Soft Matter

Reply to the Comment on "Bilayer Aggregate Microstructure Determines Viscoelasticity of Lung Surfactant Suspensions" by C. O. Ciutara and J. A. Zasadzinski, Soft Matter, 2021, 17, 5170-5182

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ARTICLE

Reply to Berret Comment on "Bilayer Aggregate Microstructure Determines Viscoelasticity of Lung Surfactant Suspensions"

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In their comment, Berret suggests that Curosurf, one of three clinical lung surfactant aqueous suspensions examined in the Soft Matter, 2021, **17** 5170-51820 [1] is a Newtonian liquid rather than a shear-thinning soft solid with a small, but measurable yield stress. We postulate that these discrepancies may be due to the size of the magnetic wire measurement probe used in their paper (Thai et al., Colloids and Surfaces B:Biointerfaces, 2019, **178**, 337-345 [3]) the diameter of which is similar in size to the Curosurf bilayer agregates (1-10 μm). The cone and plate rheometer used by Ciutara and Zasadzinski measures averaged effects over the entire macroscopic sample. Our combined results point out that the local viscoelastic properties of a moderately dense suspension may be different than its bulk properties.

1 Introduction

The clinical lung surfactants Curosurf, Infasurf and Survanta are aqueous suspensions of animal-derived lipid-protein bilayer aggregates that range in size from 1- 25 μm and are used to treat neonatal respiratory distress (NRDS) in premature infants. During treatment, a minimal amount of these surfactant suspensions is delivered to the premature infant's lungs via an intratracheal tube. Maximizing the surfactant loading is important to minimizing the volume of suspension to be delivered, which is key to treatments of the most premature and smallest infants. In our work, we examined the relationships between surfactant loading per unit suspension volume and suspension viscoelasticity and the relationship between loading and the structure of the surfactant aggregates. Curosurf, which has compact, onion-like spherical aggregates packs more surfactant in each aggregate compared to Infasurf, which consists of agglomerations of smaller vesicles with interior water cavities. Survanta forms rigid, higher aspect ratio aggregates that pack the least amount of surfactant per suspension volume. We related these variations in aggregate structure to the differences in surfactant composition. The surfactant loading is limited by the suspension viscoelasticity, which can restrict flow through the intratracheal tube and within the lung itself.

Many liquid paints, inks, consumer products and medicines such as clinical lung surfactants are suspensions of colloidal particles in Newtonian fluids like water or saline. Particles in a liquid act as obstacles, hindering the liquid's flow and therefore increasing the flow resistance or viscosity [4]. Even at low particle volume fractions, the viscosity of the suspension is increased. With increasing particle volume fraction, the probability of particle-particle interactions increases leading to transient clustering or network formation. This transient microstructure can lead to shear thinning fluids; small shear rates or shear strains may not be able to break down the microstructure while larger shear rates or strains can break down the structure, restoring the material viscosity to the Newtonian behavior of the suspending liquid [4].

The comment by Berret states that Curosurf behaves as a Newtonian fluid when analysed using 5-40 μm long, 1- 3 μm diameter rotating magnetic wires, rather than a viscoelastic soft solid with a small yield stress as reported in Ciutara and Zasadzinski when analysed in a cone and plate rheometer [1]. A Newtonian fluid is defined by having a *constant viscosity* that is independent of shear rate and near zero elastic modulus, or $G'' \gg G'$ [4]. The rheological results presented in the Ciutara paper [1] were obtained using a stress-controlled TA Instruments AR-G2 cone and plate rheometer with a 40 mm diameter and 2° angle cone. General practice suggests that the gap distance be ~ 5 times the largest particle size. Curosurf aggregates range from 1- 10 μm in diameter [1, 5] which led us to use a gap width of 49 μm. Small amplitude oscillatory shear (SAOS) was used to determine the linear viscoelastic moduli G' and G''. The oscillation amplitudes were adjusted to ensure $G'$and G'' were independent of the applied strain between 0.1-1 Hz. The critical strain, γ_c, is set at the strain that reduces G' by ≤ 10%. The 0.5% critical strain for Curosurf was small compared to ≥ 1% for Survanta and Infasurf. Figure 1 shows that while G' > G" for Curosurf for strains ≤ 0.5%, larger strains cause G" ≥ G' and these larger strains likely break down the suspension microstructure. This may be the case for the large local strains imposed by the unsteady rotation of the magnetic needle technique discussed in the comment [3].

