

# Elastic Networks of Protein Particles

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Received: 28 April 2009 / Accepted: 27 October 2009 / Published online: 11 November 2009  
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**Abstract** This paper describes the formation and properties of protein particle suspensions. The protein particles were prepared by a versatile method based on quenching a phase-separating protein–polysaccharide mixture. Two proteins were selected, gelatin and whey protein. Gelatin forms aggregates by means of reversible physical bonds, and whey protein forms aggregates that can be stabilized by chemical bonds. Rheology and microscopy show that protein particles aggregate into an elastic particle gel for both proteins. Properties similar to model systems of synthetic colloidal particles were obtained using protein particle suspensions. This suggests that the behaviour of the particle suspensions is mainly governed by the mesoscopic properties of the particle networks and to a lesser extent on the molecular properties of the particles.

**Keywords** Gelatin · Whey protein · Mesostructure · Rheology · Phase-separated · Cold gelation

## Introduction

Many materials consisting of polymer melts and colloidal suspensions show elastic behaviour. In polymer melts, elastic behaviour is caused by molecular entanglements.<sup>1</sup> In colloidal suspensions, elastic behaviour is, for example, caused by flocculation and subsequent network formation.<sup>2</sup> Elastic materials are used in many industrial applications such as thickeners, flow improvers and stabilizers of pigments.<sup>3</sup> Wheat gluten is an example of a biopolymer system with elastic properties, which allow wheat flour to retain gas during proofing and baking.<sup>4,5</sup> Gluten has self-healing properties,<sup>6–8</sup> which are uncommon in synthetic polymers.<sup>9</sup> The elastic properties of gluten are hypothesized to be a result of a glutenin particle network structure.<sup>6</sup> It is therefore of interest to understand more about the rheological behaviour of biopolymer particle systems. Limited information is available about the properties of suspensions containing protein particles. Therefore, in this study, the behaviour of suspensions containing protein particles is compared with the properties of synthetic colloidal particles found in other studies.<sup>10–17</sup>

Protein particles can be involved in several types of interactions, for example, hydrophobic, Van der Waals and hydrogen-bridge type interactions. These interactions are mainly reversible and weak. However, if these interactions exist on a larger, cooperative scale, the overall interaction can be strong. Interactions can allow the formation of disulfide bridges, leading to covalent stabilization of the resulting particle aggregate. In addition, entanglement and depletion type interactions may exist. Depending on the type of protein used to form the particles, different combinations of these interactions may exist.

In this study, gelatin and whey protein particles are used as model protein materials. These proteins have different

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intrinsic properties. Gelatin is a protein that forms aggregates via reversible, H-bridge-type bonds. The formation of these bonds is very fast, because H-bridges form within milliseconds.<sup>18</sup> Whey proteins (mainly  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin) have a high content of the amino acids glutamine, leucine and asparagine. Cystine residues are characteristic in whey proteins.<sup>19</sup> Whey protein forms aggregates on heating or acidification, which can be stabilized through disulfide bonds. The formation of these S-bridges takes seconds to minutes depending on the pH<sup>18</sup> and other properties, which is much slower than the formation of H-bridges.

Protein particles can be created by mixing a protein and a biopolymer with low compatibility.<sup>20,21</sup> The rate and onset of phase separation and gelation are important characteristics for the morphology of the protein structure produced,<sup>22,23</sup> and are critically dependent on the concentration, temperature and molar mass of the continuous phase.<sup>24–28</sup> Creation of protein particles is possible for a limited number of biopolymers using specific process conditions.<sup>22,29,30</sup> Gelatin particles are formed by inducing phase separation by temperature quenching. Whey proteins form small aggregates by mild heating of a whey protein solution. Bringing a pre-aggregated solution to its isoelectric point (ca 4.5) leads to gel formation. This process is often defined as cold gelation.<sup>31</sup>

The aim of this paper is to demonstrate that protein particle suspensions can show elastic behaviour through aggregation of protein particles. We used gelatin and whey protein to prepare particles and characterized the behaviour of the resulting particle suspensions. The results are compared with results from studies on non-biopolymer, colloidal particle systems.

## Materials and Methods

### Materials

The proteins used were gelatin type A, bloom number 175 and a gel point (for a 5% solution) at 14 °C (Bio-Rad Laboratories, The Netherlands) and whey protein (Davisco Foods International Inc., USA). All proteins were used without further purification. Both protein materials contained about 90% (w/w) protein, according to Dumas measurements (using  $N=5.55$  for gelatin and  $N=6.38$  for whey protein). The polysaccharides used were dextran (MW 2,000 kDa, Sigma Chemicals, The Netherlands) and locust bean gum (Danisco Holland BV, The Netherlands). Glucono-delta-lacton (GDL; Sigma Chemicals, The Netherlands) was used for pH regulation. Rhodamine B (Sigma Chemicals, The Netherlands) was used for confocal laser scanning microscope (CLSM) analysis. All chemicals were of analytical grade.

