The Controlled Aggregation and Tunable Viscosity of Nanostructured Lipid Carrier Dispersions

Yihui Yang^a, Alessandro Corona III^b, Surita R. Bhatia^{c,d}, Michael A. Henson^{a,*}

^aDepartment of Chemical Engineering

University of Massachusetts, Amherst

Amherst, MA 01003

^bProcter & Gamble - Household Care

Fabric and Home Care Innovation Center

Cincinnati, OH 45217

^cDepartment of Chemistry

Stony Brook University

Stony Brook, NY 11794-3400

^dCenter for Functional Nanomaterials

Brookhaven National Laboratory

Upton, NY 11793

Abstract

Applications of solid lipid nanoparticles (SLNs) in drug delivery and the encapsulation of bioactive, lipophilic compounds have been hindered by the tendency of SLN suspensions to undergo uncontrolled aggregation due to polymorphic transformation of the lipid crystals. Second generation lipid nanoparticle systems have been developed by mixing

* Phone: 413-545-3481; Fax: 413-545-1647; E-mail: henson@ecs.umass.edu

* submitted to Colloids and Surfaces A

liquid lipid with solid lipid to form more stable nanostructured lipid carriers (NLCs). In this study, we investigated the effect of chemical formulation on the aggregation behavior and rheology of NLC dispersions. We found that NLC suspension viscosity could be modified by an order of magnitude by controlling particle aggregation with different surfactant and/or oil concentrations. The viscosity could be tuned by decreasing the amount of surfactant and/or oil to achieve a desired level of particle aggregation. Oscillatory sweep tests showed that non-aggregated and aggregated dispersions exhibited typical behaviors of a viscoelastic liquid and a viscoelastic solid, respectively. Modeling results suggested a stronger particle-particle bonding force and a higher aggregation efficiency as the amount of surfactant and/or oil was decreased. Both experimental and modeling results indicated that aggregated samples had an interconnected network structure, while no indication of network formation was observed for non-aggregated samples. Collectively these results suggest that controlled NLC aggregation can be exploited to develop dispersions with tunable viscosity for applications such as rheology modification.

Keywords: Solid lipid nanoparticles; Nanostructured lipid carriers; Particle aggregation; Rheology

1. Introduction

Solid lipid nanoparticles (SLNs) hold considerable promise for the delivery of pharmaceutical and other bioactive compounds [1-8]. Compared to alternative colloidal delivery systems, SLNs offer several advantages for encapsulation of bioactive components including improved physical stability, protection against chemical degradation, no biotoxicity, precise control over release rates, and easier manufacturing scale-up [1, 3, 4]. On the other hand, a major obstacle to the industrial use of SLNs is their strong tendency to undergo uncontrolled aggregation during storage at room temperature and the associated rejection of the bioactive compound from the solid lipid matrix [1, 2]. Several studies have indicated that particle aggregation is driven by the creation of hydrophobic surfaces when the lipid undergoes a polymorphic transformation from unstable α -form crystals into stable β -form crystals. These hydrophobic patches cannot be covered due to surfactant immobility and induce interparticle attraction that eventually results in uncontrolled aggregation [9-13]. More ordered crystal packing that accompanies the polymorphic transformation leads to partial rejection of the encapsulated ingredient from the solid lipid matrix [1, 2, 6].

The second generation of lipid nanoparticles, commonly termed nanostructured lipid carrier (NLC), was developed by mixing a liquid lipid (carrier oil) with the solid lipid prior to particle formation [6, 14-19]. The incorporation of carrier oil into the solid lipid matrix improves physical and chemical stability, loading capacity, and allows for controlled release by disrupting the crystal packing structure [16]. Many studies have been performed to investigate the effects of the solid lipid/carrier oil system on drug

loading and release properties [20-29]. Moreover, the stability of the dispersion against aggregation has been shown to be highly improved [15, 30].

Rheological properties such as viscosity are critical parameters of lipid nanoparticle dispersions for typical applications such as pharmaceutical, and cosmetic products [31-37]. A common method to produce lipid nanoparticle systems with the desired rheological properties involves incorporating the SLN or NLC dispersion into topical vehicles such as creams or hydrogels [38-40]. This approach has several disadvantages, including limited SLN/NLC loading, possible incompatibilities with the added viscosity enhancer and more complex manufacturing steps [34, 41]. Consequently, the creation of NLC dispersions with rheological properties that can be tailored without the use of additional ingredients is highly desirable.

