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Citation: *The Journal of Chemical Physics* **140**, 214905 (2014); doi: 10.1063/1.4880723

View online: <http://dx.doi.org/10.1063/1.4880723>

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Formation of liquid crystalline phases in aqueous suspensions of platelet-like tripalmitin nanoparticles

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(Received 6 March 2014; accepted 19 May 2014; published online 6 June 2014)

Suspensions of platelet-like shaped tripalmitin nanocrystals stabilized by the pure lecithin DLPC and the lecithin blend S100, respectively, have been studied by small-angle x-ray scattering (SAXS) and optical observation of their birefringence at different tripalmitin (PPP) concentrations φ_{PPP} . It could be demonstrated that the platelets of these potential drug delivery systems start to form a liquid crystalline phase already at pharmaceutically relevant concentrations φ_{PPP} of less than 10 wt. %. The details of this liquid crystalline phase are described here for the first time. As in a previous study [A. Illing *et al.*, *Pharm. Res.* **21**, 592 (2004)] some platelets are found to self-assemble into lamellar stacks above a critical tripalmitin concentration φ_{PPP}^{st} of 4 wt. %. In this study another critical concentration $\varphi_{PPP}^{lc} \approx 7$ wt. % for DLPC and $\varphi_{PPP}^{lc} \approx 9$ wt. % for S100 stabilized dispersions, respectively, has been observed. φ_{PPP}^{lc} describes the transition from a phase of randomly oriented stacked lamellae and remaining non-assembled individual platelets to a phase in which the stacks and non-assembled platelets exhibit an overall preferred orientation. A careful analysis of the experimental data indicates that for concentrations above φ_{PPP}^{lc} the stacked lamellae start to coalesce to rather small liquid crystalline domains of nematically ordered stacks. These liquid crystalline domains can be individually very differently oriented but possess an overall preferred orientation over macroscopic length scales which becomes successively more expressed when further increasing φ_{PPP} . The lower critical concentration for the formation of liquid crystalline domains of the DLPC-stabilized suspension compared to φ_{PPP}^{lc} of the S100-stabilized suspension can be explained by a larger aspect ratio of the corresponding tripalmitin platelets. A geometrical model based on the excluded volumes of individual platelets and stacked lamellae has been developed and successfully applied to reproduce the critical volume fractions for both, the onset of stack formation and the appearance of the liquid crystalline phase. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4880723>]

I. INTRODUCTION

The variety of liquid crystalline phases and phase transitions has been studied extensively for different kinds of suspensions of platelet-like shaped colloidal particles in the last two decades. With computer simulations for hard colloidal “cut-sphere” platelets¹ and analytical calculations,^{2,3} the appearance of nematic and columnar phases in such suspensions has been predicted. Over the last 15 years nematic and columnar phases were found experimentally in suspensions of sterically or charge stabilized Gibbsite,^{4–6} nontronite,⁷ nickel hydroxide,⁸ and other inorganic platelets. Recently, also a smectic B phase has been reported for suspensions of Gibbsite platelets.⁹

For the Gibbsite suspensions it was found that on a microscopic level the formation of the nematic phase is preceded by the formation of nematic droplets (tactoids) in the isotropic phase. Finally these tactoids coalesce at sufficiently high concentrations to a nematic phase on a macroscopic scale.^{10,11} Grains of nematically ordered platelets have been also recently described by Yamaguchi and coworkers. For small grain sizes Yamaguchi *et al.*¹² classified them as a concentrated liquid-like isotropic phase. If the grains become large

enough they can be assigned to a nematic phase.¹² Recently, also columnar droplets, that can be regarded in a similar way as the precursor of the columnar phase, were observed in Gibbsite suspensions. Thereby, the platelets arrange in hexagonally ordered columnar stacks inside these droplets.¹³

For suspensions of organic platelets the formation of a large-scale single domain nematic phase consisting of highly oriented single platelets and platelet stacks has been reported for lozenge-shaped, micron-sized polystyrene-block-poly(L-lactide) single crystals.¹⁴

Here we study suspensions of platelet-like shaped tripalmitin nanoparticles stabilized by different lecithins. Such triglyceride suspensions, prepared by high pressure melt homogenization, have been studied in the context of potential drug delivery systems for more than 20 years.^{15,16} In the course of the sample preparation the emulsion droplets crystallize predominantly into thin platelets upon cooling. The platelets possess diameters in the colloidal size range and are characteristic for the stable triclinic β -modification of tripalmitin.¹⁶

Since the platelets possess thicknesses of only a few unit cells along the crystallographic [001] direction, the 001 Bragg

FIG. 1. 2D SAXS pattern recorded for a native tripalmitin suspension (10 wt. % tripalmitin) stabilized by DLPC. The preferential orientation of the platelet stacks (angle ϕ) results in the same anisotropy of the scattered intensity for the stack-related interferences at small scattering angles (the first three orders are marked by numbers) and the strong 001-Bragg reflection, since the stacking direction is collinear with the reciprocal lattice vector $\mathbf{G}_{001}$. The black stripes in the image originate from gaps between the three detector panels and from the beamstop.

reflection is strongly broadened and it can be observed in the typical low- Q regime of SAXS (cf. Figure 1) as the d_{001} spacing of 4.02 nm for β -tripalmitin is fairly large.^{17,18} Above a critical tripalmitin (PPP) concentration φ_{PPP}^s , interference maxima with a lamellar order arise in SAXS patterns (first three orders marked in Figure 1). They can be attributed to a self-assembly of individual platelets in stacked lamellae (named “stacks” hereafter).^{19,20} Within these stacks the platelets arrange in parallel to their large (001) faces, with repeat units typically in the range between 30 nm and 60 nm, depending on tripalmitin concentration.²⁰ The stack formation is a fully reversible process. Upon dilution the stacks disintegrate and the inter-particle interference maxima in the SAXS patterns vanish.^{19,20}

Within TEM micrographs the stacks were found to be sharply defined small objects with sizes of a few hundred nm.^{19–21} At concentrations of 15 wt. % tripalmitin and above, Illing *et al.* found in TEM micrographs that the stacks even form three-dimensional domains over a few microns.²⁰ Furthermore, Illing *et al.*²⁰ observed a size fractionation with respect to the platelet diameters in the micrographs. Platelets with larger diameters and, thus, with larger aspect ratios preferentially self-assemble in stacks.²⁰ In nematic phases such a behavior has been also found in simulations of polydisperse, infinitely thin platelets,²² and experimentally for Gibbsite suspensions.^{23,24} For the latter a size fractionation between the isotropic and the nematic phase with respect to the platelet thicknesses has been observed leading to a density inversion of the isotropic and nematic phases, where thinner platelets (i.e., with a larger aspect ratios) cumulate preferentially in the upper nematic phase leaving the thicker platelets in the lower isotropic phase.

In this study we report on isotropic to liquid crystalline phase transitions in tripalmitin nanosuspensions at pharma-

ceutically relevant concentrations of 10 wt. % tripalmitin and below. These phase transition are characterized as functions of the tripalmitin concentration φ_{PPP} by critical tripalmitin concentrations φ_{PPP}^c , representing the onset of the formation of a preferential orientation of the platelets and stacks. Parenteral administration requires suspensions which possess a low viscosity even when squeezed through narrow needles.^{21,25} The liquid crystalline phases can adversely affect the rheological properties and the particle aggregation behavior and, through this, the (long-term) stability of such suspensions upon administration and storage. Two different lecithins are used for the stabilization of the dispersions, namely the pure lecithin DLPC and purified soybean lecithin (S100), the latter being often used for pharmaceutical purposes.^{15,16,20,21,26,27}

The two critical concentrations, φ_{PPP}^s for the stack formation and φ_{PPP}^c for the onset of the preferential orientation of the platelets and stacks (i.e., the isotropic to liquid crystalline phase transition), are determined by SAXS and optical polarization measurements via the study of dilution series of the corresponding dispersions. In addition, the domain sizes of the liquid crystalline phase within the dispersions are studied by spatial 2D SAXS scans of large samples.

The distribution of the platelet thicknesses, the concentration-dependent amount of stacks, and an estimate for the size of the stacks are determined from the SAXS patterns using the x-ray and neutron powder pattern simulation analysis (XNPPSA) method described in Refs. 17 and 18.

A geometric model based on the spherical exclusion volumes of individual platelets and stacks is developed and used to reproduce the experimentally observed critical concentrations φ_{PPP}^s and φ_{PPP}^c and to estimate the size of the platelet stacks.

II. MATERIALS AND SAMPLE PREPARATION

A. Materials

Tripalmitin (Dynasan 116, >95% purity for the fatty acid fraction) was kindly provided by Sasol GmbH, Witten, Germany. 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC, purity >98%) was purchased from Lipoid GmbH, Ludwigshafen, Germany. S100, a purified soybean lecithin ($\geq 94\%$ phosphatidylcholine), that is rich in (poly-) unsaturated C_{18} fatty acids (about 65% 18:2, 13% 18:1, 5% 18:3 of the total fatty acid fraction), was kindly provided by Lipoid GmbH. Sodium glycocholate (NaGC) was purchased from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany. All purity specifications refer to the manufacturer specifications and all substances were used without any further purification.

B. Sample preparation

Here and in the following all sample concentrations are given in wt. % unless otherwise specified. Since the weight density for tripalmitin and the stabilizers are close to the weight density of the aqueous dispersion medium,

FIG. 2. Schematic representation of the highly customized Ganesha 300 XL+ SAXS instrument. The x-ray source (consisting of the x-ray generator (G) and the mirror optics (M)) and the collimation system (CS) including up to four fully automated four blade slit systems (S_1 , S_2 , S_3 , and S_4) can be moved horizontally on a rail system in beam direction. This allows to adjust the available space for sample environments placed in air (S) between the CS and detector tube (DT). The slit system S_4 is not shown in the schematic and is installed only when the sample environment inside the detector tube is used. The collimation system and the DT are evacuated and sealed with $5\text{ }\mu\text{m}$ thick mica windows (MWs). The collimation line can be tilted and shifted with respect to the horizontal plane, allowing GISAXS and reflectometry measurements of horizontally aligned specimen including liquid surfaces. Samples and even bulky sample environments can alternatively be placed at the position marked with VS inside the 600 mm wide and more than 2000 mm long evacuated detector tube, with the CS being directly (without a window) connected to the DT. Inside the detector tube a water cooled and vacuum-proof Pilatus3 300 K detector, with a maximum frame rate of 500 Hz and a readout time of about 1 ms, can be moved in all three spatial dimensions. Beamstops (BS) and a pin diode (PD) can be positioned in front of the detector. The maximum accessible s -range is between 0.003 and about 5 nm^{-1} . The whole system is fully automated and controlled by the SPEC instrument control software.

concentrations given in wt. % are virtually the same as given in vol. %. The aqueous suspensions were prepared by high-pressure melt homogenization according to the procedure described in Ref. 18. The native tripalmitin suspensions were prepared using 10% tripalmitin, 2.4% lecithin (DLPC or S100) and 0.6% NaGC in purified water. The anionic co-stabilizer NaGC is crucial to prevent the gelation of the dispersions upon the crystallization of the emulsion droplets to platelet-shaped nanocrystals.¹⁵

III. EXPERIMENTAL

A. Photon correlation spectroscopy (PCS)

The particle size d_{PCS} (z -average) and polydispersity index PdI were determined using the cumulant method from the correlation function measured with a photon correlation spectrometer (Brookhaven Instruments Corporation, Holtsville, NY, USA), consisting of a Mini-L 30 compact diode laser (30 mW, $\lambda = 637\text{ nm}$) and a BI-200 SM goniometer carrying the photo multiplier tube at a scattering angle of 90° . A few droplets of the sample were dispersed in ultrapure water in a glass cuvette until a suitable count rate is obtained. The dispersions were measured as a suspension at room temperature (22°C) and, after heating the suspension to 70°C , as an emulsion at 40°C , well above the crystallization temperature.

