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Investigation on the flow behavior of dispersions of solid triglyceride nanoparticles

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Abstract

The flow behavior of low concentrated dispersions of solid lipid nanoparticles consisting of either trimyristin, tripalmitin or tristearin and different ionic and nonionic stabilizer blends were investigated using a rheometer with cone and plate apparatus and an Ubbelohde type capillary viscometer. The data demonstrate a remarkable influence of the matrix material, the stabilizer composition and the presence of small amounts of sodium chloride on the formulations' rheological properties. A significant increase in dispersion viscosity was found in the triglyceride sequence trimyristin < tripalmitin < tristearin. This effect can be clearly attributed to an increase in particle shape anisometry with increasing length of the lipid's fatty acid chains. Surfactants, which are present during the crystallization of the dispersed lipid seem to have an additional effect on the nanoparticle shape. Beyond this, ionic surfactants and other salts affect the dispersion viscosity by altering the dimension of the electrical double layer ("second electroviscous effect").

Keywords: Solid lipid nanoparticles; Rheology; Particle shape; Second electroviscous effect

1. Introduction

Aqueous dispersions of crystalline lipid nanoparticles (solid lipid nanoparticles, SLN) have gained much attention in the last years as novel carrier systems for the administration of drugs, e.g., via the parenteral, dermal or peroral route (Yang et al., 1999; Müller et al., 2000; Westesen, 2000).

As for other disperse liquid and semisolid systems, the rheological properties of lipid nanosuspensions influence their potential for pharmaceutical applications in a fundamental way. Dermal administration forms should exhibit comparatively high viscosity and a plastic or thixotropic flow behavior to enable sticking onto the skin for a sufficient time, such as ointments or creams do. Parenteral administration forms, on

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the other hand, usually should exhibit low viscosity to enable easy withdrawal from the container (high syringeability) and easy and painless injection (high injectability) even through narrow needles. Thus, it is important for the development of such administration forms to investigate the influence of formulation parameters on the systems' rheology.

So far only limited data on the rheological characteristics of lipid nanosuspensions are available, focusing especially on formulations with high lipid content (>20% m/m) for topical application. Such investigations revealed that the lipid concentration and the particle size basically determine the rheological properties of dispersions of solid lipid nanoparticles (Lippacher et al., 2000, 2002). Increasing the amount of dispersed lipid was found to shift the flow properties from simple plastic flow characteristics to thixotropy, which makes such high concentrated systems interesting for dermal application.

Here we present experimental investigations dealing with low concentrated triglyceride nanosuspensions (triglyceride content $\leq$20% m/m). Their rheological properties were investigated in dependence on the kind and concentration of the matrix lipid, suspension stabilizers and added electrolytes. An attempt was made to explain the observed differences in flow behavior on a physical base. Furthermore, we demonstrate how rheological methods can be employed for estimating the shape (anisometry) of dispersed colloidal drug carriers.

2. Materials and methods

2.1. Sample preparation

Different triglyceride nanosuspensions were produced by melt homogenization. The triglyceride (Dynasan 114: trimyristin, Dynasan 116: tripalmitin or Dynasan 118: tristearin, Condea, D-Witten) and phosphatidylcholine-rich soybean phospholipid (Lipoid S100, Lipoid, D-Ludwigshafen) were melted at a temperature ~10 K above the triglyceride melting point. A solution of sodium glycocholate (Sigma, D-Steinheim), cetylpyridinium chloride (Caesar & Loretz, D-Hilden) or poloxamer 188 (Lutrol F68, BASF, D-Ludwigshafen) in pure water (specific resistance: ~18 MΩ cm) containing 2.25% m/m glycerol was heated to the same temperature. After mixing both liquids using a high-speed stirring device (Ultra-Turrax, Jahnke & Kunkel, D-Staufen) the crude emulsion was passed through a heated (T = 85 °C) high-pressure homogenizer (Micron Lab 40, APV Gaulin, D-Lübeck) for four times at 1200 bar and once at 1500 bar. Finally, the emulsions were allowed to cool down to room temperature, filled into glass vials and stored at temperatures of about 5–8 °C. Under these conditions all formulations transform into dispersions of crystalline particles (Bunjes et al., 1996). If not indicated otherwise, all samples contain the ingredients in a fixed mass ratio triglyceride:phospholipid:co-stabilizer of 100:24:6.