### Preparation of Protein Particles

Protein particles were prepared using cold gelation in a phase-separating biopolymer system.<sup>22,23,29–34</sup> A 10% (w/w) gelatin stock solution was prepared by stirring a gelatin solution for 2 h at 50 °C. A 10% (w/w) stock solution of dextran was prepared by stirring for 1 h at 80 °C. The dextran and gelatin stock solutions were kept at 50 °C before mixing (approximately 2 h). A mixture of gelatin (5% (w/w)) and dextran (5% (w/w)) was gelled by cooling from 50 to 30 °C in approximately 1 h. After 16 h, the mixture was cooled further to 25 °C in approximately 30 min.

A 9% (w/w) whey protein stock solution was prepared by stirring for 2 h at 25 °C followed by heating the solution at 68 °C for 2.5 h. Heating the whey protein samples resulted in the formation of small protein aggregates of 40–100 nm<sup>35</sup>, without forming a gel. A 1% (w/w) locust bean gum stock solution was prepared by stirring at 80 °C for 1 h. The whey protein and locust bean gum stock solutions were cooled to 25 °C before mixing. A mixture of whey protein (3% (w/w)) and locust bean gum (0.45% (w/w)) was gelled by adding GDL (0.20% (w/w)), as a result of a gradual decrease in pH.

After incubation of the protein–polysaccharide mixtures for 16 h, the samples were diluted with distilled water and the continuous phase was removed by centrifugation (15 min at 2,000×g). The pellet was re-dispersed in distilled water up to the original volume and then centrifuged at 2,000×g for 15 min. The pellet was re-dispersed to obtain a sample with the required concentration ( $6.5\pm 0.6\%$  (w/w)). The samples were used within 1 day after processing. Three samples per protein were prepared for analysis unless stated otherwise.

### Analysis of Protein Particles

#### *Shape and Size of Suspensions*

*Confocal Laser Scanning Microscopy* CLSM was used to analyze the shape and spatial distribution of the protein particles. After processing, the sample was transferred into two well-chambered cover glasses (Nunc, Naperville, IL, USA). Rhodamine B was added to a concentration of  $2\times 10^{-3}\%$  (w/w) for non-covalent labelling of the proteins. The samples were visualized with an LSM 510 microscope (Zeiss, Oberkochen, Germany). The 543-nm laser line was used for excitation to induce a fluorescent emission of Rhodamine B, detected between 600 and 650 nm. Image analysis of two images per sample obtained at  $\times 10$  magnification was used to calculate the average volume fraction occupied by the particles using Image-J software. The average particle diameter was measured by calculating the mean diameter of eight particles, four particles per sample.

**Size Distribution** The particle size distribution of a highly sheared diluted protein particle suspension was analyzed by laser diffraction using a Mastersizer (Malvern Instruments Ltd. 2000, Worcestershire, UK) particle size analyzer. The refractive index used was 1.347 for gelatin particles and 1.334 for whey protein particles. The average particle diameter was calculated from the measurements of two samples.

#### Rheological Characterization of Suspensions

**Shear Rate Sweeps** Shear rate sweeps were carried out at 25 °C in a cone/plate geometry (angle 4°/diameter 50 mm). The polysaccharide was almost all removed by washing and the sample was diluted with water until a protein concentration of  $6.5 \pm 0.6\%$  (w/w). After equilibrating the sample for 15 min, the shear rate was increased logarithmically over the range  $1\text{--}300\text{ s}^{-1}$ . One measurement consisted of 21 steps with ten measuring points of 10 s for each step, a total duration of 35 min per measurement. From the measurements, the shear stress and viscosity were calculated as a function of shear rate.

**Steady Shear Measurements** Steady shear measurements were performed in the concentration range  $1\text{--}5\%$  (w/w) protein at 25 °C in a cone/plate geometry (angle 1°/diameter 75 mm). One sample per concentration was measured. After equilibrating the sample for 15 min, the viscosity was measured at a shear rate of  $0.001\text{ s}^{-1}$  for 8,000 s. Each sample was measured twice with an equilibration time of 20 s between the two measurements. From the measurements, the viscosity was calculated as a function of time.

