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► **To cite this version:**

Abdellatif Dahi, Bah Mohamed-Lemine Abdellahi, Mohamed Fadel Deida, Nicolas Hucher, Catherine Malhiac, et al. Chemical and physicochemical characterizations of the water-soluble fraction of the *Commiphora Africana* exudate. *Food Hydrocolloids*, 2019, 86, pp.2-10. 10.1016/j.foodhyd.2017.10.032 . hal-02181664

HAL Id: hal-02181664

<https://univ-lemans.hal.science/hal-02181664v1>

Submitted on 25 Oct 2019

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Chemical and physicochemical characterizations of the water-soluble fraction of the

Commiphora Africana exudate

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Abstract

The present study focuses on the chemical and physical characterization of the water-soluble fraction of the *Commiphora Africana* exudate. The chemical analysis proved that this fraction is mainly composed of carbohydrates and hydrophobic amino acids. Size exclusion chromatography (SEC) revealed the presence of three distinct polysaccharide populations that are all bonded to protein. In addition, both SEC and rheology demonstrated that the gum owns highly compact structure that suggests an AGP-like polymer. *commiphora Africana* gum exhibits excellent surface activity at concentration 2 orders of magnitude lower than the reference, acacia gum and was attributed to the fact that the three populations contribute to the surface tension lowering. This remarkable property has been confirmed by preliminary experiments on model emulsions and suggests a very promising application in formulation.

Keywords

Commiphora Africana exudate; water-soluble fraction; chemical composition; Arabinogalactan-Protein-like polymer; Surface activity; compact structure

1. Introduction

Hydrocolloids own many industrial applications in pharmaceutic, cosmetic or food products. Their applications are based on their functional properties, for example, their ability to form viscous solutions at low concentration, or their ability to stabilize emulsions. The possible surface activity of several food gums has been studied in order to evaluate their

potential emulsifying properties (Dickinson, 2003; Garti & Reichman, 1993; Phillips & Williams, 2004; Phillips & Williams, 2009).

Among hydrocolloids, naturally produced ones show multiple benefits: they are biodegradable, less toxic and have activity over wide temperature, pH and ionic strength ranges. This may explain the intensive search of new natural gums and the effort to characterize their functional properties (Grein et al., 2013); (Ozaki, Oki, Suzuki, & Kitamura, 2010); (Steinhorn, Sims, Carnachan, Carr, & Schlothauer, 2011); (Vinod et al., 2008).

Commiphora Africana [A. rich] Engl. is a tropical and subtropical plant of the genus *Commiphora* (Burseraceae) and widespread over Africa. Hence, *Commiphora Africana* are found across the south part of Sahel, from West (Mauritania) to East (Ethiopia). The *Commiphora* genus is known to produce resinous exudates largely studied because of their historical medicinal use (Shen, Li, Wang, & Lou, 2012). Hence, there is a rich literature concerning the chemistry of Myrrh, from *Commiphora Myrrha*, opopanax from *Commiphora Molmol* or *Commiphora Mukul* (Hanus, Rezanka, Dembitsky, & Moussaieff, 2005) which are known to contain large amount of various hydrocolloids. In many case, these exudates are used by traditional medicine, including their oral medications (Lemenih, Abebe, & Olsson, 2003); (Abdellahi, 2013). Concerning *Commiphora Africana* although, to our knowledge, only an old study by Jessenne (Jessenne, Bezanger-Beauquesne, Pinkas, & Troitin, 1974), took interest in the chemical composition of its exudate. Unfortunately, the authors could not certify the origin of their studied gum (Bdellium) because, as stated in the PhD thesis of Jessenne (Jessenne, 1974), it came from a local traditional market. Recently, Gundidza et al. (Gundidza et al., 2011) conducted a viscosimetric analysis of a gum resin from *Commiphora Africana*, unfortunately without a previous purification of the hydrocolloids parts.

In this paper we will present the first chemical and physicochemical analyses of the water-soluble part of the *Commiphora Africana* exudates after intensive purification. The aim of this study is to elucidate the fine structure of this fraction and to determine the relationship between the structure and the functional properties, which, to the best of our knowledge, has never been done so far.

2. Materials and methods

2.1 Materials

Commiphora Africana (CA) gum was extracted from air-dried exudates of the CA tree in Africa. The studied trees are located in the province of Rosso (Wilaya Trarza) in the south of

Mauritania, on the right bank of the Senegal River. The exudates (nodules), were collected manually, two weeks after wounding, during the month of April. Nodules were air dried and stored in polyethylene bags. They present a dark reddish brown colour.

Monosaccharides and L-amino-acids, used as standards, were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France) (purity $\geq 99\%$). All other chemicals used were of analytical grade and ultrapure water (18.2 m Ω resistivity) was used in all experiments.

2.2 CA gum Extraction and purification

CA exudates (nodules) were frozen with liquid nitrogen and powdered in a high-speed mechanical blender. 10 g of the obtained powder was poured in water (1000 cm³) and stirred for 30h at 70°C. The suspension was clarified by centrifugation at 2000 g for 10 min to remove insoluble part and the resultant solution was freeze-dried. The resulting powder was again dissolved in water at room temperature and precipitated with absolute ethanol. The clear supernatant was sucked out after centrifugation at 20°C for 5min at 2000 g, and the insoluble fraction (gum) was dried under vacuum to remove traces of solvents. The dried solid was again dissolved in water, and finally freeze dried. This gave a cotton-like and slightly white CA gum. The overall yield was 38% \pm 2%.

2.3 Preparation of CA gum solutions

CA gum was dispersed in water at the target concentration (weight %). Dispersions were gently stirred at 35°C for 4 h, and left at 3 \pm 1°C overnight for complete hydration. Then, filtration using syringe filter (0.45 μ m, cellulose acetate membrane) gave clear solutions.