A few previous studies have focused on the influence of key SLN properties (particle size, physical state of the lipid, emulsifier amount) on the rheological properties of the dispersion as well as understanding how the formulation can be modified to produce dispersions with the desired rheological properties without adding further ingredients [32-34]. Our previous study on NLC aggregation stability demonstrated that aggregation can be essentially eliminated by increasing the oil content of the particle and/or the amount of surfactant [30]. We hypothesized that the rheological properties of NLC dispersions could be tuned by controlling the degree of aggregation since properties such as viscosity should depend on the aggregated particle network structure [34, 42, 43]. To the best of our knowledge, the rheological properties of NLC dispersions with controlled aggregation have not been investigated.

In this study, we investigated the effect of formulation variables (dispersed phase, carrier oil and surfactant concentrations) on particle aggregation and rheological properties of NLC dispersions. Tristearin and triolein were selected as the solid lipid and carrier oil, respectively, since we have used this model system in our previous work on NLC dispersion stability [30]. A previously developed model for weakly aggregated dispersions [42, 43] was fit to our experimental data to provide insights into the aggregation effect. We believe that our study represents the first comprehensive investigation focused on controlling aggregation to tune the rheological properties of NLC dispersions and therefore an important step towards developing rational NLC design strategies for bioactive compound delivery and rheology modification.

2. Materials and methods

2.1 Materials

Tristearin and triolein were purchased from TCI America (Portland, OR). The surfactant Tween 60 was supplied by Procter and Gamble. All materials were used as received.

2.2 NLC preparation

NLC dispersions were prepared by using the hot homogenization method [1]. The dispersed phase was prepared by mixing tristearin and triolein at a temperature of 85°C, approximately 10°C above the melting point of tristearin. The aqueous phase was prepared by mixing deionized nanopure water with Tween 60 surfactant followed by heating to 85°C. Different NLC formulations consisting of 5-30wt% total lipid phase were prepared. The triolein content in the lipid phase varied from 5wt% to 50wt%. The

mass ratio of surfactant to dispersed phase varied from 1% to 20%. Coarse oil-in-water emulsions were prepared by mixing the lipid and aqueous phases together at 85°C using a high speed blender (Ultra-Turrax Model T18, IKA-Works Inc.) for 5 minutes at 16,000 rpm. Fine emulsions were prepared by passing the coarse emulsion through a high pressure homogenizer (Emulsiflex C-3, Avestin Inc.) 4 times at 800 bar with the temperature was maintained at 85°C. The coarse and fine emulsions were cooled in a refrigerator for 12 hours to obtain NLC dispersions, which were subsequently stored at room temperature.

2.3 NLC characterization

2.3.1 Particle size distribution

Particle size distributions of NLC dispersions were measured by static light scattering (Mastersizer 2000, Malvern Instruments). Refractive indices of 1.54 for the particles and 1.33 for water were used to calculate particle size distributions [44].

2.3.2 Rheology

The rheological measurements were performed with a stress-controlled rheometer (TA Instruments AR 2000) using an aluminum cone and plate geometry with a 20 mm diameter, 4° cone with a gap of 120 μm . All measurements were carried out at a temperature of 20°C. Continuous flow measurements were performed by increasing the shear rate from 0.001 s^{-1} to 100 s^{-1} and measuring the resulting viscosity. For some dilute samples, data at the lowest shear rates were below the limit of sensitivity of the rheometer's torque transducer; these data points are not shown. Additionally, a few more concentrated samples were observed to display some type of phase separation at the

highest shear rates, with one phase exhibiting opacity, a cream-like consistency, and a sudden increase in the viscosity. These data are also not shown. Oscillation stress sweep tests were performed at a frequency of 1 Hz in a stress range of 0.01-100 Pa. Oscillation frequency sweep tests were carried out over a range from 0.1 Hz to 10 Hz at stresses of 0.02 Pa and 4 Pa.