#### Rheomicroscopy

A rheomicroscope, which is a combination of a light microscope and a rheometer equipped with a quartz parallel plate geometry, was used to observe the particle structure during steady shear. The viscosity of one sample was measured at a shear rate of  $0.001\text{ s}^{-1}$  for 8,000 s and at a shear rate of 0 and  $100\text{ s}^{-1}$  for 10 s.

**Strain Sweeps** Strain sweeps were performed at 25 °C in a plate/plate geometry (diameter 50 mm). The polysaccharide was almost all removed by washing, and the sample was diluted with water until a protein concentration of  $6.5 \pm 0.6\%$  (w/w). After equilibrating the sample for 15 min, the strain was increased logarithmically from 0.01% to 300% at a frequency of 1 Hz. The limit of linearity of the suspension was determined from the amplitude sweep. From the measurements, the elastic ( $G'$ ) and viscous ( $G''$ ) moduli and the loss tangent ( $\tan\delta$ ) were calculated as a function of the strain.

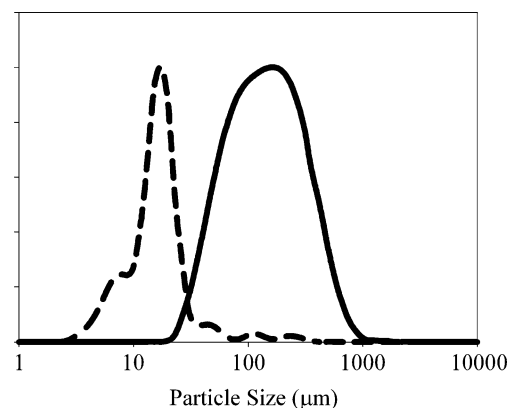
**Frequency Sweeps** Frequency sweeps were performed at 25 °C in a plate/plate geometry (diameter 50 mm). The polysaccharide was almost all removed by washing, and the sample was diluted with water until a protein concentration of  $6.5 \pm 0.6\%$  (w/w). After equilibrating the sample for 15 min, the frequency was increased logarithmically from 0.01 to 100 Hz at a strain of 0.1%. This range was within the linear viscoelastic region, as determined by preliminary strain sweep experiments. From the measurements, the elastic ( $G'$ ) and viscous ( $G''$ ) moduli and the loss tangent ( $\tan\delta$ ) were calculated as a function of the angular frequency.

Wall slip is often observed in microgel suspensions. We checked the steady shear measurement using a rheomicroscope to verify these experiments, but when interpreting the rheological results it is important to be aware of possible wall slip.


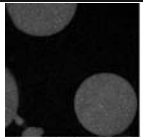
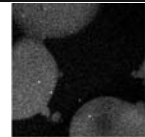
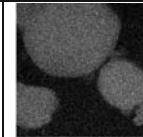
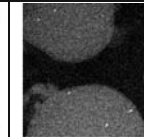
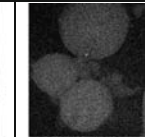

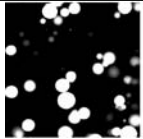
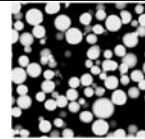
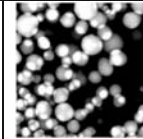
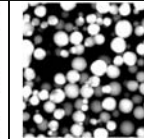
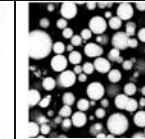
The shear rate sweep measurements were carried out using a Paar Physica MCR 501 (Anton Paar, Austria) stress-controlled rheometer; for rheomicroscopy, a Paar Physica MCR 300 (Anton Paar, Austria) stress-controlled rheometer was used; for all other rheological characterizations, a Paar Physica MCR 301 (Anton Paar, Austria) stress-controlled rheometer was used.

## Results

The gelatin and whey protein particles produced were characterized using CLSM and laser granulometry. A characteristic size distribution profile of the protein particles is presented in Figure 1, showing that the gelatin particles ( $137 \pm 19\text{ }\mu\text{m}$ ) were larger than the whey protein particles ( $18 \pm 1\text{ }\mu\text{m}$ ). The gelatin and whey protein particles retained their spherical shape for at least 1 day after processing. An overview of the CLSM pictures for the protein particles is presented in Figure 2. These pictures



**Fig. 1** Characteristic particle size distribution curves for gelatin (solid line) and whey protein (dashed line) particles analyzed by laser diffraction

protein source	size ( $\mu\text{m}$ )	centrifugation		after shear test		
		before	after	Rotational	Oscillatory	
					Strain	frequency
Gelatin 	120±17					
Whey protein 	20±4					

**Fig. 2** Overview of the size and structure of gelatin and whey protein particles before and after centrifugation and after the rheological tests. The average particle diameter was calculated from particle size analysis

were also used to determine the typical particle size for each protein used. For gelatine, an average particle size of about  $120\pm 17\ \mu\text{m}$  was found, which was slightly smaller than the particle size obtained by laser granulometry. For whey protein, the size measured by CLSM analysis was somewhat larger ( $20\pm 4\ \mu\text{m}$ ).