2.4 Chemical composition analysis

Moisture and ash content of the CA gum were measured by Thermal Gravimetric Analysis (Setaram Setsys 12) realized between 30 and 900°C at a heating rate of 10°C/min under nitrogen.

The total protein content was estimated by the Bradford protein assay taking bovine serum albumin (BSA) as standard.

2.5 Specific optical rotation

The specific optical rotation ($[\alpha]_{589}^{25^\circ\text{C}}$) of a 2.5 g/L gum solution was measured at 25°C with a Perkin Elmer polarimeter (model 214 MC) at wavelength 589 nm, using a 1 dm length cell and a Na lamp.

2.6 Monosaccharides composition

Hydrolysis of the *CA* gum was conducted through acidic conditions by dissolving the solid in 200 μL 4 M trifluoroacetic acid into a sealed tube flushed with N_2 and heated at 100°C for 4 h. The solution was then dried under vacuum, dissolved in methanol and dried again twice before precolumn derivatization.

For the precolumn derivatization adapted from Tapie et al. (Tapie, Malhiac, Hucher, & Grisel, 2008), a 4-(3-methyl-5-oxo-2-pyrazolin-1-yl) benzoic acid (PMPA) solution was prepared immediately before use by the following procedure: PMPA crystals were dissolved in 0.25 M NaOH methanol-water (1:1) up to the point at which the pH stabilized at 7.8-8 and then completed with methanol-water (1:1) to obtain a final concentration of PMPA of 0.15 M. The monosaccharides, either pure references or from the hydrolysis of the *CA* gum, were incubated with 1 mL of PMPA solution (0.15 M) at 70°C for 2 h. After incubation, samples were acidified by 150 μL of an aqueous 2M HCl solution and shaken up to precipitate most of the excess of derivatization agent. After a few minutes, samples were centrifuged for 10 min at 5 000 g. Supernatant was sucked out and filtered through 0.2 μm nylon membrane.

Separations were carried out with an Agilent 1200 LC system (Agilent Technologies, Massy, France) consisting of a binary pump, autosampler, and column oven. A Sigma-Aldrich Acsentis Express C18 column (2.7 μm 100x2.1 mm I.D. Sigma-Aldrich, Saint-Quentin Fallavier, France) was used at 25°C . Mobile phases were 20mM ammonium formate (pH 4.75) (A) and methanol (B) with a flow rate of $0.4\text{ mL}\cdot\text{min}^{-1}$ and a gradient profile as follows: 0 min 10% B; 10 min 10% B; 15 min 30% B; 20 min 30% B; 25 min 70% B; 25.1 min 10% B. Re-equilibration time was 7 min and injection volume 5 μL . An Agilent ESI-MS ion trap 6340 (Agilent Technologies, Massy, France) was used for identification and quantification. Mass spectrometry detection was performed in positive mode with optimal conditions determined by direct infusion.

2.7 Amino acid composition of the protein part

The overall amino acid composition of the protein part was determined by GC/MS as described by Sasaki et al. (Sasaki et al., 2008). Freeze-dried sample of *CA* gum (1mg) was hydrolysed with HCl 6M at 100°C for 20h. A 0.6N HCl solution in methanol was added to the liberated amino acids or pure amino acids used as reference and these solutions were heated at 100°C during 15 min. After drying under N_2 stream, the residue containing amino acids methyl esters were treated with pyridine/MeOH/ Ac_2O (300 μL 1:1:4), then heated at 100°C

during 1 hour. The solutions were directly analysed by GC/MS. GC/MS analyses were performed on a Shimadzu GCMS-QP2010SE equipped with a split injector (1/50) heated at 250°C. The carrier gas was set up at 1 mL min⁻¹. The amino acid acetate methyl esters were separated on a capillary column ZB-5MS-Phenomenex (L=30 m; id = 0.25 mm; film thickness = 0.25 µm). The oven temperature was programmed at 50°C (hold 2 min) to 90°C (20°C min⁻¹, 1 min), then to 280°C (5°C min⁻¹) then held for 2 min.

Electron Impact (EI) spectra were obtained at 70 eV at 200°C. The amino acid acetate methyl esters were identified using NIST database and by comparison with pure reference of amino acid acetate methyl esters (MS spectra and retention time).

2.8 Size exclusion chromatography

SEC analysis were performed using a shimadzu HPLC system (Shimadzu, Japan) coupled to four detectors: multi-angle laser light scattering (MALLS) operating at eighteen angles (Dawn Heleos II, Wyatt, CA, USA), differential refractometer (Optilab T-rEX, Wyatt, CA, USA), on-line viscosimeter (VISCOSTAR II, Wyatt, CA, USA) and UV-VIS detector activated at 280 nm (SPD-20A, Shimadzu, Japan). The system was composed of one Shodex OHPAK SB-G pre-column followed by three columns in series (Shodex OHPAK SB 806 HQ, OHPAK SB 805 HQ and OHPAK SB 804 HQ).

Aqueous solution of CA gum (1mg.ml⁻¹) was dissolved overnight in milliQ water, then 50 mL were injected and eluted through the system with 100 mM LiNO₃ solution containing 0.02% NaN₃ at a constant flow rate of 1 mL.min⁻¹ at room temperature.

2.9 FT-IR spectroscopy

IR spectra of the CA gum were measured by a Perkin Elmer Frontier FT-IR spectrometer using the ATR mode (Diamond/ZnSe crystal). 8 scans were collected for each measurement over the spectral range 4000–650 cm⁻¹ with a resolution of 8 cm⁻¹.

