Emulsions Stabilized by Gum Arabic: How Diversity and Interfacial Networking Lead to Metastability

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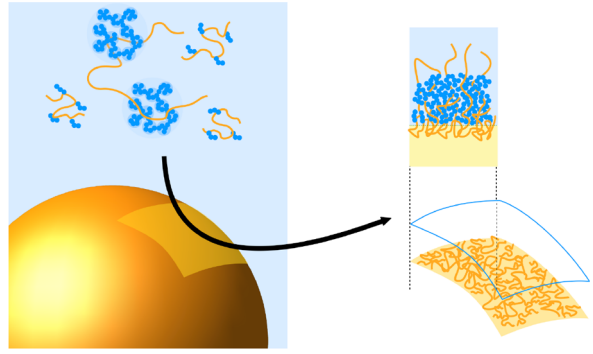
ABSTRACT: Gum arabic is a natural hydrocolloid composed of a diversity of amphiphilic species consisting of protein chains covalently linked to multiscale porous polysaccharides. Gum arabic is notably used as a food additive (E414) to provide metastability to oil-in-water emulsions, even after extensive dilution. Here, we investigate the mechanism underlying the emulsion stabilizing properties of gum arabic, using a combination of scattering and chromatographic analyses and the design of a harvesting method to collect adsorbed species. Increasing the interfacial packing of amphiphilic species leads to their irreversible interfacial aggregation, which is driven by hydrophobic interactions between protein chains. This aggregation is promoted by the size diversity of amphiphilic species, with smaller species first aggregating at intermediate interfacial packings, followed by larger species at higher packings. The resulting adsorbed layer can be considered as a shell composed of a two-dimensional protein network, irreversibly cross-linked through hydrophobic interactions, which is covalently linked to hyperbranched polysaccharide chains displaying severe conformational changes compared to their bulk structure. This shell is strongly anchored at the oil−water interface by the protein network and provides steric repulsions through the hydrated polysaccharides. Consequently, if such a shell is adequately formed during emulsification, emulsions stabilized by gum arabic may resist extensive mechanical stresses and display a long-term metastability even after drastic environmental changes. This paves the way toward more rational uses of gum arabic as an emulsion stabilizer in formulations and processes.



INTRODUCTION

Gum arabic is a natural product harvested from acacia trees exudates in sub-Saharan countries. Since ancient times, this hydrocolloid has been used in a large diversity of applications, which has prompted a research effort to describe its composition, structure, and functional properties. Gum arabic is described as a complex mixture of covalently linked protein and polysaccharides macromolecules with various size, hydrophobicity, and protein content.[1−4] We recently proposed a multiscale porous structure for these protein/polysaccharides species and a chromatographic analysis of their diversity.[4]

Several studies have specifically investigated the role of gum arabic as an emulsion stabilizer, since gum arabic is commonly used to stabilize beverage emulsions. Importantly, such an application requires emulsions to retain metastability even after extensive dilution. We recently demonstrated that this property stems from an irreversible adsorption of amphiphilic species from gum arabic to oil−water interfaces.[5] This irreversibility originates from the protein content of gum arabic, which plays a pivotal role on its amphiphilic behavior.[5,6] Treating gum arabic with protease enzymes thus degrades its emulsifying activity.[6] However, emulsions stabilized by gum arabic are extremely resilient to salinity and pH changes, in contrast to protein stabilized emulsions.[7,8] Literature ascribes this difference to the presence of hyperbranched polysaccharides, which can provide steric repulsion since they are highly hydrated polymers[9] and thus hinder droplet coalescence.

Still, several studies have also pointed out the importance of populating interfaces with a broad spectrum of amphiphilic species from gum arabic. For instance, Ray et al. used gum arabic fractions obtained from size exclusion chromatography (size) or hydrophobic interaction (protein content) and showed that using a combination of both the small and high molecular weight species of the gum yielded the optimal emulsion metastability.[10] This important observation suggests that the origin of gum arabic emulsifying properties arises from a combination of macromolecules and is not simply restricted to its highest molecular weight fraction (so-called AGPs, for arabino-galactan proteins).[6,11] Based on a chromatographic analysis of the aqueous phase after emulsification, Padala and co-workers observed that species from the whole size distribution of the gum could adsorb at the oil−water interface

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in various proportions.[12] In a recent work,[5] we characterized in detail both composition and packing within the adsorbed layer of gum arabic stabilized droplets. We showed that a large composition diversity is indeed present, which only weakly depends on the physicochemical parameters during emulsification. Furthermore, amphiphilic species are irreversibly adsorbed. However, large variations of the packing, or interfacial coverage, were observed. Typically increasing salinity and decreasing pH promote larger packings, since gum arabic is a weak polyelectrolyte due to the glucuronic acid content in polysaccharides. Noteworthy is that modifying pH or ionic strength after emulsification yielded no significant changes in metastability.

Interestingly, it is well-known through its millennia of practical use that gum arabic solutions form cohesive films upon drying.[13] This film-forming property actually corresponds to the natural function of gum arabic, which acts as a plaster on acacia tree wounds. Interfacial rheology studies showed the ability of gum arabic to form cohesive films at interfaces.[14,15] Nevertheless, there is at present no available multiscale structural insight into the underlying mechanism of film formation or any evidence of its occurrence and relevance in emulsion stabilization.

The lack of knowledge regarding the mechanism through which gum arabic stabilizes emulsions poses a challenge for both the numerous industries that use it and the design of substitutes that aim at avoiding a number of issues related to the use of gum arabic (price fluctuation, variability, heterogeneity, ethical harvesting).[16−18]

This work aims at elucidating the mechanism through which gum arabic stabilizes emulsions using both direct and indirect experimental approaches to probe the structure of oil−water interfaces, in relationship with their composition and therefore the formulation parameters. It follows two other works devoted to the composition and structure of gum arabic solutions[4] and to the interfacial composition and packing of gum arabic stabilized emulsions.[5]

The Results section starts with providing a correlation between emulsion metastability and interfacial packing. Several experiments are then presented to understand such a correlation, first by directly evidencing structural changes of adsorbed species at the interface and second by harvesting these species, which evidence their interfacial networking at high interfacial packing. We then investigate what species from gum arabic form such networks and how networking impacts their multiscale structure. Finally, we provide direct evidence that interfacial networking is key to emulsion metastability.

The Discussion section proposes a mechanism for interfacial networking and its relationship to emulsion metastability, opening up new possibilities for a rational formulation strategy of gum arabic stabilized emulsions.

RESULTS

Emulsion Metastability Correlates with Interfacial Packing. We first report some observations regarding the abrupt changes in emulsion metastability upon varying interfacial packing through varying pH, gum concentration, and ionic strength. Strikingly, micrometric hexadecane-in-water emulsions produced at native pH and ionic strength under a gum concentration of 10 g/L coalesced within the day. In contrast, micrometric (6 μm) hexadecane-in-water emulsions produced at pH 3.5 and higher concentrations (20 and 40 g/L) withstood ultracentrifugation at 250 000g for 5 min without any macroscopic phase separation. Another striking observation was made using a 6 g/L solution of gum arabic at pH 3.5, using a stir-bar rotating at 1000 rpm for 10 min. This produced millimetric droplets with a narrow size distribution, which have remained metastable since their formation 4 years ago. This millimetric emulsion withstood extensive dilution, temperature changes, and bacterial growth.

This drastic metastability difference correlates with variations in interfacial packing. Figure 1 provides a quantitative overview of this correlation by displaying the Sauter diameter $d[3,2]$ over time, as a function of both gum concentration and interfacial concentration. The corresponding emulsions were produced using a rotor/stator mixer (Dispax) rotating at 15 000 rpm during 3 min, with a dispersed oil volume fraction of 20 v/v% and a pH adjusted to 3.5. Interfacial coverages in milligrams per square meter (mg/m^2) were then estimated using the amount of nonadsorbed species from the water phase. Following emulsification, all droplet size distributions are similar for all samples, which translates into a plateau in the initial $d[3,2]$ variation. However, at longer times, the lower the gum concentration is, the faster emulsions coarsen. Importantly, lowering the gum concentration directly correlates with lowering interfacial packing. This experiment thus illustrates the drastic sensitivity of emulsion metastability to interfacial packing. This calls for an investigation of the interplay between interfacial packing, interfacial structure, and metastability, which is described in the following subsections.

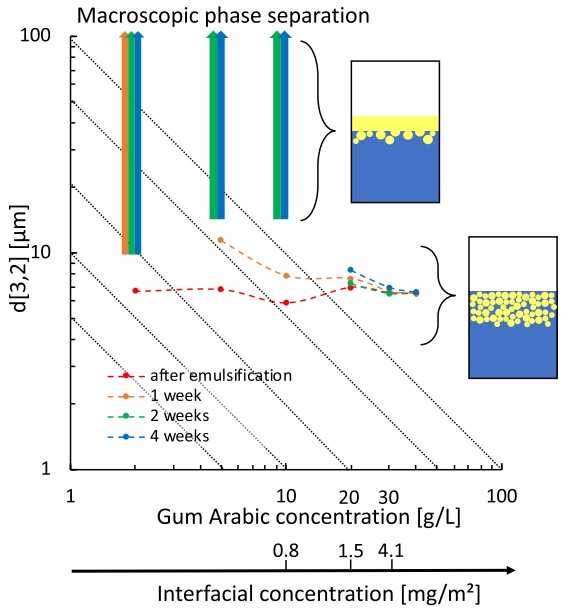


Figure 1. Time variation of the Sauter diameter, $d[3,2]$, of emulsions stabilized with increasing concentration of gum arabic (oil volume fraction: φ = 20% in hexadecane; pH = 3.5; prepared using a Dispax rotor/stator unit). Interfacial concentrations, or packings, were evaluated for three gum aqueous concentrations. In minutes following emulsification, all droplet size distributions and thus $d[3,2]$ are similar over the whole gum concentration range. Over time, emulsions prepared with the lower gum concentrations phase separated in contrast with emulsions prepared at the higher gum concentrations that retained metastability.

SANS Probes Structural Modifications of Amphiphilic Species at Oil−Water Interfaces. We first investigated the interfacial structure of oil droplets stabilized by gum arabic,

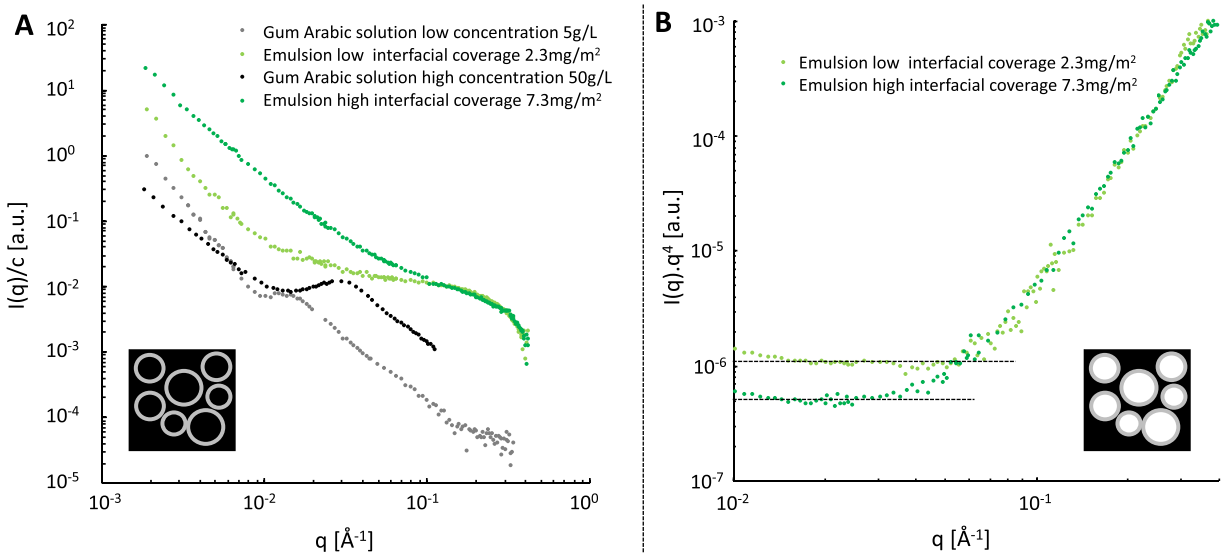


Figure 2. (A) Concentration normalized small angle neutron scattering spectra. Dark green and light green symbols refer to the scattered intensity of emulsions in contrast-matched conditions at interfacial packing of 7.3 and 2.3 mg/m^2, respectively. Black and gray symbols correspond to the scattered intensity from gum arabic solutions in D_2O at 50 g/L (Dror et al.[19]) and 5 g/L, respectively. (B) Kratky representation of the scattered intensity of the same emulsions in plain contrast conditions. The Porod law is followed at low q values, until a limit value corresponding to the interfacial thickness.