To calculate the average volume fraction occupied by the particles using Image-J software, we assumed that the plane section of the two CLSM images were representative of the sample. The volume fractions occupied by the particles were  $46\pm 1\%$  and  $53\pm 1\%$  for gelatin and whey protein, respectively. These volume fractions were well below random close packing (which occurs at a volume fraction of 63%), when jamming phenomena occur.<sup>36</sup>

CLSM was also used to check the physical stability of the individual protein particles. It was found that both gelatin and whey protein particles could withstand all deformation forces exerted during preparation and rheological analysis.

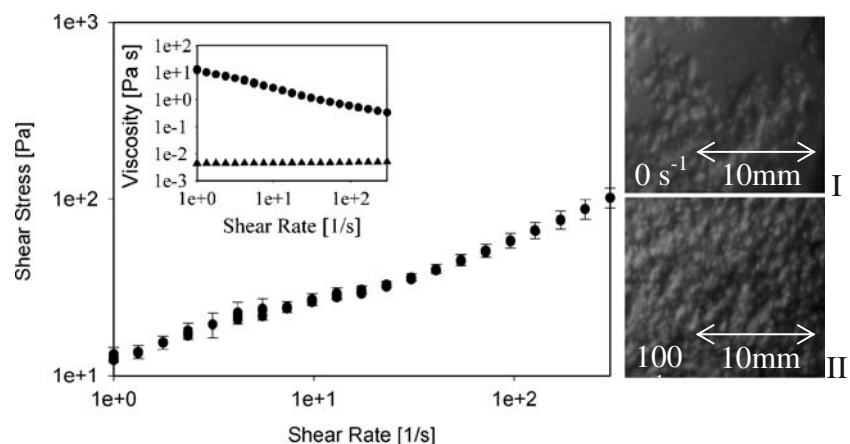
Rheology can be used to obtain information about particle interactions present in a particle suspension. Figures 3 and 4 show the shear stress as a function of shear rate for gelatin and whey protein particle suspensions

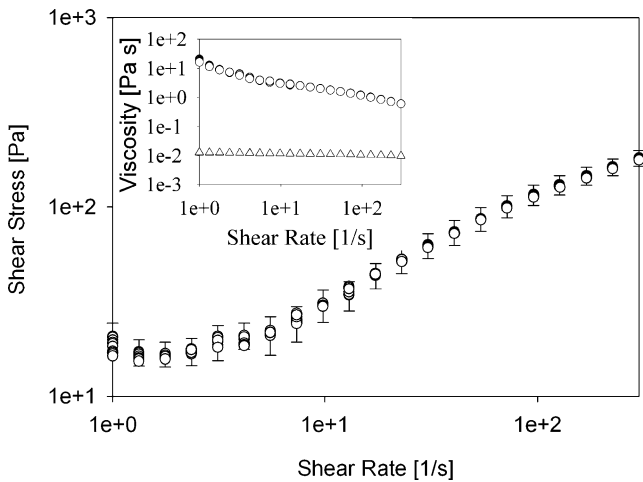
in the range  $1\text{--}300\ \text{s}^{-1}$ . The insets show the viscosity for gelatin and whey protein particles. The graph in the inset also shows the viscosity of 2.5% (*w/w*) dextran and 0.225% (*w/w*) locust bean gum (50% of the weight percentage needed for the preparation of protein particles). This graph shows that polysaccharide behaves at this concentration as a Newtonian liquid. As a result of washing, it is expected that the polysaccharide concentration in the suspension will be much lower, also leading to Newtonian behaviour. The viscosity of the protein particle suspensions is high compared with the continuous phase viscosity.

In addition, the viscosity profile of the whey protein particle suspensions show a yield stress followed by shear-thinning behaviour. We did not observe a yield stress for gelatin, but, extrapolation of the shear rate sweep for gelatin particle suspensions to zero indicated a yield point of 12 Pa. The yield point for whey protein particle suspensions is approximately 16 Pa. When the shear rate was increased beyond  $5\ \text{s}^{-1}$ , gelatin particle suspensions showed a shear rate dependency comparable to whey particle suspensions.