2.10 Viscosity measurements

Gum solutions at different concentrations (0.01 to 165 g/L) were prepared in water. Their dynamic viscosity was measured using a rolling Ball Viscometer (Anton PaarAMVn) allowing measurements at different angles (15 to 85°). The rolling Ball Viscometer uses the simple Höppler principle to measure viscosity. The basic concept is to measure the elapsed time required for the ball to roll under gravity through a sample-filled tube inclined at an angle. First, the tube (1.6 mm diameter) was filled with a gum solution. Then, it was placed in

the thermostatic measurement chamber and equilibrated to a constant temperature maintained within $\pm 0.1^\circ\text{C}$ for about 15 min. Thereafter, rolling times of solution were measured at different temperature (10, 20, 30, 40 and 50°C). At each temperature, the angle of tube from the horizontal was varied progressively from 30, 40, 50, 60, 70 to 80 degrees in order to study the flow behaviour of CA gum in solution. At each angle, the measurement was carried out 4 times to determine an average. Viscosities were directly given by the viscometer according to the rolling times.

The intrinsic viscosity $[\eta]$ was determined at 20°C using the Huggins equation (1) (Huggins, 1942) by plotting η_{sp}/C against the polymer concentration, and subsequently extrapolating to zero polymer concentration (2):

$$\frac{\eta_{sp}}{C} = [\eta] + k_H [\eta]^2 C = \frac{\eta - \eta_s}{\eta_s C} \quad (1)$$

$$[\eta] = \lim_{C \rightarrow 0} \frac{\eta_{sp}}{C} \quad (2)$$

where η and η_s are the viscosity of the solution and the solvent respectively, η_{sp} is the specific viscosity and C and k_H are the polymer concentration and the Huggins coefficient, respectively.

2.11 Surface tension measurements

Surface tension measurements were made using Du Noüy ring tensiometer (Krüss k11, Germany) with platinum ring at room temperature (25°C). Gum solutions at concentrations ranging from 0.01 to 35 g/L were used.

2.12 Evaluation of emulsifying properties

Oil-in-water emulsions were prepared by mixing 0.35 g of orange essential oil, provided by Laboratoire Rosier Davenne (Montfavet, France) and 4.65 g of CA gum solutions at 10 and 50 g/L. For comparison, commercial *Acacia Senegal* gum (food grade), provided by Alland & Robert (Port Mort, France) was also tested in the same conditions.

First, gum solutions were prepared and kept at rest for 24h. The oil phase was then dispersed in the gum solutions under strong shear using a T10 basic ULTRA-TURRAX® (IKA, Germany) at 14450 rpm for 5 min at 20°C . All emulsion samples were kept at 20°C after preparation and sheltered from the light after emulsification.

Characterization of size and size distribution of emulsions during aging were conducted using static laser diffraction apparatus SALD-7500nano (Shimadzu, Japan).

3. Results and discussion

3.1 CA gum main characteristics

The main composition of CA gum is summarized in Table 1. Two major groups of exudates can be distinguished, on the one hand the resin which are soluble in organic solvents (e.g. pine resin) and on the other hand the gum which are soluble in water (e.g. acacia gum). This first shows that when compared to other well characterized exudates, in the exudate from *Commiphora Africana* trees the water-soluble gum is a minor fraction, representing only about 40% of the total content. This is in accordance with the characteristics of exudates from the other *Commiphora* species where 30-60% of this exudates are water-soluble gum, and 25-40% are ethanol soluble resin (Hanus et al., 2005). So the *Commiphora Africa* exudates can be considered as a resin-gum exudate. This low water-soluble fraction points out the importance of purification prior to any measurement and thus data obtained without proper purification have to be taken with extreme caution. CA gum showed to be mostly composed of carbohydrates (80%) with a low content of inorganic matter but a relatively high content of proteins (Table 1). Hence, when compared to many others gums from trees (acacia gums: 1.4-2.2% (Idris, Williams, & Phillips, 1998), Tragacanth Gums: 2-4 % (Balaghi, Mohammadifar, & Zargaraan, 2010) or Barijeh gum: 4.3% (Mohammadzadeh Milani et al., 2007), the 10.2% measured as protein content in CA gum could have been related to impurities through unwashed free proteins. But SEC measurement (Figure 1), and especially from the UV-Vis detector, showed that the UV signal, generally the signature of the presence of amino acid, is very similar to the RI signal on the whole range of elution time. Hence, this suggests that the carbohydrates own covalently bonded protein with similar ratio, which will be discussed in more detail later.

3.2 FT-IR

The FTIR spectrum of CA gum is given in Figure 2. Two characteristic peaks of polysaccharides were observed at 3286 and 2934 cm^{-1} , which were related to stretching vibration modes of OH bound to carbons and C-H stretching vibration, respectively. The bands at 1637 cm^{-1} and 1539 cm^{-1} are attributed to the amide I and amide II bands of the polypeptidic backbone of glycoproteins and proteoglycans (Barth, 2007). However, polysaccharides containing uronic acids will also give bands close to 1637 cm^{-1} and to 1413 cm^{-1} that were attributed to the vibration of C=O and the deprotonated carboxylic function respectively (Boulet, Williams, & Doco, 2007). The region between 1200 and 600 cm^{-1}

represents the so-called carbohydrate fingerprints. This region is typical of the skeletal stretching vibrations of the backbone and side chains of polysaccharides, which is dominated by ring vibrations overlapped with stretching vibrations of (C–OH) side groups and (C–O–C) glycosidic bond vibrations (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). All these observations are in accordance with the presence of both polysaccharides and proteins in CA gum. (Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015)