2.2. Determination of particle size

Particle size (z-average) and polydispersity index (PI) of each cold stored formulation were determined within 1 day after preparation using photon correlation spectroscopy (PCS, Zeta Plus, Brookhaven Instruments, Holtsville, USA). Dispersions were analyzed after appropriate dilution with dust-free water and particle sizes were calculated applying the cumulant method. Since this algorithm is based on the assumption of spherical particles, the z-average values do not represent a real dimension of the platelet-like particles (Westesen and Siekmann, 1997), but characterize the dispersions by a mass proportional value averaged over all particles.

Typically, the z-averages of the investigated formulations are in the narrow range between 130 and 170 nm, polydispersity indices are between 0.14 and 0.19.

Additionally, particle sizes were also determined by laser diffraction in combination with PIDS technology (Polarization Intensity Differential Scattering, LS-230, Beckman-Coulter, D-Krefeld). This method is especially useful for the detection of particles in the micrometer range. Results are calculated according to the Mie theory (refractive index of water: 1.33 and of particles: 1.45), and they are given as LD95% and LD99% values representing the mass weighted particle size not exceeded by 95 and 99% of all particles.

2.3. Rheological measurements

Rheological measurements were performed at 25 ± 0.1 °C using a Bohlin rheometer CVO (Bohlin Instruments Germany, D-Mühlacker). The instrument was

equipped with a cone/plate apparatus 40 mm/4°. For each sample continuous variation of the shear rate γ (0–150 s^{-1}) was applied and the resulting shear stress σ was measured. Viscosities η of dispersions with Newtonian flow properties were calculated according to the relation $\eta = \sigma/\gamma$. Apparent viscosities of dispersions with non-Newtonian flow properties are calculated as the average of all viscosity values measured at shear rates γ between 30 and 80 s^{-1}. For the measuring system the lower limit for accurate viscosity determination was about 6 mPa s (manufacturer's information). Dispersions with lower viscosity and proven Newtonian flow behavior were additionally investigated at 25 ± 0.1 °C using an Ubbelohde type capillary viscometer (0.63 mm diameter, Schott Geräte, D-Hofheim a. Ts.).

2.4. Electron microscopy

Electron micrographs were taken using a CEM 902A transmission electron microscope (Zeiss, D-Oberkochen) operating at 80 kV. Samples were frozen as thin films in liquid propane (Jet Freezer JFD 030, BAL-TEC, FL-Liechtenstein), freeze fractured at 173 K and 5×10^{-6} mbar (BAF400, Bal-Tec, FL-Liechtenstein) and shadowed with platinum/carbon at 45°. The replicas were mechanically stabilized by vertical deposition of pure carbon.

3. Results and discussion

3.1. Effect of matrix lipid

For the investigation the three saturated, monoacid triglycerides trimyristin (TM), tripalmitin (TP) and tristearin (TS) were used as matrix materials and a combination of phospholipid and sodium glycocholate was used as dispersion stabilizer. The viscosity of TM nanosuspensions increases with the lipid content in the concentration range of 2–20% m/m TM. Within the investigated shear range dispersions of up to 12% m/m TM exhibit Newtonian flow behavior, whereas higher lipid concentrations cause a shift to plastic flow behavior with a lower flow limit (yield stress). In principle, the same flow characteristics were also observed in TP and TS formulations. In these cases, Newtonian flow properties were found up to 10% m/m TP and 8% m/m TS, respectively. At a concentration of 20% m/m TP or 18% m/m TS the ascending and descending shear curves do not overlap anymore indicating thixotropic behavior.