B. Micro differential scanning calorimetry (μ DSC)

μ DSC heating and cooling scans were taken with a Micro DSC III micro-calorimeter (Setaram, Caluire-et-Cuire, France). The samples were heated from 6°C up to 70°C and subsequently cooled down to 6°C at scan rates of 0.1 K/min . About 250 mg of the native suspension and the same amount of water were filled in a sample and reference cell, respectively.

C. Small angle x-ray scattering (SAXS)

SAXS patterns of the tripalmitin suspension at different dilution levels and a H_2O reference sample were mea-

sured at 22°C with a highly customized micro-focus Ganesha 300 XL+ instrument (SAXSLAB ApS/JJ X-ray Systems ApS, Skovlunde, Denmark) that has been installed recently at our institute (cf. Figure 2). A 30 W x-ray generator (GeniX 3D, Xenocs, Sassenage, France) with an ultra low divergence single bounce mirror optics provides a slightly focused monochromatized beam at $\text{Cu K}\alpha$ wavelength of $\lambda = 1.5418\text{ \AA}$ with a total x-ray photon flux of about $3 \times 10^7\text{ ph/s}$. For the measurements the beam was collimated by S_1 (tungsten blades) and S_3 (scatterless blades) with aperture sizes of $0.7 \times 0.7\text{ mm}^2$ and $0.4 \times 0.4\text{ mm}^2$ at a distance of $L_c = 1.5\text{ m}$ (cf. Figure 2). This realizes a beam size of about $0.5 \times 0.5\text{ mm}^2$ at the sample position with a total photon flux of $1.94 \times 10^7\text{ ph/s}$. The sample holders are mounted in air directly behind the evacuated collimation system and in front of the evacuated detector tube. The patterns were recorded with a 2D Pilatus3 300K detector (Dectris AG, Baden, Switzerland) located at a sample-detector distance of 804 mm. The sample-detector distance and the beam center are calibrated using a silver behenate standard. The sample transmission (measured with a PIN-diode), the sample thickness, and the calibration factor for the scattered intensity (determined from a measurement of a glassy carbon secondary standard²⁸ kindly provided by the 15ID-D USAXS beamline at the Advanced Photon Source, Argonne, IL, USA) are used to convert the scattered intensity to an absolute scale ($[\text{I/cm}^2/\text{sr}]$).²⁹ The H_2O scattering was subsequently subtracted from the 2D patterns of the suspensions. The 1D scattering functions $d\Sigma/d\Omega(s)$, where $s = Q/(2\pi) = 2/\lambda \sin(2\theta/2)$ (scattering angle 2θ), are obtained by an azimuthal average of the 2D patterns with the program *fit2dcorr*³⁰ an extension for the program *fit2d*.³¹ Similarly, the azimuthal distributions of the scattered intensity along the 001 powder ring are obtained by a radial integration of the 2D patterns within a narrow band of the 001 ring.

For dilution series the tripalmitin suspensions (2%–10% tripalmitin) and H_2O were measured in a reusable quartz capillary (home made capillary holder with 1 mm diameter capillary, Hilgenberg GmbH, Malsfeld, Germany) at 20°C for 0.5 h to 3 h depending on the concentration.

For the native DLPC-stabilized suspension the 2D distribution of the preferential platelet orientations was

investigated. Therefore, the sample and a water reference were measured in a circular sample cell with a diameter of 20 mm, a thickness of 1.2 mm and two 5 μm thick mica windows. The entire sample cell was spatially scanned in steps of 1.85 mm in vertical and horizontal direction, respectively. For each position a SAXS pattern was measured for 12 min.

In this study we intend to study the structure of the suspensions in their native form, avoiding shear stress as far as possible. Therefore, all sample holders were filled using either a syringe without a needle or a pipette to avoid strong shear rates which might cause a shear induced formation of a liquid crystalline phase. However, shear effects cannot be completely avoided. For example placing a mica window on top of the sample cells could certainly induce some shear stress on the liquids in the cells.

IV. SIMULATIONS AND FITS FOR THE SAXS PATTERNS, XNPPSA METHOD

In previous studies it has been demonstrated that the x-ray and neutron powder pattern simulation analysis (XNPPSA) method can reproduce simultaneously the SAXS and SANS patterns of dilute tripalmitin suspensions.^{17,18} The broadened 001 Bragg peak and the stack-related interference maxima in the small-angle scattering patterns allow the determination of the distribution of the platelet thicknesses and to estimate the amount of particles being assembled in stacks. This is achieved by a fit of a linear combination of the computed scattering intensities from different structural elements to the small-angle scattering patterns. In case of triglyceride suspensions these are platelet-shaped nanocrystals with different thicknesses or stacks with different numbers of platelets per stack. Other elements like vesicles can be included, too.

Using the SAXS patterns from the dilution series of the two native tripalmitin suspensions stabilized by DLPC and S100, respectively, the distribution of the platelet thicknesses can be extracted and the concentration-dependent amount of platelets that self-assemble in stacks as well as the mean number of platelets per stack can be estimated.

The computer simulations for the SAXS patterns were performed with the C++ program *XNDDiff* on the high performance computing cluster Woodcrest at the Regionales Rechenzentrum Erlangen (RRZE) in a similar way as in recent studies on dilute tripalmitin suspensions.¹⁸ The simulation data is fitted to the SAXS patterns with the Mathematica program *BatchMultiFit*.³²

In the simulations the platelets possess a parallelepipedal shape. The triclinic unit cell parameters of β -tripalmitin and the atomic positions of the tripalmitin molecules are taken from Ref. 33.

The lecithin stabilizer layer that covers the surface of the platelets is modeled by two shells. The outer shell of thickness d_{osl} represents the hydrophilic phosphatidylcholine headgroups including additional water molecules, and the inner shell of thickness d_{isl} represents the hydrophobic acyl chains. The electron densities ρ_{isl}^X and ρ_{osl}^X for the inner and outer shell, respectively, are taken from a previous study³⁴ on DLPC- and S100-stabilized tripalmitin suspensions: $\rho_{isl}^X = 267 \text{ 1/nm}^3$ and $\rho_{osl}^X = 506.5 \text{ 1/nm}^3$ (DLPC),

$\rho_{isl}^X = 274 \text{ 1/nm}^3$, and $\rho_{osl}^X = 471.7 \text{ 1/nm}^3$ (S100). There, the structure of the stabilizer layer was determined by a combination of SAXS and SANS data. Due to the lack of SANS data in the study presented here, only SAXS patterns are used in the XNPPSA method. While SANS data are indispensable for a reliable determination of details of the structure of the stabilizer layer,¹⁸ the gain of information with regard to the quantification of stacks in the suspensions can be considered to be rather low. This is because of the much higher instrumental smearing in SANS, which further smears out the already broad stack-related peaks in the patterns.

The values of the stabilizer layer thicknesses d_{isl} and d_{osl} were rechecked for dilute suspensions (3% tripalmitin) with the reported electron densities of the two shells from the combined SAXS and SANS study.³⁴ For both suspensions we obtained $d_{isl} = 7 \text{ \AA}$ and $d_{osl} = 3.5 \text{ \AA}$ which are virtually the same as the reported values (DLPC: $d_{isl} = 7 \text{ \AA}$ and $d_{osl} = 3.5 \text{ \AA}$ S100: $d_{isl} = 7 \text{ \AA}$ and $d_{osl} = 3 \text{ \AA}$).

A. Modeling dilute suspensions without stacks

The scattering patterns of sufficiently dilute tripalmitin suspensions, that lack of any stack-related interference maxima, are modeled by individual tripalmitin platelets only. In the simulations the scattering patterns of $N_{sp} = 5$ ensembles, each consisting of 500 single platelets, are computed. All platelets in the i th ensemble ($i = 1, \dots, N_{sp}$) share a common thickness of i tripalmitin unit cells along the crystallographic [001] direction. The length of a β -tripalmitin unit cell in the direction perpendicular to the (001) lattice plane is given by $d_{001} = 4.02 \text{ nm}$. Thus the thickness h_i of platelets in ensemble i reads as $h_i = i \cdot 4.02 \text{ nm}$, where the stabilizer layer is not yet included. The two lateral diameters of the parallelepipedal platelets in the ensembles follow a Gaussian distribution with a mean diameter of 200 nm and a variance of 50 nm.

According to the XNPPSA method^{17,18} the modeled macroscopic scattering cross sections ($d\Sigma/d\Omega$) can be fitted as a linear combination of the scattering contributions ($d\Sigma/d\Omega$) _{sp} ^{i} of the five simulated ensembles and a background term $A > 0$

$$\left(\frac{d\Sigma}{d\Omega}\right) = \sum_{i=1}^{N_{sp}} c_i \left(\frac{d\Sigma}{d\Omega}\right)_{sp}^i + A \quad \sum_{i=1}^{N_{sp}} c_i = 1 \quad (1)$$

to the SAXS patterns. Thereby, the fitted linear coefficients c_i can be interpreted as the volume fraction of all platelets in the suspension that possess a thickness of i tripalmitin unit cells. The c_i must fulfil the completeness relation¹⁸ given on the right-hand side of Eq. (1).