3.3 Monosaccharides composition by HPLC

The composition of the monosaccharides obtained by hydrolysis of CA gum is given in Table 2. The analytic procedure allowed quantifying the derivated monosaccharides and, if any, the di or three-saccharides not fully hydrolysed. The two major monosaccharides found were arabinose and galactose (47% and 28%, respectively). Also these two monosaccharides are usually present in exudates of *Commiphora* species (Hanus et. al. 2005). Then galacturonic acid represent about 7% of the total monosaccharide. Finally, mannose, xylose, glucosamine and fucose are also present at relatively high percentage (between 4 and 5%) which is quite unusual for plant gums, especially for glucosamine. Indeed, for instance, Wiendl et. al. (Wiendl, Müller, & Franz, 1995) reported small amount of mannose (less than 3%) in Myrrh from unprecise botanic origin among with larger amount of 4-O-Methylglucuronic acid (up to 43.9%). Fucose was also identified by TLC in gum resin of *Commiphora Mukul* (Bose & Gupta, 1964). If Jessenne et. al. (Jessenne et al., 1974) reported galacturonic acid from gum of *Bdellium*, there were no evidence that this gum samples came from identified botanic origin, even if stated “probably from *Commiphora Africana*”. This is the only paper, to-date, that discussed the chemical composition of exudate from *Commiphora Africana*. Glucosamine is far less common in plant exudates, but already reported in botanic species from *Acacia*, *Araucaria* and some others (Anderson, Hendrie, & Munro, 1972) and is closely related to the linkage of proteins to carbohydrate in glycoproteins and proteoglycans.

3.4 Amino acids composition

To our knowledge, the amino acid composition of the proteins from *Commiphora Africana* gum had never been determined. The main amino acids of *Commiphora Africana* are given in Table 3. The major amino acids therefore are Hydroxyproline (Hyp 22.7%), Proline (18.1%), Alanine (14.9%), PhenylAlanine (8.7%), Leucine (7.1%), Serine (6.2%), Glutamic and aspartic acids (6.1% and 5.3%). Hyp is also described to be the main amino acid in Myrrh (*Commiphora molmol*) exudate. (Wiendl, Müller & Franz 1995). Hyp is also the main amino

acid in case of Arabinogalactan-Protein (AGP) structure such as AGP fraction of acacia gum. Furthermore, this amino acid is often described as the link between the protein fraction and the polysaccharide fraction (Mahendran, Williams, Phillips, Al-Assaf, & Baldwin, 2008).

One can notice a high percentage of phenylalanine which can explain the strong absorbance at 280nm (Figure 1). Furthermore, the major identified amino acids were hydrophobic, which is usually related to compact globular proteins.

3.5 Viscosity measurements

The viscosity of many liquids depends exponentially on temperature, and the most appropriate model that best explains the temperature dependence of the viscosity of macromolecules is the Arrhenius equation which is expressed as:

$$\eta = \eta_{ref} \exp\left(\frac{E_a}{RT}\right) \quad (3)$$

where η (Pa.s) is viscosity, R is the gas constant ($8.31 \text{ J.K}^{-1}.\text{mol}^{-1}$), T (K) is temperature, E_a (J/mol) is the Arrhenius activation energy and η_{ref} (Pa.s) is the viscosity of the solution at the reference temperature ($T_{ref}=20^\circ\text{C}$, in this study).

The values of activation energy evolved slightly from 17 to 23 KJ.mol^{-1} for CA gum solutions with concentrations from 20 to 165 g/L. It is well known that the activation energy reflects the flexibility and interactions of macromolecules. For instance, acacia and cashew gums branched polysaccharide with few interactions, have comparable E_a values of respectively 15 kJ.mol^{-1} (Varfolomeeva, Grinberg, & Tolstogusov, 1980) and 15.6 kJ.mol^{-1} (de Paula & Rodrigues, 1995) for a 20 g/L solution. On the contrary, linear polymers strongly bonded by intra and intermolecular interactions involving hydrogen bonding show high activation energies. For example, xanthan gum exhibits E_a value about 70-80 kJ.mol^{-1} for concentration ranging from 1 to 10 g/L (Renou, Petibon, Malhiac, & Grisel, 2013). It appears clearly that the viscosity behaviour of CA gum is closer to branched polysaccharides, than to linear ones. Accordingly, the intermolecular interactions between polysaccharide chains in CA gum are weak.

On the other hand, CA gum solutions from 20 to 110 g/L exhibits at 20°C viscosities ranging from 1.06 to 10.3 mPa.s. These very low (for this range of concentration) values are similar to that of acacia gum in solutions up to 100 g/L (Glicksman, 1969). The low and newtonian (as will be seen below) viscosity at higher concentrations is a second sign of weak intermolecular interactions between polymer chains. All these results indicate probably a polymer structure in CA gum of high compactness.

3.5.2 Intrinsic viscosity

The intrinsic viscosity is a measure of the hydrodynamic volume occupied by a macromolecule, which is closely related to both the conformation and size (weight) of the macromolecular chain in a specific solvent (Lai & Chiang, 2002). Intrinsic viscosity of CA gum was determined according to the Huggins equation (3), as explained before. To this end, dilute solutions was used, ranging from 20 to 80 g/L. The obtained $[\eta]$ value for CA gum was 20.3 ml.g^{-1} . Again, this low value was close to that found for acacia gum (Al-Assaf, Phillips, & Williams, 2005) (Lopez-Torrez et al., 2015).

3.5.3 Flow behaviour of CA gum

From viscosity measurements, it was observed that the flow times for all solutions (20 to 165 g/L) decrease proportionally with the tube angle from 30 to 80°, proving that solutions remain Newtonian even at concentrations as high as 165 g/L.

The power law equation below (4) relating specific viscosity and concentration can be used as an indicator for estimating the conformation of polymer chains (Lai, Tung, & Lin, 2000):

$$\eta_{sp} = aC^b \quad (4)$$

where a and b are constants. Figure 3 shows this power-law plot for CA gum solutions at different temperature.. From this plot, it can be seen that both the slope (b value) and intercept (a value) remain stable, irrespective to the temperature. This means that the polymer chains adopt the same conformation over the studied temperature range. It has been established that b value greater than one is associated with a spherical or compact conformation, while a value of b less than one is associated with rod like conformation (Lai & Chiang, 2002). From plots, the b value was around 1.75 ± 0.05 (an average of all the slopes). Therefore, the molecular conformation of CA gum is more spherical like than rod like. Again, this result is in good accordance with polymer structure in CA gum of high compactness.

