Electronic Supplementary Information

Molecular behavior of fluid gels – the crucial role of edges and particle surface for macroscopic properties

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S1 Materials and methods

S1.1 Preparation of quiescently cooled gels (*Ex situ*)

To verify the amplitude test of the quiescently cooled gels prepared *in situ* directly in the rheometer (as described in section 2.2.2 Preparation of quiescently cooled gels), they were compared with gels prepared outside the rheometer (*ex situ*) using amplitude sweep measurements (see Figure S2). Therefore, disposable aluminum plates with a diameter of 25 mm and a height of 3 mm, and Teflon molds with an inner diameter of 25 mm and a height of 6 mm that were slipped over the aluminum plates were used. To produce the gels with defined height and diameter, the still hot agarose solution was pipetted into the Teflon mold and evenly distributed with a razor blade. The set gels were then cooled to room temperature and cured overnight in the refrigerator at 4 °C. To minimize water evaporation, the molds were covered with disposable weighing dishes that were greased around the edges to seal them airtight. The next day, the molds were carefully removed and the samples were adjusted to room temperature before measurements.

S1.2 Rheological measurements of the quiescently cooled gels (*Ex situ*)

For the quiescently cooled gels prepared *ex situ*, amplitude oscillatory measurements were performed on a Discovery HR-3 Rheometer (TA Instruments) using a 25 mm diameter plate at a constant temperature of 25 °C and a soak time of 120 s. The amplitude sweeps were carried out at a constant frequency f = 1 Hz, and storage (G') and loss (G'') moduli were measured as a function of strain γ ranging from 0.001 % to 1000 %. To avoid slippage effect of the samples, sandpaper with a grit size of P80 was used and glued on both plates with double sided adhesive tapes.

Since the height of the gels differed from the intended 3 mm during cooling, the gap size between the two plates needed to be varied to ensure sufficient adhesion and to prevent the sample from slipping

during measurements. Therefore, sufficient pressure had to be applied on the sample between the plates. However, squeezing the sample should be avoid. Hence, an axial force of about 0.15 N for the 0.5 wt% gels, 0.5 N for the 1 wt%, and 1 N for the 2 wt% gels, was applied in order to obtain appropriate contact for the amplitude sweep. This indicates that the gels were not completely flat and that more normal force had to be applied the stiffer the samples were to achieve better contact between the upper plate and the sample.

S1.2 Size exclusion chromatography (SEC)

Size exclusion chromatography measurements were performed on a PSS SECcurity^2 system (PSS, Polymer Standards Service Mainz, Germany) equipped with a refractive index detector and a UV detector (280 nm). Chromatographic separation was performed by using a SUPREMA Linear XL column (10µm, 8 x 300 mm) (PSS). All experiments were performed in 0.1M NaNO3 at a flow rate of 1 ml/min and 60°C. The sample with a concentration of 1 mg/ml was first heated at 90°C for 3 h, then cooled to 60°C and finally filtered through a 0.45 syringe filter prior to measurement. An injection volume of 50 µl was used. Calibration was carried out with pullulane stadards (molar masses between 180 g/mol and 800000 g/mol) provided by PSS. All data were recorded and evaluated by using PSS WinGPC UniChrom.

S2 Results

S2.1 The significance of the trend of tan(δ) of fluid gels compared to quiescently cooled gels

In order to obtain further information from the amplitude sweeps (Figure 13), tan(δ) was plotted against the deformation strain as shown in Figure S1. These values of tan(δ) are higher for the fluid gels than for the gels cooled under quiescent condition. This is explained by the fact that the elastic behavior of the unsheared gels is extremely dominant over the viscous behavior, which is due to the aforementioned three-dimensional continuous network of aggregated double helices in thick bundles of junction zones that lead to strong gels. The fluid gels and the corresponding gels prepared under quiescent conditions were turned upside down to demonstrate the solid-like properties (see Figure S1 (a and b)). Furthermore, taking into account the smaller error bars for the fluid gels, a slight increase in tan(δ) can be seen with increasing strain. This indicates an increase in G'', and thus an increase in the deformation energy transferred to the environment before deforming parts of the inner structure to a certain extent, and finally disrupting the connection between the particles. The values of tan(δ) at a strain γ = 0.01 of the different fluid gel concentrations are significantly different ($p < 0.05$, one-way ANOVA). However, if considering the small deformation strains below γ = 0.01 %, it can be observed that the smallest tan (δ) value are resulting for the 2 wt% fluid gels followed by the 0.5 wt% gels, which show a higher value, and finally the highest tan(δ) for the 1 wt% fluid gels. These observations are consistent with the results on tribology, textural analysis and rheology measurements such as amplitude sweep, frequency sweep and flow sweep. They are attributed to the high volume fraction and percolation of the densely packed gel particles of the 2 wt% sample on the one hand, and to the loose surface structure and less densely packed gel particles of the 0.5 wt% and 1 wt% samples on the other hand.[1] In addition, the reason why the 1 wt% fluid gel samples show a higher tan(δ) than the

