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Research paper

Continuing yet mostly reversible structuration of gum arabic in the presence of oil at interface

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ABSTRACT

To understand the long-term self-association of gum arabic (GA) in water, the time-course development during aggregation of GA molecules with or without an oil/water interface was studied. A simple experimental setup, using a rheometer was employed. The transparent cone-plate geometry was filled with gum arabic solution, and the periphery of the geometry was covered with oils or left uncovered. The observed time-course increase in shear modulus revealed that the continuous structuration of GA molecules occurs in the presence of oil at the interface. The network structure of the GA molecules was mostly re-dispersible with a flow test. In addition, the degree of structuration of GA aggregates depended on the type of oil. The physical properties of the aqueous GA solution, as measured by the rheological experimentation, were found to be strongly influenced by the interfacial conditions, namely air/water or oil/water.

1. Introduction

Emulsion plays a pivotal role in beverage and salad dressing applications. The oily flavor compounds are dispersed into the aqueous phase through emulsification (Given, 2009; Mao, Roos, Biliaderis & Miao, 2017; Molet-Rodríguez, Salvia-Trujillo & Martín-Belloso, 2018). Small-molecule surfactants (sucrose esters, Tweens, and SDS) or biopolymers (proteins and polysaccharides) are often employed (Molet-Rodríguez et al., 2018). However, with increasing consumer demand for natural products in foods (Dickinson, 1993), synthetic small-molecule surfactants are being replaced with natural emulsifiers, especially using biopolymers (McClements & Gumus, 2016; McClements, Bai & Chung, 2017; Ozturk & McClements, 2016).

Gum arabic (GA), a tree-based proteoglycan secreted from Acacia trees (*Acacia senegal*), is a typical biopolymer used in food applications (Phillips & Williams, 2000; Rideal & Youle, 1891). Owing to its amphiphilic nature, GA serves as a powerful emulsifying agent in beverage applications. Such amphiphilic nature originates from the hydrophilic negatively charged highly branched polysaccharides (~98 wt.%) and hydrophobic peptide residues (~2 wt.%) (Randall, Phillips & Williams, 1988). Unlike the protein-based emulsifiers, GA can form stable micron or submicron oil droplets over a wide range of pH, ionic strength, and temperature (McClements et al., 2017). However, one of its drawbacks is the high surfactant-to-oil ratio, as a highly concentrated (5–20 wt.%) GA solution is necessary for emulsification. Moreover, several studies have reported that the long-term emulsion stability of GA is inferior to that of other types of tree-based gums, such as gum ghatti and modified starch (Chanamai & McClements, 2001; Ido et al., 2008).

In a previous investigation (Isobe et al., 2020), the authors attempted to clarify the key parameter that can further improve the long-term emulsion stability of GA on a structural basis. It was found that the mode of adsorption of GA molecules onto the oil surface is dependent on the interfacial tension of oils. Further, it was also observed that interfacial adsorption behavior may be governed not by the primary structure of GA but by the secondary aggregating structure of GA molecules formed through self-association. This insight was obtained over a relatively short period, having a surface age less than 2000s. Hence, the practically important long-term behavior of the GA's self-association remains unclear.

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This study aims to understand the long term self-association of GA molecules, ranging up to one day in a practically important concentrated GA solution. The structuration of GA molecules was monitored with a simple rheological experimental setup: cone-plate geometry. Generally, to avoid the undesirable evaporation of water from the liquid sample in cone-plate geometry, two conditions are chosen for a cone-plate geometry, (i) covered with semi-closed high-humidity conditions (Sato & Breedveld, 2005) or (ii) covered with oils (Boger & Rama Murthy, 1969). However, as mentioned above, GA molecules can adsorbe onto the oil/water interface. Therefore, the structuration of GA, observed under two different conditions, i.e., semi-closed or oil-covered conditions, can be different. Here, to understand the effect of oils around cone-plate geometry, the structuration of GA molecules, with or without oils around the cone-plate geometry, was monitored and compared. The obtained insights should be beneficial in understanding the time-course change in the quality of oil/water-based products. For example, the required rigorousness to shake the salad dressing prior to use after a certain period of shelf time can also be gauged with the help of this study.