On the other hand, shape factor value was determined to be 2.54, from equation 5:

$$\nu = \frac{[\eta]}{V_E} \quad (5)$$

where V_E , the voluminosity, was determined following Antoniou et al. methodology (Antoniou, Themistou, Sarkar, Tsianou, & Alexandridis, 2010)

A shape factor value of 2.5 suggests that the polymer coils are spherical in nature (Joseph, Devi, & Rakshit, 1991). This indicates that the effective molecular shape of CA gum

is spherical. To verify this, the Krieger & Dougherty model (Krieger & Dougherty, 1959) was also applied to the data. This model was developed to characterize the volume fraction dependence of the relative viscosity of hard spheres and is frequently used for food systems, using equation (6):

$$\eta_{rel} = \left(1 - \frac{\phi}{\phi_{max}}\right)^{-[\eta]_0 \phi_{max}} \quad (6)$$

with ϕ and ϕ_{max} the volume fraction and maximum packing volume fraction, respectively, of suspended particles, and $[\eta]_0$ the intrinsic viscosity which theoretically depends on particle shape. The ϕ/ϕ_{max} term in the equation can then be simply substituted by C/C_{max} , where C is the polymer concentration. According to hard sphere theory, the ϕ_{max} and $[\eta]_0$ parameters of Krieger & Dougherty equation for mono-dispersed spherical systems are 0.64 and 2.5, respectively. Thus, this equation was applied to the experimental data of CA gum (Figure 4). For reasons of clarity and ease of understanding, our results were compared to a linear and spherical star-like polymer (32 arms, considered as relatively dense system) (Vlassopoulos, 2004). A deviation from the Krieger & Dougherty model (hard spheres) was found for the CA gum. This result indicates that CA gum doesn't exhibit a hard-sphere behaviour. CA gum is also not linear because its behaviour diverges for linear one and is in between linear and the star-like polymer conformation. To conclude, the polymer chains in the CA gum adopts a relatively compact structure. This explains both the low viscosifying properties of CA gum, and the weak intermolecular interactions between chains as evidenced previously by the low Ea values. To get deeper into the polymer structure of CA gum, size-exclusion chromatography was used and results are discussed in the next section.

3.6 Polymer conformation in solution

Size exclusion chromatography measurements have been carried out with using UV, RI, LS and viscosimetric detectors and the chromatograms are displayed in Figure 1. One can clearly see that the system is composed of three main populations which are quite well defined at 21, 24.5 and 27.4 min elution times, which correspond to 8 000 000, 950 000 and 150 000 g/mol, respectively as determined by the LS detector. The main population in number is eluted at 27.4 min, thus corresponding to the latter molecular weight. All the average parameters (on the whole elution time range) are summarized in Table 4. Mw is about 1 300 000 g.mol⁻¹ while Mn is 260 000 g.mol⁻¹, proving that the system is highly polydisperse as it is often observed for natural polysaccharide from plants (Eagles, Stephen, & Churms,

1993); (Phillips, 2009). In addition, thanks to viscosimetric detector, the intrinsic viscosity was determined and is very close to the one measured by the rolling ball viscometer. The hydrodynamic radius is 14.1 nm, which is quite small for molecule of such a high molecular weight, showed again that the molecules are highly compact. This observation is consistent with the viscosimetric analysis that shows that CA gum behaves as a highly branched polymer.

The UV signal measured at 280 nm showed a maximum absorbance that correlate with the three main populations at 21, 24.5 and 27.4 min elution times. This suggests that the three population own covalently bonded protein with similar ratio, proving that bonded proteins are homogeneously distributed between the three populations. This is an unusual property, very different to what is observed for gums containing glycoproteins such as acacia gum for instance (Phillips, 2009) but that were already found for closely related Myrrh gum (*Commiphora molmol*) (Wiendl et al., 1995).

3.7 Surface tension

The surface activity of CA gum was characterized by measuring the tension at air/water interface according to the Du Noüy's ring method at 25°C. Figure 5 shows the variation of the surface tension of water as a function of CA gum concentration. Surface tension of ultrapure water at 25 °C was 71.90 mNm⁻¹. One can observe a rapid lowering of the surface tension from 71.90 to 50.43 mN.m⁻¹ for concentration ranging from 0 to 7 g/L. For higher CA gum concentrations, surface tension remained constant. Critical aggregation concentration (CAC), determine as the concentration at which the surface tension starts to decrease, was determine at 0.2 g/L of CA gum. Hence, CA gum reduced the surface tension more efficiently than classic commercial pectin (63 mN.m⁻¹ in apple pectin) (Lutz, Aserin, Wicker, & Garti, 2009) or *Acacia tortuosa* (53 mN.m⁻¹) (Muñoz et al., 2007). In addition, this very low CAC is about 135 times lower than the reference gum (*Acacia Senegal*), for which CAC is close to 27 g/L in the same conditions of measurement (Grein et al., 2013). This may open a promising application of CA gums as efficient surface agent.

3.8 Emulsifying properties

Preliminary experiments on simple emulsions (orange essential oil/water) were conducted on CA gum and *Acacia Senegal* gum for comparison. Figure 6 shows the volume droplet size distribution for o/w emulsions containing 10 and 50 g/L of gum (CA and *Acacia Senegal*), and their monitoring over time at 20°C.

At 50 g/L, the droplet size distribution of the freshly prepared emulsions is very close for both gums. The main population can be seen with a volume mean diameter ($D_{4,3}$) of 12 μm . However, for the *CA* gum, a small population with a $D_{4,3}$ of 4 μm slowly disappeared after few days, probably through Ostwald ripening. In general, and for both gums, no changes in the droplet size distribution nor in the $D_{4,3}$ were observed during the experiment (up to 10 days).