For the simulation of stacks in Sec. IV B a number weighted distribution of the nanocrystal thicknesses is required. Assuming that the platelet diameters and thicknesses are uncorrelated, number weights n_i can be estimated from the c_i via¹⁸

$$n_i = \frac{c_i}{i} \cdot \left(\sum_{i=1}^{N_{sp}} \frac{c_i}{i}\right)^{-1}. \quad (2)$$

Furthermore, the distributions of the c_i and n_i can be used to calculate the volume and number averaged platelet thicknesses, $\langle i \rangle_V$ and $\langle i \rangle_N$ (both in units of $d_{001} = 4.02$ nm), respectively, as¹⁸

$$\langle i \rangle_V = \frac{\sum_i c_i \cdot i}{\sum_i c_i} \quad \langle i \rangle_N = \frac{\sum_i n_i \cdot i}{\sum_i n_i} = \frac{\sum_i c_i}{\sum_i \frac{c_i}{i}}. \quad (3)$$

Thus the number weighted mean platelet thickness $\langle h \rangle_N$ that includes the stabilizer layers is given by

$$\langle h \rangle_N = \langle i \rangle_N \cdot d_{001} + 2 \cdot (d_{isl} + d_{osl}). \quad (4)$$

B. Modeling suspensions with stacks

With increasing triglyceride concentration stack-related interference maxima are observed which are accounted for by including platelet stacks in the simulations. The macroscopic scattering cross sections $(d\Sigma/d\Omega)_{st}^i$ of four ensembles, each consisting of 100 stacks, are computed. Thereby, all stacks in ensemble i possess i platelets per stack and i runs from 2 to a maximum number of platelets per stack N_{st} . $N_{st} = 5$ was chosen in the simulations and fits, since for larger values the fit quality was found not to be improved significantly. For the distribution of the platelet thicknesses in the stacks the number weights n_i derived from the analysis of the dilute dispersions are used. The same applies for the electron densities and the shell thicknesses of the stabilizer layer and for the distribution of the platelet diameters.

The mean repeat distance d_{st} of the platelets in the stacks can be estimated approximately from the position of the interferences in the small s range of each particular experimental SAXS pattern. The repeat distances in the simulations are subject to a Gaussian distribution with the mean distance d_{st} and the variance σ_{st} . For each dilution level, simulation runs with different σ_{st} are carried out in steps of 0.5 nm in the range between 0.5 and 8 nm. The values for σ_{st} were determined from the best quality fits.

The macroscopic scattering cross section for a suspension consisting of both, ensembles of individual platelets with different platelet thicknesses ($N_{sp} = 5$) and ensembles of stacks with different numbers of platelets per stack ($N_{st} = 5$), can be written as

$$\left(\frac{d\Sigma}{d\Omega}\right) = \psi \sum_{i=1}^{N_{sp}} c_i \left(\frac{d\Sigma}{d\Omega}\right)_{sp}^i + (1 - \psi) \sum_{i=2}^{N_{st}} d_i \left(\frac{d\Sigma}{d\Omega}\right)_{st}^i + A. \quad (5)$$

Thereby, ψ ($0 \leq \psi \leq 1$) is the total volume fraction of all remaining individual platelets and, thus, ψc_i the volume fraction of platelets with a thickness of i tripalmitin unit cells. Accordingly, $(1 - \psi)d_i$ represents the volume fraction of all stacks consisting of i platelets and $1 - \psi$ represents the total volume fraction of all platelet stacks. Using Eq. (5) in the fitting of the simulation data to the SAXS patterns, the individual values for d_i and additionally $1 - \psi$ can be obtained. The c_i are fixed to the values obtained from the dilute suspension without stacks. Similarly as in Eq. (1), a completeness

relation for the volume fractions of all considered structural elements,

$$\psi \sum_{i=1}^{N_{sp}} c_i + (1 - \psi) \sum_{i=2}^{N_{st}} d_i = 1, \quad (6)$$

must be fulfilled in the fits.

A volume weighted estimate for the mean number of platelets per stack $\langle i_{st} \rangle_V$ can be obtained via

$$\langle i_{st} \rangle_V = \frac{\sum_{i=2}^{N_{st}} i \cdot d_i \cdot (1 - \psi)}{\sum_{i=2}^{N_{st}} d_i \cdot (1 - \psi)} = \frac{\sum_{i=2}^{N_{st}} i \cdot d_i}{\sum_{i=2}^{N_{st}} d_i}. \quad (7)$$

In all fits a scaling factor for the calculated SAXS patterns is included. It allows the SAXS curve to vary within $\pm 10\%$ bounds to account for small errors in the absolute intensity calibration, the determination of capillary thickness and sample concentration.

V. ESTIMATION OF THE CRITICAL CONCENTRATIONS

The maximum volume fraction φ_k^* of dispersed individual ($k = 1$) platelets and stacks, consisting of $k > 1$ platelets, is estimated using a simple geometric model, that considers the close packing of the spherical exclusion volumes of the platelets and stacks, respectively. The calculated value for φ_1^* should reproduce the critical total lipid concentration φ^{st} at the onset of the stack formation which is determined experimentally (the total lipid concentration φ including all stabilizers is given by the sample composition as $\varphi = 1.3 \cdot \varphi_{PPP}$). Furthermore, it will be demonstrated in the following how the maximum of all $\{\varphi_k^* | k > 1\}$ is linked to the critical concentration φ^{lc} at which the isotropic to liquid crystalline phase transition occurs. The latter analysis allows to estimate the number of platelets per stack which can be compared with the corresponding value of $\langle i_{st} \rangle_V$ which is obtained from the XNPPSA.

Assuming a monodisperse distribution of the platelet diameters in the dispersions under study oversimplifies the problem and results in values for φ_1^* being too large.²⁰ Therefore, the model proposed here introduces a log-normal distribution for the platelet diameters D with mean diameter μ and variance $\tilde{\sigma}$. The platelet thickness h is assumed to be monodisperse or to follow a narrow symmetric distribution with mean value $\langle h \rangle$, respectively. A small polydispersity in the thicknesses of the platelets has, incidentally, only a minor influence on the final result for φ_k^* since the platelets are rather thin. Because of the elongated shape of some platelets (cf. Sec. VI A), it is well justified to model the cross section by an elliptic shape with a long axis D and a short axis $r \cdot D$, where $0 < r \leq 1$. $r = 1$ corresponds to platelets of cylindrical shape.

The repeat distances d_{st} observed in stacks are considered to be monodisperse. This assumption is well justified by observations made from freeze-fracture TEM studies²⁰ and the results from the analysis of the SAXS patterns using the XNPPSA method presented here.

It has been observed experimentally that platelets with large aspect ratios preferentially arrange in stacks²⁰ and in a

FIG. 3. Schematic representation of the concentration dependent arrangement of platelet-like shaped triglyceride nanocrystals in aqueous dispersion. (a) A dilute system with a total lipid fraction well below the critical concentration $\varphi^{st} \simeq \varphi_1^*$. (b) Random close packing of the spherical exclusion volumes at $\varphi^{st} \simeq \varphi_1^*$. (c) A fraction of platelets self-assemble in stacks for concentrations above φ^{st} . (d) At concentrations above $\varphi^{lc} \simeq \max\{\varphi_k^* | k > 1\}$ formation of liquid crystalline domains is observed.

nematic phase,²² respectively. Accordingly, a cut-off diameter α is introduced, taking into account, that platelets with diameters larger than α can self-assemble in stacks, while platelets with smaller diameters do not self-assemble in stacks and remain as single platelets in the suspension.

The maximum volume fraction φ_1^* of isolated platelets within a stack-free dispersion (cf. Figure 3(a)) is considered to correspond to the volume fraction of the sum of the spherically excluded volumes of all platelets when closely packing these virtual spheres (cf. Figure 3(b)).²⁰ Below φ_1^* , the platelets may freely rotate in the excluded volumes. For concentrations above φ_1^* some platelets start to self-assemble in stacks leaving others their full entropy of free rotation. Experimentally, $\varphi_1^* = \varphi^{st}$ can be identified as the minimum total lipid volume fraction of dispersions exhibiting stack-related interferences in their SAXS patterns.

In the simplest case of individual monodisperse platelets, the condition for the close packing of their excluded volumes can be written as^{20,35}

$$n \cdot V_{ex} = \frac{N}{V_d} V_{ex} = \frac{\varphi_1^*}{V_p} V_{ex} = \frac{2}{3} \frac{\varphi_1^* D}{r h} = \varphi_{cp}, \quad (8)$$

where $n = N/V_d = \varphi_1^*/V_p$ denotes the number density of platelets, given by the number of platelets N per dispersion volume V_d . The volume of a particle of elliptic cylindrical shape (cylinder of height h and elliptic cross-section $\pi/4 \cdot r D^2$) is given by $V_p = \pi/4 \cdot h r D^2$ and the corresponding excluded sphere volume by $V_{ex} = \pi/6 \cdot D^3$. For the calculation of φ_{cp} the volume fraction of a close packing for monodisperse spheres, 0.74, is used.²⁰ If D and h are known,

φ_1^*/r can be calculated from Eq. (8) for the monodisperse case.

For stacks consisting of $k > 1$ platelets, a similar approach is used to estimate the maximum volume fraction φ_k^* via a close packing of their spherical excluded volumes. As for individual platelets, below φ_k^* the stacks may orientate freely in the suspension. For concentrations above φ_k^* the stacks would increasingly start to order in a more parallel arrangement, finally forming liquid crystalline domains.

Experimentally, the critical concentration φ^{lc} for the isotropic to liquid crystalline phase transition can be determined by detecting the birefringence of the dispersions located between two crossed polarizers or by the appearance of an anisotropic distribution of the scattered intensity of the 001-Bragg reflection and the stack-related interference maxima in their SAXS patterns. φ^{lc} should correspond to the maximum of all maximum volume fractions φ_k^* for stacks of $k > 1$ platelets.

The calculation of φ_k^*/r for the more realistic case of individual platelets with polydisperse platelet diameters and the corresponding calculation for stacks can be found in the Appendix. Thereby, all relevant geometric parameters are normalized to the mean value μ of the log-normal distribution of the platelet diameters. $\tau = D/\mu$ and $\sigma = \tilde{\sigma}/\mu$ denote the normalized platelet diameter and the polydispersity of the platelet diameter, respectively. $\eta = \langle h \rangle/\mu$ defines the normalized mean platelet thickness and, thus, the inverse η^{-1} the mean platelet aspect ratio. $\xi = d_{st}/\mu$ normalizes the mean repeat distance d_{st} between the platelets in the stacks with μ .

For given values of the polydispersity σ , mean aspect ratio η^{-1} , total lipid volume fraction φ of the platelets, and r , a dimensionless density⁴ $n\langle D^3 \rangle$ for the platelets in the suspensions can be calculated from the number density n as

$$n\langle D^3 \rangle = \frac{4}{\pi} \frac{\varphi}{r} \frac{\langle D^3 \rangle}{\langle D^2 \rangle \langle h \rangle} = \frac{4}{\pi} \frac{\varphi}{r} (1 + \sigma^2)^2 \frac{\langle D \rangle}{\langle h \rangle}, \quad (9)$$

$$= \frac{4}{\pi} \frac{\varphi}{r} \frac{(1 + \sigma^2)^2}{\eta}, \quad (10)$$

where the log-normal distribution function f_L from Eq. (A1) is used to calculate the averages of the powers of the platelet diameters D . The values of $n\langle D^3 \rangle$ can be compared with the isotropic to nematic phase transition densities, that were observed in other studies on suspensions of platelet-like nanoparticles.

