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## Shear-induced aggregation and break up of fibril clusters close to the percolation concentration

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**Abstract** To probe the behaviour of fibrillar assemblies of ovalbumin under oscillatory shear, close to the percolation concentration,  $c_p$  (7.5%), rheo-optical measurements and Fourier transform rheology were performed. Different results were found close to  $c_p$  (7.3%), compared to slightly further away from  $c_p$  (6.9 and 7.1%). For 6.9 and 7.1%, a decrease in complex viscosity, and a linear increase in birefringence,  $\Delta n'$ , with increasing strain was observed, indicating deformation and orientation of the fibril clusters. For 7.3%, a decrease in complex viscosity was followed by an increase in complex viscosity with increasing strain, which coincided with a strong increase in  $\Delta n'$ , dichroism,  $\Delta n''$ , and the intensity of the normalized third harmonic ( $I_3/I_1$ ). This regime was followed by a second decrease in complex viscos-

ity, where  $\Delta n'$ ,  $\Delta n''$  and  $I_3/I_1$  decreased. In the first regime where the viscosity was decreasing with increasing strain, deformation and orientation of existing clusters takes place. At higher oscillatory shear, a larger deformation occurs and larger structures are formed, which is most likely aggregation of the clusters. Finally, at even higher strains, the clusters break up again. An increase in complex viscosity,  $\Delta n'$ ,  $\Delta n''$  and  $I_3/I_1$  was observed when a second strain sweep was performed 30 min after the first. This indicates that the shear-induced cluster formation and break up are not completely reversible, and the initial cluster size distribution is not recovered after cessation of flow.

**Keywords** Percolation concentration · Rheo-optics · Fourier transform rheology · Ovalbumin

### Introduction

Structural changes of complex fluids under steady shear flow were investigated for different type of materials, such as suspensions, associating polymers and polymer solutions [1, 2, 3, 4, 5, 6]. Under specific conditions, depending on the material, a shear-thickening regime is observed followed by shear-thinning behaviour [3, 4, 6]. Shear-thickening is often the result of shear-induced formation of intermolecular structures (clusters), leading to an increase in the effective volume fraction [1, 2, 3, 5,

7]. Clusters convected by shear flow may aggregate upon contact, due to attractive interactions of particles on the periphery of the clusters. Cluster-cluster collisions that occur along the streamlines of shear flow lead to the formation of larger clusters [7]. In polymer solutions, the size of these clusters depends upon the concentration [4]. Shear-induced stretching of the structures can also contribute to a shear-thickening effect [4, 8, 9]. Increasing the shear rate increases the stress on these structures, and once a critical stress is exceeded, they break up, giving rise to shear-thinning behaviour [3, 4, 6].

A class of systems suitable for investigating structural changes of a complex fluid under shear flow are protein aggregate systems, for example ovalbumin. Ovalbumin is a major globular protein component in egg white, and has multi-functional properties, such as its ability to foam and to form gels upon heating [10, 11, 12, 13]. Under specific protein concentration, ionic strength, pH, and heating conditions, monomeric ovalbumin can form aggregates. Fibrillar structures can be formed upon heating a monomeric ovalbumin solution at pH 2 and low ionic strength at 80 °C for 1 h [14, 15]. TEM micrographs showed that semiflexible ovalbumin fibrils of about 250 nm were present at low ionic strength. A gel is formed above a critical ovalbumin concentration, depending on ionic strength, heating condition and pH. For ovalbumin, the critical percolation concentration decreased with increasing ionic strength, and ranged from 7.9 to 4.8%, for ionic strengths of 0.01–0.035 M [15].