At 10 g/L, the droplet size distribution of the freshly prepared emulsions is also very close for both gums. The main population can be seen with a $D_{4,3}$ of 19 μm . In case of *CA* gum, both the droplet size distribution and the mean droplet size remain stable over time, evidencing a powerful emulsifying capacity of *CA* gum at 10 g/L. Indeed, this moderate concentration is already at 50 times the CAC of *CA* gum. In the case of *Acacia Senegal* gum, a demixing was observed within one hour after the emulsion preparation (as seen in the picture caption figure 6) and thus no monitoring was possible afterwards. At 10 g/L, *Acacia Senegal* gum could not create a stable emulsion probably because the used concentration (10 g/L) is below the CAC of this gum (close to 27 g/L according to (Grein et al., 2013)). These preliminary results demonstrate the excellent emulsifying properties of *CA* gum, which is efficient at much lower concentrations than acacia.

4. Discussion

All of the chemical analyses of *CA* Gum lead to a proposed structure for this hydrocolloid that is clearly related to arabinogalactan-like glycoprotein (AGP) structures. First, we showed that the two major monosaccharides were arabinose and galactose, and that, minor ones, galacturonic acid and glucosamine, are usually related to glycoproteins and proteoglycans (Nguema-Ona et al., 2014). Secondly, The *CA* gum protein fraction is rich in Hyp and its overall composition is also compatible with the AGP nature of the polysaccharides, as stated for acacia gum (Idris et al., 1998).

Besides, the amino acids are mainly hydrophobic, with a large amount of proline, which is consistent with a globular structure of the resulting proteins (Lesser & Rose, 1990); (Schulz, 1977). This will most likely lead to a compact AGP-like structure, which is consistent with the high compactness that we determined for *CA* gum.

The present study shows that *CA* gum lowers the surface tension of water down to 50.43mN/m with less than 10 g/L of *CA* gum. Therefore *CA* gum possesses excellent capacities to lower the surface tension using concentrations at least 100 times lower than acacia gum for the same performance, which would open up promising opportunities in

formulation. In general, the capacity of food biopolymers to lower the surface tension is related to their chemical structure, concentration, and molecular conformation at the interface (Dickinson, 2011); (López-Franco et al., 2012). More precisely, both the accessibility of the protein moieties to the interface and the net surface charge of adsorbing molecules control the interfacial activity of polymers (Vasile, Martinez, Pizones Ruiz-Henestrosa, Judis, & Mazzobre, 2016). Some reports have shown that a relatively high protein content is required to provide good interfacial properties. Hence, the interfacial properties of acacia gum were, for example, attributed to the arabino-galactan protein fraction and its high molecular weight in the gum sample (Dickinson, 2011); (Phillips, 2009). In addition, contrary to acacia gum, for which protein are mainly located in one fraction (AGP), for CA gum, the 3 fractions possess covalently bonded protein. Thus, all fractions contribute to the surface tension lowering which may explain the much better surface activity of CA gum.

The preliminary experiments on the emulsifying properties of CA gum showed that 1) CA gum presents excellent stabilizing properties as good as *Acacia Senegal* gum then 2) CA gum is even more efficient than *Acacia Senegal* gum at low concentration, thanks to its much lower CAC. These preliminary results prove the effective ability of CA gum to stabilize interfaces and thus its potential interest in formulation.

5. Conclusion

The chemical composition of the water-soluble fraction of the *Commiphora Africana* exudate has been determined for the first time without ambiguity. This gum exudate is mainly composed of glycoproteins, which is composed of 79.5 % carbohydrates and 10.2 % proteins. The major monosaccharides found were arabinose (47 %) and galactose (28%) together with galacturonic acid (7%) and minors one. The major amino acids found were hydroxyproline (22.7 %), proline (18.1 %) and alanine (14.9 %). Rheological measurement along with size exclusion chromatography lead to a proposed structure of an highly compact AGP-like polymer. Whereas the *Commiphora Africana* gum is composed of three main populations (ranging from 8 000 000 to 150 000 g.mol⁻¹), they all possess covalently bounded protein. Hence, in accordance with what is expected for AGP-like polysaccharides, the gum showed a great capacity to lower the surface tension in water (down to 50.43 mN.m⁻¹ for a 7 g/L solution). In addition, preliminary experiments on simple emulsions gave encouraging results. In further works we will conduct a complete characterization of stabilization capacities of the *Commiphora Africana* gum, in order to confirm its promising efficiency as surface agent.

ACKNOWLEDGMENT

This project was supported by the Ministère des affaires étrangères Française (French Foreign Policy). Bah M-L Abdellahi was recipient of a Doctoral fellowship from the Coopération Française.

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Table 1: CA gum main characteristics

Extraction yield (from 10g of nodules) (w%)	38 ± 2
Moisture (w%)	6.6 ± 0.1
Ashes (w%)	3.73 ± 0.01
Proteins (w%)	10.2 ± 0.6
Carbohydrates (w%)	79.5 ± 0.6
pH (solution 5 w%)	4.9 ± 0.1
Optical rotation (°·dm ⁻¹ ·g ⁻¹ ·cm ³)	-36.6 ± 3.4

Table 2: Monosaccharide composition of the CA gum polysaccharides from HPLC-ESI-MS measurement

Monosaccharide	Proportion % (n=5)
Arabinose	46.90 ± 0.45
Galactose	28.10 ± 0.45
Galacturonic acid	6.87 ± 0.47
Mannose	4.96 ± 0.28
Xylose	4.61 ± 0.21
Glucosamine	4.33 ± 0.19
Fucose	4.24 ± 0.05