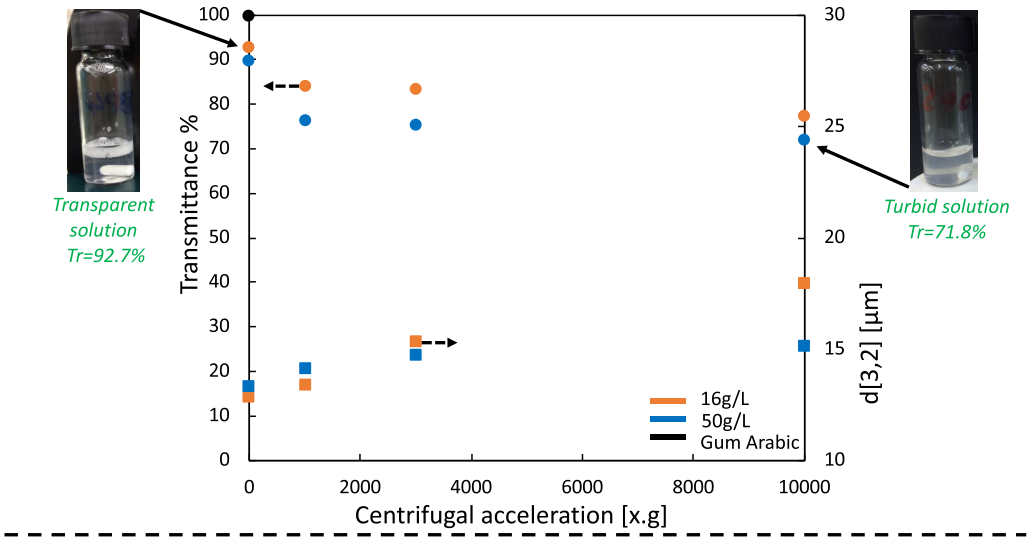
which requires the use of an in situ and nondestructive method. To this purpose, we carried out small-angle neutron scattering (SANS) measurements, which allow to access length-scales of typically 1−100 nm. We performed these measurements on creamed emulsions, which are thus more concentrated, using two types of contrasts: First, contrast-matched conditions for which the scattering length density of the oil phase is adjusted on that of the water phase, which only highlights the adsorbed material since we also rinsed the aqueous phase prior to measurement. Second, plain contrast conditions in which the droplet contribution dominates since the strongest contrast is between oil and water.

Two macroemulsions stabilized by gum arabic were prepared at two different interfacial packings, which were determined through a separate weighting experiment as 2.3 and 7.3 mg/m^2. Figure 2A displays the SANS scattering spectra of these two emulsions in contrast-matched conditions using mostly deuterated hexadecane as the oil phase and deuterated water as the continuous phase. These emulsions were rinsed prior to measurements, and the ionic strength was thus assumed to be close to that of pure water. We have thus also reported in Figure 2A two scattering spectra of gum arabic at two concentrations in D_2O, in no added salt conditions. The detailed study of gum arabic structure in solution using small-angle scattering and chromatographic techniques, can be found in one of our recent work.[4] We have chosen to display the concentration normalized scattered intensity, which requires one to calculate an equivalent volume concentration in the case of emulsions. This calculation is possible since the interfacial packing, droplet size, and oil volume fraction are known quantities. We obtain equivalent concentrations of 0.92 and 0.61 g/L for 7.3 and 2.3 mg/m^2 respectively, which are thus much smaller than the corresponding concentrations of the bulk solutions (5 and 50 g/L).

The first observation is that, over the whole probed q range, concentration-normalized scattered intensities are much higher for emulsion samples than for solution samples. Then the scattered intensity profiles along q can be discussed in three regimes. At low q values, both the high interfacial coverage emulsion and the high concentration solution display a $q^{-2.2}$ slope. The low interfacial coverage emulsion similarly matches the low concentration solution with a q^{-3} slope. The absence of any finite size is consistent with solution spectra, which evidence large length-scale structures, and droplet proximity, which allows the overlap of such structures between several droplets. At intermediate q values, a first significant difference is the absence of any structure peak for emulsion samples. We showed that this structure peak originated from the repulsive interactions between charged glucuronic acid moieties present in the polysaccharide parts.[4] This structure peak thus disappeared upon increasing ionic strength and/or increasing gum concentration. Furthermore, the two emulsion samples have a different signature, with the high coverage emulsion exhibiting a q^{-1} slope and the low coverage emulsion corresponding to a nearly constant intensity. At high q values, the two emulsions display nearly identical signatures, highlighting a well-defined nanometric length scale. The scattering intensity of these emulsions is notably higher than the gum solutions in the high q range. Interestingly, the two concentration-normalized intensities overlap for both emulsions in this region. This overlap suggests a similar local structure in both cases over the corresponding length scales.

To sum up our observations, there is a large difference in the scattered intensity value and profile of gum arabic at interfaces compared to gum arabic in solution. Furthermore, the interfacial packing impacts the scattered intensity profile. These two major observations highlight structural modifications of amphiphilic species upon adsorption at oil−water interfaces.

Figure 2B displays the SANS spectra of these two emulsions in plain contrast conditions, with hydrogenated hexadecane and deuterated water, using the Kratky representation $I(q)q^4$ as a function of q. At low q, the horizontal slope is typical of the Porod law but is lost at a q value that depends on the sample and represents the interfacial thickness. For the 2.3 mg/m^2 interfacial packing, the corresponding q value for this transition



Gum concentration used to prepare the emulsion [g/L]	Centrifugal acceleration during each rinsing step [x.g]	Interfacial concentration Γ [mg.m^{-2}]	Protein content [wt%]
16	1	6.6	10.8
50	1	9.6	12.2
16	10000	7.8	14.7
50	10000	9	16.3

Figure 3. Transmittance of recovered adsorbed species at 10 g/L (circles) and emulsion Sauter diameter (squares) as a function of centrifugal separation conditions. Red symbols: emulsions prepared with a 16g/L gum arabic solution. Blue symbols: emulsions prepared with a 50 g/L gum arabic solution. The Sauter diameter was measured after three rinsing steps. The black circle corresponds to the measured transmittance of a native gum arabic solution at 10 g/L.

is 4.5×10^{-2} Å^{-1}, while it is 3×10^{-2} Å^{-1} for the 7.3 mg/m^2 one. Using a simple estimation via $2\pi/q$, we obtain, respectively, 13 and 20 nm for these two samples. Increasing interfacial packing thus leads to thicker films.

Harvesting Amphiphilic Species Adsorbing at Oil–Water Interfaces Evidence Interfacial Aggregation. We now examine the reversibility of these adsorption-driven structural changes, which first requires the collection of amphiphilic species adsorbing at oil–water interfaces. To this purpose, we developed an emulsion-mediated separation method. This separation method starts with the preparation of pentane-in-water emulsions using gum arabic as the stabilizer. Emulsions were either left to cream in a separating funnel or centrifuged. The aqueous phase was then removed, and the resulting concentrated emulsion was rinsed with Milli-Q water. Finally, the whole emulsion was freeze-dried, which removes both pentane and water and yields adsorbed species as a residual powder. Surprisingly, the dissolution of this powder in water yielded solutions of increased turbidity compared to the initial gum arabic solutions at the same concentration. In order to discard impurities or process artefacts as the origin of this turbidity, two main tests were conducted. First, aqueous gum arabic solutions were freeze-dried and the resulting gum arabic powder was dissolved in water. We obtained the exact same solution as the initial one. Second, we freeze-dried a pentane-in-water emulsion without any stabilizer and verified there was no residue. This test was only successful for pentane oil of sufficient purity. Additionally, different emulsification conditions and gum amounts drastically modify the turbidity of the solutions obtained by dissolving residues from the emulsion-mediated separations.

The turbidity of the solution must thus be ascribed to the presence of colloidal aggregates, which are not present in the original gum solutions but form at the oil–water interface. These aggregates are therefore a signature of structural changes at the oil–water interface.

The solution's turbidity strongly depends on the formulation and separation conditions, ranging from nearly native gum transparent solutions to milky solutions. The transmittance of the solutions has been measured, using a spectrophotometer, for two gum concentrations (16 and 50 g/L at pH 3.5) in the emulsion and different separation conditions: under gravity in a separative funnel and under enhanced gravity using a centrifuge. Figure 3 shows that both the solution transmittance at 800 nm and the emulsion Sauter diameter vary with concentration and separation conditions (see Figure 1 in the Supporting Information for the transmittance measurements over the whole 200–800 nm range). Importantly, centrifugation resulted in an increase of the droplet diameter (see Figure 2 in the Supporting Information), which corresponds to a decrease of the overall interfacial area. We previously showed[5] that this decrease in surface area coincides with the desorption of weakly adsorbed amphiphilic species from the interface, therefore leading to an increase of protein content at the interface.