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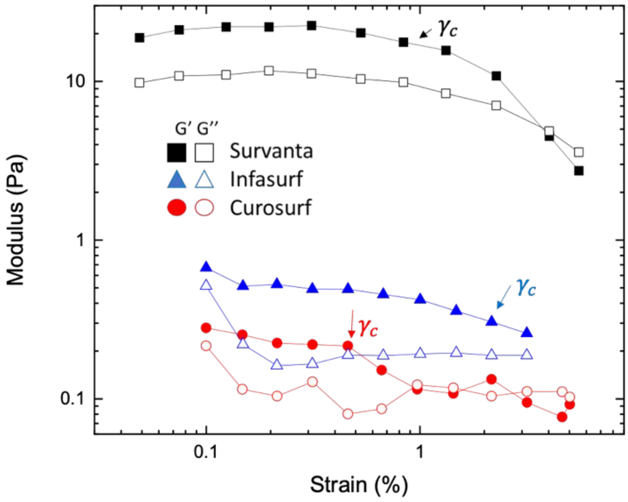

Figure 1. (From Supplementary Materials of Ciutara, Fig S1 [1]) Strain amplitude sweep for linear oscillatory shear experiments. The critical strain, γ_c, is determined as being the strain amplitude that causes the maximum G' to decrease 10%. G' for Curosurf decreases significantly with increasing strain showing that the structures responsible for the elasticity break down for relatively small strains. For strains ≥ 1%, G" ≥ G' for Curosurf. All SAOS measurements for Curosurf were conducted with ≤ 0.5% strain to produce accurate and reproducible results [1].

Figure 2 shows measurements of the elastic (G') and viscous (G'') moduli for the three surfactant suspensions [1]. We find that all three clinical surfactant suspensions had G' > G" if the appropriate strain was used to ensure that the system was in the linear viscoelastic regime (Figure 1). This result is consistent with significant microstructure in the suspension that stores elastic energy over this frequency range, which is commonly the case for moderately dense suspensions [4]. $|\eta*| = \sqrt{(G'^2 + G''^2)}/\omega$ for Survanta is also around two orders of magnitude larger than that of Curosurf or Infasurf, similar to the trend in steady shear viscosity [1]. No direct measurements of G' were presented by Berret and co-workers in their Comment. The Supplemental Materials of the Thai et al. paper [3] states that a 10% strain amplitude was used for their measurements presented in Fig. 1 of the Berret comment. From Figure 1, this strain amplitude is much larger than the 0.5% critical strain amplitude for Curosurf. As can be seen in Figure 1, G' decreases with increasing strain amplitude for all three surfactant suspensions, with G" becoming greater than G'. Thai et al did estimate G' from their wire rotation measurements using a Maxwell model as stated toward the end of their paper [3]:

> "An analysis on six different wires yields τ = 0.1 s and from the relationship G' = η/τ and an elastic modulus G' = 0.2 Pa."

From Fig. 2 our measured G' for Curosurf ranges from 0.1 to 0.2 Pa as a function of frequency. Hence, we agree with Thai et al [3] about the small, but finite value of G'. This value of G' is also consistent with Curosurf having G' > G" over this frequency range, consistent with a soft viscoelastic solid, and not a Newtonian liquid (G" >> G').

Figure 3 shows the steady shear viscosity of Survanta, Infasurf and Curosurf at 37°C along with the data for the "Ramp-up" viscosity vs shear rate in Figure 2D of the comment. Our data shows that all three surfactants are shear-thinning; the viscosity of Survanta decreases by more than four orders of magnitude over the tested shear rates, while that of Infasurf and Curosurf decrease by two orders of magnitude. Figure 2D of the comment shows that Berret finds similar shear thinning behaviour for Curosurf and that our results agree qualitatively and quantitatively with their results

