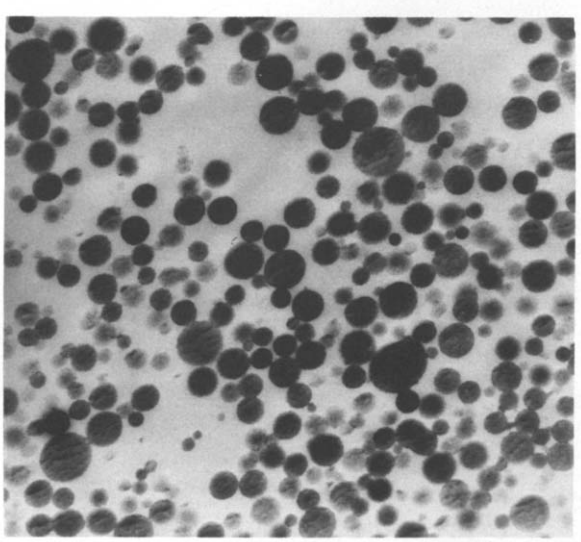

Fig. 1. Micrograph of a parenteral fat emulsion obtained with the microcapsule technique. The droplets are well defined. 16,000×.

Correspondence: J. du Plessis, Department of Pharmaceutics, Potchefstroom University for CHE, Potchefstroom, 2520 South Africa.

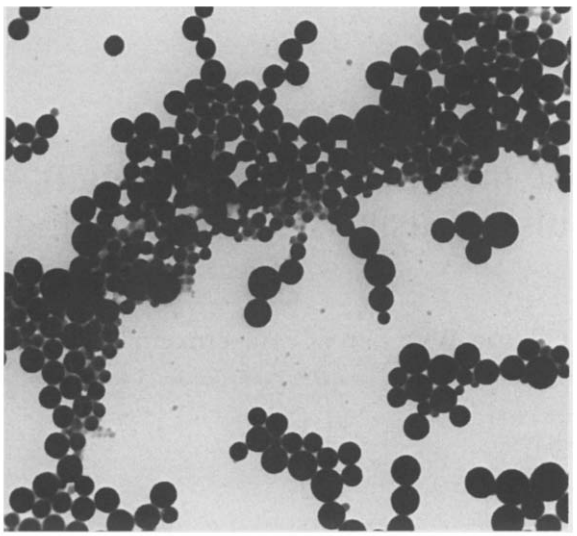

Fig. 2. Micrograph of a parenteral fat emulsion obtained with the negative staining technique. The droplets are well defined. 16,000×.

corresponds very well with the results obtained by the microcapsule technique and the particle size distribution of the emulsion reported in the literature as was shown by Du Plessis et al. (1986). The droplet size was thus not influenced by the sample preparation. A comparison of the results of the two methods are shown in the micrographs in Figs. 1 and 2.

This method has the following advantages to the microcapsule technique. There was a 4× reduction in cost. It took about 30 min to obtain results compared to the 30 h of the microcapsule technique (Du Plessis et al., 1986). It is possible to determine the actual diameter of the droplets because sectioning of samples is not necessary as in the case of the microcapsule technique. The possibility to get a lower mean volume diameter, because all the fat droplets are not cut through the centre of the droplets, is therefore minimised. More samples can be prepared to obtain more accurate results. A more clearly defined field is obtained making statistical selection of representative fields easier. This simplification and improvement of the TEM examination of PFE should rationalize research in this area significantly.

References

Du Plessis, J., Tiedt, L.R., Van Wyk, C.J. and Ackermann, C., A new transmission electron microscope method for the determination of particle size in parenteral fat emulsions. *Int. J. Pharm.*, 34 (1986) 173–174.