2.4 Mathematical model

A mathematical model developed to predict the steady shear viscosity of weakly aggregated dispersions [42, 43] was selected to fit our experimental data.

$$\eta = \mu \left(1 - \frac{\phi_a}{\phi_m}\right)^{-2.5\phi_m} + \frac{\sigma^*(\phi_p, \phi_a)}{\gamma} \quad (1)$$

$$\sigma^*(\phi_p, \phi_a) \approx \alpha \frac{F_c h_c}{a^3} \phi_a^2 \left(\frac{\phi_p}{\phi_a}\right)^{(5-2d_f-d_l)/(3-d_f)} \quad (2)$$

The steady shear viscosity of the dispersion is described by Eq. (1), where η is the viscosity of the dispersion, μ is the viscosity of the continuous phase, ϕ_a is the volume fraction of aggregates, ϕ_p is the volume fraction of particles, ϕ_m is maximum packing fraction, γ is the shear rate and σ^* can be interpreted as the yield stress of the system at fixed ϕ_a . The first term on the right hand side of Eq. (1) is the hydrodynamic contribution to the viscosity, while the second term is the structural contribution. The yield stress σ^* is approximated using Eq. (2), where α is the capture efficiency or the fraction of particle collisions resulting in rigid interaggregate chains, F_c is the bonding force between particles, h_c is the equilibrium value of the gap width between particles, a is the particle radius (we used the surface-averaged radius in our simulations), d_f is the fractal dimension of aggregates and d_l is the chemical dimension of chains [43]. In our study,

experimentally determined values of η , μ , ϕ_p and a were inputs to the model and ϕ_a was calculated from other parameters. The parameters F_c , h_c , d_f , d_l , ϕ_m and α were used to fit our experimental data by minimizing the following objective function using the MATLAB code `lsqnonlin`:

$$\Psi = \sum_{i=1}^n \left(\frac{\eta_{model,i} - \eta_{exp,i}}{\eta_{exp,i}} \right)^2 \quad (3)$$

where n is the number of data points, η_{model} is the calculated viscosity value and η_{exp} is the measured viscosity value. We performed preliminary sensitivity analysis to select model parameters that had a strong effect on viscosity predictions and were expected to vary with the formulation. The dataset for each formulation was fit individually to obtain a new set of parameters such that parameter trends could be analyzed. The function `lsqnonlin` did not provide information such as parameter confidence intervals, which was deemed acceptable for this problem due to the small sample size (e.g. each sample was fit individually).

3. Results

3.1 Effect of formulation variables on NLC dispersion aggregation and viscosity

The effect of dispersed phase concentration on the viscosity of stable (non-aggregating) NLC dispersions was studied at a constant ratio of solid lipid (tristearin)/carrier oil (triolein)/surfactant (Tween 60). Each sample was labeled with three different numbers: dispersed phase concentration, oil content in the dispersed phase and total surfactant content. For example, the sample labeled “5%L-10%O-1%S” contained 5wt% total dispersed phase (lipid phase) and 1wt% surfactant with the 5wt% dispersed phase

prepared by mixing 90wt% tristearin and 10wt% triolein. The particle size distributions (PSDs) from samples collected at different times are shown in Fig. 1 (a-c). In each plot, the black line is the PSD of the emulsion before cooling, while the red and blue lines are the PSDs when the dispersion was first removed from the refrigerator and right before the viscosity measurement, respectively. The PSDs of these three samples with dispersed phase concentrations of 5wt%, 10wt% and 20wt% were similar. No significant PSD changes were observed during storage, as indicated by the PSDs at different times largely overlapping. Therefore, we concluded that these samples exhibited negligible particle aggregation. The viscosity of each sample as a function of shear rate is presented in Fig. 1 (d). The results showed a shear thinning behavior for all three samples, as the viscosity decreased with increasing shear rate and reached an approximately constant value at high shear rates. As expected [32], the dispersion viscosity increased significantly as the dispersed phase concentration increased from 5wt% to 20wt%. Additionally, the sample a dispersed phase concentration of 20 wt% has a low shear viscosity similar to the 10 wt% sample, but maintains a higher viscosity at high shear rates. The difference between the low shear and high shear viscosity in dispersions is usually indicative of the importance of non-hydrodynamic effects; that is, the degree of structuring. It is likely that interparticle interactions play a larger role in the 20 wt% dispersions, perhaps leading to stronger ordering in these samples.