Particle aggregation can lead to the formation of a macroscopic sample-spanning network. Rheomicroscopy indeed shows that gelatin particles at rest form a particle

**Fig. 3** Shear stress as a function of the shear rate (*up-sweep*) of gelatin particle suspensions (6.5%). The *inset* shows the viscosity as a function of shear rate (*up-sweep*) of gelatin particle suspensions 6.5% (*triangle*) and a dextran solution 2.5% (*circle*). I A rheomicroscopy image of gelatin particles at rest; II a rheomicroscopy image of gelatin particles at a shear rate of  $100\ \text{s}^{-1}$





**Fig. 4** Shear stress as a function of the shear rate (*up-sweep*) of whey protein particle suspensions (6.5%). The *inset* shows the viscosity as a function of the shear rate (*up-sweep*) of whey protein particle suspensions 6.5% (*triangles*) and a locust bean gum solution 0.225% (*circles*)

network (Figure 3 (I)). The application of shear breaks up the network, leading to unclustered particles (Figure 3 (II)).

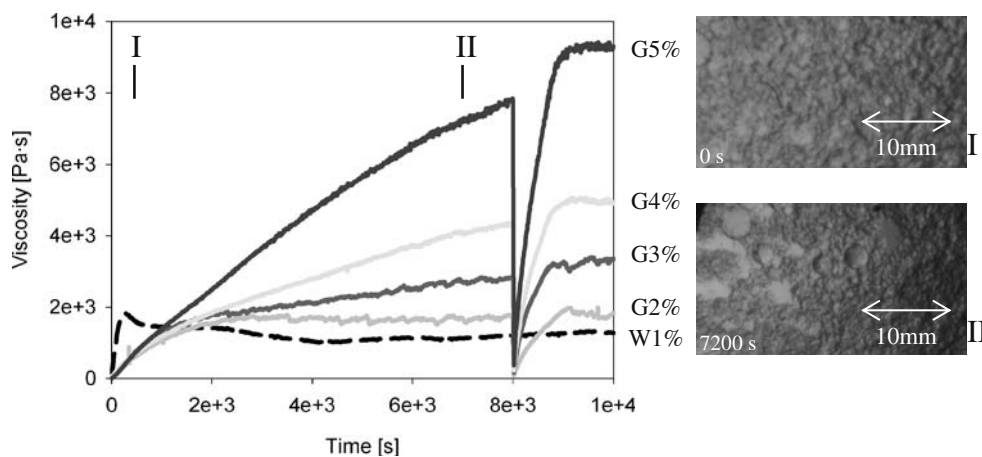
To investigate the effect of particle interactions at near-static conditions, we measured the shear stress as a function of shear time at a constant low shear rate ( $0.001 \text{ s}^{-1}$ ). The gelatin and whey protein particle suspensions were measured at different protein concentrations (gelatin 2%, 3%, 4% and 5% (*w/w*) and whey protein 1%, 2%, 3%, 4% and 5% (*w/w*)). Figure 5 shows that the viscosity of the gelatin particle suspension increased over time with low shear rate indicating rheopectic behaviour. An increase in the protein concentration gave an increase in the final shear stress for gelatin. The time needed to reach the final shear stress increased with gelatin concentration. The viscosity profile

of the whey protein particle suspension did not show a clear correlation with the protein concentration (results not shown). For clarity, only one whey protein concentration is shown in Figure 5. Whey protein particle suspensions showed an initial increase in the shear stress with time, followed by a decrease in the shear stress. Resuming the shear after 20 s resulted in a constant shear stress.

Microscopic pictures of a gelatin suspension were used to investigate the structure formation of the gelatin particles during constant deformation of  $0.001 \text{ s}^{-1}$ . At 0 s, the gelatin particles were distributed homogeneously (Figure 5 (I)). After 7,200 s, clusters of gelatin particles were present (Figure 5 (II)). This particle aggregation suggests a significant interaction between the gelatin particles.