2. Materials and methods

2.1. Materials

Commercial gum arabic (*Acacia senegal*, GA) in spray-dried form was supplied by San-Ei Gen F.F.I., Inc., Japan. Pure water (Milli-Q) was used in this study. Reagent-grade dodecane (Wako Chemical Co., Ltd., Japan) was used without further purification. Further, commercial colza oil (purchased at Carrefour, France) was utilized as the vegetable oil. GA was dissolved in pure water at room temperature to obtain a 10 wt.% GA solution, which was stored at 4 °C until further use.

2.2. Multi-angle dynamic light scattering

Multi-angle dynamic light scattering (DLS) was performed using an in-house DLS instrument, as detailed in Supplementary Materials (Figure S1). A green laser (MSL-FN-532, wavelength: 532 nm, power: 100 mW, Changchun New Industries Optoelectronics Technology Co. Ltd., China), stepper motor (NEMA23, 57HS25), CCD camera (AcA640–100 gm/gc, Basler AG, Germany), photomultiplier (SPCM-AQR-13, Excelitas Technologies, USA), and a correlator (Flex03-LQ-1, Correlator.com, USA) were employed. The GA solution sample was placed in a glass tube having a diameter of 8 mm, and a wall thickness of 1 mm (LS Instruments AG, Switzerland). DLS measurements were performed at diffraction angles ranging from 30° to 150°

2.3. Rheometry

Rheological measurements were performed using an MCR301 instrument (Anton Paar, GmbH). The GA solution was filled in the transparent cone-plate geometry (gap: 0.36 mm, cone diameter: 49.923 mm, cone angle: 0.983°, and cone truncation: 98 μm, CP50–1/PC, Anton Paar, GmbH). The oils were poured between the GA solution and the stainless-steel ring wall, as summarized in Fig. 2. Each run consisted of four steps: (1) flow sweep test, wherein the shear rate was increased from 0.01 to 1000 s^{-1}), (2) a series of oscillatory tests with an angular frequency of 1.03 rad/s and shear strain of 0.5%, at certain time intervals up to 20 h, (3) flow sweep test wherein the shear rate was increased from 0.01 to 1000 s^{-1}, and (4) an oscillatory test at an angular frequency of 1.03 rad/s and shear strain of 0.5%. Using a strain sweep test (Figure S2), it was confirmed that at a shear strain of 0.5%, the loss and storage moduli were constant. Therefore, the oscillatory test at a shear strain of 0.5% did not destroy the network structure made of GA molecules. All the experiments were performed at 25 °C. Moreover, the reproducibility of the measurements was checked by refilling the samples and performing the same experiments at least three times, with less than 5% difference among the runs. Additionally, to remove the effect originating from the air/GA solution interface, and to confirm rotational rheometry measurements, dynamic viscosity was measured using a rolling ball viscometer (Lovis 2000 M, Anton Paar, GmbH).

3. Results and discussion

The rheological experimental setup under semi-closed high-humidity conditions is depicted by Fig. 1. The GA solution was filled in the transparent cone geometry, and the water was filled in the water channel of a stainless-steel ring placed around the cone-plate geometry. In this manner, a sufficiently high humidity was maintained to avoid water evaporation from the GA solution. The entire setup was covered with a lid composed of two parts, and a hole in the middle to allow free movement of the shaft of the upper cone geometry. It was confirmed that, under this semi-closed environment, the increase in GA concentration was negligibly small, i.e., less than 0.2 wt.% even 24 h after filling. This indicates that the effect of evaporation was negligible.