Fig. 1 Effect of dispersed phase concentration on the viscosity of non-aggregating NLC dispersions: (a)-(c) particle size distributions (PSD) of samples prepared with 5wt%, 10wt% and 20wt% dispersed phase concentrations; (d) steady shear viscosities of the three samples.

Next the effect of surfactant concentration on particle aggregation and dispersion viscosity was investigated. Three samples were prepared using 10wt% total dispersed phase, 10wt% oil in the dispersed phase, and 0.25wt%, 1wt% and 2wt% surfactant. The

PSD behaviors of the three NLC dispersions are shown in Fig. 2 (a-c). After 3 days significant aggregation was evident in the sample with 0.25wt% surfactant. By contrast, the sample with 1wt% surfactant showed little aggregation after 5 days and negligible aggregation was observed after 2 days when the surfactant content was increased to 2wt%. Our previous study on the aggregation stability of NLC dispersions showed that such samples remained stable and the PSD was unchanged beyond the first two days of storage at room temperature [30]. The long term stability of the PSD of the 0.25wt% surfactant sample is shown in Fig. 2 (a). The results showed no substantial change between the PSDs measured on days 3 and 128, especially with respect to large aggregates that increase dispersion viscosity. In our previous study [30], we showed that the PSD changed primarily during the first few days for stable NLC dispersions. We attributed the type of small PSD changes observed between days 3 and 128 in Figure 2a to measurement error, as the PSD analyzer imparts a small amount of shear during the measurement. Therefore, these results demonstrate that controlled aggregation of NLC dispersions can be achieved by varying the amount of surfactant. The steady shear viscosities of these three dispersions are shown in Fig. 2 (d). Despite having the same dispersed phase volume fraction, the three samples produced substantially different viscosity profiles. The 0.25wt% surfactant sample exhibited low shear viscosities that were more than two orders of magnitude larger than those at 2wt% surfactant. It should be noted that this sample contains some large aggregates ($\sim 100\text{ }\mu\text{m}$) that are on the order of the size of the rheometer gap ($120\text{ }\mu\text{m}$). Large aggregates may jam in the rheometer gap, which usually appears in the data as a sudden apparent increase in the viscosity, which we do not observe for this particular sample. The 1wt% surfactant sample

produced viscosities between the other two samples, suggesting that viscosity was directly affected by the degree of aggregation. These results indicated that the surfactant concentration can be adjusted to obtain controlled aggregation and tunable viscosity behavior.

Fig. 2 Effect of surfactant concentration on the aggregation and viscosity of NLC dispersions: (a)-(c) PSDs of samples prepared with 10wt% dispersed phase concentration

and 10wt% oil containing 0.5, 1 and 2wt% surfactant; (d) steady shear viscosities of the three samples.

Samples with the same dispersed phase and surfactant concentrations but different oil contents in the lipid phase were prepared to investigate the effect of oil content on dispersion aggregation and viscosity. Three samples were prepared using 10wt% dispersed phase concentration, 1wt% surfactant concentration and oil contents in the lipid phase of 5wt%, 10wt% and 30wt%. PSDs collected for sample are presented in Fig. 3 (a-c). Paralleling the results for surfactant concentration (Figure 2), substantial aggregation was observed in the 5wt% oil sample, little aggregation was observed in the 10wt% oil sample and negligible aggregation was observed in the 30wt% oil sample. The long term stability of the PSD of the 5wt% oil content sample is shown in Fig. 3 (a). The PSDs exhibited only small differences when measured on days 3 and 130. When combined with the results in Figure 2 (a) and in our previous paper [30], these results indicate that NLC samples that were stable after 2-3 days remained stable to additional aggregation for much longer periods. The steady shear viscosities of the three dispersions are shown in Fig. 3 (d). Qualitatively similar results to those observed by changing the surfactant concentration were obtained. More specifically, low shear viscosities were increased by more than an order of magnitude by reducing the oil content from 30wt% to 5wt%. Collectively these results suggested that the surfactant concentration and/or the oil content could be reduced to obtain controlled aggregation and tunable viscosity behavior at a constant dispersed phase concentration.

Fig. 3 Effect of oil content on the aggregation and viscosity of NLC dispersions: (a)-(c) PSDs of samples prepared with 10wt% lipid concentration and 1wt% surfactant containing 5, 10 and 30wt% oil; (d) steady shear viscosities of the three samples.