Interfacial Networks Are Composed of Protein-Rich Species from the Gum. We now turn to assessing the composition of these colloidal aggregates, which also coincides

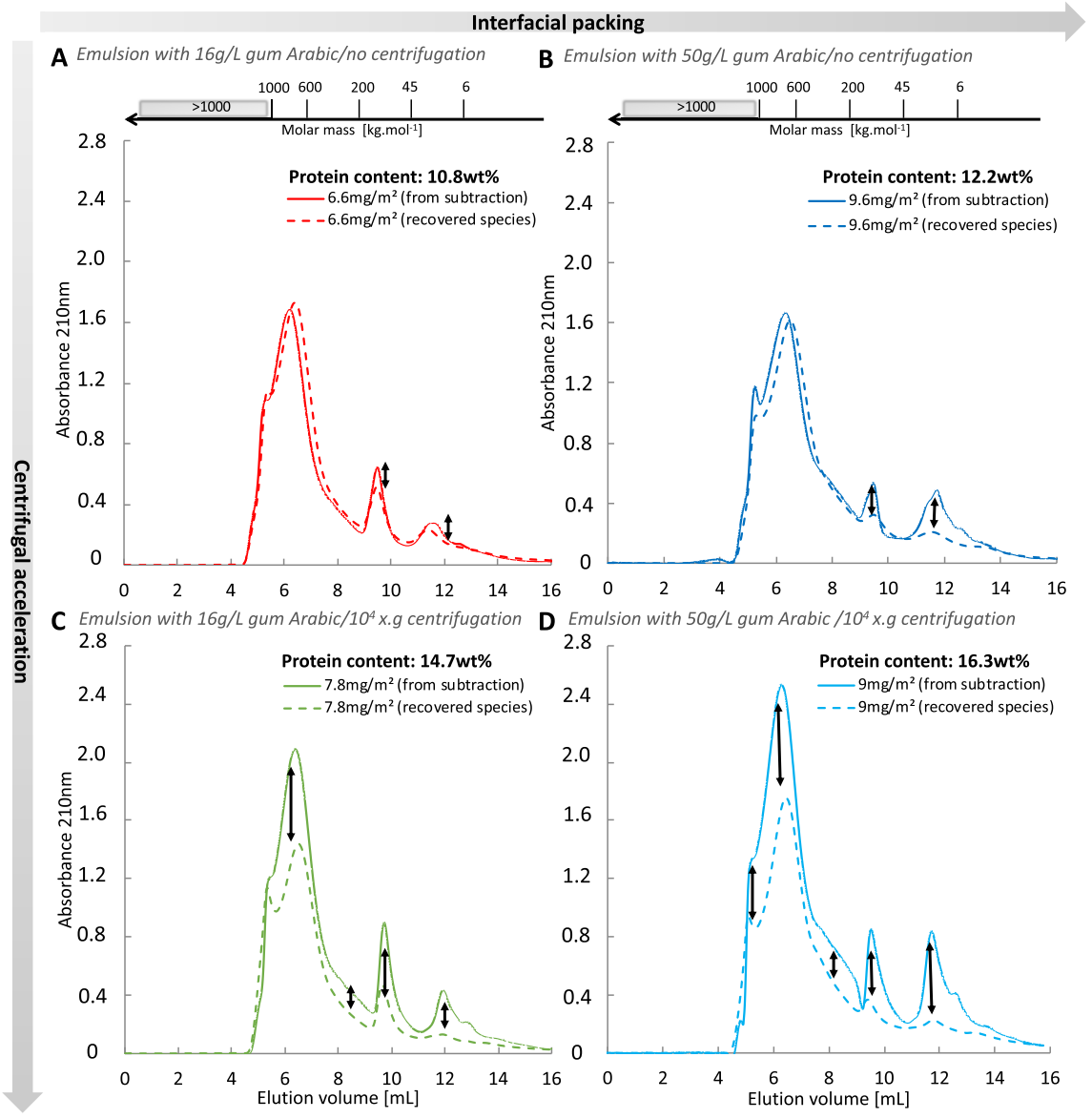


Figure 4. Comparison between calculated (continuous line) and measured after filtration (dotted line) size exclusion chromatograms of adsorbed species. (A) Emulsion prepared with a 16 g/L gum arabic solution without centrifugation. (B) Emulsion prepared with a 50 g/L gum arabic solution without centrifugation. (C) Emulsion prepared with a 16 g/L GA solution, centrifuged at $10^4 g$. (D) Emulsion prepared with a 50 g/L gum arabic, centrifuged at $10^4 g$. Black arrows highlight differences between calculated and measured chromatograms and correspond to species that were filtered out before measurements, because they were aggregated upon adsorption.

with the composition of interfacial networks. We observed that these aggregates were filtered out using 200 nm pore size membranes and thus decided to compare chromatograms of solutions before and after filtration to deduce the composition of these aggregates with respect to the different species within gum arabic. We used a dual chromatographic analysis of both the nonadsorbed and adsorbed species, using size exclusion (Figure 4) and hydrophobic interaction chromatography separations (see Figure 3 in Supporting Information). Chromatograms of adsorbed species were obtained by subtracting, from the native gum chromatogram, the chromatogram of nonadsorbed species, recovered from the aqueous phase. These calculated chromatograms were compared to chromatograms of recovered adsorbed species that were redissolved in water (yielding cloudy solutions) and filtered at 200 nm. The difference corresponds to the aggregates' composition. Two parameters were investigated: the native gum concentration in solution (16 and 50 g/L) and the centrifugal acceleration ($1g$ and $10^4 g$) applied during phase separation (leading to four pairs of chromatograms for each chromatographic technique). As a general trend, the chromatogram's area of recovered species (dotted line on the graphs) is of lower intensity than that of the calculated chromatograms (continuous line). This difference corresponds to the mass loss resulting from filtering out aggregates. This method therefore provides a relevant tool to unravel the composition of the aggregates forming at the oil−water interface.

In size exclusion chromatography, molecules of higher molecular weight are eluted first. The scale of molecular weight of gum species related to their elution volume appears on top of the chromatograms in Figure 4. Chromatograms of Figure 4A and B correspond to the case of gravity-driven separation, whereas chromatograms of Figure 4C and D correspond to the

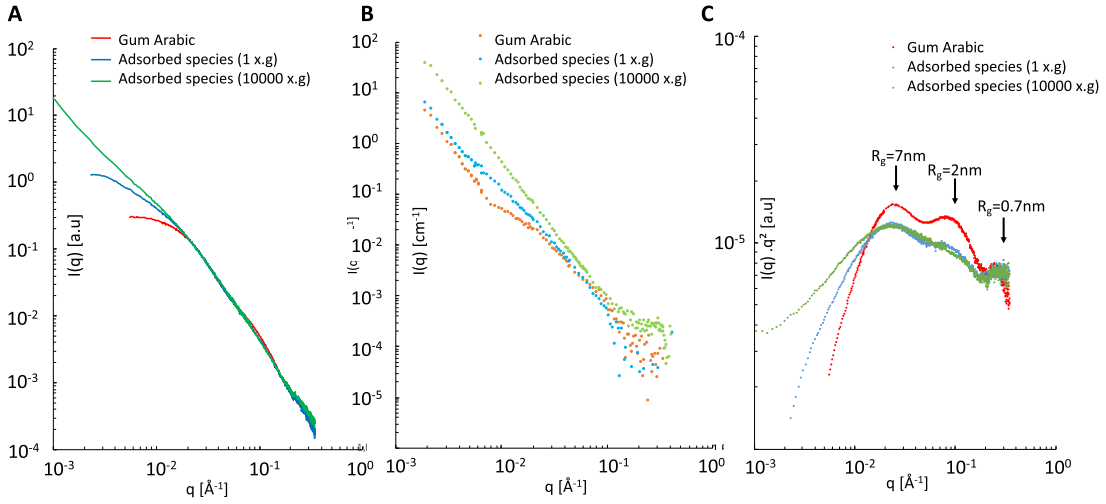


Figure 5. Small angle scattering spectra of gum arabic and recovered adsorbed species (from emulsions prepared with 50 g/L gum arabic solutions), dissolved in 20 mM NaCl aqueous solution at a 5 g/L concentration. (A) SAXS, (B) SANS, and (C) Kratky-plot type (from SAXS data) evidencing three characteristic length scales in the native gum that are lost upon interfacial aggregation.

case where the two liquid phases were centrifuged at $10^4 g$, hence submitted to coalescence events.

Since the UV detection signal at 210 nm is mostly sensitive to the peptide bonds,[20,21] the area under the calculated chromatograms of the interfacial film (continuous line) is proportional to the interfacial protein content, which was independently measured for each sample. The order of magnitude of the protein content at the interface is 14 wt % (between 10.8 and 16.3 wt %), compared to the 2.5 wt % of the native gum, which highlights the predominant role of protein chains in the adsorption process.

For an emulsion prepared with the lowest gum concentration (16 g/L) and without centrifugation (Figure 4A), only a weak difference between both chromatograms is observed for the smaller size macromolecules, eluted around 9.5 and 11.5 mL (6−200 kg mol^{-1}). A similar behavior can be noticed for the emulsion stabilized with a 50 g/L gum arabic solution and without centrifugation (Figure 4B): smaller macromolecules preferentially aggregate. Comparison between Figure 4A and B shows that the aggregation rate is increasing as the interfacial coverage and protein content increase (black arrows on the graphs).

When these emulsions were submitted to centrifugation during the rinsing steps, the composition of the interfacial film changed with a noticeable increase in protein content. Figure 4C and D shows that interfacially induced aggregation concerns almost all of the range of the size spectrum of macromolecules. With the centrifugation and increase of the surface concentration, high molecular masses (elution volume between 4.5 and 7 mL) (>1000−600 kg/mol) and intermediate molecular masses (elution volume between 7 and 9.5 mL) (600−200 kg/mol) also aggregate. As in the case with no centrifugation, the comparison between Figure 4C and D shows that each class of molecular weight aggregates more upon increasing surface concentration (between 16 and 50 g/L), in particular for species eluted at 5 and 9.5 mL (Figure 4D).

Interfacial Aggregation Induces Multiscale Structural Changes. For hydrocolloids, a nonintrusive and representative structural characterization can be adequately performed using scattering methods such as SANS and small angle X-ray scattering (SAXS). While highly similar, SAXS and SANS substantially differ with respect to the origin of contrast. In SAXS, the scattering contrast originates from differences in electronic densities, whereas it originates from differences in nucleus types in SANS and is particularly sensitive to isotopic H/D substitution. A dual characterization using both techniques is thus a powerful tool to investigate the heterogeneous mesostructure of hydrocolloids. We recently used SAXS and SANS to characterize gum arabic in aqueous solutions, to which the reader can refer for further details.[4] In the present work, we rather focus on comparative differences exhibited by interfacially recovered gum arabic, at different aggregation levels, compared to native gum arabic.

Figure 5A and B, respectively, displays the SAXS and SANS spectra of aqueous solutions of both gum arabic and amphiphilic species recovered from gum arabic stabilized emulsions, either centrifuged or not, using a a 50 g/L gum arabic concentration. For the three samples, the gum mass concentration is 5 g/L and the concentration of NaCl is 0.02 M. With both SAXS and SANS techniques, a large increase of the scattered intensity is observed at low q values (large length scales) for recovered gum compared to the native gum. This increase is maximal for gum collected from the interfaces of the centrifuged emulsion. This low q scattering intensity increase is consistent with the corresponding turbidity increase observed in Figure 3. However, for a given sample, SAXS and SANS intensities in the low q range significantly differ in magnitude and follow different power laws, while they are similar in the high q range (small distances). As shown before,[4] SANS spectra highlight polypeptides while SAXS spectra represent all species in their relative proportions, which favors polysaccharides. Differences in power law are thus indicative of structural differences between the two types of species.