First, to dissociate the pre-formed aggregates of GA molecules, the GA solution was subjected to a flow sweep test, as shown in Fig. 2. The viscosity flow curve clearly shows the shear thinning behavior, i.e., decrease in viscosity with an increase in shear rate. The viscosity reached an almost constant value of approximately 3.5 mPa•s at a high shear rate (~1000 s^{-1}), which corresponds well to the dynamic viscosity measured by a rolling-ball viscometer.

Further, right after the flow sweep test shown in Fig. 2, a frequency sweep test was performed. The frequency dependence of the shear moduli in the 10 wt.% GA solution observed under semi-closed high-humidity conditions is shown in Fig. 3. The storage modulus was 1.5 times higher than the loss modulus, up to an angular frequency of 10 rad/s. This suggests that 10 wt.% GA solution behaved as a weak gel (Winter & Mours, 1997), where the aggregates of GA molecules form the network structure. Since this measurement was performed just after a flow sweep test (Fig. 2), the network structure of GA molecules was formed within a few minutes, suggesting a fast structuration that proceeds on a minute time scale. Above 10 rad/s, the viscous and elastic moduli follow a power law with an exponent of 2, owing to the inertia of the upper cone geometry (Auffret et al., 2009; Krieger, 1990; Läuger & Stettin, 2016; Yao, Larsen & Weitz, 2008) and fluid inertia (Böhme & Stenger, 1990).

To monitor the time-course change on an hour time scale in shear modulus, namely storage and loss modulus, in semi-closed high-humidity conditions, oscillatory tests were performed at a certain time interval (Fig. 4a). The shear moduli increased, up to 3–5 h, and decreased to a steady state at 15 h (Fig. 4a). This fluctuation before stabilization is possibly due to the stabilization of humidity in the experimental setup. At steady state, while the loss modulus is close to the initial value (~0.03 Pa), the storage modulus (~0.11 Pa) was approximately twice as high as the initial value (~0.05 Pa). This suggests that a relatively slow structuration of GA molecules proceeds on an hourly time scale. These structured GA molecules were subjected to flow sweep tests (Fig. 4b). As in Fig. 2, the viscosity reached almost the same level of 3.5 mPa•s at high shear rate (1000 s^{-1}), indicating the complete dissociation of GA molecules by shearing. However, the thinning behavior was more prominent than that observed in the initial state, shown by the open triangles in Fig. 4b. The viscosity at the lowest shear rate (0.01 s^{-1}) increased by three times from the initial state. This also corroborates the structuration of GA molecules with time, leading to the thixotropic nature of the aqueous GA solution (Li et al., 2011).

Further, the effect of oil at the interface in the cone-plate geometry was examined, as shown in Fig. 5. The GA solution was filled in the transparent cone geometry, and oil was gently poured between the GA solution and stainless-steel ring wall. The transparent appearance of the cone geometry allowed the visual confirmation that there was no penetration of oil beneath or onto the cone geometry. Thus, the oil contacted the GA solution and the lower edge of the cone geometry.