For substantially aggregated samples (e.g. Figures 2 (a) and 3 (a)), the effect of the dispersed phase concentration on viscosity was also studied. For samples with 5wt% and 10wt% dispersed phase concentrations, the mass ratio of lipid/oil/surfactant was held constant. However, the sample prepared with 20wt% dispersed phase concentration using

the same mass ratio was visually observed to form a gel during storage. In an attempt to achieve roughly the same degree of aggregation as the other two samples, the amount of surfactant used in the 20wt% dispersed phase concentration sample was doubled. All three samples exhibited a substantial but controlled amount of aggregation as shown in Fig. 4 (a-c). The steady shear viscosities of the three dispersions are shown in Fig. 4 (d). Similar to non-aggregated samples (Fig. 1), the dispersed phase concentration appeared to be an important parameter that affected the viscosity of aggregated dispersions. However the interpretation of the aggregated results was more difficult because the samples exhibited different degrees of aggregation, which perhaps resulted in the 10wt% and 20wt% dispersed phase concentration samples producing similar viscosities below 1 s^{-1} . Additionally, the rheometer geometry is likely impacting the data at high shear rates for the sample with 1 wt% surfactant (Fig. 4c and 4d). This sample contains some aggregates that are larger than the instrument gap, and an anomalous increase in the viscosity is seen for this sample at high shear rates. It is likely that there is some jamming of the particles in the gap at higher shear rates. Although not shown here, we found that controlled aggregation was difficult to achieve at dispersed phase concentrations greater than 30wt%.

Fig. 4 Effect of dispersed phase concentration on the viscosity of aggregated NLC dispersions: (a)-(c) PSDs of samples prepared with 5wt%, 10wt% and 20wt% dispersed phase concentrations and the amounts of oil and surfactant indicated; (d) steady shear viscosities of the three samples.

3.2 Oscillatory sweep experiments

To gain a better understanding of the microstructure of our NLC dispersions, oscillation stress and oscillation frequency sweep experiments were performed on non-aggregated and aggregated NLC dispersions with 5wt% and 10wt% dispersed phase concentration. An oscillation stress sweep test at a constant frequency of 1 Hz was performed for each sample to determine the linear viscoelastic region over which the aggregated network structure was intact. These tests yielded the complex modulus (G^*) as a function of stress at a constant frequency. The complex modulus is a measure of the total resistance of the sample to strain. The linear viscoelastic region is the range of stress over which G^* and

the phase angle δ are independent of the applied stress [34]. The stability of the system is also reflected in the change of phase angle δ from the elastic region ($\delta < 45^\circ$) to the viscous region ($\delta > 45^\circ$).

A representative oscillation stress sweep is shown in Fig. 5 (a) for an aggregated sample with 10wt% dispersed phase. The linear viscoelastic region extended to a stress of 0.06 Pa, at which point the complex modulus decreased dramatically with increasing stress. As the stress was increased, the phase angle increased from a value less than 10° (elastic region) to a value around 80° (viscous region). The results indicated the destruction of the internal network structure of the aggregated dispersion. A sample with more stable structure would be expected to have a wider linear viscoelastic region than a sample with a weaker structure [34]. The oscillation stress sweep data for four representative NLC dispersions are shown in Fig. 5 (b). All four samples had the same oil content of 10wt% and either 5wt% or 10wt% dispersed phase concentration. The two samples prepared with a 40:1 dispersed phase/surfactant ratio (labeled 5-10-0.125 and 10-10-0.25) exhibited a substantial degree of aggregation, while the two samples prepared with a 5:1 dispersed phase/surfactant ratio (labeled 5-10-1 and 10-10-2) exhibited a negligible amount of aggregation. The stress sweep data showed that both aggregated samples have much larger G^* than the non-aggregated samples. For the non-aggregated samples, there was no significant change of G^* over the range of measurement and the fluctuations at low stress were due to limitations of the equipment. For the aggregated samples, the size of the linear viscoelastic region increased as the dispersed phase concentration was increased from 5wt% to 10wt%. For both types of dispersions, the 10wt% samples produced larger G^* values than the 5wt% samples. The stress values of 0.02 Pa and 4 Pa

were selected in the linear viscoelastic regions of the aggregated and non-aggregated samples, respectively, for the oscillation frequency sweep tests presented below.