In the gum arabic SAXS spectra displayed in Figure 5A, a finite size is observed at low q values where the scattered intensity is reaching a plateau value, whereas a q^{-3} slope is observed in the SANS spectrum, corresponding to collapsed chains. In the adsorbed species spectra, both SAXS and SANS spectra indicate that no finite size is observed over the probed length scale. However, for the adsorbed species from the centrifuged emulsion (green curves), the SAXS curve yields a

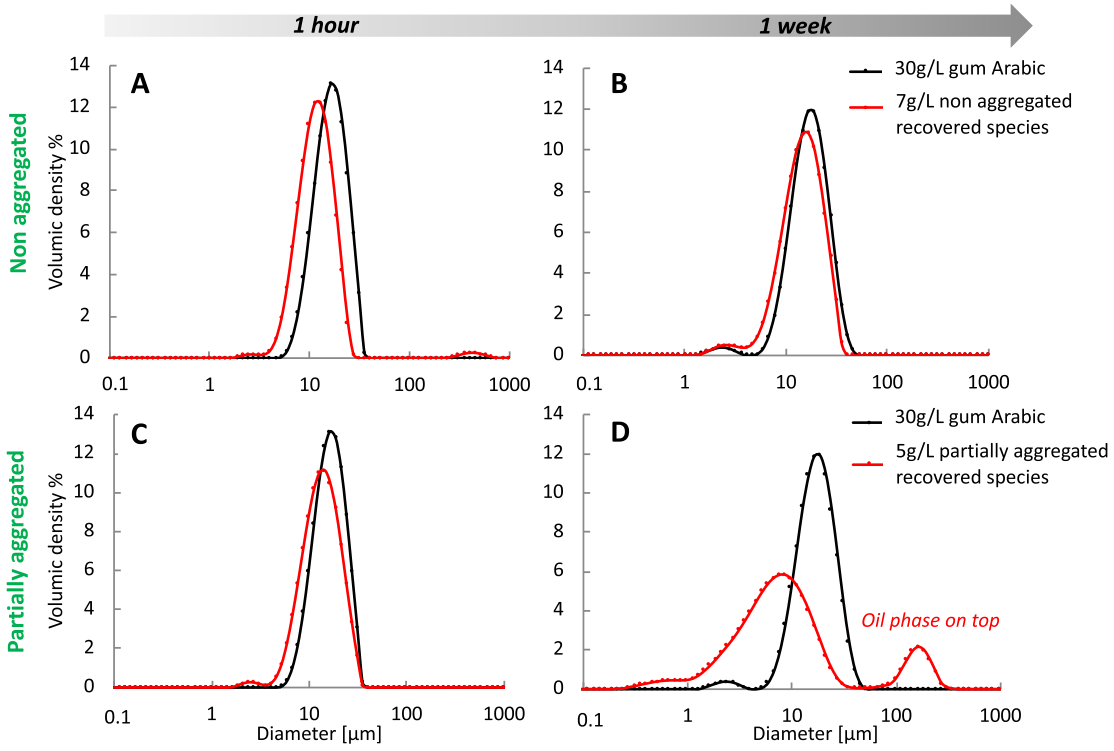


Figure 6. Time evolution of size distribution of hexadecane-in-water emulsion (20 v/v%) stabilized by gum arabic (black) or species recovered from the interface (red) nonaggregated (A and B) or partially aggregated (C and D). The emulsions aqueous phase was prepared at pH 3.5 prior to emulsification. A concentration of 30 g/L was used for the aqueous phase of the emulsion stabilized by native gum, and the concentration for the aqueous phase using recovered species from the interface was calculated using an equi-nitrogen ratio compared to the gum solution (protein contents: gum arabic = 2.5%, nonaggregated species = 10.8%, partially aggregated species = 16.3%).

$q^{-1.5}$ slope while the SANS curve yields a $q^{-2.8}$ slope. The SAXS slope indicates a swelled structure in a good solvent, which is consistent with hydrophilic polysaccharides chains. The SANS slope suggests a fairly collapsed structure in a bad solvent, in accordance with the signature of hydrophobic polypeptide chains. This difference shows that both hydrophilic and hydrophobic moieties optimize their conformation in solution, resulting in a difference in mesoscopic organization. Like native species, the aggregates are porous rather than dense.

Since the signal-to-noise ratio was much better in the SAXS spectra than in the SANS spectra, a closer examination of the scattering behavior at high q values is possible. Figure 5C displays the SAXS spectra in a Kratky plot ($I(q)q^2$ vs q), commonly used to investigate intrinsically disordered proteins.[22,23] For a given contrast, the area under the curve is proportional to the total volume of the scattering material. Three oscillations are visible on the spectrum of gum arabic at 0.246, 0.854, and 2.54 nm^{-1} corresponding, respectively, to gyration radii of 7, 2, and 0.7 nm, using the relationship $q_{peak}R_g = 3^{1/2}$. We proposed in our previous study a multiscale mass fractal structure for the hyperbranched polysaccharides, containing three length scales associated with these three oscillations.[4]

SAXS spectra of adsorbed species strikingly display an intensity collapse at these small length scales, which counterbalances the intensity gain observed in the low q region. Furthermore, the first and last oscillations remain, but the intermediate one is drastically reduced on the spectrum of adsorbed species collected from a noncentrifuged emulsion and absent for species collected from a centrifuged emulsion. This observation suggests an irreversible conformation change of the hyperbranched polysaccharides after adsorption at an oil−water interface. Moreover, circular dichroism measurements were performed on the same sample of recovered species (see Figure 6 in the Supporting Information), and results indicate that the secondary structure of the protein moieties was not lost (optical activity intact) when recovering species from the interface. This observation dispels the potential contribution of secondary protein structures in the observed SAXS spectra.

Interfacial Networking Correlates with Enhanced Emulsion Metastability. So far, we have shown evidence of interfacial networking of gum arabic amphiphilic species, in formulation conditions promoting large interfacial coverages. In a previous study,[5] we evidenced that high interfacial coverages correlated to excellent emulsion metastability in gum arabic stabilized emulsions. This was also supported by systematic centrifugation experiments on two emulsion samples with two different interfacial coverages, as displayed in Figure 2 of the Supporting Information. Indeed, the emulsion with the highest interfacial coverage withstood better centrifugation, which corresponds to a lower degree of coalescence. Interestingly, both emulsions reached a steady-state size distribution after 1 min of coalescence. Only the increase of centrifugal acceleration resulted in changes on the size distribution. Therefore, these centrifugation experiments evidence an equilibrium repulsion barrier, and also a self-stabilizing response mechanism of these emulsions when submitted to a mechanical stress.

All these observations suggest the importance of interfacial networking for the metastability of these emulsions. We thus

performed an experiment to test this hypothesis, which consists of comparing the metastability of three emulsions stabilized by (1) native gum arabic, (2) amphiphilic species from gum arabic in no interfacial aggregation conditions (see panel (A) from Figure 6), and (3) amphiphilic species from gum arabic in interfacial aggregation conditions (see panel (C) from Figure 6). Since the proportions of amphiphilic species are different in each case, a choice must be made regarding gum concentration. We chose an equi-nitrogen approach in which the nitrogen concentration, which corresponds to the protein content, is kept constant. Indeed, the protein content roughly correlates with the amount of amphiphilic species. Emulsion size distributions were monitored over time through static light scattering, as displayed in Figure 6.

A first observation is that, 1 h after emulsification, all size distributions are similar, despite a much lower stabilizer concentration in the two emulsions prepared with recovered gum arabic than that in the reference case with native gum arabic, which supports the equi-nitrogen approach. At longer times, the emulsion prepared from nonaggregated species displays the same metastability as the reference emulsion, despite again a much lower stabilizer concentration, which confirms the relevance of the equi-nitrogen approach. On the contrary, the emulsion prepared from partially aggregated species coarsens and some oil phase separates on top of it. Such a drastic difference highlights the pivotal role of interfacial structuration when discussing gum stabilized emulsion metastability. Amphiphilic species possessing a high protein content are crucial to emulsions metastability, since a similar metastability is attained with more than four times less gum arabic when using only amphiphilic species. However, these amphiphilic species must retain their native conformation in order to adsorb and stabilize emulsions.

Rinsing Emulsions Enhance Their Metastability. Since gum arabic amphiphilic species are strongly adsorbed to oil−water interfaces, emulsions can be rinsed extensively and still exhibit a high metastability, a property that was used for interfacial characterization.[5] Interestingly, we actually observed that rinsing the emulsion prior to its centrifugation significantly improved its metastability as shown in Figure 4 of the Supporting Information. As rinsing improved metastability, droplet coalescence was hindered and the resulting interfacial aggregation was less pronounced as shown in Figure 5 of the Supporting Information.

DISCUSSION

Gum arabic comprises a large variety species of various size and amphiphilicity, yet all consist of a protein backbone grafted with polysaccharide chains. When an aqueous solution of gum arabic is brought into contact with hydrophobic interfaces, for instance, through emulsification, a selection occurs between the different species, which we recently investigated.[5] One major conclusion from that work is that adsorption selects species with the highest protein content but barely impacts their size diversity. This leads to the conclusion that protein backbones are indeed responsible for the amphiphilic behavior of gum arabic species, which discards the alternate explanation put forward by Sanchez et al. regarding the possible role of polysaccharides.[13] Yet this protein content is distributed over a large array of sizes and hydrophobicities.[4,5] This discussion will demonstrate that the hydrophobicity of some protein segments, combined with such a size diversity, is at the core of gum arabic stabilizing properties. We first examine a mechanism for interfacial networking based on hydrophobic aggregation of protein chains at high interfacial packings. We then evidence that interfacial networking is the pivotal mechanism underlying emulsion metastability when using gum arabic as a stabilizer. Finally, we turn to practical considerations in formulation and processing of these mechanistic insights.

Interfacial Networking Occurs at High Interfacial Packings through the Hydrophobic Aggregation of Protein-Rich Species. When dissolved in water, gum arabic does not undergo aggregation, although its amphiphilicity, which stems from its protein chains, still manifests itself through self-association into large-scale structures.[4] This contrasts with the interfacially promoted aggregation that takes place above a certain surface packing threshold, which means that oil−water interfaces give access to a conformational pathway enabling aggregation.

Both direct SANS measurements on emulsions and indirect SAXS/SANS measurements on recovered adsorbed species evidence such conformational changes. SANS measurements in contrast matched conditions, which highlights the adsorbed layer, show an increase in contrast that is nearly proportional to the protein content, consistently with our structural interpretations.[4] They also evidence structural differences over a large q range compared to bulk solutions. Notably, only the smallest characteristic length of the multiscale porous structure is retained. This loss is irreversible at high surface packing, as shown by the SAXS/SANS spectra of recovered species.

The SANS spectra in contrast-matched conditions also display an absence of correlation peaks, contrasting with the corresponding bulk spectra. This can be easily understood by estimating the local concentration. Indeed, the correlation peak originates from ionic repulsions between glucuronic moieties from polysaccharides.[19] At large polysaccharide concentrations, above 10 wt %, this correlation peak collapses as shown by Dror et al.[19] For the 2.3 mg/m^2 interfacial packing, an estimate of the thickness is 13 nm, while it is 20 nm for the 7.3 mg/m^2 interfacial packing. This leads to, respectively, 177 and 365 g/L, which are indeed above 10 wt %.

The existence of a threshold for interfacial aggregation can be explained from geometrical considerations. Indeed, using the mean molecular weight of the broad size distribution of the adsorbing species within gum arabic, we obtain an area per species of, respectively, 169 nm^2 for 6.6 mg/m^2 (weakly aggregated) and 116 nm^2 for 9.6 mg/m^2 (strongly aggregated).[5] From the hydrodynamic radius of hyperbranched polysaccharides of 9 nm, we obtain a disk area of 254 nm^2. Actually, an even larger area of a mean amphiphilic species is expected as a 9 nm spheroid corresponds to a molar mass of about 300 kg/mol,[4] while the mean molar mass of amphiphilic species in gum arabic is 670 kg/mol.