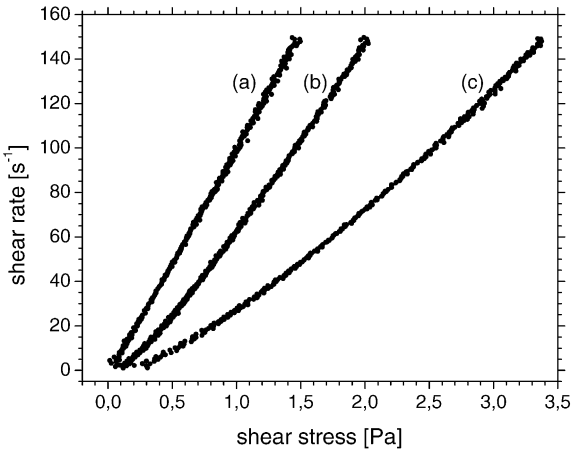

Fig. 1. Flow curves of nanodispersions containing 12% m/m triglyceride each: (a) trimyristin, (b) tripalmitin, (c) tristearin. The up- and down-curves exactly overlap within the instrument's resolution.

When comparing formulations with equivalent lipid content, but differing in the matrix lipid, in any case an increase of viscosity in the sequence TM < TP < TS was observed (Fig. 1). For dispersions with 20% m/m triglyceride these viscosity differences are also macroscopically visible: the TM sample easily flows under the influence of gravity, the TP sample flows very slowly and the TS sample does not flow at all.

The increase of dispersion viscosity with increasing lipid content, in the general form, is predicted by the following equation (Krieger, 1972):

$$\eta = \eta_0 \left(1 - \frac{\varphi}{p}\right)^{-[\eta]p} \quad (1)$$

where η is the viscosity of the dispersion, η_0 the viscosity of the dispersion medium, $[\eta]$ the intrinsic viscosity, φ the volume fraction of the disperse phase, p the volume fraction of disperse phase at most dense packing, e.g. 0.74 for spheres or $\pi/4$ for cylinders and can be explained by gradual increasing mechanical interparticle interactions until a final locking of the dispersed particles into a rigid structure occurs when φ approaches p. Electron micrographs of some selected TP formulations give an impression on the different, concentration-dependent packing densities of platelet-shaped nanocrystals in dispersions (Fig. 2).

