EFFECT OF LACTOSE ON RHEOLOGY OF MILK PROTEIN DISPERSIONS

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ABSTRACT

A systematic study of the flow behaviour of commercially available AME100 milk protein dispersed in water and the effect of lactose on the rheology of the system was performed at various concentration with *Ubbelohde* viscometry in the dilute regime and with rotational shear rheometry in the semidilute and concentrated regime. Intrinsic viscosity and overlap threshold of the lactose free system was quantified.

Concentrated dispersions showed enhanced dependency of viscosity on concentration, pronounced shear thinning and elastic properties were evident. Addition of small amounts of milk protein to lactose solutions resulted in a decrease of solution viscosity while in semidilute and concentrated dispersions the viscosity was raised to a higher extent than would have been expected through the increase of solvent viscosity upon lactose addition. Findings show that changed protein-protein and protein-solvent interactions in the presence of lactose in the solvent affects the flow behaviour of the investigated system.

1 INTRODUCTION

The study of the flow behaviour of protein dispersions is of considerable interest in the production of protein products. It provides information necessary for the optimal design of flow processes such as pumping, piping, spray drying, extruding, etc., and can be used for quality control of both the manufacturing process and the final products.

Although all proteins are built from amino acids, the flow behaviour of their dispersions may differ greatly due to factors such as shape, size, charge, heat treatment and solvent composition.

Milk protein is a mixture of different protein types originated from mostly cow milk. The caseins are with a portion of about 80 % quantitatively the most important fractions. They are amphiphilic and mostly random coil polypeptides with a molecular weight of 19'000 to 23'000. In milk, caseins exist as highly hydrated micelles. Processed to sodium caseinate the native structure of the casein micelles is disrupted through acid and alkaline treatments. Sodium caseinate is soluble above pH 5.5, has good water holding properties and forms high viscous solutions. The whey proteins, the remaining 20 % of milk proteins, are with a molecular weight of 14'000 to 18'300 smaller in size. They are globular proteins, have a more organized structure and are more sensitive to heat and pH than caseins and caseinates. Whey proteins have low water absorption properties. Their dispersions remain rather low viscous even at high concentrations. Physicochemical and functional properties of milk proteins have been reviewed by Kinsella [1].

Many food systems are composed of more than a single ingredient. Besides protein, components such as hydrocarbon oils, polysaccharides or sugars are present in protein based foods. The flow behaviour of the protein dispersions can be altered by the presence of such ingredients. This paper focuses specifically on the effect of added lactose on the rheology of milk protein dispersions. Thus, the change of solvent quality caused by the addition of sugar to a protein dispersion and as a consequence thereof the change of interaction mechanisms in the system is a determining factor for the flow behaviour of the system.

The protection against globular protein unfolding that is conferred by high sugar/protein ratios is a well-known phenomenon with consequences for heat stability [2]. Several workers contributed with their research to the understanding of the influence of sugars on the physicochemical properties of protein systems. The phenomenon of protein stabilization has been interpreted as follows. Arakawa and Timasheff reported in [3] that the direct contact between protein and water is thermodynamically unfavourable in the presence of sugars. Their findings can be correlated directly with an enhancement of hydrophobic interactions [4]. Sugar addition leads to a diminished water activity. This change of the system is considered to make water-protein interactions less effective [5]. Direct sugar-protein interactions through hydrogen bonding may lead to a change in protein surface hydrophilicity [6]. However, for a caseinate containing system Dickinson and Matia Merino [7] found enhanced casein-casein interactions in the presence of added sugars. In a spectroscopic study Alexander et al. [8] measured smaller micelles in lactose free milk and partly attributed this to the ability of lactose

to induce self-assembly of whey proteins. Against this background, the influence of lactose on the rheology of specific milk protein dispersions still needs to be properly characterized.

2 MATERIALS AND METHODS

2.1 Materials

The experiments were carried out using commercially available spray dried milk protein powder AME100 supplied by EMMI Milch AG (Dagmersellen, Switzerland). In this protein powder the casein fraction is present as sodium caseinate and the whey proteins are partially denatured. Edible lactose Esprion 070 was supplied by DMV International (The Netherlands).

2.2 Sample preparation

For the *Ubbelohde* viscometer measurements standard solutions of 1 g/100 ml were prepared as follows: milk protein powder was dissolved at a temperature of 55 °C. Water and lactose solutions with concentrations of 5, 7.5 and 10 g anhydrous lactose per 100 g total mass were used as solvents. The standard solutions were further diluted with their solvent to obtain solutions at concentrations of 0.1, 0.15, 0.25, 0.5 g per 100 ml. Fresh samples were stored for a maximum of 2 days at 5 °C before measurement.