VI. RESULTS AND DISCUSSION

In the first part of this section the characterization of the native tripalmitin suspensions by PCS, micro-calorimetric measurements and freeze-fracture TEM studies is presented. SAXS measurements and optical birefringence experiments were conducted on a dilution series for the determination of the critical concentrations of the formation of stacks and liquid crystalline phases, respectively. These studies are discussed in Sec. VI B. The domain sizes and the degree of order of the liquid crystalline phase have been studied by spatially resolved SAXS measurements of a native

DLPC-stabilized suspension (cf. Sec. VI C). Using the XNPPSA method, it was possible to determine the thickness distribution of the platelets, the amount of stacks, and the mean number of platelets per stack within the suspensions (cf. Sec. VI D). Finally, the experimental results are discussed in Sec. VI E in the light of the geometric model which has been presented in Sec. V and in the Appendix.

A. Sample characterization by PCS, μ DSC, and freeze-fracture TEM

The correlation functions measured by PCS for native tripalmitin suspensions and emulsions stabilized by DLPC and S100, respectively, (data not shown) can be modeled by monomodal polydisperse particle size distributions. The measured harmonic z -averages d_{PCS} and polydispersity indices PdI are listed in Table I. In the Rayleigh approximation,³⁶ d_{PCS} represent the intensity ($I \propto V^2 \propto d^6$) weighted average diameters $\langle d^6 \rangle_N / \langle d^3 \rangle_N$,³⁷ where $\langle \cdot \rangle_N$ indicates the number average.

d_{PCS} and in particular the PdI for the dispersions stabilized by S100 are significantly lower than for the dispersions stabilized by DLPC. Obtaining reliable information on the distribution of the platelet diameters is, however, difficult from the PCS data.

Because of the polydispersity and the intensity weighted average for d_{PCS} , the number weighted mean diameter for the emulsion droplets is always smaller than d_{PCS} . In the case of suspensions the situation is even more complex, since for platelets d_{PCS} can be used only as a crude approximation for the diameters and underestimates the platelet diameters. Using the d_{PCS} values for the diameter of the emulsion droplets and the mean platelet thicknesses of 10.3 nm (DLPC) and 11.5 nm (S100), obtained later with the XNPPSA method (cf. Table II in Sec. VI D 1), upper limits for the mean platelet diameters can be estimated to be 225 nm (DLPC) and 186 nm (S100), respectively, assuming the same volume of the spherical droplets and the cylindrical platelets.

The micro-calorimetric heating and cooling curves for both native suspensions are displayed in Figure 4. The heating curves are characterized by a fractionated melting behavior over a wide temperature range between 38 °C and the bulk melting point of tripalmitin at about 65 °C. The partially overlapping melting peaks can be interpreted as a se-

TABLE I. PCS measured z -averages d_{PCS} and polydispersity indices PdI for the native tripalmitin dispersions (10 wt. % tripalmitin), measured as a suspension and an emulsion, respectively. Furthermore, the enthalpies of fusion ΔH_{fus} and crystallization ΔH_{cry} derived from the μ DSC heating and cooling curves of the suspensions in Figure 4, respectively, are listed.

		PCS		μ DSC	
		d_{PCS} (nm)	PdI (-)	ΔH_{fus} (J/g)	ΔH_{cry} (J/g)
DLPC	Suspension	144.4	0.200	18.1	-17.2
	Emulsion	91.9	0.195	...	...
S100	Suspension	121.3	0.161	18.6	-18.0
	Emulsion	84.2	0.149	...	...

TABLE II. Volume ($\langle i \rangle_V$) and number ($\langle i \rangle_N$) weighted mean platelet thicknesses and thicknesses for the inner (d_{isl}), outer (d_{osl}), and total (d_{tot}) stabilizer layer of the platelets stabilized by DLPC and S100, respectively. The values for $\langle i \rangle_V$ and $\langle i \rangle_N$ are obtained using Eq. (3) and are given in units of $d_{001} = 4.02$ nm which corresponds to the thickness of one unit cell in the direction perpendicular to the (001) lattice planes. Finally, the mean platelet thicknesses including the stabilizer layer, $\langle h \rangle_N = \langle i \rangle_N \cdot d_{001} + 2 \cdot d_{tot}$, are listed.

	$\langle i \rangle_V$ (d_{001})	$\langle i \rangle_N$ (d_{001})	d_{isl} (Å)	d_{osl} (Å)	d_{tot} (Å)	$\langle h \rangle_N$ (nm)
DLPC	2.40	2.03	7.0	3.5	10.5	10.3
S100	2.79	2.33	7.0	3.5	10.5	11.5

quence of size-dependent melting events, where each peak corresponds to an ensemble of platelets that shares a common thickness.^{38,39}

The small melting peak that is observed for both suspensions in the heating curve between 38 °C and 45 °C is linked to small tripalmitin nanoparticles with an unknown modification.⁴⁰ The melting peaks between 45 °C and 65 °C can be attributed to the thickness dependent melting of tripalmitin platelets in the β -modification. In agreement to the previous study, these melting peaks are shifted by about 2 K to lower temperatures for the suspension stabilized by DLPC as compared to the S100-stabilized suspension. This already suggests, that the thicknesses of the DLPC-stabilized platelets are slightly smaller than those of the S100-stabilized platelets.

The total enthalpies of fusion ΔH_{fus} and crystallization ΔH_{cry} obtained by integration of the heating and cooling curves with respect to their baselines are listed in Table I. Since the enthalpy of fusion for pure β -tripalmitin is 188 J/g,⁴¹ the listed values for ΔH_{fus} confirm that most of the tripalmitin has crystallized in the β -modification.

The amount of the unknown modification with respect to the total tripalmitin content is rather low. The enthalpy of fusion for the melting peak between 38 °C and 45 °C amounts for both suspensions only to about 14% of the total enthalpy of fusion.

The cooling curves exhibit only one sharp crystallization peak at 24.5 °C (DLPC) and 22.5 °C (S100), respectively. The values for ΔH_{cry} in Table I are both above 18 J/g and, thus,

FIG. 4. μ DSC heating and cooling runs of native tripalmitin suspensions stabilized by DLPC and S100, respectively.

(a)

(b)

FIG. 5. Freeze fracture electron micrographs taken from native tripalmitin suspensions stabilized by (a) S100 and (b) DLPC. The black scale bars mark a length of 200 nm.

indicate a predominantly direct crystallization of the emulsion droplets into the β -modification for slow cooling.

Freeze-fracture TEM micrographs for native DLPC- and S100-stabilized suspensions are displayed in Figure 5. The characteristic platelet-like shape of tripalmitin nanoparticles in their β -modification is observed for both samples. The platelets are polydisperse in size and shape. Some appear to have a rather elongated elliptic shape of their large base areas.

The larger diameters of the platelet cross sections typically range between 100 and 300 nm. A mean platelet diameter of roughly 200 nm agrees with the estimated upper limits for the mean platelet diameters obtained from the PCS measurements.

B. SAXS studies on dilution series

The azimuthally averaged SAXS patterns for the tripalmitin suspensions stabilized by DLPC and S100 for different dilution levels ranging from 2% up to the native concentration of 10% tripalmitin are displayed in Figures 6(a) and 6(b), respectively. In Figures 7(a) and 7(b) the corresponding azimuthal distributions of the scattered intensities along the 001 powder rings are displayed, respectively.

For both suspensions the stack-related interference maxima can be observed in the SAXS patterns above a critical tripalmitin concentration φ_{PPP}^{st} of about 4%. This agrees with the observation for the tripalmitin suspensions studied by Illing *et al.*²⁰ The distinct stack-related interference maxima at higher concentrations are more clearly observed in the DLPC-stabilized suspensions than for the S100-stabilized suspensions. This may result from a higher volume fraction $1 - \psi$ of stacks in the suspension, stacks of more regular arranged platelets (i.e., smaller values for σ_{st}) or stacks with a larger number of platelets (i.e., larger values for $\langle i_{st} \rangle_V$) and will be discussed in Sec. VI E.

(a)

(b)

FIG. 6. SAXS patterns taken at a temperature of 22 °C for the suspensions stabilized by DLPC (a) and S100 (b) at different dilution levels (2%–10% tripalmitin). The red lines represent the best fits obtained by the XNPPSA method. The insets show the volume weighted distributions of the platelet thicknesses obtained from the XNPPSA fits to the dilute suspensions with 3% tripalmitin.

FIG. 7. Azimuthal distribution of the scattering intensity along the 001 Bragg peak powder ring in the SAXS patterns of the dilution series of the suspensions stabilized by DLPC (a) and S100 (b). The black and red data points correspond to azimuthal angles in the ranges $[0, 180]^\circ$ and $[180, 360]^\circ$, respectively, where the latter have been shifted by -180° . The gaps between data points at about 20° and 160° (black points) and at about 240° and 300° (red points) stem from gaps in the detector area (cf. Figure 1).

From the azimuthal distribution of the intensity along the 001 Bragg peak displayed in Figures 7(a) and 7(b) a second critical tripalmitin concentration φ_{PP}^{lc} can be extracted. An anisotropic distribution of the scattered intensity along the ring indicates the existence of domains with a preferential orientation of the platelets due to the formation of a liquid crystalline phase. For the DLPC-stabilized suspension the patterns become anisotropic already at a tripalmitin concentration of 7%. For the S100-stabilized suspension a similar behavior is detected above a tripalmitin concentration of 9%.

From the photographs in Figure 8 taken for different dilution levels of the DLPC-stabilized suspension between

crossed polarizers it can be observed that the suspension turns birefringent at slightly higher concentrations as one could have expected from the SAXS data. Starting at 8% (might be visible only in the electronic version), the suspension becomes clearly birefringent at 9% tripalmitin. For the suspension stabilized by S100 (data not shown) a weak brightening, can be observed for the native suspension (10% tripalmitin). The critical concentrations for the isotropic to liquid crystalline phase transition determined by SAXS are lower than those detected by the optical measurements. The difference of about 1% tripalmitin is due to the fact that by SAXS the formation of small and isolated liquid crystalline domains on a microscopic level can be detected. In optical birefringence images the liquid crystalline phase becomes visible only at a larger number or size of such domains.

In contrast to Gibbsite suspensions no clear phase separation between the isotropic and the liquid crystalline phase can be observed here, which can be most probably attributed to the small weight density difference between the tripalmitin platelets (1.04 g/cm^3 for bulk β -tripalmitin) and the aqueous phase (1.0 g/cm^3).