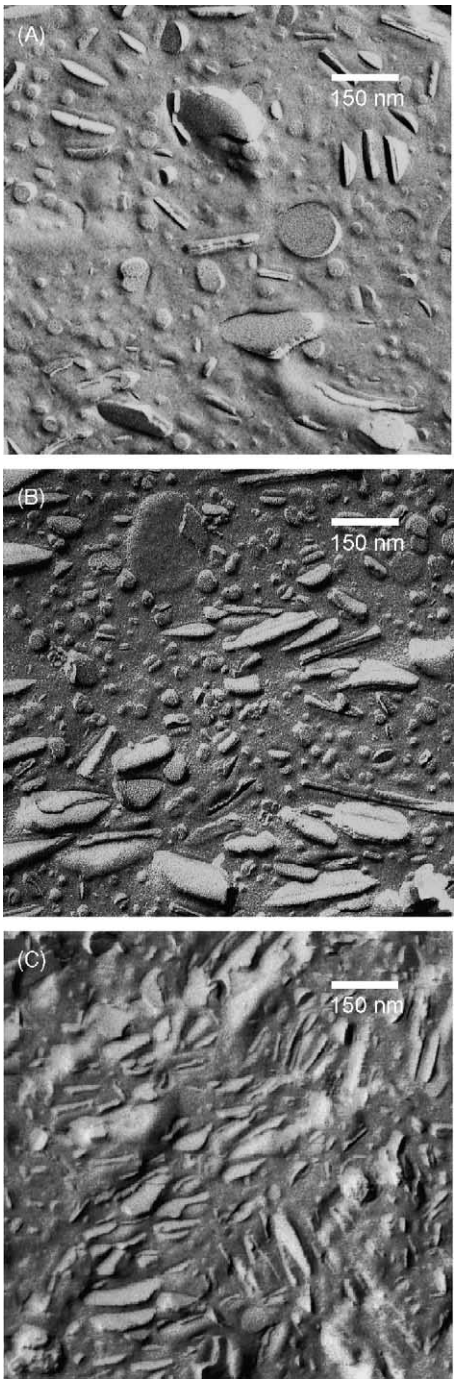

Fig. 2. Transmission electron micrographs of freeze fractured tripalmitin dispersions with (A) 8% m/m TP, (B) 10% m/m TP and (C) 20% m/m TP.

Parameters p and $[\eta]$ strongly depend on the shape of the dispersed particles. Since cold-stored nanocrystals of saturated, monoacid triglycerides generally exhibit a platelet-like shape (Siekmann and Westesen, 1992; Westesen and Siekmann, 1997), p can be assumed to have a constant value of approximately $\pi/4$ (value for all flat cylindrical particles). The intrinsic viscosity $[\eta]$ equals to 5/2 in the case of perfectly spherical particles and becomes larger with increasing particle anisometry, i.e. with increasing ratio between diameter D and thickness h (Everett, 1992).

Therefore, the observed increase of viscosity η of equally concentrated dispersions in the matrix lipid sequence TM < TP < TS can only be due to an increase in $[\eta]$ and, hence, it is a clear indication for a successively increasing anisometry of nanocrystal shape. This result is in good agreement with earlier X-ray studies showing a decrease of the average nanocrystals thickness and a simultaneous increase in particle size (PCS z-average) in the triglyceride sequence trilaurin < trimyristin < tripalmitin (Unruh et al., 2002).

3.2. Effect of dispersion stabilizer

Dispersions of 8–15% m/m tripalmitin and a phospholipid/cetylpyridinium chloride stabilizer combination are more viscous than corresponding formulations containing the same stabilizer concentration, but a phospholipid/sodium glycocholate stabilizer blend (Fig. 3). Whereas the transition from Newtonian to plastic flow behavior for bile salt stabilized dispersions occurs at about 10% m/m TP, this point is lowered to about 8% m/m in the case of cetylpyridinium chloride stabilized formulations.

Increasing concentrations of sodium glycocholate (0.2–1.5% m/m) in combination with constant amounts of phospholipid distinctly decrease the viscosities of 10% m/m tripalmitin formulations as displayed in Fig. 4.

These observed viscosity differences are most likely due to one of the following reasons or a combination thereof:

(1) The co-stabilizer induces differences of the nanocrystal shapes overlaying the influence of the matrix lipid. During recrystallization from the melt the developing triglyceride β-polymorph crystals preferentially grow at the (1 1 3) and (2 1 3) faces

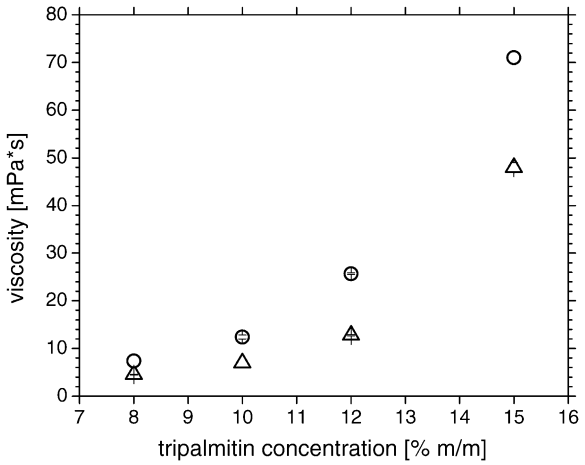

Fig. 3. Viscosities and apparent viscosities of tripalmitin nanodispersions stabilized with either phospholipid/sodium glycocholate (Δ) or phospholipid/cetylpyridinium chloride (◯). Each point represents the mean ± S.D. (n = 2–3). Error bars are usually smaller than the symbols.

