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A new transmission electron microscope method for the determination of particle size in parenteral fat emulsions

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Fat emulsions are used in parenteral nutrition in the prevention of essential fatty acid deficiency as well as a source of energy. Fat droplets larger than 6 μm given intravenously are known to cause adverse reactions, particularly emboli in the lungs (Geyer et al., 1951). An easy and quick method to determine the particle size of the fat droplets should therefore be of much value in the production and quality control of parenteral fat emulsions (PFE).

Various methods and apparatus for the determination of particle size in PFE are described (Davis, 1982) but they have certain disadvantages compared to transmission electron microscope (TEM) methods. None of these other methods can be used to determine the distribution of particles from 0.01 to larger than 20 μm as is found in unstable PFE. Dilution of the sample is done with electrolyte solution and water, respectively, when Coulter Counter methods and photon correlation spectroscopy are used (Burnham et al., 1983; Davis, 1982; Hardy et al., 1982). These procedures may cause instability of the emulsion or lead to methodological artifacts.

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Shoefl (1968) and Pamperl and Kleinberger (1982) reported TEM methods which could be used. These techniques, however, did not yield the same satisfactory results in our laboratories as have been reported. The method of Schoefl (1968) gave coalesced droplets while no droplets could be detected with the method of Pamperl and Kleinberger (1982). However, an adapted form of the microcapsule technique of Henstra and Schmidt (1974) proved to be applicable to PFE.

This technique involved the enclosure of the emulsion in small agar capsules and was used to prepare ultra-thin sections of the PFE. A thin glass rod (approximately 0.5 mm diameter) was dipped in a hot agar solution (3% in water). After removal the rod was covered with a thin layer of agar. This layer was allowed to solidify and the agar tube was cut with a razor blade (Fig. 1a). The glass rod was used as a piston to draw the emulsion into the tube (Fig. 1b). Care was taken that no air bubbles were trapped in the microcapsule. The lower end of the tube was dipped into a drop of the hot agar solution on a small spatula (Fig. 1c). With the open end of the tube hanging free over the edge of a microscope slide the other end was also closed (Fig. 1d).

The emulsion-filled agar capsules were placed in small stoppered bottles and fixed for 2 h in a

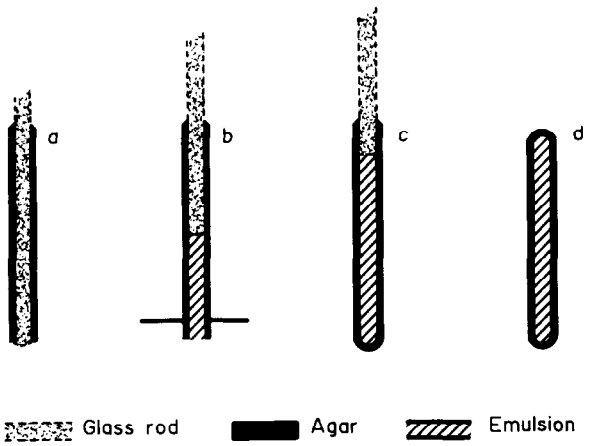

Fig. 1. The steps in the preparation, filling and closing of agar microcapsules.

2% osmium tetroxide solution. The capsules were dehydrated with mixtures of alcohol and water with increasing percentages of alcohol (70–100%). The capsules were embedded in Spurr's resin. The embedded capsules and their contents were clearly visible in the resin and trimming of the blocks was done in the usual manner. No special precautions or post-staining was necessary when the ultra-thin cuts were made with the ultramicrotome.

Well-defined droplets were obtained (Fig. 2).

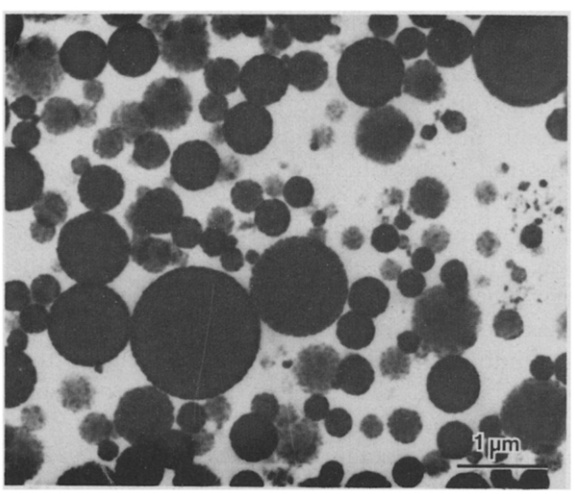

Fig. 2. Micrograph of a parenteral fat emulsion obtained with the microcapsule technique. The droplets are well defined (25,000×).

The particle size distribution of the fat droplets could be determined with a Zeiss particle size analyzer. The mean particle size for two PFE (Travamulsion 10% and Intralipid 10%) were 210 nm and 260 nm, respectively. This corresponds very well with the results of Burnham et al. (1983), Davis (1982) and Hardy et al. (1982), although their results were obtained with photon correlation spectroscopy, laser light scattering and photon correlation spectroscopy, respectively.

This method for the determination of drop size has the following advantages: no dilution or centrifugation which may influence the stability of the emulsion is performed. Therefore the size and form of the droplets are most probably not changed during analysis. Only small samples of emulsion and a short fixation time is required. Drop sizes of 0.01 µm to larger than 20 µm could be determined at once. This is a more rapid and accurate method than any other currently available TEM method.

References

Burnham, W.R., Hansrani, P.K., Knott, C.E., Cook, J.A. and Davis, S.S., Stability of a fat emulsion based intravenous feeding mixture. *Int. J. Pharm.*, 13 (1983) 9–22.

Davis, S.S., The stability of fat emulsions for intravenous administration. In *Advances in Clinical Nutrition — Proceedings of the 2nd International Symposium*, Mtp Press, Lancaster, 1982, pp. 213–239.

Geyer, R.P., Watkin, D.M., Matthews, L.W. and Store, J., Parenteral nutrition. XI. Studies with stable and unstable fat emulsions administered intravenously. *Proc. Exp. Biol.*, 77 (1951) 872–876.

Hardy, G., Cotter, R. and Dawe, R., The stability and comparative clearance of TPN mixtures with lipids. In *Advances in Clinical Nutrition — Proceedings of the 2nd International Symposium*, Mtp Press, Lancaster, 1982, pp. 241–260.

Henstra, S. and Schmidt, D.G., The microcapsule technique, an embedding procedure for the study of suspensions and emulsion. *Application Note for LKB — Produkter AB*, Sweden, (1974) 1–4.

Pamperl, H. and Kleinberger, G., Morphologic changes of Intralipid 20% liposomes in all-in-one solutions during prolonged storage. *Infusion Therapy*, 8 (1982) 86–91.

Schoefl, G.I., The ultrastructure of chylomicra and of the particles in an artificial fat emulsion. *Proc. R. Soc. London Ser. B*, 169 (1968) 147–152.