Table 3: Amino acids composition of the CA gum proteins from GC-MS measurement

Amino acids	Proportions % (n=3)
HydroxyProline	22.7 ± 4.0
Proline	18.1 ± 1.4
Alanine	14.9 ± 2.0
Phenylalanine	8.7 ± 0.8
Leucine	7.1 ± 0.8
Serine	6.2 ± 1.1
Glutamic Acid	6.1 ± 1.5
Aspartic Acid	5.3 ± 1.8
Threonine	4.9 ± 0.9
Glycine	4.3 ± 0.7
Isoleucine	1.2 ± 0.6
Valine	0.9 ± 0.2

Table 4: CA gum characteristics from SEC measurement

Elution time (min)	19.1 - 28.5
Mw (g/mol)	1 303 000
Mn (g/mol)	258 600
Polydispersity	5
Rh (nm)	14.1
η (ml/g)	22.8
Rg (nm) (form 19.1 to 24.3 min)	57.7

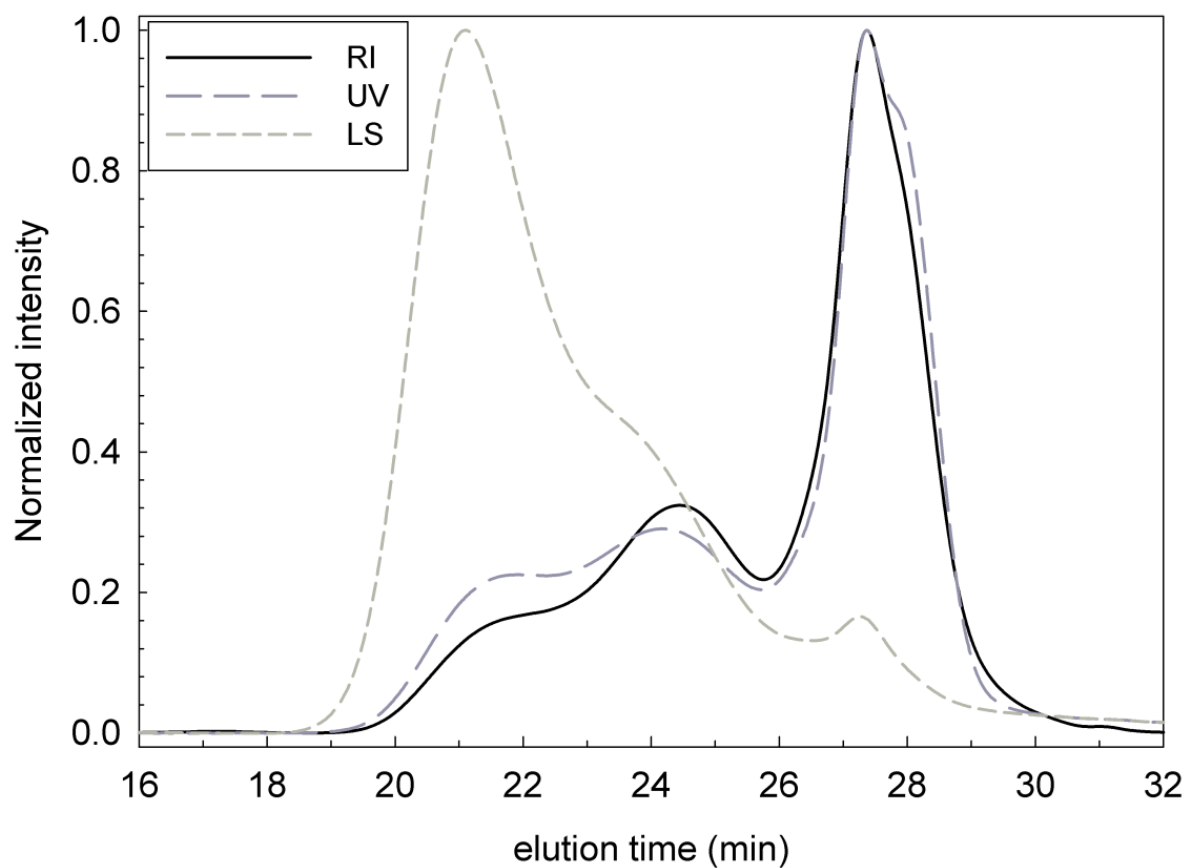


Figure 1. Chromatogram from SEC measurement. Detectors signals from differential refractometer (RI), UV-VIS detector activated at 280nm (UV) and multi-angle laser light scattering (LS).