From these findings we infer a concentration-dependent behavior for the platelets as it is illustrated in Figure 3. For total lipid concentrations below the critical concentration φ^{st} , the directors of the individual platelets (cf. Figure 3(a)) are randomly distributed and the spherical exclusion volumes of the platelets do not overlap. For φ^{st} the exclusion volumes reach a close packing (cf. Figure 3(b)). Above φ^{st} a fraction of the platelets starts to self-assemble in randomly orientated stacks (Figure 3(c)). For even higher concentrations the packing density of these stacks is not sufficient any more, and the exclusion volumes of the stacks would reach a close packing, too. Thus, beyond this second critical concentration φ^{lc} the stacks and remaining individual platelets would increasingly form liquid crystalline domains where on average the platelets in these domains share a common preferential orientations (cf. Figure 3(d)).

C. Domain sizes and phase characterization

The size of the liquid crystalline domains in the native DLPC-stabilized native suspension were studied by 2D SAXS scans of tripalmitin nanosuspension stabilized by DLPC and located within a circular sample holder similar to the one displayed in Figure 8(e). For these scans a beam diameter of about 0.5 mm (FWHM) at sample position and a horizontal and vertical step size of 1.85 mm , respectively, were chosen.

An assembly of the recorded 2D images is shown in Figure 9(a). All SAXS measurements exhibit an anisotropic scattering pattern. For each pattern the scattered intensity along a semicircle of the 001 ring has been modeled by a set of Gaussian peaks and an additional constant. From the azimuthal position of the Gaussian peaks and their amplitudes a map of the preferential orientations at each scan position is plotted in Figure 9(b) where the orientation and the length of the dashes indicate the preferential orientation of the domains and the magnitude of the anisotropy, respectively.

From Figure 9(b) the existence of domains that laterally share a similar preferential orientation over several

FIG. 8. (a)–(d) Photographs taken at 22 °C under crossed polarizers from the DLPC-stabilized suspensions (6%, 7%, 8%, and 9% tripalmitin) in a spectroscopic glass cuvette (0.5 mm path length). At 8% the sample starts to become birefringent. In (e) a photograph of the native DLPC-stabilized tripalmitin suspension (10%) located between two crossed polarizers in a circular sample holder. The sample thickness amounts to about 1.2 mm.

millimeters can be anticipated. In some distinct areas of the sample two different preferential orientations could be distinguished within the same sample volume.

One has to bear in mind that only platelets and stacks satisfying the Bragg condition can contribute to the 001 reflection intensity and the stack-related interferences. Although all patterns in Figures 9(a) possess an anisotropic intensity distribution along the powder rings of the 001 Bragg peak and the stack-related peaks, respectively, another constant intensity contribution is detected along the rings. This clearly indicates that beside liquid crystalline domains with a preferred orientation such that the 001 Bragg peak can be observed, additional liquid crystalline domains and/or single particles oriented in Bragg condition, but apart from that oriented isotropically, are present in each of the small sample volumes studied. Thus, in accordance with the observations from TEM micrographs of Illing *et al.* on similar tripalmitin suspensions,²⁰ the birefringent suspensions are assumed to consist of many small liquid crystalline domains of only several microns in size, where the stacks and remaining individual platelets share a common orientation. These small micro-domains may have partially random, but on average one or two preferential orientations. Such regions of an overall preferred orientation of domains can extend beyond some hundred cubic millimeters.

The azimuthally averaged scattering patterns and the stack-related peak positions, i.e. the repeat distances d_{st} , do not change significantly throughout the scanned positions (data not shown), indicating a rather homogeneous sample with respect to the inter-particle distances.

From Figure 9(a) it can also be inferred that the sample holder itself affects the orientation of the platelets in the vicinity of the rim of the cylindrical sample cell. The dashes point often in normal direction to the circular rim of the sample cell and thus platelets close to the rim of the sample holder align preferentially in parallel to the surface of the sample holder.

High resolution SAXS measurements (sample-to-detector distance $d = 2116$ mm, data not shown) were conducted for the central sample area of a tripalmitin nanosuspension in order to check for the existence of a columnar phase. However, no side-side correlations could be found up to the maximum detected d -value of 280 nm. Thus,

tripalmitin nanosuspensions at concentrations above φ^{lc} form nematic liquid crystalline domains which are partially aligned over large spatial regions. No indications for the occurrence of columnar phases could be found.

D. The mesoscopic structure of the dispersions

1. Dilute suspensions (3%)

For both suspensions the volume weighted distribution of the platelet thicknesses are determined using the XNPPSA method. For that purpose a dilution level of 3% tripalmitin is used since at that concentration no stack-related interference maxima can be found in the SAXS patterns (cf. Figure 6). The corresponding XNPPSA fits using Eq. (1) in Sec. IV are also displayed in Figure 6. The resulting distribution functions of the volume fractions c_i for platelets with a thickness corresponding to i tripalmitin unit cells are displayed in the insets of Figures 6(a) and 6(b).

The volume and number weighted mean platelet thicknesses (without the stabilizer layer), $\langle i \rangle_V$ and $\langle i \rangle_N$, respectively, calculated using the volume fractions c_i and Eq. (3), are listed together with the fitted inner, outer, and total stabilizer layer thicknesses in Table II. The number weighted mean platelet thicknesses $\langle h \rangle_N$, which include the stabilizer layers, are listed too.

The distributions of the c_i and the mean values $\langle i \rangle_V$ and $\langle i \rangle_N$ confirm the observation from the micro-calorimetric measurements namely that the DLPC-stabilized platelets are thinner than those stabilized with S100. For the DLPC-stabilized suspension $\langle i \rangle_N$ is about $0.3 \cdot d_{001} = 1.2$ nm thinner than for the S100-stabilized suspension.

The smaller thicknesses and slightly larger diameters of the DLPC-stabilized platelets lead to larger platelet aspect ratios. The increased aspect ratio of the particles leads to a more pronounced stack formation for the DLPC-stabilized platelets which is in full agreement with the findings in a previous study.¹⁸ The larger aspect ratios of the particles also result in a lower critical concentration φ^{lc} for the isotropic to liquid crystalline phase transition in the case of the DLPC-stabilized suspension compared to the S100-stabilized suspension.

FIG. 9. (a) SAXS patterns of an DLPC-stabilized native tripalmitin suspension collected at different positions inside the circular sample holder. The diameter of the sample area is 20 mm. The sample was scanned in steps of 1.85 mm for both the vertical and horizontal direction. (b) 2D distribution of the preferential orientations of the platelets as extracted from the SAXS patterns in (a). The orientation of the lines represent the preferential orientation of the platelets and the lengths of the lines correspond to the magnitude of the anisotropy.

2. Higher concentrations

In the next step we determine the amount and the size of the platelet stacks for suspensions with tripalmitin concentrations of $\varphi_{PPP}^s \geq 4\%$.

Using the previously determined distributions of the platelet thicknesses, simulations, and fits including platelet stacks were carried out. The mean repeat distances d_{st} for the simulations were taken from the positions of the stack-related maxima in the SAXS patterns in Figure 6 and are listed as a function of the tripalmitin concentration φ_{PPP} (or total lipid concentration φ , including the stabilizers) in Table III. As for the suspensions with 3% tripalmitin, the best fits for higher

TABLE III. Mean platelet repeat distances d_{st} in stacks taken from the SAXS patterns of the dilution series in Figure 6. φ_{PPP} and φ denote the tripalmitin and the total lipid (including the stabilizers) concentrations, respectively. For the best XNPPSA fits to the data in Figure 6, the variances σ_{st} of the repeat distances, the stack volume fractions $1 - \psi$, and the mean number of platelets per stack $\langle i_{st} \rangle_V$ are listed. Using the parameters for σ , η , and r from Sec. VI E, the dimensionless densities $n(D^3)$ are computed from Eq. (10). Values determined at the critical concentrations φ_{PPP}^{lc} are printed in bold.

	$\varphi_{PPP}(\%)$	4	5	6	7	8	9	10
	$\varphi(\%)$	5.2	6.5	7.8	9.1	10.4	11.7	13.0
DLPC	d_{st} (nm)	55.8	50.7	45.8	43.2	40.5	38.5	35.7
	σ_{st} (nm)	2.0	4.5	2.5	1.5	2.0	1.5	1.5
	$1 - \psi$	0.21	0.33	0.42	0.56	0.71	0.73	0.98
	$\langle i_{st} \rangle_V$	2.2	4.3	3.1	3.2	3.7	3.5	3.7
	$n(D^3)$	1.4	1.8	2.1	2.5	2.8	3.2	3.5
S100	d_{st} (nm)	55.6	52.0	46.0	43.4	43.0	38.1	37.0
	σ_{st} (nm)	2.5	4.0	4.5	4.5	4.0	4.0	4.5
	$1 - \psi$	0.08	0.20	0.33	0.44	0.46	0.79	1.0
	$\langle i_{st} \rangle_V$	4.0	4.0	3.7	4.0	3.5	3.5	4.1
	$n(D^3)$	1.2	1.5	1.8	2.2	2.5	2.8	3.1

tripalmitin concentrations in Figure 6 can reproduce the data very well, even for the native DLPC-stabilized suspension with its highly deformed 001 Bragg peak.

The fitted values for the variances σ_{st} of the repeat distances d_{st} , the volume fractions of all stacks $1 - \psi$, and the volume weighted mean numbers of platelets per stacks $\langle i_{st} \rangle_V$ are listed for the best fits in Table III, too.

The curve progressions of $1 - \psi$ and $\langle i_{st} \rangle_V$ obtained from the best fits are displayed as a function of the concentration in Figures 10(a) and 10(b), respectively. In particular for low tripalmitin concentrations between 4% and 6%, where the SAXS patterns exhibit only weak and broadened stack-related peaks, high quality fits could be obtained in a broad range of σ_{st} . Corresponding error bars and mean values for $1 - \psi$ and $\langle i_{st} \rangle_V$ are included in Figure 10. The error bars represent the range of the fitted values for $1 - \psi$ and $\langle i_{st} \rangle_V$ obtained from all high quality fits. Exemplary for the DLPC-stabilized suspension, the fitted values for $1 - \psi$ and $\langle i_{st} \rangle_V$ are displayed in Figures 11(a) and 11(b), respectively, for the different tripalmitin concentrations φ_{PPP} and for the probed range of the σ_{st} . In these figures, white dots mark high quality fits which deviate only slightly from the best fit, marked by a black point for each particular tripalmitin concentration.