The samples used in rotational rheometer measurements were all based on a concentrated dispersion of 30 wt-% milk protein. Milk protein powder was dispersed in water in a stirring apparatus. Stirring was performed during a period of 4 hours at a temperature of 55 °C in a low-pressure environment (0.5 bar) to remove dispersed air. Dispersions with concentrations between 5 and 20 wt-% milk protein and 0, 5, 10 and 15 wt-% anhydrous lactose in the solvent were prepared by gentle stirring at room temperature until dissolution was complete. Fresh samples were stored for a maximum of 2 days at 5 °C before measurement.

2.3 Rheological measurements

Viscosity of low viscous milk protein solutions and lactose solutions were measured with a calibrated KPG-*Ubbelohde* viscometer No. 1 (capillary constant K=0.01) at a temperature of 20 °C. The processor viscosity system PVS 1 (Lauda, Lauda-Königshofen, Germany) was used for the time measurements. Three measurements with a standard deviation < 0.5 seconds were used for the calculation of the average measurment time. The wall shear rate in the capillary was estimated according the *Hagen-Poseuille* law as given in Eq. (1):

$$\dot{\gamma}_w = \frac{4 \cdot \dot{V}}{\pi \cdot d_k^3} \quad (1)$$

Density of the solutions was measured with a DMA 38 density meter (Anton Paar, Graz, Austria) to compute the dynamic viscosity of the samples.

Viscosity measurements of semi-dilute and concentrated samples were carried out in a Rheometric Scientific DSR rheometer using a couette geometry with a gap of 1.25 mm and a diameter ratio between inner and outer cylinder of 0.92. A solvent trap was used to avoid solvent evaporation during measurements. Measurements were made in triplicates at decreasing shear stresses. The data points were collected in most accurate steady state sensing mode in order to get equivalent runs for all systems under investigation. The temperature was kept constant at 20 °C throughout the measurements.

Additional measurements on dispersions with highest investigated concentrations were made with a Rheometric Scientific ARES rheometer using a cone plate geometry with 40 mm in diameter and an angle of 0.04 rad. After loading, the edges of the sample were covered with mineral oil to prevent solvent evaporation during the measurement. Viscosity and first normal stress measurements were made in triplicates at decreasing shear rates. A holding time of 5 minutes at each shear rate was maintained before data collection in order to get equivalent runs for each sample. The temperature was kept constant at 20 °C throughout the measurements. Oscillatory measurements were carried out in the linear viscoelastic regime using the same geometry.

3 RESULTS AND DISCUSSION

3.1 Lactose free milk protein samples

Fig. 1 shows the reduced viscosity η_{sp}/c measurements of milk protein AME100 dispersed in water with the corresponding Huggins equation (Eq. 2).

$$\frac{\eta_{sp}}{c} = [\eta] + k_H \cdot [\eta]^2 \cdot c \quad (2)$$

The approximated intrinsic viscosity of the dissolved milk protein is $[\eta] = 17.2\ cm^3\ g^{-1}$. For rigid hard spheres without solvent interaction the intrinsic viscosity is according to Einstein [9] $[\eta] = 2.5\ cm^3\ g^{-1}$. The intrinsic viscosity of flexible macromolecules differs from this value due to solvatization and interaction of the macromolecules with the solvent. Intermolecular interactions are not expected as long as the increase of η_{sp}/c is linear with concentration. All solutions were slightly turbid what suggests the presence of molecular assemblies. Light microscopy supports this indication. Very few small aggregates

were evident in the images. Nevertheless, their impact on the intrinsic viscosity might be negligible.

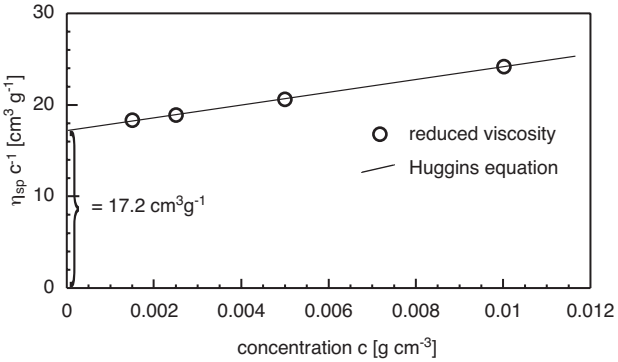

Figure 1: Reduced viscosity of milk protein AME100 at 20 °C versus concentration approximated with Huggins equation ($\eta_{sp}/c = [\eta] + k_H [\eta]^2$ c).