(a)

(b)

Fig. 5 Oscillation stress sweep tests: (a) aggregated dispersion with 10wt% dispersed phase, 10wt% oil and 0.25wt% surfactant. (b) aggregated (5wt% dispersed phase-10wt% oil-0.125wt% surfactant and 10wt% dispersed phase-10wt% oil-0.25wt% surfactant) and non-aggregated samples (5wt% dispersed phase-10wt% oil-1wt% surfactant and 10wt% dispersed phase-10wt% oil-2wt% surfactant).

Oscillation frequency sweeps were conducted to study the viscoelastic behavior of aggregated and non-aggregated dispersions and the network structure formed by particle interactions. An oscillation frequency sweep measures the response of the dispersion as a function of frequency at a constant stress. From these experiments, we obtained the storage modulus G' (elastic response), which indicated the energy stored in the system after an applied stress, and the loss modulus G'' (viscous response), which reflected the energy loss after an applied stress. If performed within the linear viscoelastic region, oscillatory frequency sweeps provide information about the system structure under non-destructive conditions [34]. The dispersions were measured without significantly disrupting their structure, as may occur in a continuous shear flow experiment [45].

Fig. 6 (a) presents the oscillation frequency sweep data for two non-aggregated samples with 5wt% and 10wt% dispersed phase (again labeled 5-10-1 and 10-10-2). These two samples produced similar data including an increase of G' and G'' with increasing frequency. The fluctuations of G' at low frequencies were due to the low torque limit of the equipment. At low frequencies G'' was larger than G' , while at higher frequencies G' was larger than G'' . For two aggregated dispersions (again labeled 5-10-0.125 and 10-10-0.25), very different rheological behavior was observed as shown in Fig. 6 (b). More specifically, the aggregated samples produced much larger values of both moduli than the

non-aggregated samples and both G' and G'' of the aggregated samples exhibited only weak dependency on frequency. Because the aggregated samples produced G' values approximately one order of magnitude larger than the corresponding G'' values over the whole frequency range, these samples satisfy the rheological definition of a gel. That is, G' is greater than G'' over the entire measureable frequency range, and G' is fairly independent of frequency. However, it is a very weak gel, with $G' = 20\text{-}30$ Pa. These samples also were observed to flow upon sample inversion; that is, if a vial of these samples is turned upside down, the gel is not strong enough to support its own weight, and the sample will slowly flow. This has been observed with other weak gels of biopolymers and block copolymers; although G' is greater than G'' and is frequency-dependent, the samples do not appear visually to be gels [46].

(a)

(b)

Fig. 6. Oscillation frequency sweep tests: (a) non-aggregated samples (5wt% dispersed phase-10wt% oil-1wt% surfactant and 10wt% dispersed phase-10wt% oil-2wt% surfactant); (b) aggregated samples (5wt% dispersed phase-10wt% oil-0.125wt% surfactant and 10wt% dispersed phase-10wt% oil-0.25wt% surfactant)

To better visualize the effect of formulation variables on the degree of particle aggregation, the state diagram shown in Fig. 7 was generated. The diagram only includes samples with 10wt% oil content, as all samples prepared with 30wt% and 50wt% oil content were stable against particle aggregation (not shown). Additionally, the states shown in Fig. 7 represent samples at rest; this diagram does not account for samples that may gel or phase separate at high shear rates. We referred to samples with no PSD change during storage or with an area of the second peak at large size less than 3% of the total volume as non-aggregated samples. Samples that exhibited aggregation but with an

area of the second peak less than 15% were classified as marginally aggregated samples. If the area of the second peak was larger than 15vol% but the sample did not visually form a gel, we used term controlled aggregation. As we mentioned above, even some of the controlled aggregated samples satisfy the rheological definition of a gel, albeit a weak gel as the samples visually still appear to be fluids. Here, we use the term strong gel to refer to samples that visually appear to be gels; that is, those samples that do not visually flow and support their own weight when inverted. However, we also expect these samples to behave rheologically as gels, with the storage modulus G' mainly independent of frequency and larger than the loss modulus G'' for all frequencies measured.