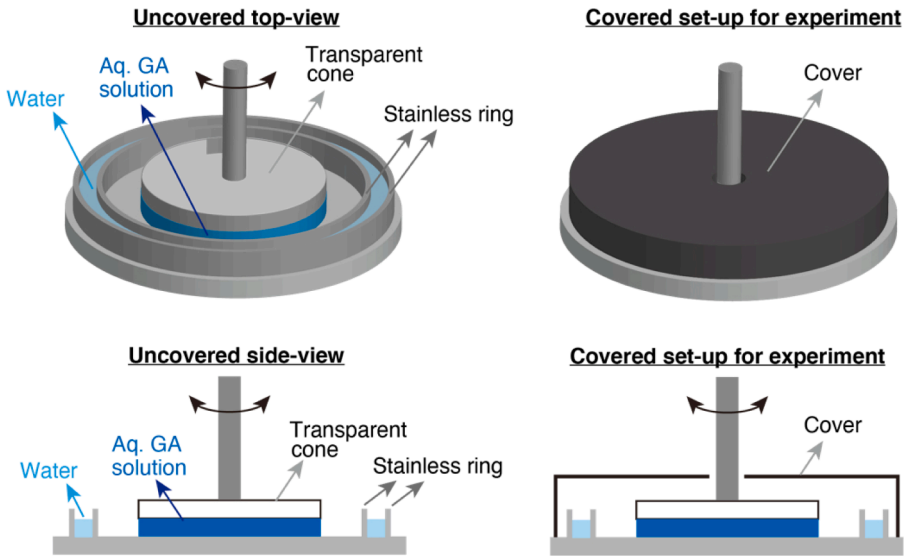


Fig. 1. Schematic representation of the experimental set-up used on rheometer for semi-closed high-humidity condition.

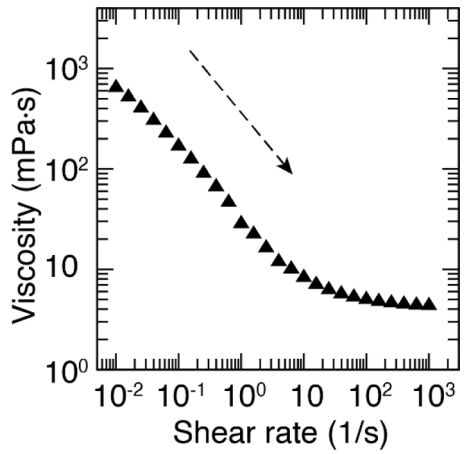


Fig. 2. Viscosity flow curve of GA solution just after the filling in an experimental set-up with semi-closed high-humidity condition (Fig. 1). The measurement was performed by a flow sweep test, wherein the shear rate was increased from 0.01 to 1000 s^{-1}.

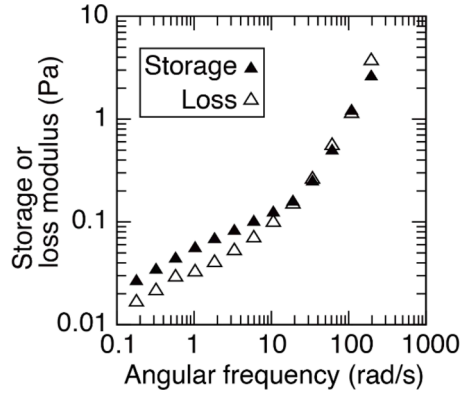


Fig. 3. Frequency dependence of shear moduli observed in semi-closed high-humidity condition. This measurement was performed just after a flow sweep test (Fig. 2). Each point in the figure was obtained in a stationary viscosity condition.

With this setup, the time-course change in shear modulus, namely storage and loss modulus, was monitored by performing oscillatory tests at a certain time interval. The obtained shear moduli cannot be discussed quantitatively. However, the monitoring of the structure of GA molecules was successful as follows.

Before starting to monitor the time-course change in shear modulus, a flow sweep test was performed to dissociate the pre-formed network structure of aggregated GA molecules (open circle in Fig. 6b). A typical shear thinning behavior was observed, as in the semi-closed high-humidity condition shown in Fig. 2 and Fig. 4b. Using the following oscillatory tests at a certain time interval, the formation of the network structure of GA molecules from the completely dissociated state was monitored. The time-course changes in the storage (G') and loss (G'') moduli of the GA solution filled with vegetable oil are shown in Fig. 6a. Both G' and G'' showed a similar trend, i.e., a steep rise up to 2 h followed by a slow but continuous increase up to 20 h. This continuously increasing trend of shear moduli was opposite to the case with semi-closed high humidity conditions. This infers that the structuration of aggregated GA molecules in the oil-covered geometry developed more highly than in the case of semi-closed high-humidity conditions.

To determine whether this structure can be dissociated, a flow sweep test was performed (filled circle in Fig. 6b). The viscosity decreased with increasing shear rate, and reached an almost constant value of approximately 10 mPa•s at a high shear rate (~1000 s^{-1}). This indicates that the GA solution recovered the initial state. However, the viscosity at the lowest shear rate (3000 mPa•s at 0.05 s^{-1}) was thirty-times higher than that of the initial flow sweep test (100 mPa•s), whereas the increase in viscosity was only three times in the case of semi-closed high humidity conditions (Fig. 4b). Therefore, this also corroborates the higher development of structured GA molecules in the oil-covered geometry in comparison with the semi-closed high humidity condition.