At higher concentrations the flow behaviour of the AME100 milk protein dispersions changes from Newtonian to shear thinning behaviour. Shear viscosity versus shear rate of dispersions in water at various concentrations are depicted in Fig. 2. With increasing concentration there is an increasing deviation from Newtonian behaviour and an increasing dependency of the concentration on the viscosity of the samples. At all concentrations a Newtonian plateau can be observed in the low shear rate domain.

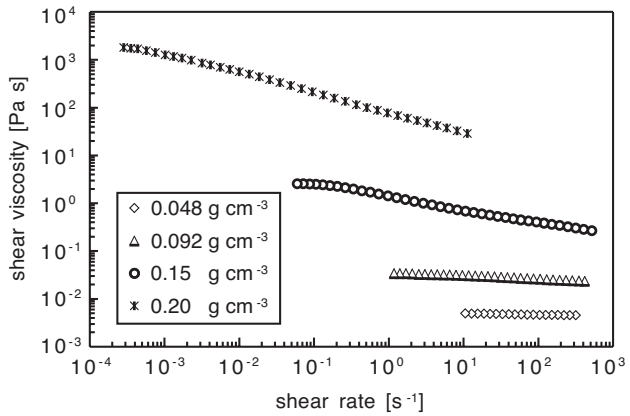

Figure 2: Shear viscosity of milk protein AME100 versus shear rate at various concentrations, measured at 20 °C.

In Fig. 3, which shows log(η_{sp}) versus log(c [η]), two linear domains can be found in the double logarithmic plot; the first for small and the second for high c [η]-values. In between there is a transition region. At low c [η] the specific viscosity is proportional to c [η] $^{1.09}$. In this domain the system is dilute and no contact between the single protein molecules or molecular assemblies is evident. The concentration above which the data points deviate from this relation is referred to as overlap threshold. For monodisperse polymers in good solvents an approximate value of the overlap threshold c* can be calculated with Eq. 3 [10].

$$c^* \approx \frac{1.46}{[\eta]} \quad (3)$$

Accordingly, the overlap threshold for AME100 milk protein dispersed in water would be expected at a concentration of 0.085 g cm $^{-3}$. However, the value determined from the viscosity measurements lays below 0.048 g cm $^{-3}$. This deviation is not surprising since milk protein is a polydisperse mixture of several casein and whey protein fractions, and the factor of 1.46 should not be understood restrictive. Thus, the calculated value gives a rough estimation for the investigated system.

Above c* the system can be described as semidilute.The protein molecules contact and begin to contract. Intermolecular interactions start to dominate the flow behaviour of the system. In the region where the specific viscosity of the AME100 milk protein dispersions is proportional to c [η] ^{22} no further contraction is expected. The dispersions are concentrated and show an emphasized dependency of the viscosity on the concentration of the dispersed proteins.

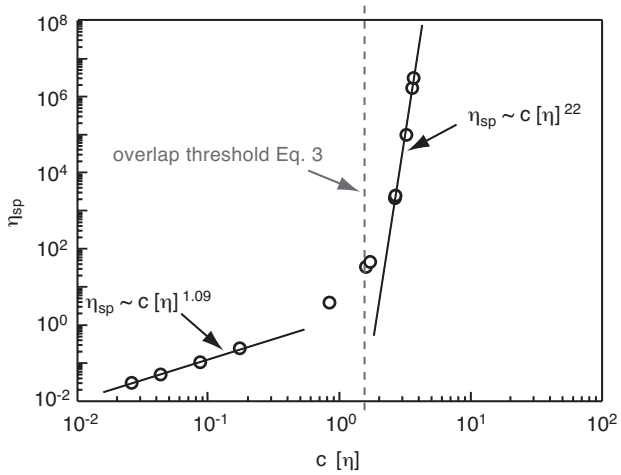

Figure 3: Specific viscosity of milk protein AME100 at 20 °C; log $\eta_{sp} = f$(log c[η]).

3.2 Effect of lactose

Fig. 4 shows the results from the measurements of the low concentrated AME100 milk protein solutions with various portions of lactose in the solvent versus protein concentration. The open symbols refer to calculated values assuming no interaction between milk protein and lactose. In the case of no interaction the viscosity of the milk protein dispersed in water would simply be increased by the relative viscosity of the solvent. Up to a concentration of 0.0025 g cm $^{-3}$ the viscosity obtained from viscosity measurements lays below the calculated value and even below the viscosity of the pure lactose solutions which is 1.1492 mPa s, 1.2406 mPa s, and 1.3485 mPa s for lactose solutions with 5 wt-%, 7.5 wt-%, and 10 wt-%.

