ESRF	**Experiment title:** Self−assembly of Triglyceride Nanocrystals in Aqueous Dispersions	**Experiment number**: SC−890
Beamline: BM26B	**Date of experiment**: from: 16−11−2001 to: 19−11−2001	**Date of report**: 05−04−2002
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Report:

Aqueous dispersions of triglyceride nanocrystals are under investigation as drug carrier systems for numerous therapeutic applications, such as intravenous administration of poorly water soluble drugs [1]. The development of such colloidal drug carriers requires detailed information on the ultrastructure of the systems, their formation and changes on storage. Some dispersions of tripalmitin and trimyristin, which contain strongly anisometric particles, tend to form discrete, stack−like self−assemblies of particles (stacked lamellae). These structures can be detected by SAXS due to a characteristic interference pattern resulting from the lamellar order of particles. Since the scattering intensity of the interferences is low, it is essential to use synchrotron radiation to perform those experiments. From the positions of the interference maxima the mean length of a repeat unit d (particle thickness plus interparticle distance) can be calculated. Typical values for tripalmitin dispersions are in the range of 30 nm < d < 60 nm. The relative intensity of the stack−related interferences (RDSF), on the other hand, describes the fraction of particles associated into stacked lamellae [2,3,4].

In the current project it was intended to investigate lamellar particle arrangement in tripalmitin and tristearin nanodispersions of different sample composition. Due to the broad s−range accessible with the BM26A instrument, it was expected to detect the 1st order and all higher orders of the stack−related interference.

For this study nanodispersions of tripalmitin and tristearin were produced in our laboratory by high−pressure homogenization of emulsions of the molten lipid and an aqueous stabilizer solution. Subsequent cooling to room temperature yielded nanosuspensions of platelet−like crystals with mean particle sizes of about 120−160 nm (PCS z−average). Some dispersions were diluted after preparation with a certain amount of the aqueous phase and some dispersions were concentrated by ultrafiltration.

The dispersions were transfered into a thermostated sample cell (25°C). For each sample SAXS patterns were recorded at two sample−detector distances of 2.5 and 5 meters, respectively, using a 2D detector. The data, which were collected in ten seperate time frames of 60 sec each, were averaged after checking sample stability. The background−corrected interference patterns were fitted to a combination of peak functions in order to seperate the overlaying scattering contributions of the triglyceride crystal structure and the lamellar particle arrangement (superstructure) [cf. Fig. 1].

Stack formation was found to occur only above a triglyceride concentration of about 4 % w/w. Above this value the intensity of stack formation increases continously when increasing the triglyceride content. This is valid not only for native dispersions, but also for samples obtained by dilution of more concentrated dispersions. When dispersions of 2 and 4 % w/w tripalmitin, which do not exhibit stack formation in the native state, were concentrated by ultrafiltration to a final lipid content of 10 % w/w, they do exhibit self−assembly. These observations clearly indicate a concentration−dependent thermodynamic equilibrium between stack formation and disintegration. Parallel to the increase of particle self−assembly also a decrease of the repeat unit *d* was observed.

With tripalmitin and tristearin dispersions of 15 and 20 % w/w lipid content, we found distinct deviation from rotation−symmetrical distribution of the scattering intensities [cf. Fig. 2]. This observation can only be explained by the formation of domains of parallely arranged platelets in the diameter range of the X−ray beam [some 100 µm]. This newly observed ordering phenomenon is currently under investigation.

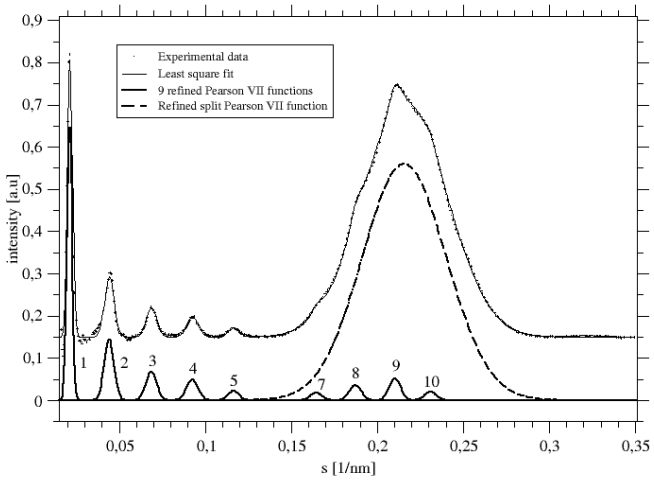

Fig. 1: SAXS pattern of a tristearin dispersion (10% w/w). The pattern was background corrected and fitted to a combination of Pearson VII functions representing the (001)−Bragg reflection of the triglyceride β−modification and the 1st to 10th order interference due to the lamellar particle arrangement. The experimental data and the least square fit are shifted vertically for better visualization.

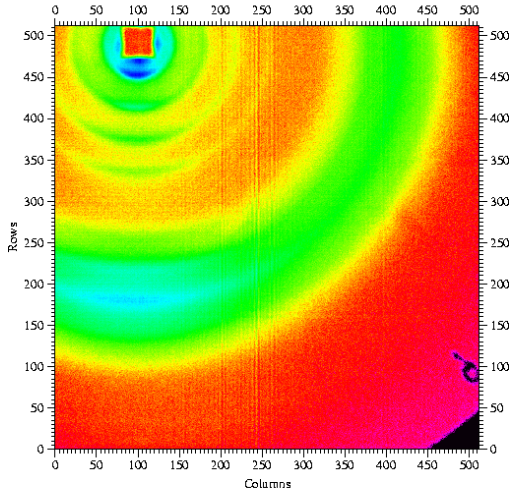

Fig. 2: 2D−detector image of a tristearin dispersion containing 15% w/w lipid matrix. The scattering intensities do not exhibit rotation−symmetrical distribution due to the texture phenomenon in the sample (cf. text).

Conclusions

The experimental results of the SAXS measurements indicate a dependency between the lipid concentration and the degree of stack formation in lipid nanosuspensions. The self−assembly phenomenon seems to represent a thermodynamic equilibrium. Triglyceride nanocrystals in high−concentrated dispersions form coherent domains of parallely aligned particles.

References

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