$1 - \psi$ increases linearly with the tripalmitin concentration for both suspensions (cf. Figure 10(a)), what is in good agreement with results found by Illing *et al.*²⁰ At the second critical concentration φ^{lc} , $1 - \psi$ reaches 0.56 and 0.79 for the DLPC- and S100-stabilized suspension. These values are in particular for the DLPC-stabilized suspension significantly lower as 1, which one could have expected from the assumption that at φ^{lc} a close packing of only stacks is reached. However, $1 - \psi$ must not necessarily reach 1 at φ^{lc} , for higher packing densities it might be favorable that platelets with smaller diameters remain as isolated platelets in the suspension, while platelets with larger diameters preferentially self-assemble in stacks (size fractionation).

FIG. 10. (a) Volume fraction of all stacks $1 - \psi$ and (b) mean number of platelets per stack $\langle i_{st} \rangle_V$ obtained from the XNPPSA fits to the SAXS patterns of different tripalmitin concentrations ϕ_{PPP} (or total lipid concentration $\phi = 1.3 \cdot \phi_{PPP}$ including the stabilizers) in Figure 6. The large symbols mark the values obtained for the best fits (cf. Table III) which in case of the DLPC-stabilized suspensions correspond to the data points marked with a black dot in Figures 11(a) and 11(b), respectively. The error bars stem from a range of fitted values of high quality fits (marked with white dots in Figures 11(a) and 11(b) for the DLPC-stabilized suspensions). The small symbols mark the mean of the fitted values from these high quality fits.

The stack volume fractions $1 - \psi$ can be correlated to the variance σ_{st} of the repeat distances and the number of platelets per stack $\langle i_{st} \rangle_V$. Therefore, the fitted parameters $1 - \psi$, σ_{st} , and d_i (and, thus, also $\langle i_{st} \rangle_V$) should be considered as rough estimates only. For tripalmitin concentrations up to 8%, $1 - \psi$ is for the DLPC-stabilized suspension by a factor of about 1.5–2 larger than for the S100-stabilized suspension. However, within their uncertainties, $1 - \psi$ (and $\langle i_{st} \rangle_V$) cannot be distinguished for the two different suspensions. For the best fits, the more pronounced stack-related peaks for the DLPC-stabilized suspensions are ascribed to a better order of the platelets in the stacks (smaller σ_{st}) rather than to a larger amount of stacks ($1 - \psi$). For tripalmitin concentrations $\geq 6\%$, σ_{st} for the DLPC-stabilized suspension is roughly half the value as for the S100-stabilized suspensions (cf.

Table III). For tripalmitin concentrations well above ϕ_{PPP}^{lc} (what particularly applies for the DLPC-stabilized suspension) the fitted values should be treated with care, since the model of isolated stacks in the simulations might be inadequate to describe liquid crystalline domains of several microns in size. On the other hand, the volumes which are considered in the simulations needs not to exceed several $(100 \text{ nm})^3$ because of the limited coherence volume for SAXS.

The mean number of platelets per stack $\langle i_{st} \rangle_V$ in Figure 10(b) typically ranges between 2.9 and 4.3 for both kinds of suspensions at tripalmitin concentrations of 6% and above. This is in good agreement with the results from Sec. VI E, where it is demonstrated that stacks consisting of 4 platelets often provide the best packing density, while for stacks with 5 platelets the packing density does not significantly increase any more or even starts to decrease.

E. Estimation of the critical concentrations

Using the geometric model of hard platelets with polydisperse diameters introduced in Sec. V and described in detail in the Appendix, the experimentally observed critical concentrations for the formation of stacks and liquid crystalline phases can be reproduced, respectively. In this context the dependence of the maximum achievable volume fraction of stacks on the number of platelets per stack could be studied and compared with the mean number of platelets per stack $\langle i_{st} \rangle_V$ obtained from the evaluation of the SAXS data in Sec. VI D 2.

In case of the DLPC-stabilized suspension the maximum achievable volume fractions φ_k^*/r , for which a close packing of the spherical exclusion volumes of individual platelets ($k = 1$) and stacks of $k > 1$ platelets is reached, are plotted in Figure 12 for different polydispersities σ of the platelet diameters and normalized platelet thicknesses $\eta = h/\mu$. Platelets with a circular cross section ($r = 1$) are used in the plots. For the mean platelet diameter $\mu = \langle D \rangle$ we use as in the XNPPSA simulations 200 nm which was estimated from the PCS measurements. In case of the φ_k^* for stacks, which we want to compare with the critical concentration φ^{lc} , we used the normalized repeat distance $\xi = d_{st}/\mu = 43.2/200$ at the critical concentration $\varphi_{PPP}^{lc} = 7\%$ (cf. Table III).

In Figure 12(a) φ_1^* ranges for an aspect ratio $\eta = \langle h \rangle_N/\mu = 10.3/200 \approx 0.05$ (using $\langle h \rangle_N$ for the DLPC-stabilized suspension from Table II as the mean platelet thickness) and moderate polydispersities $\sigma \lesssim 0.2$ between 4.7% and 6.5%. This is in good agreement with the experimentally observed first critical concentration $\varphi^{st} = 5.2\%$ ($\varphi_{PPP}^{st} = 4\%$), found in the dilution series.

From Figures 12(a) to 12(c) it turns out that an increasing polydispersity σ reduces the maximum volume fractions φ_k^* for individual platelets and smaller stacks, consisting of 2 and 3 platelets, respectively. Depending on σ and η , φ_2^* for stacks of two platelets increases by up to 85% with respect to φ_1^* (Figure 12(b)). For stacks consisting of three platelets, φ_3^* rises only by 10%–25% relative to φ_2^* (Figure 12(c)). For stacks of four platelets and polydispersities between 0.1 and

FIG. 11. (a) Total volume fraction of stacks $1 - \psi$ and (b) mean number of platelets per stack $\langle i_{st} \rangle_V$ obtained from the XNPPSA fits to the SAXS patterns of the DLPC-stabilized suspensions. For each tripalmitin concentrations φ_{PPP} the simulations and fits were performed for different values of the variances σ_{st} of the repeat distances d_{st} . For a particular tripalmitin concentration a black dot marks the best fit and white dots mark high quality fits whose target function values deviate typically by less than 2% from the value obtained for the best fit.

0.3, φ_4^* grows, if at all, only slightly with respect to φ_3^* by a few percent (Figure 12(d)). For stacks with five platelets the situation starts to change. While φ_5^* is for sufficiently high polydispersities virtually the same as φ_4^* , it decreases for smaller σ with respect to φ_4^* (Figure 12(e)).

Thus, the plots in Figure 12 indicate that the number of platelets per stack is for tripalmitin concentrations below φ_{PPP}^{lc} in the range between 3 and 5, since for larger stacks the packing density does not grow any more or already decreases.

In the next step we tried to optimize for the DLPC-stabilized suspension the polydispersity σ and the aspect ratio r such that φ_1^* and $\max\{\varphi_k^* | k = 2, \dots, 5\}$ match the experimentally observed critical total lipid concentrations φ^{st} and φ^{lc} , respectively. As in Figure 12 we used $\xi = 43.2/200$ and $\eta = 10.3/200$. For the polydispersity $\sigma = 0.21$ and circular platelets $r = 1$, the two experimentally observed critical concentrations $\varphi^{st} = 5.2$ ($\varphi_{PPP}^{st} = 4\%$) and $\varphi^{lc} = 9.1$ ($\varphi_{PPP}^{lc} = 7\%$) can be reproduced with φ_1^* and φ_4^* (stacks of four platelets), respectively (cf. column $\alpha = 0$ in Table IV).

Similar calculations were carried out for the S100-stabilized suspension at its critical concentrations of $\varphi^{st} = 5.2$ ($\varphi_{PPP}^{st} = 4\%$) and $\varphi^{lc} = 11.7\%$ ($\varphi_{PPP}^{lc} = 9\%$) using $\eta = \langle h \rangle_N / \mu = 11.5/200$ and $\xi = d_{st} / \mu = 38.1/200$ (cf. Tables II and III). Again a good agreement with the critical concentrations φ^{st} and φ^{lc} can be found when using a polydispersity σ of 0.18 and $r = 1$.

Due to the lack of a reliable statistics on the platelet diameters it is difficult to compare the obtained polydispersities with experimental data. However, most probably the values for σ , 0.21 (DLPC), and 0.18 (S100), tend to slightly underestimate the real polydispersities.

Table IV lists also for both suspensions the volume fractions φ_k^* for monodisperse platelets ($\sigma = 0$) using the same values for ξ , η , and r as above. It turns out that monodisperse platelets do not significantly increase φ_1^* . This also appears from Figure 12(a), where φ_1^* for the large diameter-to-thickness aspect ratios η^{-1} of almost 20 found here (DLPC: 19.4, S100: 17.4) does not significantly change with σ .

However, it turns out, that in particular for the second critical concentration it is crucial to include the polydispersity of the platelet diameters since for the monodisperse case the values for φ_k^* ($k > 1$) would be too large.

As already observed in Figure 12, for both suspensions, the values for φ_k^* in Table IV do not significantly increase anymore or even start to decrease, when raising the number of platelets per stack from three or four to five. Thus, the geometric model can reproduce the values for $\langle i_{st} \rangle_V$ which were found with the XNPPSA method to range for tripalmitin concentrations above 6% between 2.9 and 4.3 platelets per stack. This supports the idea that $\langle i_{st} \rangle_V$ can be regarded as a good estimate for the mean number of platelets per stack.

Table IV includes also φ_k^* calculated with size fractionation ($\alpha > 0$) and the polydispersities $\sigma = 0.21$ (DLPC) and $\sigma = 0.18$ (S100) obtained priorly without size fractionation ($\alpha = 0$). Different cut-off diameters $\alpha_j = 1 + j \cdot \sigma$, where $j = 0, \pm 1, \pm 2$, were considered. The effect of size fractionation on increasing the maximum volume fractions φ_k^* ($k > 1$) turns out to be very small for the geometry of the platelets and stacks, and polydispersities σ considered here. In case of the DLPC-stabilized suspension, a small increase for φ_k^* can be observed for $j = -1$. This corresponds to the case, when platelets with normalized diameters $\tau = D/\mu$

FIG. 12. Maximum volume fractions φ_k^* for individual platelets ($k = 1$) and stacks of $k > 1$ platelets to reach a close packing of their spherical exclusion volumes. The φ_k^* are calculated without size fractionation ($\alpha = 0$) for different diameter polydispersities σ and mean aspect ratios η^{-1} for circular platelets ($r = 1$). (a) φ_1^* , (b) relative change in % of φ_2^* with respect to φ_1^* . Similarly, the plots in (c)–(e) show the relative changes of φ_3^* to φ_2^* , φ_4^* to φ_3^* , and φ_5^* to φ_4^* , respectively. For the stacks in the plots (b)–(e) a normalized repeat unit of $\xi = 43.2/200$ was used, which corresponds to the critical tripalmitin concentration φ_{PP}^{lc} of 7% for the DLPC-stabilized suspension (cf. Table III).

larger than $1 - \sigma$ self-assemble in stacks, while all smaller ones remain as individual platelets in the suspension. Thus, for concentrations above φ^{lc} , still small amounts of the dispersed phase in the form of small individual platelets might be present in the suspension and $1 - \psi$ must not necessarily reach 1 at φ^{lc} as it was found for the XNPPSA fits in Sec. VI D 2.