Recently, we performed steady shear rheo-optical measurements on ovalbumin fibrils solutions [16]. From these results we concluded that, close to the percolation concentration ( $c_p$ ), the ovalbumin fibrils are organized in flexible clusters. The objective of the present study was to obtain more insight in the fibrillar ovalbumin structures under oscillatory shear, close to the  $c_p$ , with the use of rheo-optical measurements and Fourier transform rheology (FT-rheology). The rheo-optical measurements allow the extraction of information on the shear-induced anisotropy within the sample, in a non-invasive way. This can be used to relate to mesostructural changes within the sample upon shear. We measured birefringence,  $\Delta n'$ , and dichroism,  $\Delta n''$ , as a function of strain. To quantify the non-linearity of the stress deformation response of the fibrillar ovalbumin solutions, we made use of FT-rheology, allowing us to measure the intensity of higher harmonics in the stress response as a function of strain [17, 18, 19].

## Materials and methods

**Sample preparation** Ovalbumin was obtained from Sigma (A5503, lotnr 81K7025) with a purity of at least 99%. Ovalbumin (8%, w/w) was dissolved in bidistilled water at pH 2, and stirred overnight. The pH of the protein sample was adjusted to 2, using a 6 M HCL solution. The ovalbumin solution was centrifuged at 26,000g for 30 min at 4 °C, to remove any traces of undissolved material. Subsequently, the supernatant was filtered through a protein filter (FP 30/0.45 CA-S filter, Schleicher & Schuell). The pH of the solution was checked to be 2. The concentration of this stock solution was determined with a UV spectrophotometer at a wavelength of 278 nm.

Ovalbumin solutions of 6.9, 7.1 and 7.3% were prepared from the stock solution, and heated at 80 °C for 1 h in a water bath, while shaking. The solution was cooled on ice water to 4 °C, and stirred slowly at 21 °C for 1.5 h before measuring.

**Rheo-optical measurements** Rheo-optical measurements were performed using an ARES rheometer (Rheometric) with a Couette geometry (cup diameter, 33.8 mm; bob diameter, 30.0 mm; bob length, 20.09 mm), and an optical analysis module (OAM). The optical unit consists of a solid-state laser, a polarizer cube, a half wave plate that rotates at around 400 Hz, a beam splitter and a linear polarizer in front of the reference detector and a circular polarizer in front of the sample detector. For details on how to calculate the birefringence, dichroism and the corresponding angles of the sample from the intensity of the signals at the detectors we refer you to the monograph of Fuller [20]. The laser beam is directed downward in the direction of vorticity, through the gap between the cup and the bob. This allows information to be obtained on flow-induced orientation in the flow–flow gradient plane. The optical pathlength of the sample is 20.09 mm. The optical signal from the detector was digitized using an ADC and analysed using Labview (National Instruments). After pouring the ovalbumin sample into the Couette geometry, the sample was rested for 30 min. Dynamic measurements were performed on 6.9, 7.1 and 7.3% ovalbumin (frequency 1 Hz, strain 0.1–400%), where each strain was measured for 60 s. To determine if shear-induced structural changes of the ovalbumin solutions were reversible, the sample was kept at rest for 30 min in the Couette cell after the first measurement (test 1), and then a second strain sweep was performed (test 2). In order to qualitatively test whether sample relaxation influences the strain sweep, we also applied a second strain sweep after 10 min instead of 30 min for the 7.3% sample (sample 2). The influence of sample relaxation was expected to be most pronounced at this concentration. Since the measurements were conducted in an oscillatory mode (at 1 Hz), the birefringence and dichroism were determined by averaging their values over time. In contrast to the birefringence and dichroism, the corresponding angles average out to zero. Although the average value of the angles is zero in the oscillatory mode, they do change during the oscillation, indicating alignment. The rheological and optical measurements were performed simultaneously.