These results are rather surprising and the reasons are not known at the moment. One speculation could be that close to the protein molecules the concentration of lactose is increased and thus the concentration of lactose in the solvent is decreased and contributes less to the viscosity increase of the system. It is evident that these measurements do not allow for determining the intrinsic viscosity of the AME100 milk protein dispersed in lactose solutions, because of relative viscosities resulting at values < 1.

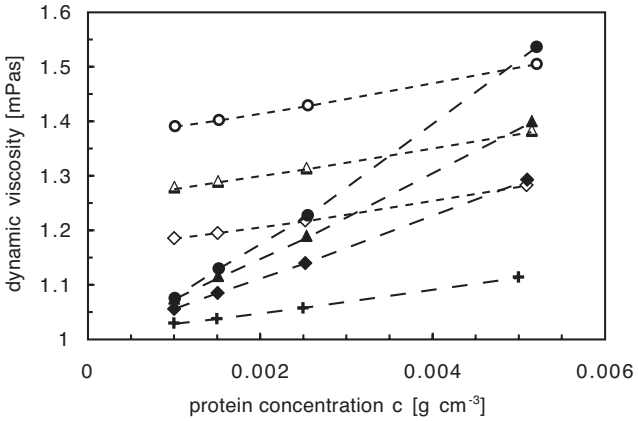

Figure 4: Viscosity of AME100 milk protein solutions versus concentration. Solvents: water (+), lactose solutions with 5 wt-% (◆), 7.5 wt-% (▲), and 10 wt-% (●). Open symbols refer to calculated values assuming no interaction ($\eta = \eta_{AME100/water} \cdot \eta_{rel,\ lactose\ solution}$). Lines are displayed to guide the eye.

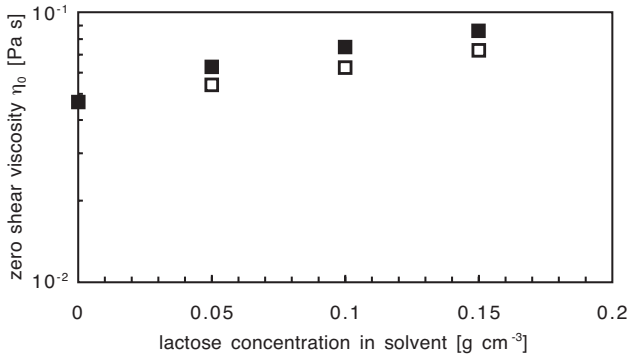

Figure 5: Zero shear viscosity of a 0.1 g cm $^{-3}$ milk protein AME100 dispersions at increasing concentration of lactose in solvent, measured at 20 °C. Open symbols refer to calculated values assuming no interaction ($\eta = \eta_{AME100/water} \cdot \eta_{rel,\ lactose\ solution}$).

At a protein concentration of 0.005 g cm $^{-3}$ the measured values are slightly higher than the ones obtained from the calculation. The same trend is obvious for dispersions in the semidilute regime at a concentration of 0.1 g cm $^{-3}$ (Fig. 5) and in the concentrated regime at a concentration of 0.21 g cm $^{-3}$ (Fig. 6). This again suggests that with the change of solvent quality through the addition of lactose interaction mechanisms in the system are altered. Whether this is mainly due to enhanced hydrophobic interactions, less effective water-protein interactions or increased sugar-protein interactions and changed protein surface hydrophilicity remains unclear in the moment.

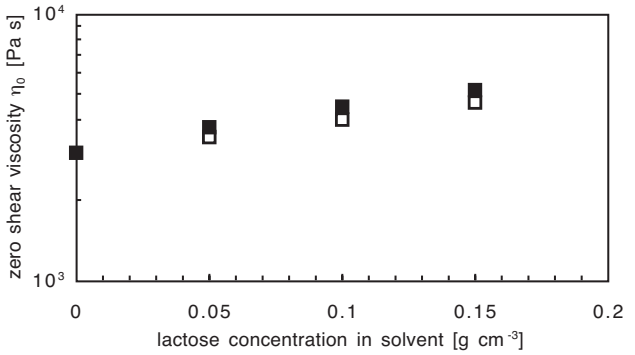

Figure 6: Zero shear viscosity of a 0.21 g cm $^{-3}$ milk protein AME100 dispersions at increasing concentration of lactose in solvent, measured at 20 °C. Open symbols refer to calculated values assuming no interaction ($\eta = \eta_{AME100/water} \cdot \eta_{rel,\ lactose\ solution}$).