For the S100-stabilized suspension the maximum volume fractions φ_k^* are for $\alpha \leq 1 - \sigma$ virtually the same as without size fractionation ($\alpha = 0$). As for the suspension stabilized with DLPC, the φ_k^* decrease considerably for $\alpha \geq 1$.

The dimensionless densities $n\langle D^3 \rangle$ are calculated for both suspensions for different total lipid volume fractions φ with Eq. (10) and listed in Table III. The same values for μ , η (DLPC: 10.3/200, S100: 11.5/200) and σ (DLPC: 0.21, S100: 0.18) from above and $r = 1$ were used.

At the second critical concentration φ^{lc} of 9.1% (DLPC) and 11.7% (S100) one obtains for $n\langle D^3 \rangle$, 2.5 and 2.8, respectively. For Gibbsite platelets with an aspect ratio of $\langle D \rangle / \langle L \rangle = 11$ ($\langle D \rangle = 168$ nm, $\langle L \rangle = 15$ nm) the isotropic-nematic phase transition was observed at a concentration of $\varphi = 15.9$ vol. %.⁴ The reported value for $n\langle D^3 \rangle = 2.5$ is in good

TABLE IV. Maximum volume fractions φ_k^* in vol. % for individual platelets ($k = 1$) and stacks of $k = 2, \dots, 5$ platelets. For each suspension the first column provides the φ_k^* for monodisperse platelet diameters ($\sigma = 0$) using Eq. (A20). For polydisperse ($\sigma > 0$) diameters the second column provides φ_k^* without size fractionation ($\alpha = 0$, using Eq. (A16)) while the 3rd to 7th columns include size fractionation ($\alpha > 0$, using Eq. (A18)) using different parameters for $\alpha_j = 1 + j \cdot \sigma$ ($j = 0, \pm 1, \pm 2$). The values for ξ were taken from Table III at the critical concentrations for the formation of the liquid crystalline phase.

k	DLPC ($\varphi^{st} = 5.2, \varphi^{lc} = 9.1$)							S100 ($\varphi^{st} = 5.2, \varphi^{lc} = 11.7$)						
	$\sigma = 0.21$							$\sigma = 0.18$						
	$\sigma = 0$	$\alpha = 0$	$\alpha_j = 1 + j \cdot \sigma$					$\sigma = 0$	$\alpha = 0$	$\alpha_j = 1 + j \cdot \sigma$				
1	5.7	5.2	α_{-2}	α_{-1}	α_0	α_1	α_2	6.3	5.9	α_{-2}	α_{-1}	α_0	α_1	α_2
2	10.3	7.2	7.2	7.3	6.9	6.0	5.5	11.6	8.6	8.6	8.7	7.9	6.8	6.2
3	12.5	8.5	8.5	8.7	7.8	6.4	5.6	14.7	10.5	10.5	10.5	9.0	7.2	6.3
4	12.5	9.1	9.1	9.2	8.2	6.5	5.6	15.4	11.5	11.5	11.4	9.5	7.3	6.4
5	11.4	9.0	9.0	9.2	8.3	6.6	5.6	14.7	11.7	11.7	11.6	9.7	7.4	6.4

agreement with the values observed here, since the larger aspect ratios η^{-1} of 19.4 (DLPC) and 17.4 (S100) are set-off by significantly lower φ^{lc} of 9.1% (DLPC) and 11.7% (S100).

For suspensions of lecithin stabilized triglyceride platelets, the diameter-to-thickness aspect ratio η^{-1} depends on the applied homogenization pressure and the amount and type of the stabilizer.²¹ Furthermore, the aspect ratio increases with the length of the fatty acids of the triglyceride. This promotes the formation of platelet stacks and increases the viscosity of the suspensions in the sequence trilaurin (12 : 0) < trimyristin (14 : 0) < tripalmitin (16 : 0) < tristearin (18 : 0).^{19,21,25}

Thus, not only φ^{st} , but also φ^{lc} can depend on the choice of the triglyceride and stabilizers and the preparation conditions. For tristearin suspensions it can be anticipated, that φ^{lc} might be even lower than for the tripalmitin suspensions considered here.

To prevent the formation of stacks and liquid crystalline phases in triglyceride suspensions which is expected to enhance the long term stability and flow properties of the suspensions, the sample composition and preparation must be optimised to produce less anisometric solid lipid nanoparticles. Therefore, triglycerides with shorter acyl chains should be used. Stabilizers like DLPC and ionic co-stabilizers like cetylpyridinium chloride,²⁰ which promote the stack formation, should be avoided in favor of other stabilizers and co-stabilizers such as S100 and NaGC, respectively. The observation of liquid crystalline phases in a fresh or aged formulation in its native unsheared form might be regarded as a strong hint that the formulation is not suitable for an application since it bears the risk of gelling upon parenteral administration through narrow needles and thus embolism.

VII. CONCLUSION

Liquid crystalline phases were observed in suspensions of platelet-like tripalmitin nanoparticles stabilized with either the pure lecithin DLPC or purified soy bean lecithin (S100) at tripalmitin concentrations as low as 7 wt. % and, thus, in the range of potential pharmaceutical applications.

The formation of the liquid crystalline phase above a particular critical concentration (7 wt. % and 9 wt. % for the DLPC- and S100-stabilized suspensions, respectively) is ob-

served either directly by taking photographs of the suspensions located between crossed polarizers or by 2D SAXS patterns which indicate a preferential orientation of the platelets.

From the evaluation of SAXS patterns, taken from dilution series, the following mechanism for the formation of the liquid crystalline phase can be inferred: At low tripalmitin concentrations the isolated particles are randomly oriented. Above a first critical concentration the tripalmitin platelets exhibit the well-known self-assembly into small platelet stacks (stacked lamellae). For higher concentrations the fraction of the stacks in the suspension rises linearly up to a second critical concentration where the stacks and remaining single platelets start to merge to nematically ordered domains.

A simple geometrical model that takes the spherical excluded volumes of individual platelets and stacks into account has been derived. It facilitates the calculation of the critical volume fractions for the onset of the stack formation and the transition to the liquid crystalline phase, respectively. It can reproduce the two critical concentrations very well and supports the findings from the analysis of the SAXS patterns. The stacks typically consist of three to four platelets per stack up to the second critical concentration. For larger stacks the packing density does not significantly increase anymore or even decreases.

A more detailed study of the microscopic structure and domain sizes of the liquid crystalline phase indicates that the liquid crystalline phase consists of many small micron-sized domains with a nematic order rather than of a few domains of macroscopic size in the mm to cm range. The dimensionless number densities $n(D^3)$ for the platelets at the isotropic to liquid crystalline phase transition are in good agreement with the values reported for suspensions of Gibbsite platelets.

The more pronounced stack-related interference maxima in the SAXS pattern and the lower critical concentration for the isotropic to nematic phase transition of the DLPC-stabilized suspension can be explained by the larger diameter-to-thickness aspect ratio of the DLPC-stabilized dispersions with respect to the S100-stabilized dispersions.

In this study we tried to avoid shear stress of the suspensions as far as possible. For future studies it could be interesting to study formations of liquid crystalline phases using controlled shear conditions (as they appear for example in an administration with a syringe). Additional shear

stress could lead to more pronounced birefringence patterns and lower critical concentrations for the isotropic-liquid crystalline phase transition.

ACKNOWLEDGMENTS

This work was funded by Deutsche Forschungsgemeinschaft (DFG) through Project No. UN267/2-1 and the Cluster of Excellence Engineering of Advanced Materials. We acknowledge the RRZE for granting computation time at the Woodcrest HPC cluster. Susanne Linde from the EMZ Jena is acknowledged for the preparation of the freeze-fracture specimens. We thank C. Bär for designing the special sample holders needed for the SAXS studies.

APPENDIX: ESTIMATION OF THE PACKING DENSITIES OF RANDOMLY ORIENTATED POLYDISPERSE PLATELETS AND PLATELET STACKS

In the following, platelets with an elliptic base area and a log-normal distribution of the two main axes of the elliptic base area, D and $r \cdot D$ (r : fixed aspect ratio), respectively, are considered. As mentioned in Sec. V, geometric parameters like the platelet diameters D , the variance σ in D , the mean platelet thicknesses $\langle h \rangle$ and the repeat distance d_{st} in the stacks are normalized to the mean value $\mu = \langle D \rangle$ of the log-normal distribution for D . The normalized parameters are defined as $\tau = D/\mu$, $\sigma = \sigma/\mu$, $\eta = \langle h \rangle/\mu$, and $\xi = d_{st}/\mu$. Due to the normalization we use the log-normal probability density function (PDF) f_L with mean diameter 1 and variance σ for the new dimensionless long axis variable $\tau = D/\mu$,

$$f_L(\tau; \sigma) = \frac{e^{-\frac{[\ln(\tau\sqrt{1+\sigma^2})]^2}{2\ln(1+\sigma^2)}}}{\sqrt{2\pi\tau} \cdot \sqrt{\ln(1+\sigma^2)}}. \quad (\text{A1})$$

The probability $P_L(\alpha \leq \tau \leq \beta; \sigma)$ that τ is between a lower (α) and an upper (β) diameter reads as

$$P_L(\alpha \leq \tau \leq \beta; \sigma) = F_L(\beta; \sigma) - F_L(\alpha; \sigma), \quad (\text{A2})$$

where the cumulative distribution function F_L is given by integration of f_L as

$$F_L(\tau; \sigma) = \frac{1}{2} \operatorname{erf} \left[\frac{\ln[\tau\sqrt{1+\sigma^2}]}{\sqrt{2}\sqrt{\ln[1+\sigma^2]}} \right]. \quad (\text{A3})$$

The close packing condition in Eq. (8) for the exclusion volumes of individual platelets with monodisperse platelet diameters can be generalized to polydisperse individual platelets ($k = 1$) or stacks of $k > 1$ polydisperse platelets as

$$\varphi_{cp}(\sigma) = \frac{2}{3} \frac{\varphi_k^*}{r} \frac{1}{k} \frac{\langle \delta^3 \rangle_k}{\eta \langle \tau^2 \rangle_L}, \quad (\text{A4})$$

$$= \frac{2}{3} \frac{\varphi_k^*}{r} \frac{1}{k} \frac{\int_0^\infty \delta^3 f_k(\delta; \sigma, \eta, \xi, 0) d\delta}{\eta \int_0^\infty \tau^2 f_L(\tau; \sigma) d\tau}, \quad (\text{A5})$$

where φ_k^* is the maximum volume fraction we are looking for, if σ , η , r , and ξ are given. The new variable δ represents the dimensionless diameter for the exclusion spheres and the f_k

represent their corresponding PDFs that still have to be determined. φ_{cp} in Eq. (A5) is actually not a constant anymore. Because of the polydispersity of the platelet diameters, the sphere diameters of the excluded volumes become polydisperse, too. To estimate the range for φ_{cp} one can consider the packing fraction for random close packed (RCP) hard polydisperse spheres. Over a wide range of polydispersities for log-normally distributed spheres, it was shown that the packing fraction varies only slightly between 0.64 (RCP fraction for monodisperse spheres) and about 0.74.⁴² Because the diameters of the exclusion spheres are not necessarily log-normally distributed and probably closer directed towards a mono-disperse distribution, we can use $\varphi_{cp} \approx 0.74$ (close packing of monodisperse spheres) as a good approximation (as also used in Eq. (8)).