due to kinetic effects (Skoda and van den Temple, 1967). This process leads to the platelet-like crystal habit with extremely large (0 0 1) faces. The newly created particle surfaces become covered with phospholipid and co-stabilizer molecules within short time. Phospholipid molecules seem to adsorb on or even merge with the (0 0 1) faces, whereas smaller co-surfactant molecules, like glycocholate,

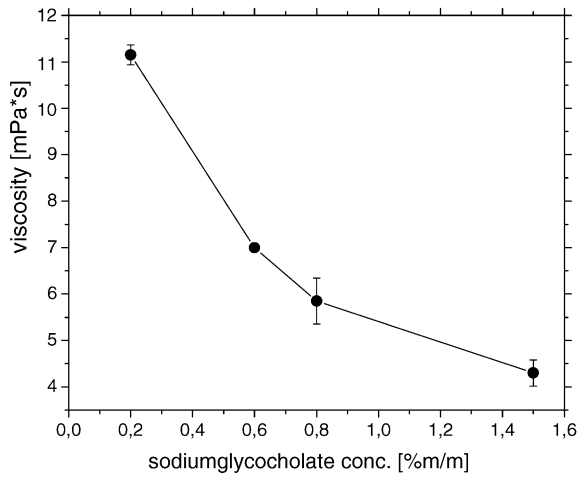

Fig. 4. Viscosities of 10% m/m tripalmitin dispersions expressed as a function of sodium glycocholate concentration. Each point represents the mean ± S.D. (n = 2–3).

preferably seem to cover the smaller lateral faces (Siekmann, 1994; Westesen and Siekmann, 1997). Thus, high concentrations of glycocholate, that lead to a rapid and effective stabilization of the lateral crystal faces against further growth, cause small aspect ratios D/h and, consequently, small $[\eta]$ values. In this context, the observation of higher viscosities with cetylpyridinium stabilized dispersions implies that cetylpyridinium ions have a significantly lower affinity to the lateral crystal faces than glycocholate ions and cause higher D/h ratios. The difference might be attributed to structural similarities (long chains of CH_2 units) between fatty acid residues of triglycerides and cetylpyridinium ions and the following incorporation of cetylpyridinium into the nanoparticles' crystal lattice structure. Due to crystallographic considerations, incorporation of cetylpyridinium into the triglyceride lattice structure is, however, only possible when the polar head groups are present at the crystallographic (0 0 1) faces, but not at the lateral (1 1 3) and (2 1 3) faces.

(2) The stabilizer influences the size of the electrical double layer. Dispersed particles in an electrolyte solution are surrounded by a double layer of adsorbed ions and solvent molecules (Hunter, 1986). Part of this double layer is tightly bound to the particles and, therefore, increases the effective volume fraction of disperse phase φ in Eq. (1) (second electroviscous effect; Krieger, 1972; Everett, 1992; Ogawa et al., 1997). In the investigated triglyceride systems the dimension of the electrical double layer mainly depends on the concentration of co-surfactant molecules and, hence, the co-surfactant concentration will have a strong influence on dispersion viscosity.