Additionally, even after the flow test, no artifacts, such as emulsion formation, were observed in the experimental set-up (Figure S3a). This ensured the validity of the data obtained with the present setup. After the flow test (Fig. 6b), an oscillatory measurement was performed to determine whether the shear modulus recovered the initial values. As shown in Fig. 6a, the storage and loss moduli were almost identical to the initial state, indicating that the dissociation of the network structure by a simple flow test was successful.

To check the reversible nature of the structuration of GA molecules, a second run of the oscillatory test was conducted at certain time intervals (Fig. 7a). As in the case of first run (Fig. 6a), the second run exhibited the following trend: a steep rise at an early stage (up to 2 h) followed by a

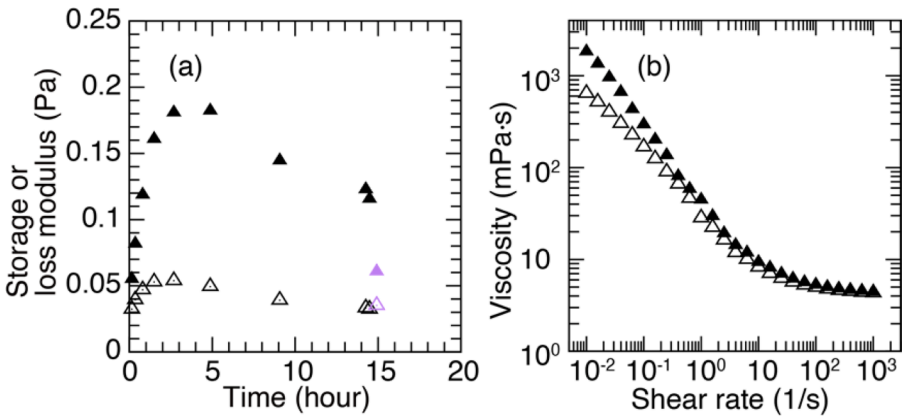


Fig. 4. (a) Time-course change in Storage (filled triangle) and loss (open triangle) modulus. Storage and loss modulus just after the complete shearing with flow sweep test are shown with filled (storage) and open (loss) circles in violet; (b) Viscosity flow curve of GA solution 14 h after the filling (filled triangles in black) and just after the filling (open triangles, same plots as in Fig. 2). The measurement was performed by a flow sweep test, wherein the shear rate was increased by steps from 0.01 to 1000 s^{-1}.

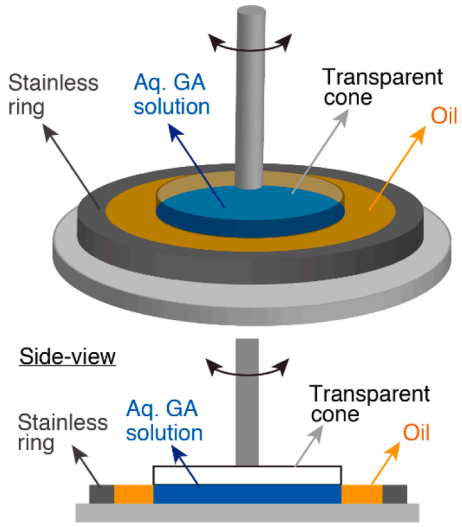


Fig. 5. Schematic presentation of the experimental set-up of the rheometer with oils.

slow but continuous increase. The rate in the second run was faster than the first run, whereas the shear modulus in the second run was approximately 30% higher than the first run. This is probably because the remaining undissociated GA species may facilitate the reformation of the network structure.