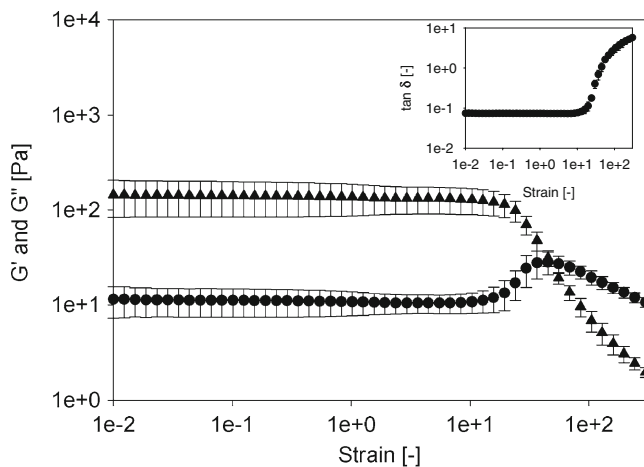
The strength and ability of the protein network to recover after deformation was measured with oscillatory frequency and strain experiments. Figure 6 shows  $G'$  and  $G''$  as a function of the strain for gelatin and whey protein particle suspensions in the strain range of 0.01–300%. Both gelatin and whey protein particle suspensions showed network fracture at higher strain values. For gelatin particles, there is fracture at a strain of 16 (maximum deviation was 1%). The whey protein particles fracture at a lower strain of 0.54 (maximum deviation was 1%). The loss factor showed a steeper increase in the non-linear regime for gelatin particle suspensions, indicating that the network was affected more abruptly at high strain values.

Figure 7 shows  $G'$  and  $G''$  as a function of the frequency of gelatin and whey protein particle suspensions in the range  $0.1\text{--}100 \text{ s}^{-1}$ . The loss and viscous moduli of both suspensions were slightly dependent on the frequency. Gelatin particle suspensions were more frequency dependent compared with the whey protein particle suspensions. Both gelatin and whey protein particle suspensions had a



**Fig. 5** Shear stress as a function of the shear time of gelatin (*solid lines*) and whey protein (*dashed line*) particle suspensions. Four different concentrations for gelatin particle suspensions (2%, 3%, 4%, 5% (*w/w*)) and one concentration for whey protein particle suspensions (1% (*w/w*)) are shown. The shear stress is measured at a constant

deformation of  $0.001 \text{ s}^{-1}$  for 8,000 s. After 8,000 s, the shear is stopped for 20 s and then continued with a constant deformation of  $0.001 \text{ s}^{-1}$ . *I* A rheomicroscopy image of gelatin particles at a constant deformation of  $0.001 \text{ s}^{-1}$  after 0 s; *II* a rheomicroscopy image of gelatin particles at a constant deformation of  $0.001 \text{ s}^{-1}$  after 7200 s

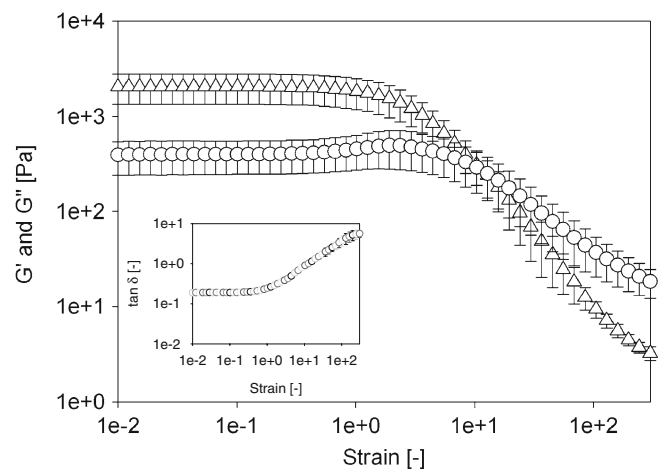


**Fig. 6** Storage modulus  $G'$  (triangles) and loss modulus  $G''$  (circles) as a function of the strain of gelatin (closed symbols) and whey protein (open symbols) particle suspensions (6–7%). The inset shows  $\tan \delta$  as a

larger value for  $G'$  than for  $G''$  over the whole frequency range. Figure 8 shows the complex viscosity as a function of the frequency in the range  $0.1$ – $100 \text{ s}^{-1}$  and the viscosity as a function of the shear rate in the range  $1$ – $300 \text{ s}^{-1}$  for gelatin and whey protein particles. The complex viscosity decreased with increasing frequency over the frequency region measured.

## Discussion

The purpose of this study was to demonstrate the unique properties of protein particles suspensions. Even though the microstructure of gelatin and whey proteins is different and the molecular properties of the proteins differ widely, the behaviour of gelatin and whey protein particle suspensions