The black data points in Fig. 7 represent non-aggregated samples. The area under the black line is the region where non-aggregated samples definitely would be formed, since decreasing dispersed phase concentration and/or increasing surfactant concentration will result in less aggregation. The green data points represent samples that formed strong gels. The area above the green line is the region where strong gelled samples definitely would be formed, since higher dispersed phase concentration and/or less surfactant will facilitate gelation. No gelation was visually observed for samples with 5wt% and 10wt% dispersed phase concentrations, although again as we showed above, some of these samples rheologically displayed significant elasticity. If dispersion occupied the area between the two lines, the sample could be in the non-aggregation regime, the marginally aggregated regime (red points), the controlled aggregation regime (blue points) or the strong gel regime depending on the formulation.

Fig. 7 State diagram with 10wt% oil content for NLC dispersions at rest. Black squares: no aggregation; red circles: marginally aggregation; blue triangles: controlled aggregation; green diamonds: strong gel formation.

3.3 Modeling results

The mathematical model described in Section 2.4 for prediction of the steady shear viscosity of weakly aggregated dispersions [42, 43] was fit to our shear rate versus viscosity data. The model fitting parameters were F_c , h_c , d_f , d_l , ϕ_m and α . Each formulation was fit individually to obtain a new set of parameters such that parameter trends could be analyzed. A representative set of model fitting results for NLC dispersions prepared with 10wt% oil concentration, 10wt% oil content and 0.25-2.0% surfactant concentration are shown in Fig. 8. The fitted models provided satisfactory viscosity predictions over the wide range of shear rates studied.

(a)

(b)

(c)

(d)

Fig. 8 Viscosity model fitting for NLC dispersions prepared with 10% dispersed phase concentration, 10% oil content and 0.25-2% surfactant concentration.

Two fitting parameters (d_l , ϕ_p) showed no obvious trends with respect to the NLC formulation (not shown). By contrast, clear trends were observed for the remaining three parameters: the bonding force F_c , the fractal dimension d_f and the aggregation efficiency

α . These trends are shown in Fig. 9 for samples prepared with 10wt% dispersed phase concentration. The fitting parameters obtained for samples prepared with 10% oil content and different surfactant concentrations are shown in Fig. 9 (a). Both F_c and α decreased as the surfactant concentration was increased, suggesting diminished bonding forces between particles and a reduced aggregation efficiency. The fractal dimension d_f increased from about 1.7 for low surfactant samples to about 2.2 for samples with high surfactant. Similar trends were observed for samples prepared with 1% surfactant concentration as the oil content was increased from 5wt% to 50wt% (Fig. 9 (b)).

We hypothesized that increasing surfactant concentration resulted in increased surfactant coverage on particle surfaces, reduced hydrophobic attractive forces between particles and weaker bonding forces. Similarly, increased surface coverage could lead to improved particle stabilization and reduced aggregation efficiency. We further hypothesized that increasing oil content increased the mobility of surfactant molecules on particle surfaces and inhibited particle shape change, both of which will resulted in weaker hydrophobic attractive forces and reduced aggregation. The observed variations in the fractal dimension parameter indicated an internal structure change of the aggregated dispersions. More specifically, the fractal dimension increase suggested the internal structure changed from porous network to more compact structure [47, 48] as the surfactant concentration and/or oil content was increased.

(a)

(b)

Fig. 9 Viscosity model fitting parameters for NLC dispersions with: (a) 10% dispersed phase concentration, 10% oil content and 0.25-2% surfactant concentration; (b) 10% dispersed phase concentration, 1% surfactant and 5-50% oil content.

4. Discussion and Conclusions

Our experimental results demonstrated that the rheological properties of NLC dispersions can be substantially modified through controlled aggregation of the lipid nanoparticles. While the viscosity can be increased through the usual approach of simply increasing the dispersed phase concentration (Fig. 1), controlled aggregation represents a potentially more cost effective approach for producing large dispersion volumes with tunable viscosity. We showed that the viscosity could be increased by a least of magnitude at moderate shear rates ($<10 \text{ s}^{-1}$) by decreasing the surfactant concentration (Fig. 2) and/or the amount of carrier oil mixed with the lipid prior to solid particle formation (Fig. 3) through controlled aggregation without changing the dispersed phase concentration. We attributed the increased viscosity of the aggregated dispersions to increased hydrophobic attraction between particles mediated by low surfactant concentrations and/or low oil contents. Both factors resulted in increased particle aggregation and the formation of an internal network structure within the dispersion. While additional increases in NLC dispersion viscosity could be obtained by increasing the dispersed phase concentration (Fig. 4), we found that controlled aggregation was difficult to achieve at total lipid concentrations exceeding 30wt%.