This evaluation is simplistic but sufficient to demonstrate that amphiphilic species must undergo conformational changes upon adsorption at surface packing promoting interfacial aggregation. Conformational changes occur on several length scales: (1) the two largest characteristic length scales of polysaccharide units, 2 and 9 nm, are lost in recovered species; (2) the interfacial layer is thicker than the largest length scale of polysaccharide units, which is visible also on direct SANS spectra in contrast-matched conditions that evidence an elongation of the polysaccharide units. This structuration mechanism is illustrated in Figure 7A. Importantly, while so far

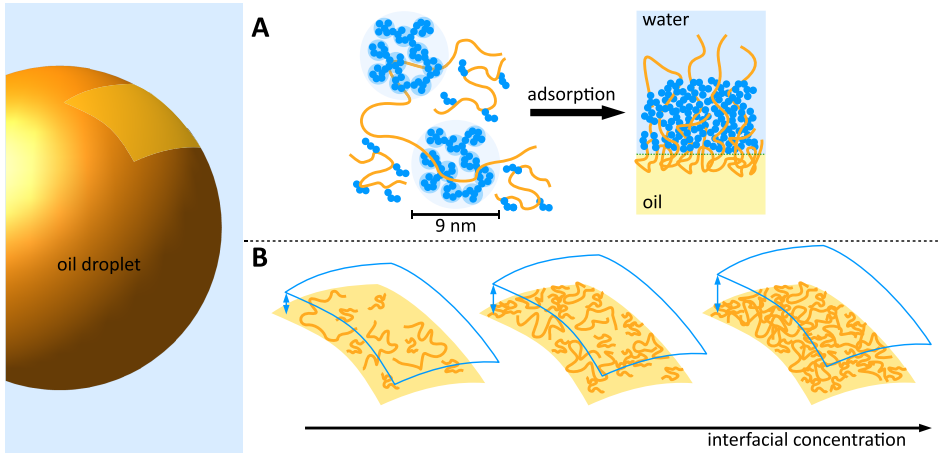


Figure 7. Schematic representation of gum arabic amphiphilic species at oil−water interfaces. Amphiphilic species consist of protein chains (orange) covalently linked to hyperbranched polysaccharides (blue), displaying a multiscale structure in solution. (A) Adsorption is driven by hydrophobic interaction of protein hydrophobic segments with the oil phase, leading to the compaction of hyperbranched polysaccharides. (B) Upon increasing the interfacial concentration of amphiphilic species, hydrophobic protein segments aggregate, leading to the buildup of a two-dimensional network. Smaller species aggregate first, followed by larger ones upon increasing concentration. Polysaccharides compact and distort, leading to an increase of the interfacial layer's thickness.

the discussion has assumed a mean molecular structure, amphiphilic species within gum arabic are a heterogeneous blend. As shown by the chromatographic analysis, interfacial aggregation occurs progressively upon increasing surface packing. Smaller amphiphilic species aggregate first and larger species aggregate only at higher surface packing, which can be promoted by centrifuging emulsions.

This size dependence can be interpreted from the following observations: smaller species are more numerous than larger ones (19−32% for smaller species vs 18−22% for the larger species);[5] they display the largest protein content; and they are prone to aggregation when separated from the rest,[4] which suggests a higher hydrophobicity. Interfacial aggregation can thus be mentally pictured as occurring first from bridging many small species into a mortar, that then include some larger bricks upon increasing surface packing, as pictured in Figure 7B.

Centrifugation operates on two levels in interfacial packing and aggregation: it removes weakly amphiphilic species[5] that are less prone to aggregation, and it promotes higher interfacial packing values by achieving partial droplet coalescence.

Emulsion Metastability Originates from a Dual Network Composed of Hydrated Polysaccharides Covalently Linked to an Interfacial Network of Adsorbed Protein Chains. We now turn toward the relationship between interfacial aggregation and emulsion metastability. We start by discarding a simple possibility, which would be bulk rheology. Indeed, the use of polymeric additives is a common strategy to build a viscoelastic network that considerably slows down droplet collisions and thus emulsion coalescence. In the case of gum arabic, viscosity changes remain moderate and more importantly extensive dilution, even by several orders of magnitude, has no impact on emulsion metastability.

Ionic repulsions arising from the charged glucuronic moieties are important for amphiphilic species adsorption and packing and thus indirectly impact emulsion metastability. However, they do not play a direct role in emulsion metastability, as shown by the resilience of gum arabic stabilized emulsions to high ionic strengths after emulsification.

Steric repulsions stemming from the entropy of hydrated hydrophilic chains[9] are likely candidates in nonionic or weakly ionic amphiphilic stabilizers. They are indeed at the basis of gum arabic stabilizing properties as shown from rinsing experiments. Rinsing improves emulsion metastability as it removes the large number of polymeric species dissolved in water and thus increases the difference in water chemical potential between the hydrated adsorbed layer and the aqueous phase. The existence of a steady state under centrifugation also supports a thermodynamic repulsion barrier, consistent with steric repulsions that stem from hydration entropy. However, as we have shown in this Article, steric repulsions alone cannot explain both the strong variability in emulsion metastability upon varying interfacial packing and the crucial importance of having small amphiphilic species that are only weak contributors to steric repulsions. Furthermore, our observations support a stabilizer-rich regime in the whole gum concentration probed, as evidenced in Figure 1 by the constant emulsion diameter right after emulsification upon increasing gum concentration. Yet, emulsion metastability drastically varies with concentration, which is paradoxical in a usual stabilizer-rich regime and supports the existence of an additional mechanism.

So far, we have shown that gum arabic amphiphilic species adsorb at oil−water interfaces to form a mixed layer consisting of a constrained hydrophilic polymeric network, built from elongated and distorted polysaccharide units, and a hydrophobic polymeric network, built from aggregated protein chains. This structural picture is perfectly consistent with the interfacial rheology study from Erni et al.,[15] who evidenced the viscoelastic behavior of adsorbed gum arabic layer, in contrast to hydrophobically modified starch that displayed only a viscous behavior.

A crucial observation is that the onset of interfacial aggregation, which relates to high enough interfacial packings, corresponds to the onset of an outstanding metastability, as emulsions then withstand even ultrahigh centrifugation. As we showed in our study devoted to surface packing as a function of formulation conditions,[5] higher surface packings systematically enhance emulsion metastability. Moreover, using the

same gum bulk concentration, but in conditions promoting widely different interfacial packing, led to large differences in emulsion metastability.

We showed above that preaggregating gum arabic amphiphilic species is extremely detrimental to emulsion metastability. Importantly, we used conditions mainly promoting the aggregation of smaller species while mostly leaving the largest protein/polysaccharide species unaggregated. Therefore, the large amphiphilic species are insufficient to explain emulsion metastability, whereas the small amphiphilic species display a pivotal role. This role can be explained by the formation of an interfacial network, which is thus at the core of emulsion metastability when using optimally gum arabic. Consequently, in gum arabic, diversity is an asset for emulsion stabilization and the broad array of species cannot be replaced by a single species such as large protein/polysaccharide conjugates.

Instead, we should rather consider the interface as a separate elastic shell strongly anchored to the droplet interface. Such a shell is very efficient to prevent any hole opening between two droplets and thus inhibit coalescence. This depiction is also consistent with the strong hysteresis observed upon preparing emulsions. Indeed, while preparation conditions largely impact what species adsorb and more importantly at which interfacial packing, we systematically observed the resilience of emulsions to environmental conditions changes after emulsification. This hysteresis stems from the irreversibility provided by hydrophobic interactions between protein chain segments, which manifest through the irreversibility of adsorption, together with the irreversibility of interfacial networking. Interestingly, such a hysteresis provides a formulation strategy consisting of preparing an emulsion in the best conditions for its metastability and then modifying these conditions to meet the final application requirements.

Mechanistic Insights Lead to Rationalizing Emulsion Formulation Using Gum Arabic. We finish this discussion by presenting a practical summary of this work together with its two companion papers,[4,5] which consists of a formulation strategy based on mechanistic and structural insights unveiled through this work. Achieving high interfacial packing is the ultimate goal as it leads to the formation of an interfacial network, which is responsible for the excellent metastability yielded by gum arabic in the right conditions. As adsorption is mainly tuned by the ionic repulsions between glucuronic acids in water, decreasing pH or increasing ionic strength are efficient means to increase interfacial packing. We showed that this could notably be used to reduce by an order of magnitude the amount of gum arabic in a product while retaining metastability.[5] Importantly, this simple strategy functions well because varying these parameters has only weak consequences on the size diversity of adsorbing species.[5] Additionally, imposing mechanical stress during the process, as demonstrated with centrifugation, is beneficial for interfacial networking, as it can promote partial coalescence, interfacial deformation, and removal of the weakest amphiphilic species. We may expect these effects to be at work in high pressure homogenization processes. We also evidenced that rinsing emulsions significantly improved metastability by removing hydrophilic polymers from the aqueous phase, which can both reduce steric repulsions and trigger depletion.

We have also shown that interfacial selection methods were an interesting mean to retain only amphiphilic species, thus avoiding large amounts of hydrophilic species in water, which could promote depletion. However, this would require the transposition of our method into an industrial solution. Finally, as we stated above, the hysteresis for adsorption and network formation suggests a general strategy based on optimizing parameters during emulsification while adjusting the composition to the final desired application afterward.

CONCLUSION

Gum arabic represents a specific category of emulsion stabilizer: its amphiphilic species are irreversibly adsorbed, and the adsorbed layer withstands ultrahigh centrifugation, in contrast to surfactants; it acts on preventing emulsion coalescence when droplets come into contact, in contrast to hydrophilic polymers that act as thickeners but only provide an apparent metastability by slowing droplet collisions; once adsorbed, the interfacial layer is nearly insensitive to formulation conditions such as pH and ionic strength, in contrast to protein stabilized emulsions; adsorption is driven by hydrophobic segments, unlike the case of particle stabilized emulsions in which wettability plays a pivotal role.

These characteristics stem from the particular structure and size diversity of gum arabic amphiphilic species, which consists of protein chains covalently linked to multiscale porous and weakly charged polysaccharides as we recently investigated.[4] Indeed, gum arabic amphiphilic species can adsorb and pack at oil−water interfaces at varying surface packing, which we showed was controlled during emulsification by pH and ionic strength (from the gum or from added ions).[5] Upon increasing surface packing, the smaller protein/polysaccharide amphiphilic species increasingly aggregate at oil−water interfaces, eventually followed by larger protein/polysaccharide amphiphilic species. Driven by hydrophobic interactions similarly to self-association in aqueous solution and adsorption at oil−water interfaces, this interfacial aggregation is irreversible. This irreversibility testifies that aggregation and thus hydrophobic interactions dominate over other contributions such as polysaccharide hydration and chain entropy, which also explains why the formed network is highly resilient to environmental changes after emulsification. Indeed, as the aggregating protein chains are covalently linked to polysaccharides, the whole interface becomes similar, at high surface packing, to a cross-linked hydrophilic layer strongly anchored to oil−water interfaces and developing elasticity, thus avoiding rupture. Such a shell indeed optimized several constraints for emulsion metastability, which explains why gum arabic stabilized emulsions may display a robust and long-term metastability. Furthermore, our findings specify the formulation and process conditions enabling and optimizing this original stabilization mechanism: high surface packing must be promoted through environmental factors screening ionic repulsions, and/or by promoting partial coalescence and interfacial deformation through mechanical processing. This mechanistic understanding paves the way to a more rational use of gum arabic in formulations and gives objective criteria of comparison with substitute candidates.

MATERIALS AND METHODS

Materials. Gum arabic powder (Acacia Senegal) was provided by Caragum International (Marseille, France), and its composition was 2.5 wt % proteins (N × 7), 11 wt % moisture, 3.16 wt % ash, and nearly no lipid. Sodium chloride (>99.5% BioXtra), hexadecane (99% ReagentPlus), pentane (99% for HPLC), and hydrochloride acid were

purchased from Sigma-Aldrich. Milli-Q purified water was used for all experiments.

Emulsion Preparation. Oil-in-water emulsions stabilized by gum arabic were prepared using either a rotor/stator device (Ultra-Turrax 1 min, 10 000 rpm; or Dispax 3 min, 15 000 rpm) or a microsyringe (for SANS contrast match measurement, passed 15 times). Alkane oils (pentane or hexadecane) were used as the organic phase. We verified that both oils yielded the same interfacial composition. Emulsions were formulated as follows: 20 or 40 v/v% oil was dispersed in a gum arabic aqueous solution. Gum arabic solutions were prepared at different concentrations and centrifuged at 19 000*g* to remove insoluble materials. Then the pH or the salt concentration of each solution was adjusted by adding small amounts of HCl at 1 mol/L or NaCl at 3 mol/L.