At the end of the second run, a flow sweep test was conducted to dissociate the network structure of the GA molecules (Fig. 7b). In the second run, the shear rate was increased up to 5000 s^{-1}, being five times faster than the first run. The flow curve showed an almost identical trend to the first run (Fig. 6b), except for the outlier at 5000 s^{-1}. This anomaly was caused by the formation of emulsion droplets, as shown in Figure S3b. This result indicates that the current experimental setup is valid and repeatable when the shear rate does not exceed 1000 s^{-1} in the flow test.

As shown above, the developed structure of GA molecules in the oil-covered geometry was observed. The origin of this higher development can be the condensation of GA molecules in the vicinity of the oil/water interface, namely the combination of the adsorption of GA molecules onto the oil/water interface and non-adsorbed concentrated GA network in the vicinity of the interface. This was reported by the use of interfacial rheology experiments that require special instruments such as the Du Noüy ring (W. Li et al., 2018) or biconical disk geometry (Erni et al., 2007). However, vegetable oil contains impurities; thus, the effect of such impurities needs to be examined (Kershaw, 1986). For this, the same experiment was performed using dodecane (Fig. 8), which is a type of liquid alkane hydrocarbon, and is often employed as a model oil in lab-scale emulsion experiments. The storage modulus increased monotonically for up to 20 h, as in the case of vegetable oil (Fig. 8a). In addition, the enhancement of shear thinning was observed (Fig. 8b). The network structure was dissociated by the flow test (Fig. 8b), and the storage and loss moduli almost recovered to their initial values (Fig. 8a). The principal difference between vegetable oil and dodecane was the magnitude of change in storage modulus after 20 h from the start: 1.2 Pa for vegetable oil and 0.4 Pa for dodecane. This difference arises from the different adsorption capacities of the GA molecules at the oil surface (Isobe et al., 2020). Consequently, it can be concluded that the effect of impurities in vegetable oil is not significant.

The continuous increase in the shear moduli observed in a cone-plate geometry covered with oils was likely due to the condensation of GA

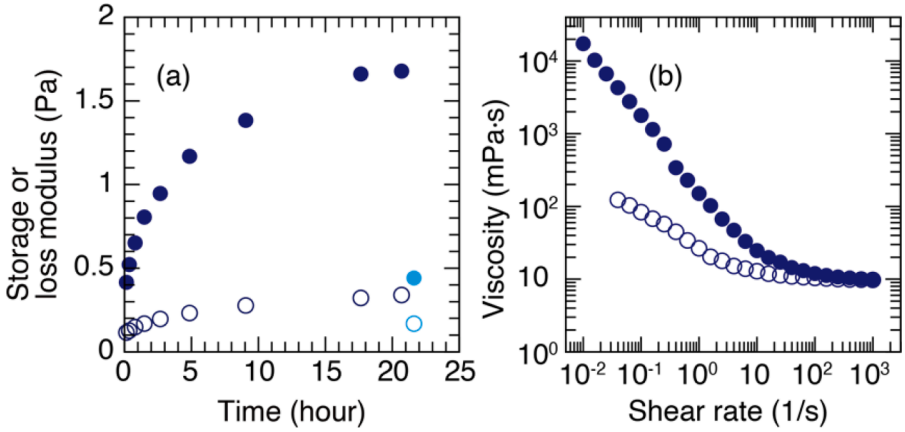


Fig. 6. Time-course change in (a) Storage (filled circles in dark blue) and loss modulus (open circles in dark blue) of GA solution filled with vegetable oil. Storage and loss modulus just after the complete shearing with flow sweep test are shown with filled (storage) and open (loss) circles in light-blue; (b) Viscosity flow curve of GA solution 22 h later from the filling (filled circles in dark blue) and just after the filling (open circles). The measurement was performed by a flow sweep test, wherein the shear rate was increased by steps from 0.01 to 1000 s^{-1}.