3.3. *Effect of added electrolytes*

After a few days of storage sodium glycocholate and cetylpyridinium chloride stabilized tripalmitin formulations (8–20% m/m TP) were mixed with concentrate solutions of NaCl (0.0035, 0.035, 0.35, 3.5 mol kg^{-1}) leading to dispersions with final salt contents of either 10^{-4}, 10^{-3}, 10^{-2} or 10^{-1} mol kg^{-1}. Dispersions containing 10^{-1} mol kg^{-1} salt exhibit macroscopic flocculates or formation of a soft, cream-like gel within 1 day. At lower salt concentrations, however, the samples

Table 1
Particle sizes of phospholipid/glycocholate stabilized nanodispersions containing 10% m/m tripalmitin after addition of sodium chloride

NaCl content (mol kg^{-1})	Day of preparation, *z*-average/PI	After 3 months, *z*-average/PI	After 12 months, *z*-average/PI	After 12 months, LD95%/LD99%
10^{-4}	153 nm/0.15	154 nm/0.17	149 nm/0.17	243/848 nm
10^{-3}	152 nm/0.16	152 nm/0.17	154 nm/0.18	239/852 nm
10^{-2}	152 nm/0.16	151 nm/0.17	151 nm/0.18	242/706 nm
10^{-1}	Not determined, flocculated	Not determined, flocculated	Not determined, flocculated	Not determined, flocculated

Measurements were performed immediately, 3 months and 12 months after preparation.

were physically stable. No significant particle growth was detected even within 1 year of storage (Table 1). The viscosities of the stable formulations were found to decrease with increasing electrolyte concentration having the most pronounced effect at high lipid contents (Fig. 5).

Addition of NaCl to a poloxamer stabilized dispersion (10% TP, 3.0% S100, 4.5% poloxamer) also leads to a slight reduction of viscosity. The dependence on the salt concentration is, however, significantly lower than for dispersions with the ionic stabilizers (Fig. 6).

The observed viscosity effect in dispersions with ionic stabilizers is probably due to a shrinking of the particles' electrical double layer by the influence of added electrolytes and corresponds to the reduction of the particles' effective volume fraction φ (Brodnyan and Kelley, 1964; Hunter, 1986; Everett, 1992). At salt concentrations around 10^{-1} mol kg^{-1} the double layer is reduced to such an extent that flocculation or gelation occurs. In poloxamer stabilized dispersions, however, the surface charges are low in any case and, therefore, the ionic strength of the dispersion medium will not influence the particles' effective volume fraction in a significant way.

3.4. Determination of particle-shape parameters

Nanocrystals of saturated, monoacid triglycerides generally exhibit a platelet-like shape (Siekmann and Westesen, 1992; Westesen and Siekmann, 1997). The

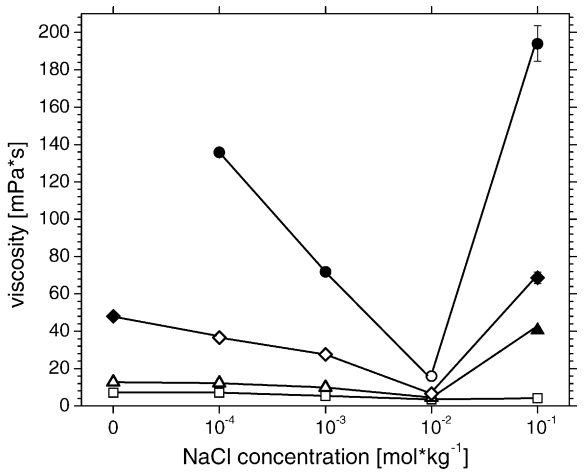

Fig. 5. Viscosities and apparent viscosities of phospholipid/glycocholate stabilized tripalmitin dispersions expressed as a function of NaCl concentration: (□) 10% m/m TP, (Δ) 12% m/m TP, (◇) 15% m/m TP, (○) 20% m/m TP. Open symbols indicate Newtonian flow properties, filled symbols indicate non-Newtonian flow properties. Each point represents the mean ± S.D. (n = 2–3). Error bars are usually smaller than the symbols.