0.5 wt% fluid gel can be explained by the presence of a higher proportion of hairy structures on the particle surfaces of the lower concentrated fluid gel, which was already observed in Ghebremedhin, Seiffert, and Vilgis.[1] The aggregated chains on the particle surface become entangled at low strain (within the LVE range), resulting in higher elasticity and thus lower tan (δ) for the 0.5 wt% than for the 1 wt% fluid gels sample. Moreover, the differences of tan (δ) at a strain γ = 0.01 of the gels cooled at quiescent conditions are not significant with respect to the concentration ($p < 0.05$). On the other hand, there is an increase not only in the error bars but also in the tan(δ). As mentioned earlier, this is due the fact that as the deformation increases, the gels break up and thus the viscous part increases. Although a trend of increasing storage and loss moduli with increasing concentration is obvious from the results shown in Figure 13 and Table 3, a one-way ANOVA revealed no statistically significant difference in tan(δ) at strain γ = 0.01 of the gels cooled under quiescent conditions. Note in Figure S1 that, in contrast to the fluid gels, the tan(δ) of the quiescently cooled gels increases with increasing concentration. However, this observation is not consistent with previously published results in which the authors reported that tan(δ) of agarose gels decreases with increasing concentration and requires further investigation.[2] Therefore, supplementary amplitude sweep measurements were performed with gels previously gelled outside the rheometer (*ex situ*) under quiescent conditions. This examination will be discussed in detail below.

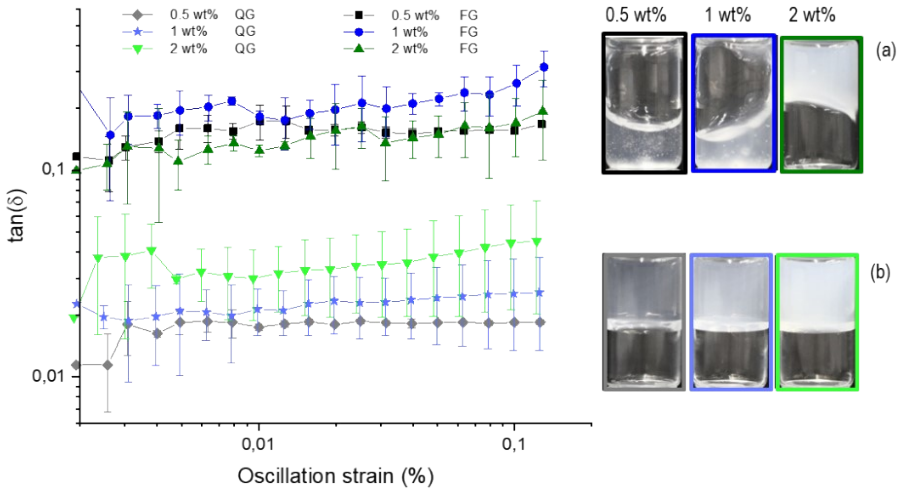

Figure S1. Amplitude sweep for the gels prepared under shear and non-sheared gels for the different agarose concentrations. Dependence of tan (δ) on strain at constant frequency (f = 1 Hz) and temperature (T = 25 °C). Fluid gels (a) and corresponding gels prepared under quiescent conditions (b) were turned upside down to demonstrate the solid-like properties.

Figure S2 show the comparison of the amplitude sweeps of the different prepared quiescently cooled gels: the gels prepared directly in the rheometer (*in situ*) and the gels prepared under quiescent conditions outside the rheometer in molds of defined diameter and height (*ex situ*). As expected, it can be seen that the storage and loss moduli increase with increasing concentration for both the *in situ* and the *ex situ* quiescently cooled gels. However, compared to the gels prepared *ex situ*, the gels prepared *in situ* show a higher value for the storage moduli for all concentrations (see Figure S2 (a)). For the loss moduli, on the other hand, the differences between the various prepared gels are smaller (see Figure S2 (b)).