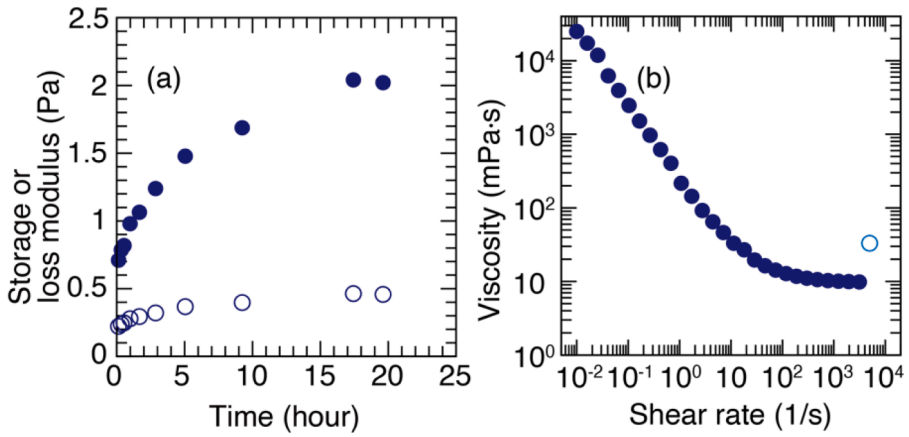


Fig. 7. Second run of time-course change in (a) Storage (filled circles) and loss modulus (open circles) of GA solution filled with vegetable oil; (b) Viscosity flow curve of GA solution 20 h later from the filling (filled circles). Open circle corresponds to the viscosity measured at the shear rate of 5000 s^{-1}. The measurement was performed by a flow sweep test, wherein the shear rate was increased from 0.01 to 5000 s^{-1}.

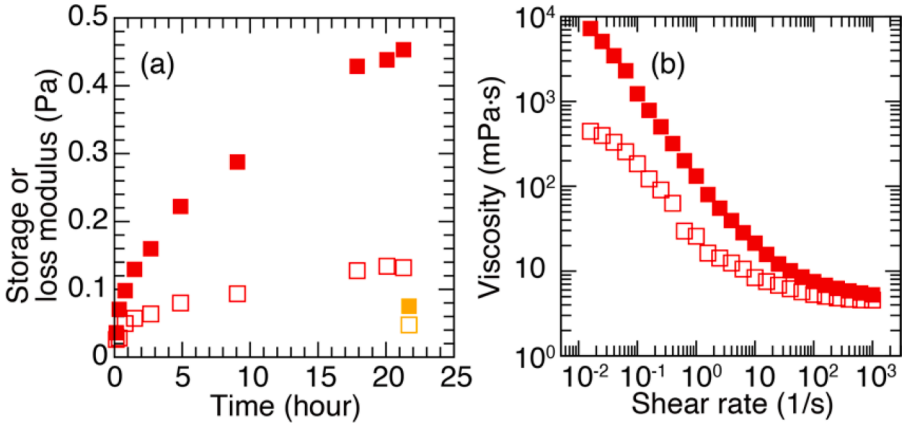


Fig. 8. (a)Time-course change in storage (filled rectangles in red) and loss modulus (open rectangles in red) of GA solution filled with dodecane. Storage and loss modulus just after the complete shearing with flow sweep test are shown with filled (storage) and open (loss) rectangles in orange; (b) Viscosity flow curve of GA solution 22 h later from the filling (filled rectangles in red) and just after the filling (open rectangles). The measurement was performed by a flow sweep test, where the shear rate was increased from 0.01 to 1000 s^{-1}.

molecules at the oil/water interface. To determine if such preferential condensation of GA molecules affects the structure of GA molecules in the bulk phase, the time-course structuration of GA was monitored by multi-angle dynamic light scattering (DLS) measurements (Fig. 9). The GA solution was filled in two test tubes, in which vegetable oil was added on top. DLS measurements were then performed in the middle of the bulk solution. Irrespective of the presence of oil on top of the GA solution, the autocorrelation functions shifted toward longer decay times and remained unchanged 2 h after filling the GA solution into the test tubes. This indicated that the structuration of GA molecules proceeds similarly, regardless of the oil on top. The size of the structured GA aggregates became larger than the observable size in this DLS setup (6 μm) within 2 h after filling.