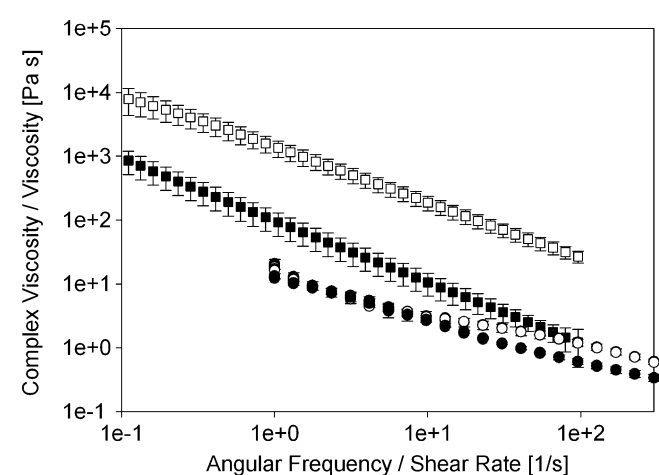
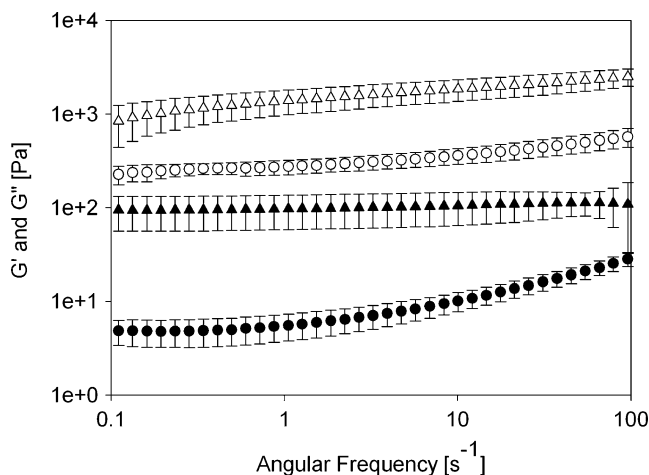


**Fig. 7** Storage modulus  $G'$  (triangles) and loss modulus  $G''$  (circles) as a function of the angular frequency  $\omega$  of gelatin (closed symbols) and whey protein (open symbols) particles (6–7%)

show similarities. Both protein systems show elastic behaviour and similar particle gel characteristics. The macroscopic behaviour of the particle gel systems seems to depend on the mesoscopic structure of the suspension rather than the specific chemical nature of the constituent material.

Gelation during phase separation was used to produce protein particles, because this technique is known as a suitable method to produce spherical protein particles.<sup>22,23,29–34</sup> However, this technique has some limitations because it requires the use of a polysaccharide to induce phase separation. We washed the protein particle suspension to remove most of the polysaccharide present in the system. However, even at this low residual concentration of polysaccharide, depletion flocculation can occur.<sup>37</sup> It was not possible to use the same polysaccharide for both

function of the strain of gelatin (closed symbols) and whey protein (open symbols) particle suspensions (6–7%). The inset shows  $\tan \delta$  as a function of strain.



**Fig. 8** Complex viscosity (squares) and viscosity (circles) as a function of the angular frequency and shear rate of gelatin (closed symbols) and whey protein (open symbols) particles (6–7%)

systems. The combination of whey protein and dextran did not result in protein particles. Studies show that it is possible to produce gelatin particles with locust bean gum.<sup>32</sup> However, in that study, the concentration of gelatin was very low. We did not succeed in producing gelatin particles with locust bean gum using higher gelatine concentrations. In addition, and probably as a consequence, it was not possible to prepare particles of whey protein with the same size as gelatin particles. The whey protein particles were always smaller (ca eight times) than the gelatin particles (see Figures 1 and 2). The large gelatin particles show a broad size distribution compared with the small whey protein particles.

The importance of particle interaction can be shown by estimating the effect on viscosity assuming that no particle interaction is present. When the protein particles behave as inert hard spheres, the viscosity can be estimated using the volume fraction indicated by CLSM images and the viscosity of the continuous phase of 50% of the polysaccharide (i.e. 2.5% (w/w) dextran and 0.225% (w/w) locust bean gum, respectively). According to the Krieger–Dougherty equation, with an intrinsic viscosity of 2.5 and maximum packing fraction of 0.63, the viscosity of the suspension would be 0.037 and 0.205 Pa s for gelatin and whey protein particles, respectively. The measured viscosity of the solutions is almost a factor of 100 larger. As the volume fraction of the particles is well below the jamming transition, this high viscosity must be linked to particle interactions.