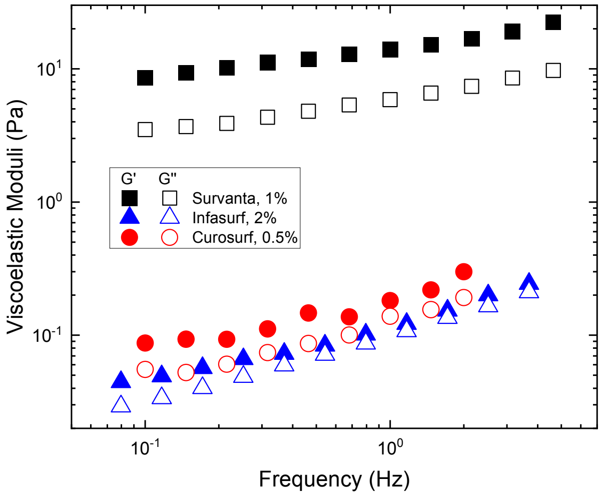

Figure 2. (From Figure 5 of Ciutara [1]) Linear shear rheology of Survanta, Infasurf and Curosurf at oscillatory strains less than or equal to the critical strain amplitude evaluated in Figure 1. All three materials had G'>G" consistent with significant microstructure in the suspension that could store elastic energy over this frequency range. $|\eta*| = \sqrt{(G'^2 + G''^2})/\omega$ for Survanta is also around two orders of magnitude larger than that of Curosurf or Infasurf, similar to the trend in steady shear viscosity (Fig. 3).

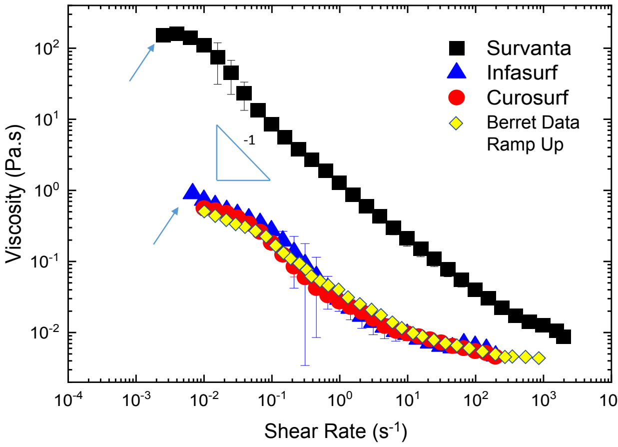

Figure 3. Steady shear viscosity vs shear rate for the clinical surfactants Survanta, Curosurf and Infasurf at 37°C from Figure 1 of Ciutara [1] and from Figure 2 of the comment by Berret. The three surfactants are shear-thinning and follow a power-law relationship $\eta = a\dot{\gamma}^m$ with $m \approx -1$ over shear rates from 10^{-2} to 10^2 s^{-1}. At the lowest shear rates, a constant viscosity plateau (arrows) indicates slip of the concentrated suspension at the walls of the cone and plate [2, 4]. See Figure 4.

(Figure 3). Shear thinning at low shear rates was observed previously in similar lung surfactant dispersions [6, 7], and in other bilayer systems [8, 9].

The shear thinning of the three surfactants followed a power-law relationship $\eta = a\dot{\gamma}^m$ over shear rates from 10^{-2} to 10^{2} s^{-1} with $-1 \le m \le -0.8$. Over these shear rates, the shear stress, $\tau = \eta\dot{\gamma}$, is roughly constant, suggesting that these suspensions are sufficiently concentrated to have a yield stress [4]. We define yield stress fluids as power law fluids with m values close to -1, instead of fluids that do not flow at all at low shear.

The yield stress in suspensions must be overcome to initiate flow by deforming the individual particles or by breaking down *long-range structure* in the suspension. Figure 4 shows the viscosity as a function of shear stress, $\tau(\dot{\gamma}) = \eta\dot{\gamma}$. All three surfactant suspensions show a nearly constant viscosity below the suspension yield stress. $\tau_{yield} \approx 1$ Pa for Survanta and 0.01 – 0.03 Pa for Curosurf and Infasurf (arrows in Fig. 4). At the yield stress the viscosity drops by orders of magnitude, followed by shear thinning at higher shear stresses. Similar results were obtained for Survanta using a sweep at constant stress (open symbols). For both systems, the constant viscosity plateau at low shear stress is consistent with wall slip below the yield stress, which is typical for soft viscoelastic solids [2]. The wall slip likely causes the magnitude of the yield stress to be under-estimated [2]. The two orders of magnitude difference in yield stress carries over from the two orders of magnitude difference in viscosity between Survanta and Infasurf and Curosurf (Fig.3). All three surfactants have the signature of a yield stress as described in textbooks such as *Colloidal Suspension Rheology* by Mewis and Wagner [4] and the classic paper by Russel and Grant [2].