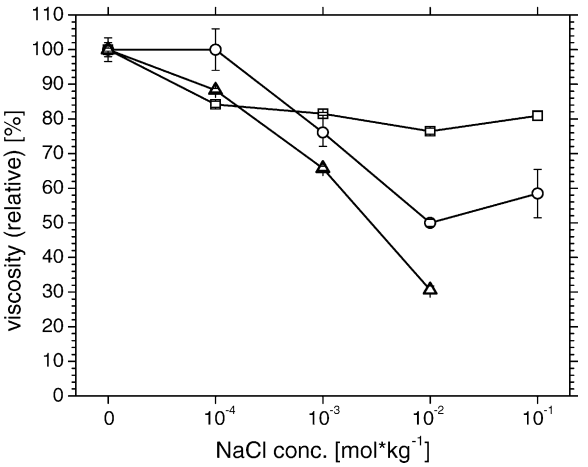

Fig. 6. Relative viscosity values of 10% m/m tripalmitin dispersions expressed as a function of NaCl concentration. The formulations were stabilized with three different surfactant blends: (□) 3% S100, 4.5% poloxamer; (○) 2.4% S100, 0.6% sodium glycocholate; (Δ) 2.4% S100, 0.6% cetylpyridinium chloride. Viscosity values of each of the electrolyte-free dispersions were set to a relative viscosity value of 100%. Each point represents the mean ± S.D. (n = 2–3).

accurate determination of particle-shape parameters, such as diameter, D and, especially, thickness, h, however, is a challenging task, due to the irregular particle shape and the fairly broad size distribution of both D and h. Electron micrographs of solid lipid nanoparticles only display a small fraction of all particles making size determination questionable from a statistical point of view. Moreover, it has to be considered that micrographs usually show particles in a more or less tilted position, which even further complicates accurate measurements of the particles' dimensions (Fig. 2).

Recently, an X-ray scattering method has been established to determine the thickness of triglyceride nanocrystals perpendicular to the (0 0 1) faces, which yields average values of h = 15 nm for TP (at PCS z-average of 140 nm) and h = 18 nm for TM (at PCS z-average of 125 nm) (Unruh et al., 1999, 2001, 2002). Combining electron microscopy and X-ray scattering, one can only roughly estimate a mean D/h ratio for TM and TP particles of about 10. However, the statistical significance of this result is limited due to the dependence on the electron microscopy data.

Applying Eq. (1), it should be possible to estimate the aspect ratio D/h of disperse nanoparticles directly from rheological data which would have the advantage of averaging the D/h value over a huge number of individual particles and, hence, giving the results a higher statistic significance. For testing this method glycocholate stabilized dispersions of TM, TP and TS (20% m/m triglyceride) were prepared. From each of the three triglyceride samples a series of aqueous dilutions containing final triglyceride concentrations between 2 and 12% m/m was prepared. This dilution procedure should guarantee the presence of a fraction of exactly the same population of particles within each of the diluted samples. Parameters $[\eta]$ for the TM, TP and TS particles, respectively, were estimated by least square fits of Eq. (1) to the experimental viscosity data of each series of dilutions with Newtonian or almost Newtonian flow properties (Fig. 7). For these calculations the mass fraction of disperse phase (consisting of triglyceride and lecithin) has been converted into volume fraction φ assuming a density of $\sim$1000 kg m^{-3} (van Langevelde et al., 1999). The aspect ratio D/h was then calculated assuming the simple particle shape model of a flat rotational ellipsoid. The intrinsic viscosity $[\eta]$ of platelet-like rotational ellipsoids under weak shear conditions ($\gamma/D_r \ll 1$, γ is the shear rate, D_r the rotational diffusion coefficient) is given by the relation (Hinch and Leal, 1972):