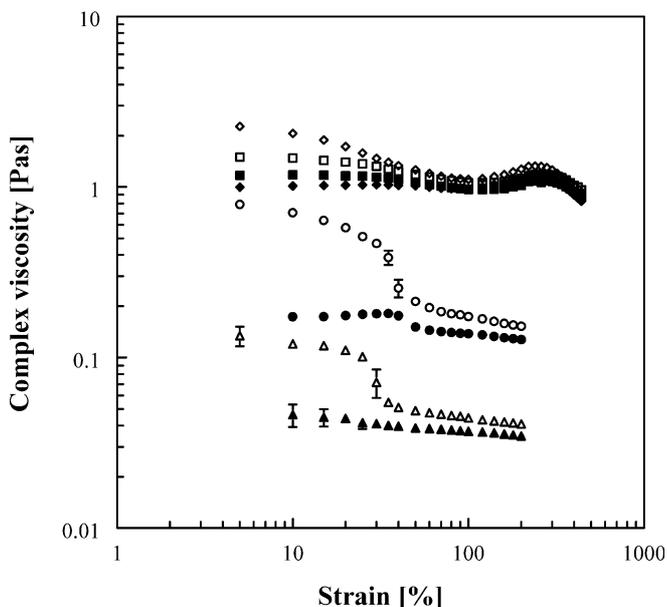
**FT-rheology** FT-rheology can be used as a tool to quantify the nonlinear response to large-amplitude oscillatory shear (LAOS), by analysis of higher harmonics in the stress response [17, 19]. For an oscillatory shear measurement with applied frequency  $\omega_1$ , in the non-linear regime the formation of mechanical odd

harmonics at  $3\omega_1$ ,  $5\omega_1$ ,  $7\omega_1$ , and so forth appear in the torque response. The amplitudes and phases of the higher harmonics within the torque signal can be detected as spectra  $I(\omega)$  in Fourier space. The torque signals were digitized using an ADC, and analyzed in Fourier space using Labview (National Instruments).

## Results

### Rheological results

Results of the complex viscosity versus strain for various ovalbumin concentrations close to the percolation concentration show a decrease in complex viscosity with increasing strain for all concentrations (Fig. 1). For 7.3%, this decrease in complex viscosity is followed by an increase in complex viscosity with increasing strain, and finally, at even higher strains, the complex viscosity decreases again (Fig. 1). This trend was different from that of 6.9 and 7.1% ovalbumin, where only a decrease in complex viscosity with increasing strain was observed. For all concentrations, a higher complex viscosity was found for the second strain sweep (test 2), which was performed 30 min after the first measurement (test 1). For 7.3%, this effect was smaller when the second strain sweep (sample 2, test 2) was made only 10 min after the first one (sample 2, test 1).

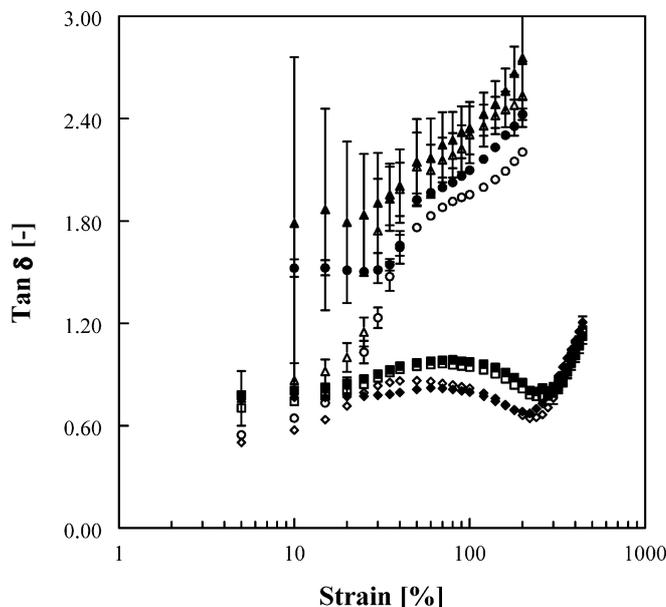


**Fig. 1** Complex viscosity versus strain for three different ovalbumin concentrations close to the percolation concentration. *Closed triangle* 6.9% test 1; *open triangle* 6.9% test 2; *closed circle* 7.1% test 1; *open circle* 7.1% test 2; *closed diamond* 7.3% sample 1, test 1; *open diamond* 7.3% sample 1, test 2; *closed square* 7.3% sample 2, test 1; *open square* 7.3% sample 2, test 2