Droplet Size Distribution. Droplet size distributions of emulsions were measured using a static light scattering instrument (Mastersizer 3000, Malvern) with a 633 nm laser wavelength. An emulsion sample was diluted in circulating water until an obscuration value between 1 and 10% was obtained, in order to avoid multiple scattering. The average droplet diameter was determined as the volume-surface mean diameter $d[3,2]$ or Sauter diameter. Each measurement was repeated in triplicate at room temperature.

Interfacial Separation. Gum arabic amphiphilic species were separated through an emulsion-mediated separation technique. Emulsions of pentane in water, stabilized by gum arabic, were prepared following the aforementioned protocol. These emulsions were then placed in a separative funnel, diluted with pentane-saturated Milli-Q water (in order to prevent pentane diffusion from oil droplets into the aqueous phase), and either left to cream under normal gravity or centrifuged. In both cases, the aqueous phase was recovered and the emulsion cream was rinsed again (three times) in order to remove all species that could be trapped between droplets without being strongly adsorbed at an interface. The aqueous phase, which contains all the nonadsorbed species, was then dialyzed against water and lyophilized. The final cream was dispersed in water and freeze-dried. A mass balance on the recovered species was carried out on each batch in order to calculate the surface concentration of the droplets.

Interfacial Film Composition. The interfacial concentration Γ (in mg/m^2) in each emulsion was determined using eq 1.

$$\Gamma = \frac{m_{ads} d_{[3,2]}}{6 V_{oil}} \quad (1)$$

The recovered mass (m_{ads}) was determined from a mass balance in the interfacial separation procedure.

In Figure 4, we compare chromatograms of interfacially adsorbed species obtained either from direct measurement using solutions of recovered species, which were filtered at 200 nm, or from indirect measurements using solutions of nonadsorbed species. In the absence of interfacial aggregation, both methods yield the same results. However, if interfacial aggregation occurs, filtration removes species that aggregated, resulting in a difference that directly corresponds to the aggregates' composition. Experimentally, this comparison requires some rescaling operations, as we injected aqueous solutions of the same concentration (3 wt %). This rescaling is possible, as we know the respective amounts of adsorbed and nonadsorbed species through performing a mass balance after the interfacial separation procedure.

Nitrogen Content Fraction Analysis. Nitrogen rate analyses were performed at the Laboratoire de Coordination Chimique (Toulouse, France). A PerkinElmer 2400 CHN series II was operated at 1050 °C, using dioxygen as carrier gas. Each sample was analyzed in duplicate. Protein fraction content was deduced from the nitrogen content through the protein conversion number calculated from the gum arabic proteins amino acid distribution.[3]

$$\%_{protein} = \%_{nitrogen} \times 7 \quad (2)$$

It is worth noting that this value slightly differs from the common value of 6.6 taken in the majority of gum arabic publications. However, we must stress that the 6.6 value does not correspond to the measured amino acid distribution of gum arabic and is therefore incorrect.

Transmittance Measurements. Solution transmittances were measured using a UV−visible spectrophotometer between 200 and 800 nm. Quartz cells were used, and the background measurement was performed using a cell filled with Milli-Q water.

Size Exclusion Chromatography. Size exclusion chromatography was used to separate arabic gum species as a function of their relative hydrodynamic volume. A 7.8 mm × 300 mm BioSuite 450 Å SEC column (Waters) packed with 8 μm porous silica beads was used. The average pore size of the silica beads was 450 Å.

The separation was performed on an Alliance HPLC unit (Waters 2695 separations module), and a 0.5 mol/L NaCl aqueous solution at 25 °C was used as the eluent phase at a flow rate of 0.8 mL/min. Each sample was filtrated with a nylon 0.2 μm membrane, and a volume of 50 μL was injected. UV detection was performed at 210 and 280 nm using a Waters 2487 UV detector. A refractive index detection was used to measure the mass percentage of each eluted moiety (Waters 410 differential refractometer). The refractive index detection was only available until 10.5 mL of elution volume due to a negative peak appearing from the difference in refractive index between the sodium chloride of the eluent phase and the water from the injected sample. The column was calibrated using branched dextran standards (Waters) (Figure 7 in the Supporting Information).[24]

Variation coefficients for each detection were calculated from three injections of the same sample. For UV detection at 210 nm, a 0.3% variation coefficient was measured. It was 0.5% for UV detection at 280 nm and 13% for the refractive index detection.

UV detection at 280 nm is more sensitive to the amount of aromatic amino acids present within the gum polypeptidic moieties (tyrosin and phenylalanine). Cyclic amino acids however are not the predominant amino acid moiety in gum arabic, as already observed in the literature.[2,10,25] UV detection at 210 nm is mostly sensitive to $\pi \rightarrow \pi^*$ transitions from the C═O bond of the amide linkages between amino acid moieties, and from those of carboxylic moieties present onto the polysaccharides backbones and of reducing sugar units at the end of polysaccharides chains.

Hydrophobic Interaction Chromatography. Hydrophobic interaction chromatography was used to separate arabic gum fractions as a function of their hydrophobic properties. A 7.5 mm × 75 mm 10 μm Biosuite Phenyl column was used (Waters), which consists of phenyl groups grafted to a methacrylic ester based polymeric resin. The average pore size of the column was 1000 Å to accommodate macromolecules with a high molecular weight. A salt concentration gradient is required to create a "salting out" effect and progressively desorb the hydrophobic species. The less hydrophobic species are eluted first with the highest salt concentration, while the more hydrophobic species are the last ones eluted.

The separation was performed on an Alliance HPLC unit (Waters 2695 separation module). The mobile phase was composed of a solution of NaCl at a constant flow rate of 0.5 mL/min. A continuous time gradient of salt concentration between 4 and 0 mol/L was applied during 22 min followed by Milli-Q water during 12 more minutes (see Figure 8 in the Supporting Information). Each sample was filtered with a nylon 0.2 μm membrane, and a 30 μL volume was injected. The UV absorbance was measured at 210 and 280 nm (Waters 2487 UV detector). We observed that the UV absorbance (especially at 210 nm) was very sensitive to NaCl concentration. The baseline was not constant along the separation time. In order to correct this deviation, the chromatogram of Milli-Q water injected in same elution conditions was subtracted to each measured chromatogram.

Variation coefficients for each detection were estimated from comparison of three injections of the same sample. A 6% (respectively, 11%) variation coefficient was observed at 210 nm (respectively, 280 nm).

Small Angle Neutron Scattering. SANS experiments were performed on the PAXY small angle diffractometer located at the Orphee reactor in Saclay, France. Samples were dissolved in 20 mM NaCl solution in D_2O. Spectra were recorded using four different

spectrometer configurations: λ = 6 Å (incident wavelength), d = 1 m (distance of the sample to the detector), λ = 6 Å and d = 3 m, λ = 8.5 Å and d = 5 m, and λ = 15 Å and d = 6.7 m. The wave vector q range covered was from 6.5×10^{-3} to 4.5×10^{-2} Å^{-1}. Contributions from the Quartz Helma cells and ambient noise were subtracted. Data was normalized using the H_2O spectra and set to absolute scale thanks to normalization factor measurements.[26] The incoherent background subtraction was performed using the sample transmission at high q values and a calibration of D_2O/H_2O solutions at different ratios (transmission as a function of scattered intensity). Several power laws were observed, and the typical uncertainty on each power law was evaluated to ±0.1.

Small Angle X-ray Scattering. SAXS experiments were performed on the ID02 instrument at the ESRF synchrotron facility in Grenoble, France. Samples were prepared using 1.5 mm disposable quartz capillaries. Measurements were taken at 1.5 and 7 m. Azimuthal averaging was performed after mask subtraction, and data was normalized by the transmission. One-dimensional spectra were subtracted by the capillary filled with water or the buffer solution. Several power laws were observed, and the typical uncertainty on each power law was evaluated to ±0.1

Circular Dichroism. Circular dichroism measurements were performed on gum arabic and gum SEC fractions on a Jasco J-815 CD spectrometer at IMRCP laboratory in Toulouse, France. A quartz cell with a 10 mm path length was used. Measurements were performed between 250 and 185 nm at 25 °C. A concentration of 0.025% was used for gum arabic. Results were normalized by both concentration and path length, in order to be comparable.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.9b02541.

Transmittance spectra; droplet size evolution; hydrophobic interaction chromatograms; evolution of GA stabilized emulsion size distribution; CD measurements (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Vandevelde, M.-C.; Fenyo, J.-C. Macromolecular Distribution of Acacia Senegal Gum (Gum Arabic) by Size-Exclusion Chromatography. *Carbohydr. Polym.* **1985**, *5* (4), 251−273.

(2) Randall, R. C.; Phillips, G. O.; Williams, P. A. Fractionation and Characterization of Gum from Acacia Senegal. *Food Hydrocolloids* **1989**, *3* (1), 65−75.

(3) Renard, D.; Lavenant-Gourgeon, L.; Ralet, M.-C.; Sanchez, C. Acacia Senegal Gum: Continuum of Molecular Species Differing by Their Protein to Sugar Ratio, Molecular Weight, and Charges. *Biomacromolecules* **2006**, *7* (9), 2637−2649.

(4) Atgié, M.; Garrigues, J. C.; Chennevière, A.; Masbernat, O.; Roger, K. Gum Arabic in Solution: Composition and Multi-Scale Structures. *Food Hydrocolloids* **2019**, *91*, 319−330.

(5) Atgié, M.; Masbernat, O.; Roger, K. Emulsions Stabilized by Gum Arabic: Composition and Packing within Interfacial Films. *Langmuir* **2019**, *35* (4), 962−972.

(6) Randall, R. C.; Phillips, G. O.; Williams, P. A. The Role of the Proteinaceous Component on the Emulsifying Properties of Gum Arabic. *Food Hydrocolloids* **1988**, *2* (2), 131−140.

(7) Chanamai, R.; McClements, D. J. Comparison of Gum Arabic, Modified Starch, and Whey Protein Isolate as Emulsifiers: Influence of PH, CaCl2 and Temperature. *J. Food Sci.* **2002**, *67* (1), 120−125.

(8) Jayme, M. L.; Dunstan, D. E.; Gee, M. L. Zeta Potentials of Gum Arabic Stabilised Oil in Water Emulsions. *Food Hydrocolloids* **1999**, *13* (6), 459−465.

(9) Fennell Evans, D.; Wennerstrom, H. *The Colloidal Domain: Where Physics, Chemistry, Biology, and Technology Meet*, 2nd ed.; Wiley, 1999.

(10) Ray, A. K.; Bird, P. B.; Iacobucci, G. A.; Clark, B. C., Jr Functionality of Gum Arabic. Fractionation, Characterization and Evaluation of Gum Fractions in Citrus Oil Emulsions and Model Beverages. *Food Hydrocolloids* **1995**, *9* (2), 123−131.

(11) Islam, A. M.; Phillips, G. O.; Sljivo, A.; Snowden, M. J.; Williams, P. A. A Review of Recent Developments on the Regulatory, Structural and Functional Aspects of Gum Arabic. *Food Hydrocolloids* **1997**, *11* (4), 493−505.

(12) Padala, S. R.; Williams, P. A.; Phillips, G. O. Adsorption of Gum Arabic, Egg White Protein, and Their Mixtures at the Oil-Water Interface in Limonene Oil-in-Water Emulsions. *J. Agric. Food Chem.* **2009**, *57* (11), 4964−4973.

(13) Sanchez, C.; Nigen, M.; Mejia Tamayo, V.; Doco, T.; Williams, P.; Amine, C.; Renard, D. Acacia Gum: History of the Future. *Food Hydrocolloids* **2018**, *78*, 140.