Shear stress and first normal stress difference versus shear rate of samples with a milk protein concentration of 0.2 g cm $^{-3}$ dispersed in water and 15 wt-% lactose solution are depicted in Fig. 7. At constant shear rate stresses are higher for the dispersion with lactose. A cross-over of the curves can be observed at moderate shear rate for both samples. The first normal stress difference dominates shear stress at similar stress level independent of the presence of lactose. Fig. 8 shows storage and loss moduli of samples with a milk protein concentration of 0.2 g cm $^{-3}$ dispersed in water and 15 wt-% lactose solution versus frequency. Both samples can be described as visco-elastic liquids. The addition of lactose leads to a shift of the curve to lower frequencies, in addition, storage modulus exceeds loss modulus at lower values. The viscoelastic behaviour of the dispersions does not change qualitatively with the presence of lactose in the system. However, lactose leads to stiffer samples and to higher values of elastic response.

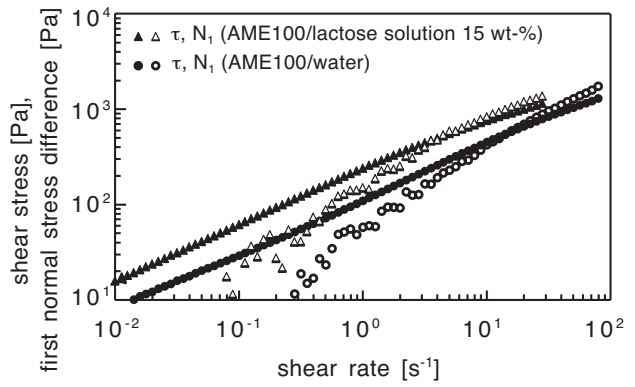

Figure 7: Shear stress and first normal stress difference of 0.2 g cm $^{-3}$ AME100 milk protein dispersions versus shear rate.

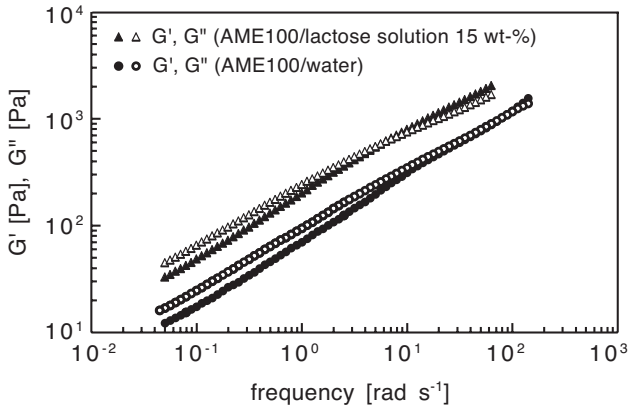

Figure 8: G' and G" of 0.2 g cm $^{-3}$ AME100 milk protein dispersions versus frequency.

4 CONCLUSIONS

The flow behaviour of AME100 milk protein dispersed in water has been studied over a wide concentration range. In the dilute regime the solution shows the typical behaviour of dilute polymer solutions.The intrinsic viscosity amounts to 17.2 cm ^{3} g $^{-1}$. The overlap threshold of the system lays below 0.048 g cm $^{-3}$. Above this concentration, in the semidilute regime the flow behaviour changes from Newtonian to shear thinning. Concentrated dispersions show pronounced shear thinning, elastic properties and a strong dependency on protein concentration.

The addition of lactose to the system affects the rheological behaviour of the milk protein dispersions to a higher extent than simply raising the viscosity of the solvent.The solvent quality is changed and thus interaction mechanisms in the dispersion are altered. The addition of only a small amount (< 0.0025 g cm $^{-3}$) of milk protein to a lactose solution with a concentration of 5 to 10 wt-% significantly decreased the viscosity of the system. Additions of higher amounts of milk protein lead to an increased viscosity, whereas this increase was higher than calculated for a system without interaction between solvent and dispersed protein molecules. The elastic properties were not changed qualitatively compared to lactose free samples with same amount of dispersed proteins.

The results show that the manner in which the influence of lactose on the flow behaviour of the investigated milk protein dispersions becomes apparent is a function of concentration of the protein dispersion. Thus, different interaction mechanisms dominate in the respective concentration regimes. In dilute solutions a layer of lactose molecules seems to be present around the surface of the proteins which leads to a decrease of lactose concentration in the solvent and could give an explanation for the results of the viscosity measurements. In the semidilute and concentrated regime mechanisms like enhancement of hydophobic and/or protein-protein interactions, less effective water-protein interactions or increased sugar-protein interactions could be responsible for the discussed effects.

5 ACKNOWLEDGEMENTS

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