To obtain more insight into the elasticity of the ovalbumin fibrils solution,  $\tan \delta$  versus strain was plotted in Fig. 2. For 6.9 and 7.1%,  $\tan \delta$  increased with increasing strain, indicating that the sample was more viscous at higher strains. For almost all strains,  $\tan \delta$  was higher than 1, which indicates a fluid-like behaviour. For 7.3% ovalbumin,  $\tan \delta$  first increased, then decreased, and finally increased again as a function of strain. This trend indicated that at lower strains the sample became more viscous, but was still gel-like because  $\tan \delta$  was smaller than 1. At strains higher than 100%, the sample became more elastic. Above a strain of about 250%, a strong increase in  $\tan \delta$  was observed indicating a more viscous sample. At very high strains,  $\tan \delta$  was larger than 1, indicating a more fluid-like behaviour.

### FT-rheology

To probe the non-linearity of the stress response of the ovalbumin samples, we used FT-rheology. For 6.9 and 7.1%, no clear higher harmonics could be detected in the Fourier spectrum in the range of strains we applied. For ovalbumin fibrils solutions made at 7.3%, third harmonics were present. Harmonics higher than the third were not found. Figure 3 shows the intensity of the third harmonic normalized by the intensity of the first harmonic ( $I_3/I_1$ ) for both samples at 7.3%. Up to a strain of about 100%  $I_3/I_1$  increased slightly. Above 100%, a



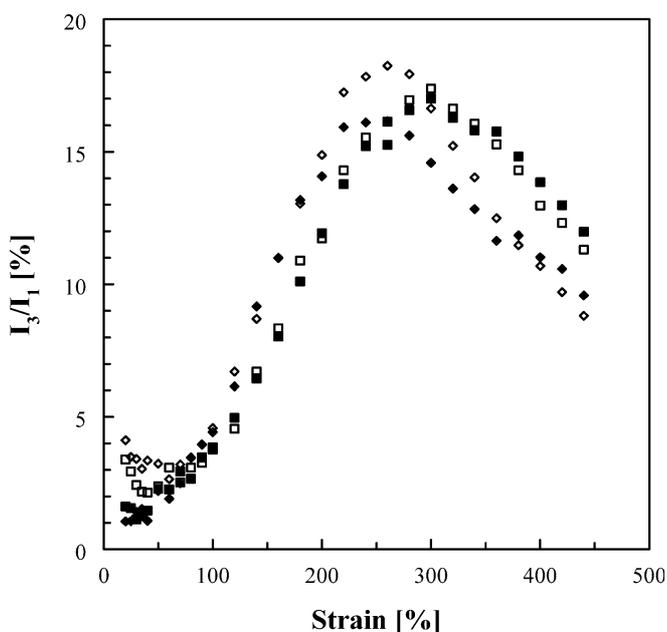
**Fig. 2**  $\tan \delta$  versus strain for three different ovalbumin concentrations close to the percolation concentration. *Closed triangle* 6.9% test 1; *open triangle* 6.9% test 2; *closed circle* 7.1% test 1; *open circle* 7.1% test 2; *closed diamond* 7.3% sample 1, test 1; *open diamond* 7.3% sample 1, test 2; *closed square* 7.3% sample 2, test 1; *open square* 7.3% sample 2, test 2

strong increase in non-linearity was observed. At strains higher than 250% a decrease in  $I_3/I_1$  was found. The time between the first and the second strain sweep seems to have influence on the increase in  $I_3/I_1$  observed in the second sweep compared to the first one. This increase is higher when the time between sweeps is larger.