Therefore, the oil does not affect the bulk properties, and the observed structure in the oil-covered cone-plate geometry should originate from the preferential condensation of GA molecules in the vicinity

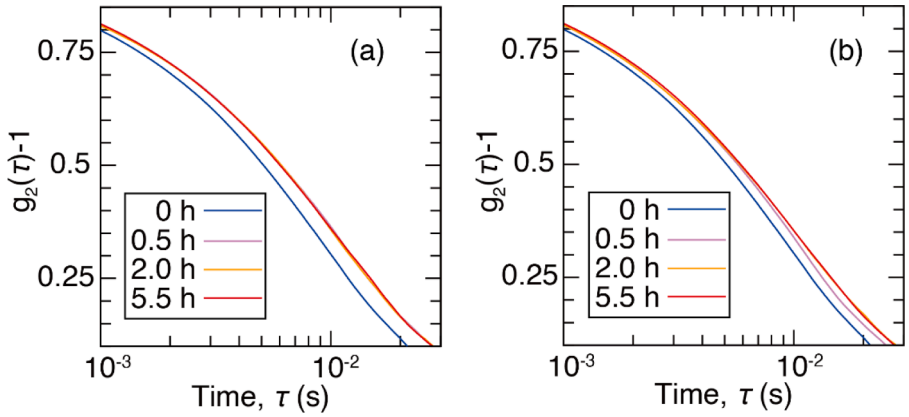


Fig. 9. Autocorrelation functions of GA solution (a) Without oil (b) With oil (vegetable oil) on top obtained by multi-angle dynamic light scattering (DLS) measurement 0, 0.5, 2, and 5.5 h after the filling in sample tube at the scattering angle of 90° DLS measurements were performed in the middle of sample tube, thereby measuring the bulk solution property. The $g_2(\tau) - 1$ is an auto correlation function.

of the oil/water interface. To clarify whether the condensation originates from either or both adsorption of GA onto the oil surface (Isobe et al., 2020; Renard et al., 2021) or the development of self-aggregation (Atgié, Garrigues, Chennevière, Masbernat & Roger, 2019), further studies based on rheological experiments combined with scattering measurements such as rheo-SANS, rheo-SAXS, and rheo-SALS (Eberle & Porcar, 2012; Pignon et al., 2021) are necessary.

5. Conclusions

The viscoelastic properties of the highly concentrated GA solution were found to be dependent on the experimental conditions. When the geometry was covered with oils to avoid water evaporation of the solution sample, a continuous increase in storage and loss modulus up to 1 day occurred. This indicates the development of a network structure composed of aggregates of GA molecules. In addition, the magnitude of the structuration was dependent on the type of oil. Therefore, with this simple experimental setup, one can detect the different modes of interfacial condensation, depending on the type of oil used. This approach can be extended to other oil/water/emulsifier systems, especially in food applications.

Author contributions

Conceptualization, N.I.; methodology, N.I., H.M., R.C., F.C., D.C.D.R., and S.D.; investigation, N.I., H.M., R.C., F.C., and D.C.D.R.; writing-original draft preparation, N.I., H.M.; writing-review and editing, N.I., H.M., R.C., F.C., D.C.D.R., and S.D.; funding acquisition, N.I. and S.D. All authors have read and agreed to the published version of the manuscript.

Supplementary Materials

Figure S1: Photograph of the dynamic light scattering system, Figure S2: Schematic presentation of the experimental setup of rheometer without oils, Figure S3: (a) Visual appearance of an experimental setup after the flow test at a shear rate of 1000 s^{-1}. The absence of an artifact was visually confirmed; (b) visual appearance of an experimental setup after the flow test at the shear rate of 5000 s^{-1}. The presence of artifacts (formation of emulsified droplets) was visually confirmed (indicated by red arrows).

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpta.2021.100181.

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