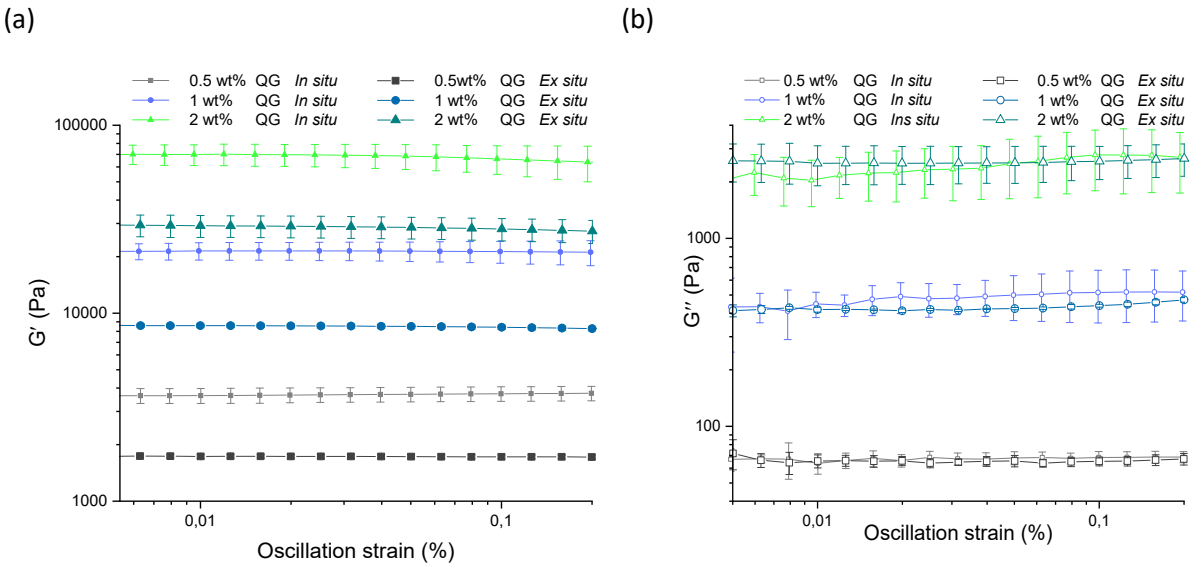

Figure S2. (a) Storage (G') and loss (G'') moduli of the amplitude sweep of the different prepared quiescently cooled gels. Gels prepared directly in the rheometer (in situ) and outside the rheometer (ex situ).

However, when comparing the tan (δ) in Figure S3 of the differently prepared quiescently cooled gels for both the same trend of an increase of tan(δ) with increasing concentration can be observe. As mentioned earlier, this is not in agreement with some previously published results. Nevertheless, on the other hand a similar trend was observed in studies on agar microgel suspensions.[3] However, the tendency observed in this work here can be explained by the fact that, in contrast to an ideal network in which each chain is continuously cross-linked, the gels produced here are not. According to the size exclusion chromatography (SEC) results in Figure S4, the molecular weight of the agarose used in this work exhibits a broad distribution. This indicates that shorter chain ends remain uncrosslinked and therefore move, leading to an increase in deformation energy and thus an increase in viscous dissipation. The relative movement of the free chain ends, which are not permanently embedded in the network, could explain this inconsistency of increasing tan (δ) with increasing concentration. Moreover, since not all agarose chains can be cross-linked, more uncross-linked chains dangle freely between the meshes at higher concentrations.

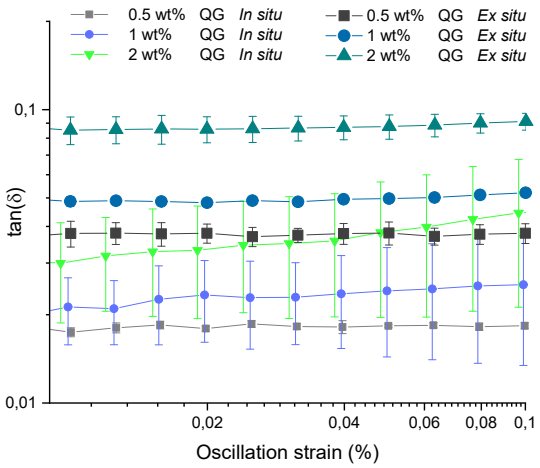

Figure S3. Tan(δ) of the amplitude sweep of the different prepared quiescently cooled gels. Gels prepared directly in the rheometer (in situ) and outside the rheometer (ex situ).

S2.2 Size exclusion chromatography (SEC)

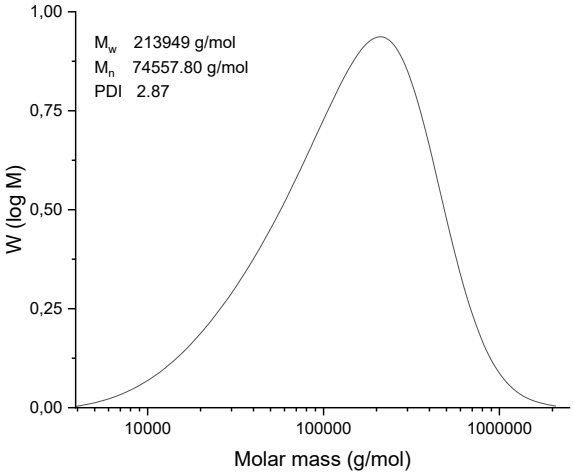

Figure S4. Molecular weight distributions of agarose estimated by SEC. Number average molar mass (M_n), weight-average molar mass (M_w) and polydispersity index (PDI) are also shown.

References

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2. K. Nishinari, M. Watase, K. Kohyama, N. Nishinari, D. Oakenfull, S. Koide, K. Ogino, P. A. Williams and G. O. Phillips, The effect of sucrose on the thermo-reversible gel-sol transition in agarose and gelatin, *Polymer Journal*, 1992, **24**, 871-877.
3. S. Adams, W. J. Frith and J. R. Stokes, Influence of particle modulus on the rheological properties of agar microgel suspensions, *Journal of Rheology*, 2004, **48**, 1195-1213.