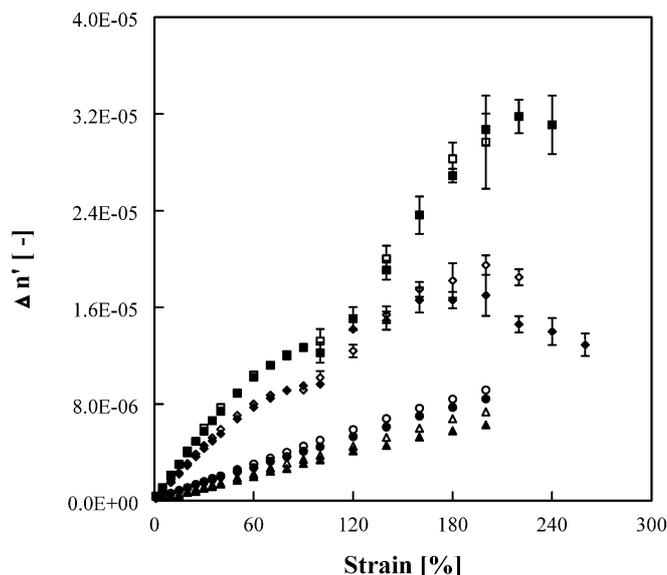
### Optical results

Figure 4 shows the results of birefringence,  $\Delta n'$ , measurements versus strain for ovalbumin fibrils solutions at 6.9, 7.1 and 7.3%. For 6.9 and 7.1%, a linear increase of  $\Delta n'$  with increasing strain was found, where for 7.1%  $\Delta n'$  was higher at equal strain compared to 6.9%. For 7.3%, a higher value for  $\Delta n'$  at equal strain was observed than for 6.9 and 7.1%. Above a strain of about 100%,  $\Delta n'$  strongly increased. At strains higher than 200%, a decrease in  $\Delta n'$  was observed. This trend was found for both samples of 7.3%. The order of magnitude of  $\Delta n'$  was  $10^{-6}$ – $10^{-5}$ . For all samples the values for  $\Delta n'$  of the second sweep (tests 2) were slightly higher compared to the first one (tests 1).

Dichroism,  $\Delta n''$ , measurements were performed for a 7.3% ovalbumin fibrils solution (Fig. 5). The order of magnitude of  $\Delta n''$  was  $10^{-9}$ – $10^{-8}$ , indicating that the value of  $\Delta n''$  is negligible compared to  $\Delta n'$  values. At low strains,  $\Delta n''$  was very small and nearly independent of strain. Above strains of 100% an increase in  $\Delta n''$  was observed, followed by a decrease in  $\Delta n''$  at strains higher than 220%. For both tests the same trend was found.



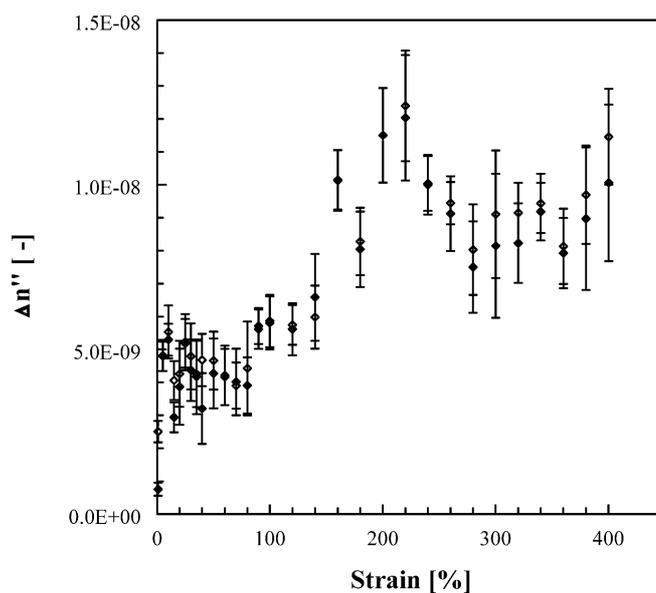
**Fig. 3** The intensity of the third harmonic normalized by the intensity of the first harmonic,  $I_3/I_1$ , versus strain for 7.3% ovalbumin. *Closed diamond* Sample 1, test 1; *open diamond* sample 1, test 2; *closed square* sample 2, test 1; *open square* sample 2, test 2