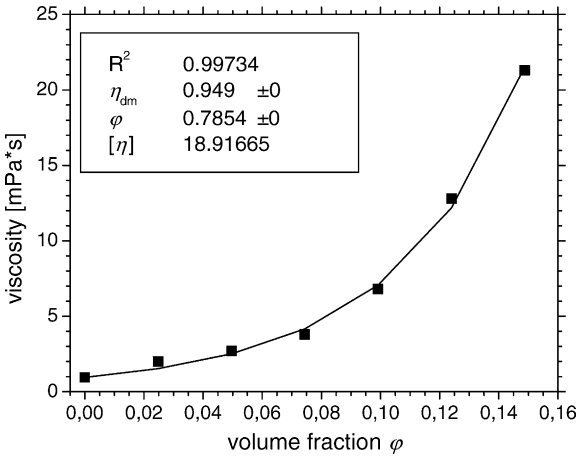

Fig. 7. Example for the approximation of the intrinsic viscosity. Experimental viscosity data (■) of different dilution steps of one single phospholipid/sodium glycocholate stabilized tristearin dispersion (20% m/m) were fitted to Eq. (1). The line represents the best-fit result.

$$[\eta] = \frac{32}{15\pi}\frac{D}{h} \tag{2}$$

where h is the platelet thickness and D the platelet diameter. The obtained aspect ratios D/h, however, are by a factor 2 larger than expected from electron microscopy and X-ray scattering (Table 2). These differences are most likely to result from the perturbing effect of the particles' electrical double layer on viscosity as described in Sections 3.2 and 3.3. Therefore, the experiments were repeated after the dispersions had been adjusted to a NaCl concentration of 10^{-2} mol kg^{-1}. As discussed above, this salt concentration reduces the particles' electric double layer as much as possible without causing dispersion instability. The D/h ratios determined under such altered experimental conditions exhibit much better conformity to the combined results of electron microscopy and X-ray scattering experiments (Table 2). These experimental results strongly corroborate the observation of an increase in particle anisometry within the triglyceride sequence TM < TP < TS. Furthermore, the D/h value of a cetylpyridinium chloride stabilized TP formulation additionally given in Table 2 confirms the assumption of an increase in particle anisometry within the stabilizer sequence

Table 2
Aspect ratios of triglyceride nanocrystals, determined in dispersions without electrolyte and after addition of 10^{-2} mol kg^{-1} NaCl

	Trimyristin/GC	Tripalmitin/GC	Tripalmitin/CP	Tristearin/GC
D/*h* ratio (electrolyte-free)	20.6	21.4	Not determined	27.8
D/*h* ratio (10^{-2} mol kg^{-1} NaCl)	10.6	12.3	14.0	13.4

Stabilizer combinations: GC – phospholipid/sodium glycocholate; CP – phospholipid/cetylpyridinium chloride.

sodium glycocholate < cetylpyridinium chloride. However, it has to be considered that the dispersions containing 10^{-2} mol kg^{-1} salt are still sufficiently stable and, therefore, at least a thin electrical double layer remains onto the particle surface. This will, of course, slightly influence the determined particle shape. Moreover, the dispersions usually contain also small fractions of vesicles and micelles consisting of phospholipids and co-stabilizers (Westesen and Siekmann, 1997), which have been neglected in the calculations. Because of all these simplifications made within the model, the calculated particle-shape parameters also should be regarded as approximations only.

4. Conclusions

Rheological properties of low-concentrated dispersions of solid lipid nanoparticles are, among other formulation parameters, significantly influenced by the kind of matrix lipid and the kind of dispersion stabilizer. Both ingredients seem to determine the dispersion viscosity via the shape of the dispersed particles. Increasing lengths of fatty acid residues generally lead to more anisometric triglyceride crystals and, hence, to higher dispersion viscosities. The hydrophilic co-stabilizer is able to modify this basic crystal shape in dependence on its chemical structure and concentration. Moreover, adsorbed surfactants can increase the dispersion viscosity by their contribution to the volume fraction of dispersed phase. Nanoparticle dispersions, which are stabilized by electrical charge, are sensitive to the addition of electrolytes. Whereas high salt concentrations induce an increase in viscosity and formation of gels, low salt concentrations, in contrast, reduce the dispersion viscosity. These investigations may help to develop formulations of solid lipid nanoparticles, which are optimized with respect to the desired rheological properties.

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