The yield stress observed in both protein particle suspensions is characteristic for colloidal suspensions that form a network.<sup>16,38</sup> The yield stress is related to the force that the network can withstand before gel rupture.<sup>39</sup> A further increase of the shear forces leads to detachment of particles and shear thinning behaviour.<sup>40</sup> Shear-induced collisions can rebuild the particle network.<sup>40</sup> This was observed for the gelatin particle suspension. Gelatin particles showed rheopectic behaviour, which indicates the formation of interactions in the system,<sup>41–43</sup> leading to the (re)formation of an interparticle structure.<sup>41</sup>

Both protein particle suspensions show strain-dependent behaviour. At low strain, the particle gels are strain independent, but at higher strains they show strain softening. A particle gel of non-biopolymer particles shows comparable strain dependency; the strain independent region is an indication of the particle interaction in the gel.<sup>40,44</sup> Gels with a high degree of interaction are brittle and break at low strain values.<sup>45</sup> As the interaction decreases, the strain-independent region increases and the gel becomes deformable.<sup>40,44</sup> Gelatin particle suspensions show high deformability, which is comparable to weakly interacted particle gels. The whey protein particle suspensions are more brittle and behave as a strong interacted particle gel.<sup>45</sup> Both protein particle suspensions show

frequency-independent deformation behaviour. A particle gel of non-biopolymer particles shows comparable independence on frequency.<sup>40,44,46,47</sup> The storage modulus is larger than the loss modulus over the whole frequency range, indicating that the particles form an elastic gel with an infinite relaxation time.<sup>40,47</sup>

The presence of structural ordering is supported by the rheomicroscopy images in Figures 3 and 5 and by the invalidity of the Cox–Merz rule ( $\eta(\dot{\gamma}) = \eta^*(\omega)$ ),<sup>40,48</sup> shown in Figure 8. The  $\eta^*(\omega)$  curves were greater than the  $\eta(\dot{\gamma})$  curves throughout the measured shear region, indicating a structured system.<sup>48,49</sup> But the  $\eta^*(\omega)$  and  $\eta(\dot{\gamma})$  curves did not show parallel behaviour, which makes it impossible to use a shift factor.<sup>50</sup> The unparallel behaviour was observed previously in particle gel systems, but no explanation has yet been found for this behaviour.<sup>51</sup>

The rheological behaviour of the suspensions described above is comparable with the rheological behaviour of particulate networks of non-biopolymer model-particles. Shear thinning<sup>52</sup> and yielding<sup>16,38</sup> were observed, and the oscillatory rheology was comparable with other particle networks. Like other particle networks, our protein particle networks are strongly elastic at small strain values as seen from the slightly frequency-dependent behaviour.<sup>10,46</sup> The strain dependence of the gelatin particle network was compared with weakly interacted particle gels,<sup>44</sup> and the strain dependency of the whey protein particle network was comparable with strong interacted particle gels.<sup>45</sup>

Generally, the particles investigated in other studies were much smaller. For example, the radius of carbon black particles was 14 nm,<sup>10</sup> and the radius of carboxylated latex particles was 90 nm.<sup>46</sup> The particles in this study were 100 (whey) to almost 1,000 (gelatin) times larger. More comparable particle sizes are observed in studies on depletion-flocculated emulsions; the particles (droplets) were only ten (whey) to 100 (gelatin) times smaller.<sup>37,52,53</sup> Generally, those model studies indicate that the minimum volume fraction necessary for elastic gel behaviour decreases with decreasing particle size.<sup>12,13</sup> This implies that the protein particles show a remarkably high degree of interaction. The nature of the interaction present in the protein systems is not yet fully understood. Remaining polysaccharide might cause depletion interactions, but other interactions such as hydrogen bonds and Van der Waals forces cannot be excluded. Most likely, a combination of these interactions accounts for the high degree of interactions present in the system.

Although the behaviour of the two systems is qualitatively the same, some differences in properties can be observed. Those differences are probably caused by specific features of the protein, such as charge density and distribution along the protein molecules, the importance of hydrophobic interactions and the ability to stabilize super-

structures formed by creating additional S–S bridges. The main differences between gelatin and whey protein particle suspensions are related to the strength of the interactions and the ability to form new interactions. Gelatin particles form a loose network that can easily be reformed provided it has sufficient time to relax. This reformation is supported by its rheopectic behaviour. The whey protein particle network shows a higher degree of structure that can withstand a small deformation. The higher degree of structure is supported by the higher yield stress and the higher value of the complex viscosity.<sup>13,14</sup>

## Conclusions

Protein particles, created from gelatin and whey protein, can form an elastic particle network in suspension as a result of the high degree of interactions present between the protein particles. The presence of a network structure is evident from the yield stress and shear thinning behaviour. Strain dependency measurements also indicate the presence of a network. The properties of both suspensions suggest that the behaviour of the protein particles in the suspension depends to a large extent on the mesoscopic properties of the protein. The differences in the behaviour of gelatin and whey protein suspensions, such as response to oscillation and low shear rate, are probably caused by difference in their microstructure and molecular properties.

**Acknowledgement** This research was financially supported by the Graduate School VLAG.

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