**Fig. 4** Birefringence,  $\Delta n'$ , versus strain for three different ovalbumin concentrations close to the percolation concentration. *Closed triangle* 6.9% test 1; *open triangle* 6.9% test 2; *closed circle* 7.1% test 1; *open circle* 7.1% test 2; *closed diamond* 7.3% sample 1, test 1; *open diamond* 7.3% sample 1, test 2; *closed square* 7.3% sample 2, test 1; *open square* 7.3% sample 2, test 2

### Discussion

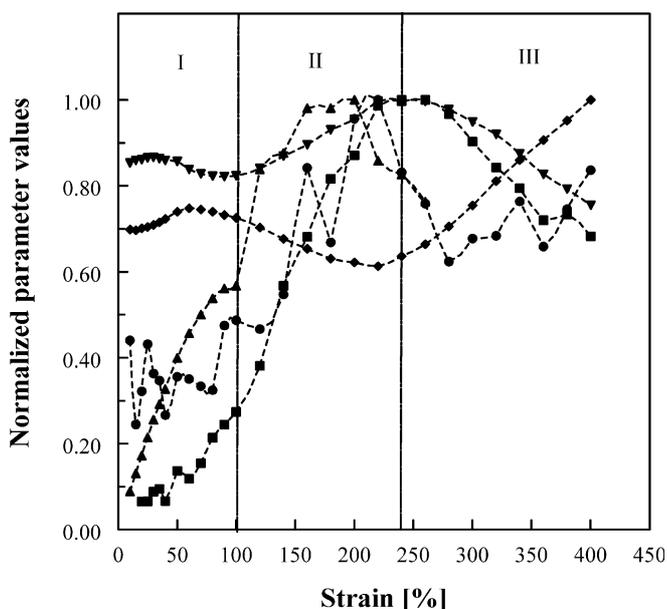
We investigated the behaviour of fibrillar assemblies of ovalbumin under oscillatory shear, close to the perco-



**Fig. 5** Dichroism,  $\Delta n''$ , versus strain for 7.3% ovalbumin. *Closed diamond* Test 1, *open diamond* test 2

lation concentration,  $c_p$  (which is 7.5%). At 7.3% the behaviour was different from the behaviour at 6.9 and 7.1%. The difference may be explained in terms of a difference in cluster size. Percolation theory assumes the presence of clusters, whose size depends on  $c_p - c$ , where  $c$  is the monomer concentration [21, 22]. For ovalbumin, fibrillar structures were formed after heating, which formed isotropic clusters. These clusters can be thought of as finite size or “local” network structures. Closer to  $c_p$ , these clusters are larger, and become infinite at  $c_p$ . The radius of a cluster signifies the correlation length, or spatial extent of the connectivity function, equal to the probability that two fibrils at distance  $r$  belong to the same cluster [21]. The difference in cluster size influences the rheological and optical behaviour under oscillatory shear, as we will discuss now. For 6.9 and 7.1% ovalbumin, a decrease in complex viscosity, an increase in  $\tan \delta$ , and a linear increase in  $\Delta n'$  with increasing strain was observed, indicating deformation and alignment of the clusters, without much hindrance between neighbouring clusters.

Normalized values for the measured parameters,  $\Delta n'$ ,  $\Delta n''$ , complex viscosity,  $\tan \delta$ , and  $I_3/I_1$  at 7.3% ovalbumin are given in Fig. 6, which can be divided in three strain regions. The first region (I), up to a strain of 100%, shows a decrease in complex viscosity, which indicates small deformations of the ovalbumin clusters.  $\tan \delta$  was slightly increased, corresponding to a slightly more viscous system, but still gel-like because  $\tan \delta$  was