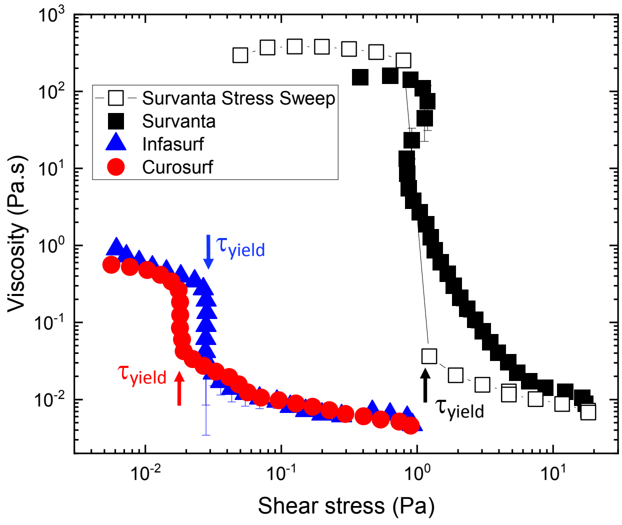

Figure 4. Viscosity vs. shear stress [1]. All three surfactants exhibit the features of yield stress fluids. The Newtonian plateau at shear stresses below the yield stress (arrows) is indicative of wall slip, which is common to concentrated suspensions [2]. The viscosity drops by more than an order of magnitude at the yield stress, and the fluid is weakly shear thinning at higher stresses. The error in measurements is within the size of the symbols used unless noted.

Concentrated foams and emulsions, which are similar in microstructure to concentrated surfactant suspensions yield at applied stresses such that $\tau_{yield} \approx {G'}/{10}$ [10, 11]. For Survanta $G' \approx 10\ Pa$ and $\tau_{yield} \approx 1\ Pa$, while for Infasurf and Curosurf, $G' \approx 10^{-1}\ Pa$ and $\tau_{yield} \approx .01\ Pa$, surprisingly consistent with this prediction for emulsions [12]. Hence, our SAOS and steady shear measurements are internally consistent and show all three lung surfactant suspensions, including Curosurf, are soft solids (G' > G") with small, but measurable yield stresses.

Reconciling the Results

How can we reconcile our macroscopic rheological results that show Curosurf is a shear-thinning soft solid with a small yield stress with Berret's claim that Curosurf behaves as a Newtonian fluid when analysed using rotating wires of dimensions on the order of the suspension particles? One possible explanation is that for shear rates > 5 s^{-1}, Infasurf and Curosurf are less shear thinning and asymptote to a nearly constant viscosity of $\sim 4 \; x\; 10^{-3}$ Pa.s similar to the continuous saline suspending fluid of viscosity ~ 10^{-3} Pa.s. The shear rates in the rotating magnetic wires are not reported and likely vary along the wire [3].

A more likely explanation is that the wire does not interact with the collective motions and interactions of the particles in suspension, but rather slips through the saline suspending fluid by slightly displacing the particles. The wire diameters are 1-3 µm, which is same magnitude as the Curosurf particle sizes of 1-10 µm. This decoupling from the suspended particles also occurs in the cone and plate rheometer at shear stresses lower than the yield stress in Figure 4. All three suspensions have a constant viscosity plateau (arrows in Fig. 3) at shear rates of 10^{-2} s^{-1} and below that can be interpreted as wall slip, which is common in concentrated suspensions [2, 4]. This explanation is also consistent with the increase in viscoelasticity in Curosurf suspensions crosslinked by alumina nanoparticles reported by Berret and co-workers [13]. The slip of the wire or the sweeping out of the Curosurf particles would be prevented by the large-scale crosslinked network induced by the nanoparticles, and as Berret and co-workers found, the suspension became viscoelastic with G' > G".

Acknowledgements

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