Oscillation stress sweep tests on aggregated samples indicated a linear viscoelastic region where the dispersions behaved like an elastic solid (Fig. 5). When the stress was increased beyond the viscoelastic region, the complex modulus decreased sharply with

increasing stress indicating the breakdown of the internal network structure. The viscoelastic region became wider as the dispersed phase concentration was increased from 5wt% to 10wt%, suggesting a more rigid structure due to increased particle interactions [34]. Oscillation frequency sweep tests performed in the linear viscoelastic region indicated that non-aggregated dispersions behaved like a viscoelastic liquid with a more viscous response at low frequencies (<2 Hz) and a more elastic response above this crossover frequency (Fig. 6). As expected, aggregated dispersions produced more complex oscillation frequency sweep results. Both the storage modulus G' and the loss modulus G'' were much larger than the corresponding values for non-aggregated dispersions, and both moduli showed only a weak dependency on the applied frequency which is typical for a viscoelastic solid [34]. The storage modulus was approximately one order of magnitude larger than the loss modulus over the entire range of applied frequencies, which indicated the presence of a gel-like structure. However, these aggregated dispersions are actually weak gels and most likely have very weak network structures. Our experimental results were collectively used to produce a state diagram that showed the effect of the surfactant concentration and the oil content on the degree of particle aggregation for NLC dispersions with 10wt% dispersed phase concentration (Fig. 7).

A previously developed viscosity model for weakly aggregated dispersions fit to the viscosity versus shear rate data for different NLC formulations (Fig. 8) produced clear trends in three fitting parameters (Fig. 9). The bonding force parameter F_c decreased as the surfactant concentration or oil content was increased, suggesting that attractive hydrophobic forces between particles were decreased. A similar trend was observed for

the aggregation efficiency parameter α as increased amounts of surfactant or oil stabilized the particles and reduced the efficiency of particle collisions. When the surfactant concentration or oil content was increased, the fractal dimension parameter d_f increased from about 1.7 for aggregated samples to roughly 2.3 for non-aggregated samples. The d_f value of 1.7 obtained for aggregated samples indicated the formation of a porous, interconnected network structure in the aqueous liquid [47], which was consistent with our results from oscillatory sweep experiments. Conversely the d_f value of roughly 2.3 obtained for non-aggregated samples suggested a more compact structure [48] and should represent the fractal dimension of individual particles.

Collectively our results have demonstrated that the viscosity of NLC dispersions can be tuned by controlling the degree of particle aggregation through appropriate selection of the surfactant concentration and/or oil content of the formulation. The controlled aggregation mechanism provides a means to dramatically increase the dispersion viscosity without altering the dispersed phase concentration. As a result, partially aggregated NLC dispersions hold promise as low cost viscosity enhancers for applications in the food, pharmaceutical and consumer product industries.

Acknowledgements

The authors wish to acknowledge Procter and Gamble for project funding, the NSF-funded MRSEC on Polymers at the University of Massachusetts Amherst, and the Center for Functional Nanomaterials, Brookhaven National Laboratory, which is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, under Contract No. DE-SC0012704.

Nomenclature

a	particle radius, m
d_f	fractal dimension of aggregates, dimensionless
d_l	chemical dimension of chains, dimensionless
F_c	bonding force between particles, N
G^*	complex modulus, Pa
G'	storage modulus, Pa
G''	loss modulus, Pa
h_c	equilibrium value of the gap width between particles, m

Greek letters

α	aggregation efficiency, dimensionless
γ	shear rate, s ⁻¹
δ	phase angle, °
η	viscosity of the dispersion, Pa·s
μ	viscosity of the continuous phase, Pa·s
σ^*	yield stress, Pa
ϕ_a	volume fraction of aggregates, dimensionless
ϕ_m	maximum packing fraction, dimensionless
ϕ_p	volume fraction of particles, dimensionless

Acronyms

NLC	nanostructured lipid carrier
SLN	solid lipid nanoparticle

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