**Fig. 6** Normalized values for measured parameters for 7.3% ovalbumin. Hereby all values are divided by the highest value of the measurement, for each parameter. *Closed up-triangle*  $\Delta n'$ ; *closed circle*  $\Delta n''$ ; *closed down-triangle* complex viscosity, *closed diamond*  $\tan \delta$ , *closed square*  $I_3/I_1$

smaller than 1. The results of FT-rheology show a gradual increase in  $I_3/I_1$ , indicating slightly non-linear behaviour. In the same region a linear increase in  $\Delta n'$  was observed, so more alignment and or deformation of the clusters takes place. The values for  $\Delta n''$  were very small and hard to distinguish from the background noise, which indicates that the clusters present were smaller than the wavelength of the laser light, which is 670 nm. All results indicate that the clusters deform and align slightly in region I.

In the second region (II), ranging from 100% to about 240% strain, a larger deformation may cause hindrance between the clusters, resulting in an increase in complex viscosity. Also, growth of the clusters can contribute to an increase in complex viscosity.  $\tan \delta$  decreased, corresponding to a more elastic gel-like behaviour. An increase in  $\Delta n''$  measurements was found, indicating that part of the laser light entering the material is scattered anisotropically by entities with a typical size of the wavelength of the laser light. This can be explained by the presence of more deformed or larger structures, with a size larger than the wavelength of the laser light (670 nm). Also a strong increase in  $\Delta n'$  was observed in this region, which can be explained by a strong alignment of the clusters, due to a large deformation. The non-linearity increased strongly in this region, which may be due to the more deformed or larger clusters. The trends found in region II can be summarised as a combined effect of large deformation and the formation of larger structures, which is most likely aggregation of the fibril clusters.

Finally, in region III, from about 240% to 400% strain, the complex viscosity decreased again.  $\tan \delta$  increased to above 1, indicating a more viscous fluid-like behaviour. The decrease in  $I_3/I_1$  indicates less non-linearity of the sample. Also, the values for  $\Delta n'$  and  $\Delta n''$  decreased. Both optical and rheological results indicate the break up of clusters at high oscillatory shear.

Another trend observed in all measurements was higher values for the complex viscosity,  $\tan \delta$ ,  $I_3/I_1$ ,  $\Delta n'$  and  $\Delta n''$  for the second strain sweep (tests 2) performed 30 min after the first one (tests 1). For all concentrations these results indicate rearrangements during rest resulting in a different initial cluster size distribution when the second measurement was started. For 7.3%, this effect was smaller, with only 10 min rest between the first and second strain sweeps, indicating that the resting time between two measurements is an important factor, which influences the cluster size distribution when starting the second measurement. For 7.3% ovalbumin, another effect may also play a role. At high oscillatory shear the clusters break up, but the size of the clusters may still be larger than the size when the first measurement was started, which will also contribute to a different cluster size distribution at the start of the second measurement.

## Conclusion

We investigated the behaviour of fibrillar assemblies of ovalbumin under oscillatory shear, close to the percolation concentration,  $c_p$  (which is 7.5%). Near  $c_p$  (7.3%) different behaviour was observed compared to slightly further away from  $c_p$  (6.9 and 7.1%). This can be explained with the use of percolation theory, which assumes the presence of clusters, with a size that depends on the distance to  $c_p$ . For 6.9 and 7.1%, the clusters present after heating will deform and align when oscillatory shear is applied. For 7.3%, we propose, based on the results of rheo-optics and FT-rheology, that the strain regime can be divided in three regions. In region I, deformation and orientation of the clusters occur up to a strain of about 100%. At higher oscillatory shear (region

II), a larger deformation occurs and larger structures are formed, which is most likely aggregation of the clusters. Finally, at strains higher than 240% (region III), the clusters break up. Both rheo-optical and FT-rheology results indicate rearrangements of the clusters in time, which indicates that the initial cluster size distribution is not recovered after cessation of flow. The results obtained from the two different techniques (rheo-optics and FT-rheology) are consistent with one another, and give more insight in the behaviour of ovalbumin fibrils under oscillatory shear close to the percolation concentration.

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