(14) Dickinson, E.; Elverson, D.; Murray, B. S. On the Film-Forming and Emulsion-Stabilizing Properties of Gum Arabic: Dilution and Flocculation Aspects. *Food Hydrocolloids* **1989**, *3* (2), 101−114.

(15) Erni, P.; Windhab, E. J.; Gunde, R.; Graber, M.; Pfister, B.; Parker, A.; Fischer, P. Interfacial Rheology of Surface-Active Biopolymers: Acacia Senegal Gum versus Hydrophobically Modifed Starch. *Biomacromolecules* **2007**, *8* (11), 3458−3466.

(16) *Gums and Stabilisers For the Food Industry*; Williams, P. A., Phillips, G. O., Eds.; Royal Society of Chemistry, 2003; Vol. *12*.

(17) Idris, O. H. M.; Williams, P. A.; Phillips, G. O. Characterisation of Gum from Acacia Senegal Trees of Different Age and Location Using Multidetection Gel Permeation Chromatography. *Food Hydrocolloids* **1998**, *12* (4), 379−388.

(18) Schmitt, C.; Turgeon, S. L. Protein/Polysaccharide Complexes and Coacervates in Food Systems. *Adv. Colloid Interface Sci.* **2011**, *167* (1), 63−70.

(19) Dror, Y.; Cohen, Y.; Yerushalmi-Rozen, R. Structure of Gum Arabic in Aqueous Solution. *J. Polym. Sci., Part B: Polym. Phys.* **2006**, *44* (22), 3265−3271.

(20) Kobayashi, M.; Utsugi, H.; Matsuda, K. Intensive UV Absorption of Dextrans and Its Application to Enzyme Reactions. *Agric. Biol. Chem.* **1986**, *50* (4), 1051−1053.

(21) Kuipers, B. J. H.; Gruppen, H. Prediction of Molar Extinction Coefficients of Proteins and Peptides Using UV Absorption of the Constituent Amino Acids at 214 Nm To Enable Quantitative Reverse

Phase High-Performance Liquid Chromatography-Mass Spectrometry Analysis. *J. Agric. Food Chem.* **2007**, *55* (14), 5445−5451.
(22) Receveur-Bréchot, V.; Durand, D. How Random Are Intrinsically Disordered Proteins? A Small Angle Study: Ingenta Connect. *Curr. Protein Pept. Sci.* **2012**, *13*, 55−75.
(23) Kikhney, A. G.; Svergun, D. I. A Practical Guide to Small Angle X-Ray Scattering (SAXS) of Flexible and Intrinsically Disordered Proteins. *FEBS Lett.* **2015**, *589* (19PartA), 2570−2577.
(24) Kato, T.; Tokuya, T.; Takahashi, A. Comparison of Poly(Ethylene Oxide), Pullulan and Dextran as Polymer Standards in Aqueous Gel Chromatography. *J. Chromatogr. A* **1983**, *256*, 61−69.
(25) Goodrum, L. J.; Patel, A.; Leykam, J. F.; Kieliszewski, M. J. Gum Arabic Glycoprotein Contains Glycomodules of Both Extensin and Arabinogalactan-Glycoproteins. *Phytochemistry* **2000**, *54* (1), 99−106.
(26) Brûlet, A.; Lairez, D.; Lapp, A.; Cotton, J.-P. Improvement of Data Treatment in Small-Angle Neutron Scattering. *J. Appl. Crystallogr.* **2007**, *40* (1), 165−177.

Supporting information.

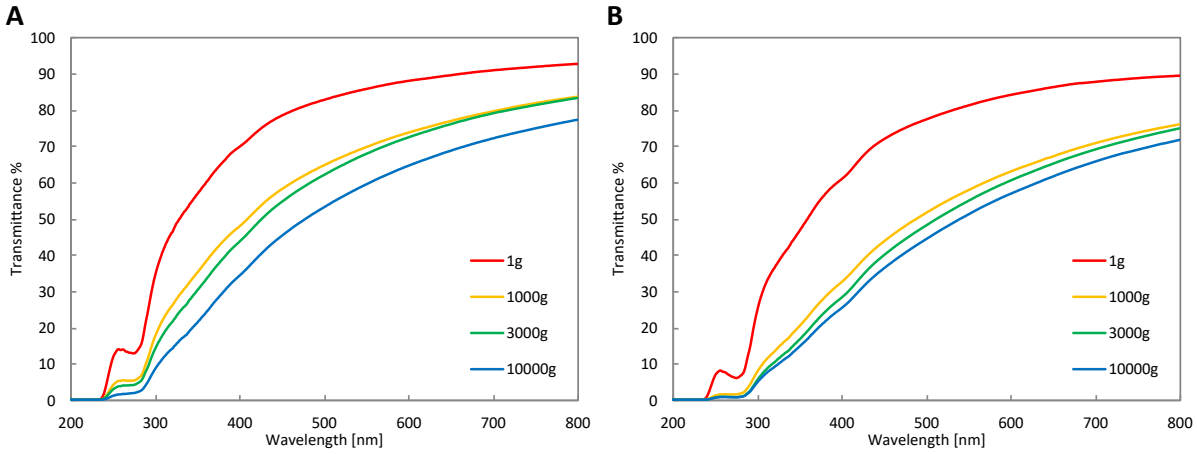

Figure 1: Transmittance of adsorbed species solution (1w/w%) between 200 and 800 nm for various centrifugal acceleration applied during rising steps (1g corresponds to the case without centrifugation). (A) Emulsion stabilized with a 16g/L GA solution, (B) Emulsion stabilized with a 50g/L GA solution.

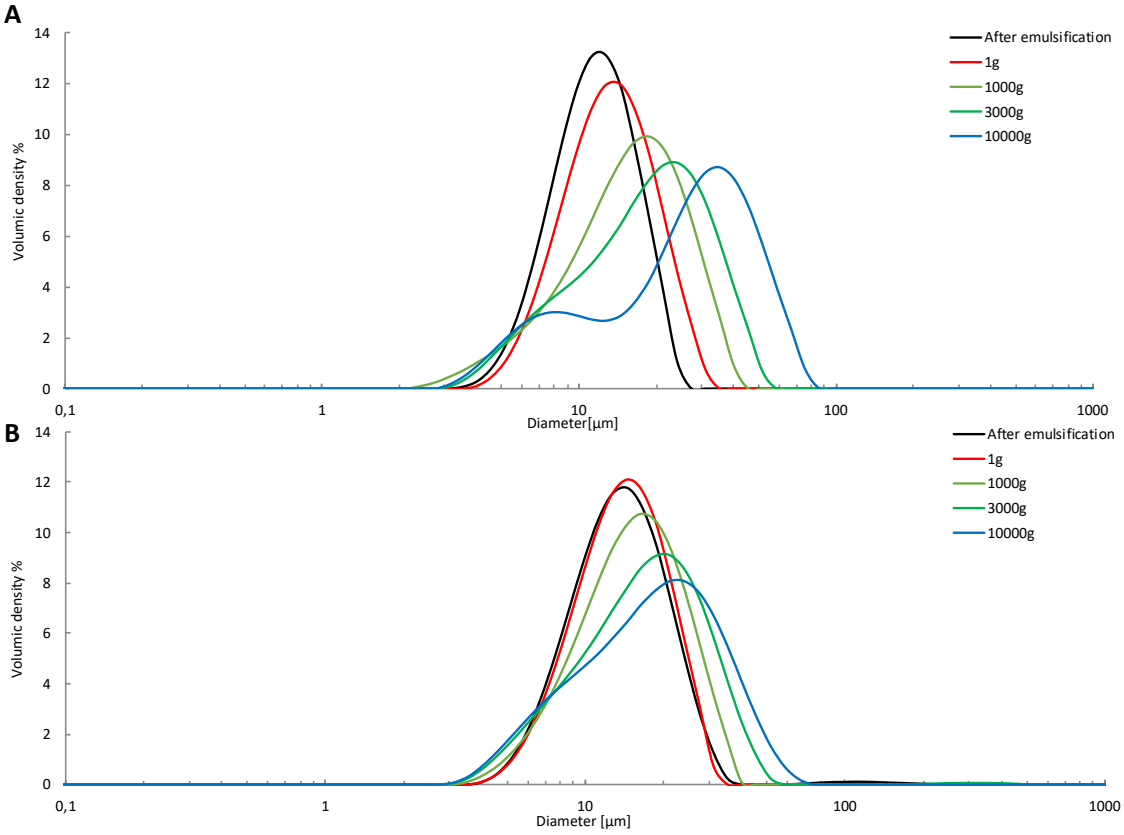

Figure 2: Evolution of droplet size distribution as a function of centrifugation acceleration: (A) emulsion stabilized with a 16g/L GA solution and (B) emulsion stabilized with a 50g/L GA solution.

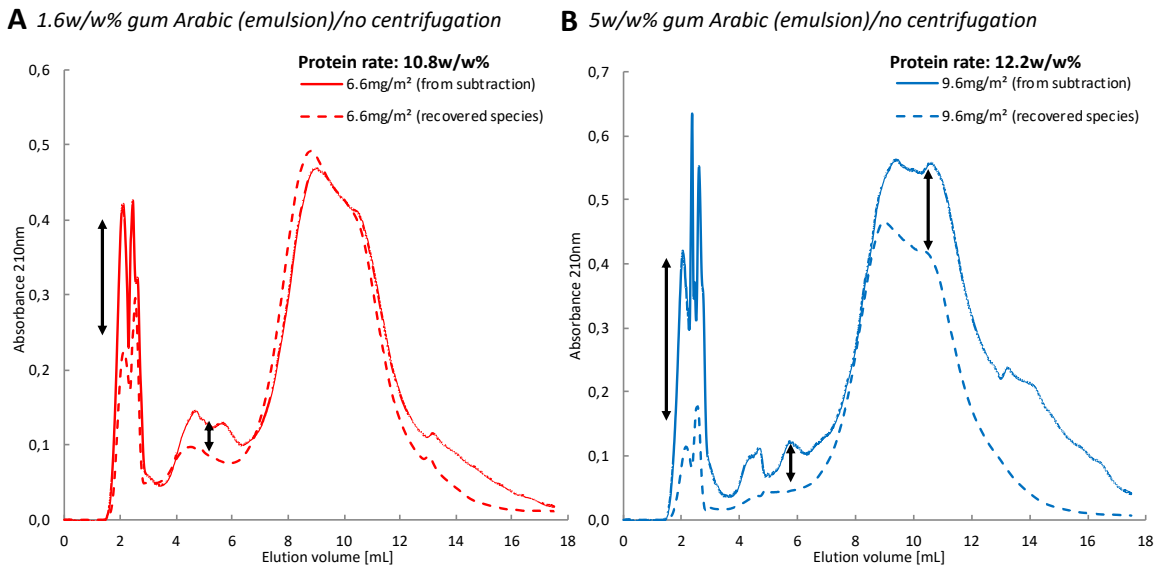

Figure 3: Hydrophobic interaction chromatograms: comparison of adsorbed species calculated spectrum (from subtraction of non-adsorbed species chromatogram to GA chromatogram) and recovered adsorbed species spectrum. (A) Emulsion stabilized with 16g/L GA solution, (B) Emulsion stabilized with 50g/L GA solution.