To find the PDFs f_k we consider in Eqs. (A6) and (A7) the probability P_k , that for a given stack of k platelets (platelets indexed by $i = 1, \dots, k$), the maximum of all distances δ_i (cf. inset in Figure 13) is lower than a given diameter δ of a sphere.

$$P_k(\alpha \leq \min_i \{\tau_i\} \wedge \max_i \{\delta_i\} \leq \delta) \\ = P_k(\alpha \leq \tau_1 \wedge \delta_1 \leq \delta, \dots, \alpha \leq \tau_k \wedge \delta_k \leq \delta), \quad (\text{A6})$$

$$= P_k(\alpha \leq \tau_1 \leq \beta_1, \dots, \alpha \leq \tau_k \leq \beta_k), \quad (\text{A7})$$

$$= \prod_{i=1}^k P_L(\alpha \leq \tau_i \leq \beta_i) = \prod_{i=1}^k P_L(\alpha \leq \tau \leq \beta_i). \quad (\text{A8})$$

Implicitly we assume in this model, that the central points of all platelets are located on a common axis. Furthermore, in Eq. (A6) we include already the effect of size fractionation by requesting that all platelet diameters τ_i in the stack are bigger

FIG. 13. Diameter distribution functions f_k for the spherical excluded volumes of stacks consisting of 2 to 5 platelets with ($\alpha = 1$, dashed line) and without size fractionation ($\alpha = 0$, solid line). $f_1(\delta)$, that is virtually identical to $f_L(D)$ for sufficiently small η , is also displayed. The parameters for σ , η , and ξ were used from the 7% tripalmitin suspension stabilized by DLPC (cf. Sec. VI E). The upper inset displays the model for a stack of 3 platelets with different diameters τ_i , the lower inset the platelet's elliptic cross-sections of the large faces. The diameter of the exclusion sphere δ is given as the maximum over all distances δ_i .

than a given value α . As illustrated in the inset of Figure 13 the δ_i are given by

$$\delta_i(\tau_i) = \sqrt{\tau_i^2 + [(i-1)\xi + \eta]^2} \quad i = 1, \dots, k. \quad (\text{A9})$$

After some transformations and using

$$\beta_i(\delta) = \sqrt{\delta^2 - [(i-1)\xi + \eta]^2} \quad i = 1, \dots, k, \quad (\text{A10})$$

the probabilities P_k can be finally expressed as in Eq. (A8) by the probabilities P_L (Eq. (A2)) of the log-normal distribution function for the individual platelets. In the last step of Eq. (A8) we can drop the index i in the τ_i . By differentiating Eq. (A8) with respect to δ , the PDFs f_k can be obtained. Using the symmetry of the arrangement of the platelets with respect to the center of the exclusion sphere the f_k can be written for even and odd k , respectively, as in Eqs. (A11) and (A12).

$$f_k(\delta; \sigma, \eta, \xi, \alpha) = \frac{2 \cdot \Theta(\delta - \delta_{min}^k)}{[P_L(\alpha \leq \tau \leq \infty)]^k} \left[\prod_{i=1}^{k/2} [P_L(\alpha \leq \tau \leq \beta_{2i})]^2 \right] \left[\sum_{j=1}^{k/2} \frac{f_L(\beta_{2j}; \sigma)}{P_L(\alpha \leq \tau \leq \beta_{2j})} \cdot \frac{d\beta_{2j}}{d\delta} \right], \quad (\text{A11})$$

$$f_k(\delta; \sigma, \eta, \xi, \alpha) = \frac{2 \cdot \Theta(\delta - \delta_{min}^k)}{[P_L(\alpha \leq \tau \leq \infty)]^k} \left[\prod_{i=1}^{\frac{k-1}{2}} [P_L(\alpha \leq \tau \leq \beta_{2i+1})]^2 \right] \left[\frac{1}{2} f_L(\beta_1; \sigma) \cdot \frac{d\beta_1}{d\delta} + \dots \right. \\ \left. P_L(\alpha \leq \tau \leq \beta_1) \cdot \sum_{j=1}^{\frac{k-1}{2}} \left(\frac{f_L(\beta_{2j+1}; \sigma)}{P_L(\alpha \leq \tau \leq \beta_{2j+1})} \cdot \frac{d\beta_{2j+1}}{d\delta} \right) \right]. \quad (\text{A12})$$

The derivatives $d\beta_i/d\delta$ in Eqs. (A11) and (A12) can be calculated from Eq. (A10). Θ denotes the Heaviside step function which ensures that f_k is 0 for all sphere diameters δ smaller than δ_{min}^k that is defined via Eq. (A9) as the smallest possible sphere diameter:

$$\delta_{min}^k = \delta_k(\alpha) = \sqrt{\alpha^2 + ((k-1)\xi + \eta)^2}. \quad (\text{A13})$$

Without size fractionation, i.e., $\alpha = 0$, δ_{min}^k reduces to $(k-1)\xi + \eta$, i.e., the length of the line connecting the platelets. The normalization factor $[P_L(\alpha \leq \tau \leq \infty)]^k$ in Eqs. (A11) and (A12) normalizes the PDFs f_k such that

$$\int_0^\infty f_k(\delta; \sigma, \eta, \xi, \alpha) d\delta = \int_{\delta_{min}^k}^\infty f_k(\dots) d\delta = 1 \quad (\text{A14})$$

holds. For $\alpha = 0$, i.e., without size fractionation, the normalization factor reduces to 1. With Eq. (A14) and using

$$\int_0^\infty \tau^2 f_L(\tau; \sigma) d\tau = 1 + \sigma^2, \quad (\text{A15})$$

we can rewrite Eq. (A5) as

$$\varphi_{cp} = \frac{2}{3} \frac{\varphi_k^*}{r} \frac{1}{k} \frac{\int_{\delta_{min}^k}^\infty \delta^3 f_k(\delta; \sigma, \eta, \xi, 0) d\delta}{\eta(1 + \sigma^2)}. \quad (\text{A16})$$

In the next step we revise Eq. (A5) to include size fractionation. This is done in Eqs. (A17) and (A18), where all platelets with (long) axis τ larger than the cut-off diameter α assemble in stacks with excluded volumes V_{ex}^{st} , and all platelets with $\tau < \alpha$ remain as individual platelets with excluded volumes V_{ex}^{sp} in the suspension. Since according to Eq. (A14) the PDFs f_k are normalized to 1 for $\delta > \delta_{min}^k$, we must introduce the weighting factor $P_L(\alpha \leq \tau \leq \infty)$ before the second integral, which is proportional to the number of

platelets that are larger than α . Since for the suspensions in this study the platelets are sufficiently thin (small η), we can also use the log-normal distribution $f_L(\tau) \approx f_1(\delta)$ for the excluded volume of the individual platelets instead of $f_1(\delta)$:

$$\varphi_{cp}(\sigma) = \varphi_k^* \frac{V_{ex}^{sp} + V_{ex}^{st}}{V_p} = \frac{2}{3} \frac{\varphi_k^*}{r} \frac{1}{\eta \langle \tau^2 \rangle_L} \left[\int_0^\alpha \tau^3 f_L(\tau; \sigma) d\tau + \frac{P_L(\alpha \leq \tau \leq \infty)}{k} \langle \delta^3 \rangle_k \right], \quad (\text{A17})$$

$$= \frac{2}{3} \frac{\varphi_k^*}{r} \frac{1}{\eta(1 + \sigma^2)} \left[\int_0^\alpha \tau^3 f_L(\tau; \sigma) d\tau + \frac{P_L(\alpha \leq \tau \leq \infty)}{k} \int_{\delta_{min}^k}^\infty \delta^3 f_k(\delta; \sigma, \eta, \xi, \alpha) d\delta \right]. \quad (\text{A18})$$

The first integral for the individual platelets in Eq. (A18) can be expressed analytically, however, the second integral for the stacks must be integrated numerically. For $\alpha = 0$, Eq. (A18) reduces to Eq. (A5).

With the parameters σ , η , and ξ for the DLPC-stabilized tripalmitin suspension (7%, cf. Sec. VI), Figure 13 depicts the PDFs f_k without ($\alpha = 0$) and with size fractionation ($\alpha = 1$, i.e., all platelets with diameters above the mean diameter μ are self-assembled in stacks). With size fractionation a sharper onset of the PDFs is observed compared to the PDFs with $\alpha = 0$.

Equation (A20), directly derived from Eq. (A5), can be used to calculate the φ_k^* for stacks of platelets with

monodisperse diameters:

$$\varphi_{cp} = \frac{2}{3} \frac{\varphi_k^*}{r} \frac{1}{k} \frac{[\delta_k(1)]^3}{\eta}, \quad (\text{A19})$$

$$= \frac{2}{3} \frac{\varphi_k^*}{r} \frac{1}{k} \frac{[1 + [(k-1)\xi + \eta]^2]^{3/2}}{\eta}. \quad (\text{A20})$$

One has to bear in mind that this simple geometric model cannot predict the critical concentrations for the appearance of stacks and the nematic phase with high precision. First of all the platelets do not really possess a cylindrical shape with elliptic or circular cross section. Furthermore, for small aspect ratios r (in particular for $r < 1/2$) a layer in the lamellae might be occupied by 2 or more platelets, which arrange in parallel to their rim faces in this layer. The same applies for high diameter polydispersities σ , where several small particles could arrange within the same stack layer. Both effects would shift φ_k^* to higher values. Also the approximation of $\varphi_{cp} = 0.74$ might cause an over-estimation of up to 10% for the φ_k^* at high polydispersities σ .

All calculations presented here were performed using the Mathematica program *polystacksweepvol*.⁴³

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