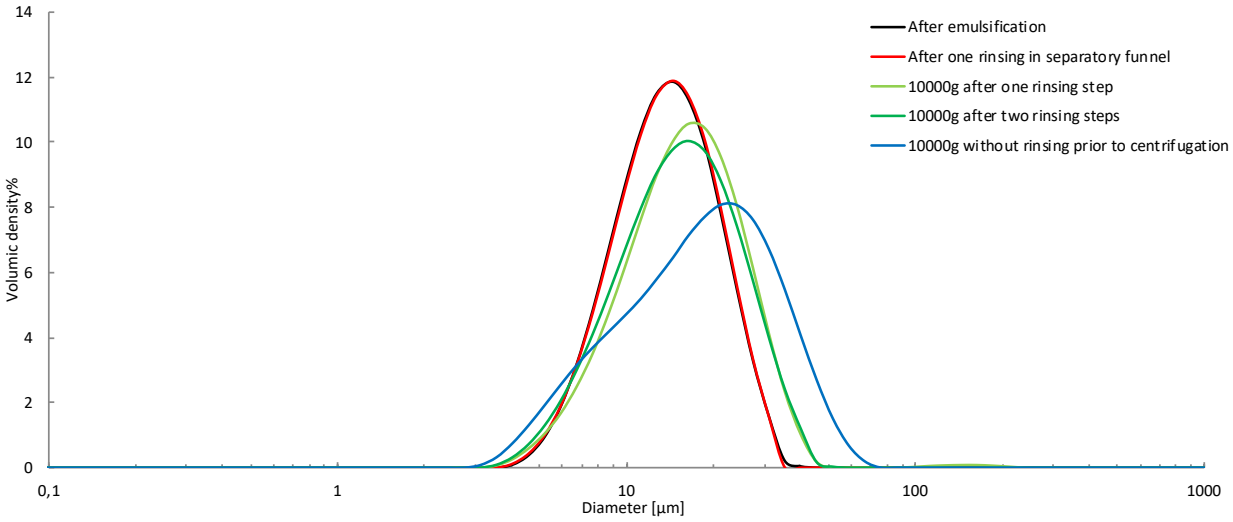

Figure 4: Evolution of GA stabilized emulsion (50g/L) size distribution for different conditions of the adsorbed species recovering process.

From Figure 4, we observed that a GA stabilized emulsion better resisted centrifugation when almost all the non-adsorbed species were removed from the aqueous phase (by a rinsing step in

a separating funnel prior to centrifugation). Therefore, more coalescence events occur when droplets are packed in the presence of non-adsorbed macromolecules. This observation proves that non-adsorbed macromolecules of the aqueous phase not only do not favor droplet stabilization, but to the contrary promote coalescence.

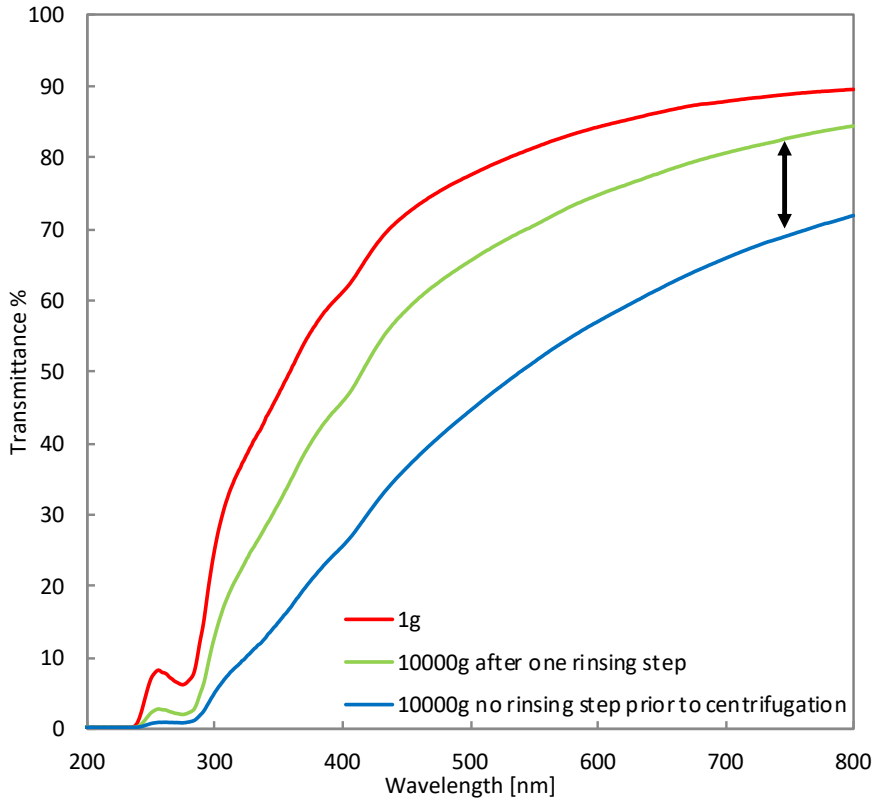

Figure 5: Transmittance of adsorbed species solutions (1w/w%) between 200 and 800 nm at different conditions of the interface species recovering process. (emulsions prepared with a 50g/L GA solution).

Measurement of recovered species solution transmittance revealed that they were less aggregated when removing the non-adsorbed species from the aqueous phase prior to centrifugation as can be seen in Figure 5. This result agrees with the size distribution evolution of Figure 4, as less coalescence occurs when the emulsion is rinsed.

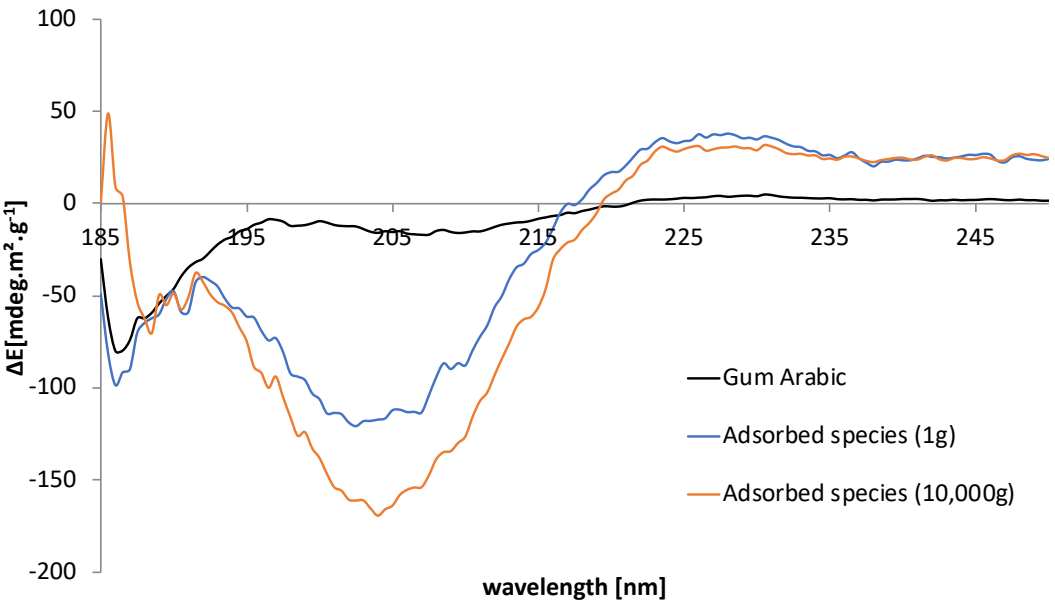

Figure 6: Circular dichroism measurements of Arabic gum and recovered species from the interface. Results were normalized by the cell path length and the sample concentration.

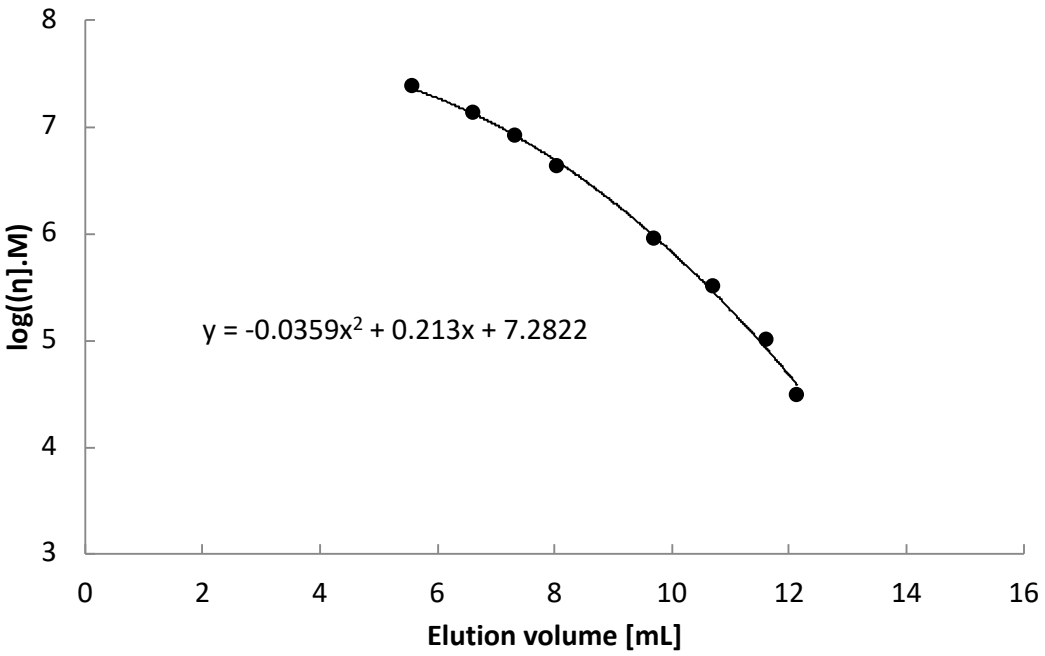

Figure 7: Universal calibration curve (realized with branched dextran of known molecular weight) for size exclusion separation on Biosuite 450Å SEC column (waters) with a low flow rate of 0.8mL/min and 0.5M NaCl as the eluting solution.

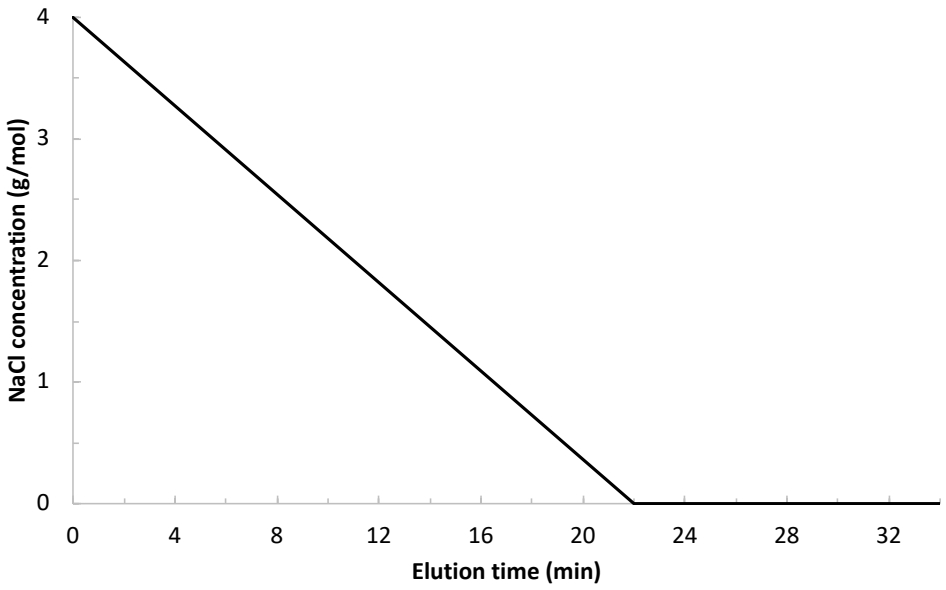

Figure 8: NaCl elution gradient used for hydrophobic interaction chromatographic separation.