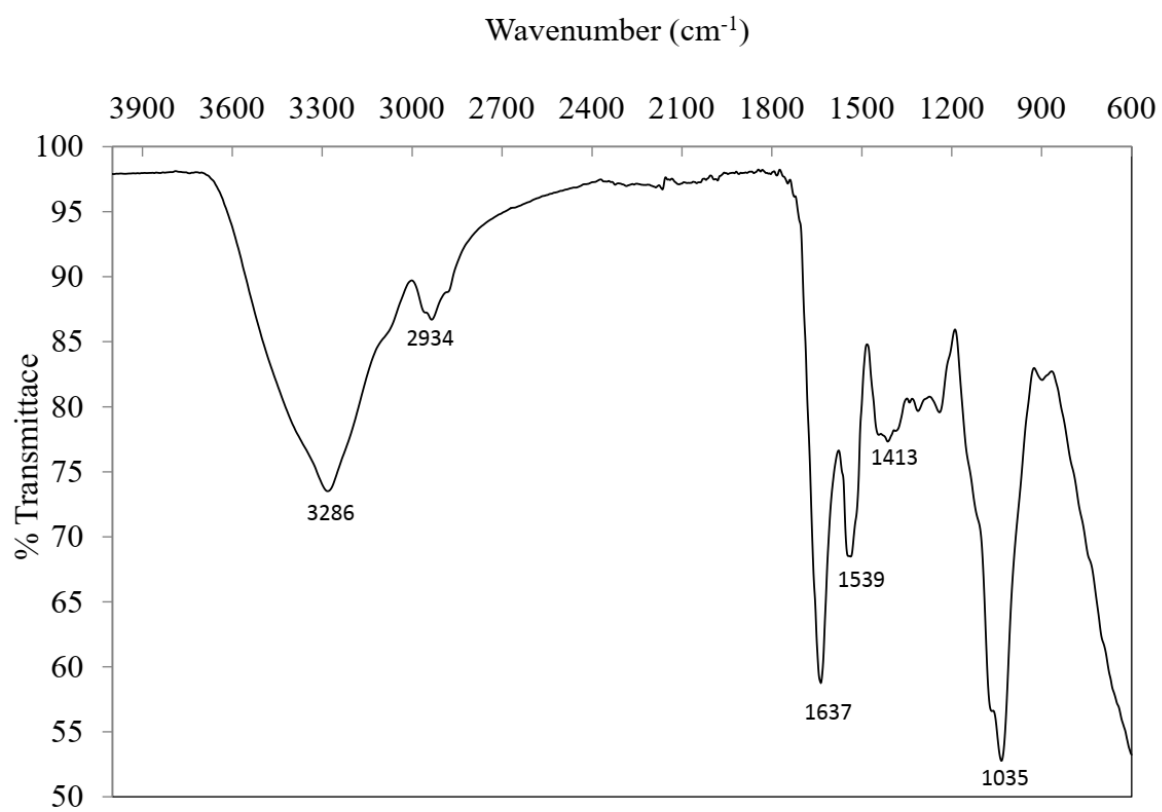


Figure2. ATR FTIR spectrum of CA gum.

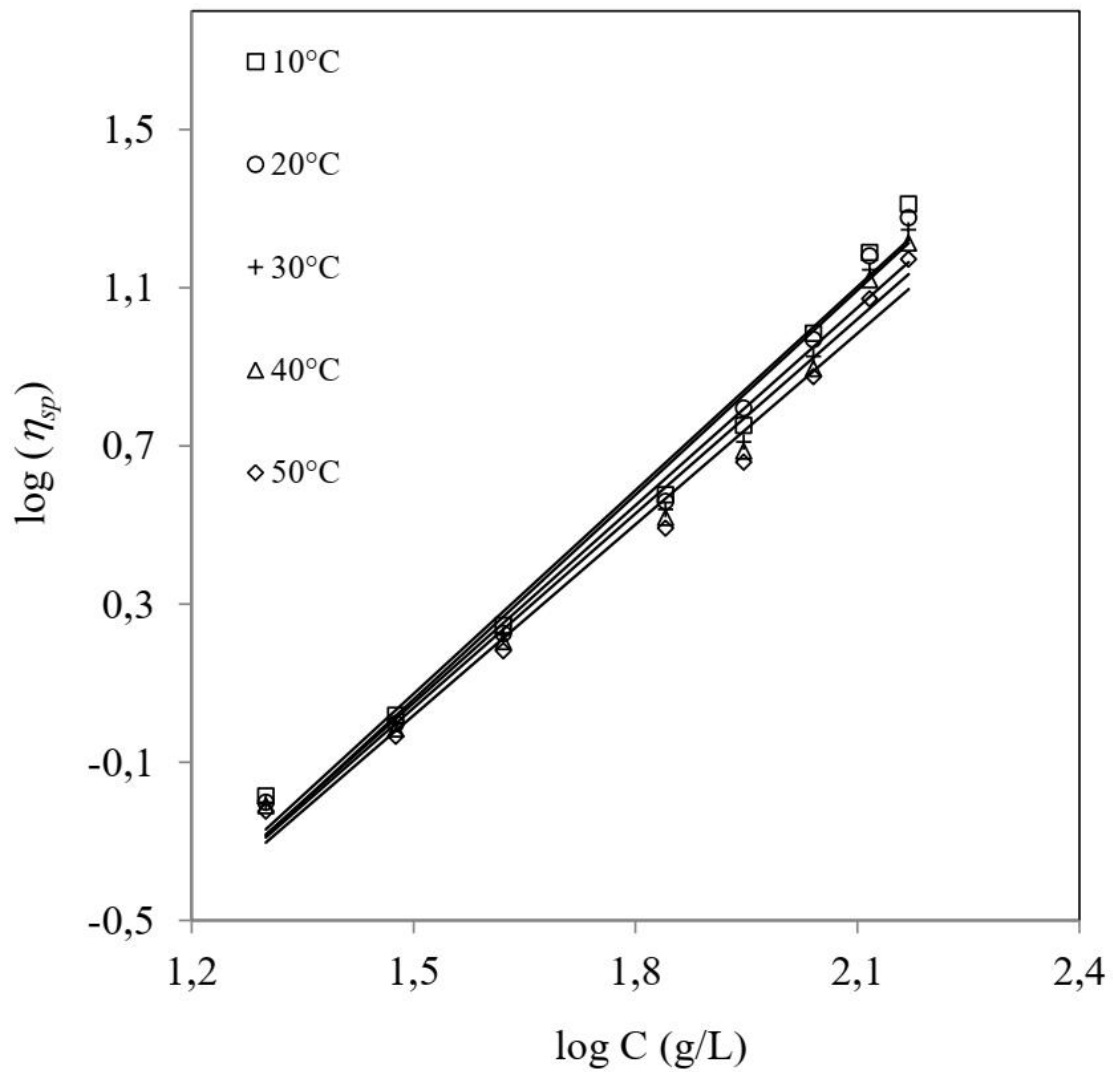


Figure 3. Specific viscosity as a function of CA gum concentration (g/L) at different temperatures from 10°C to 50°C.

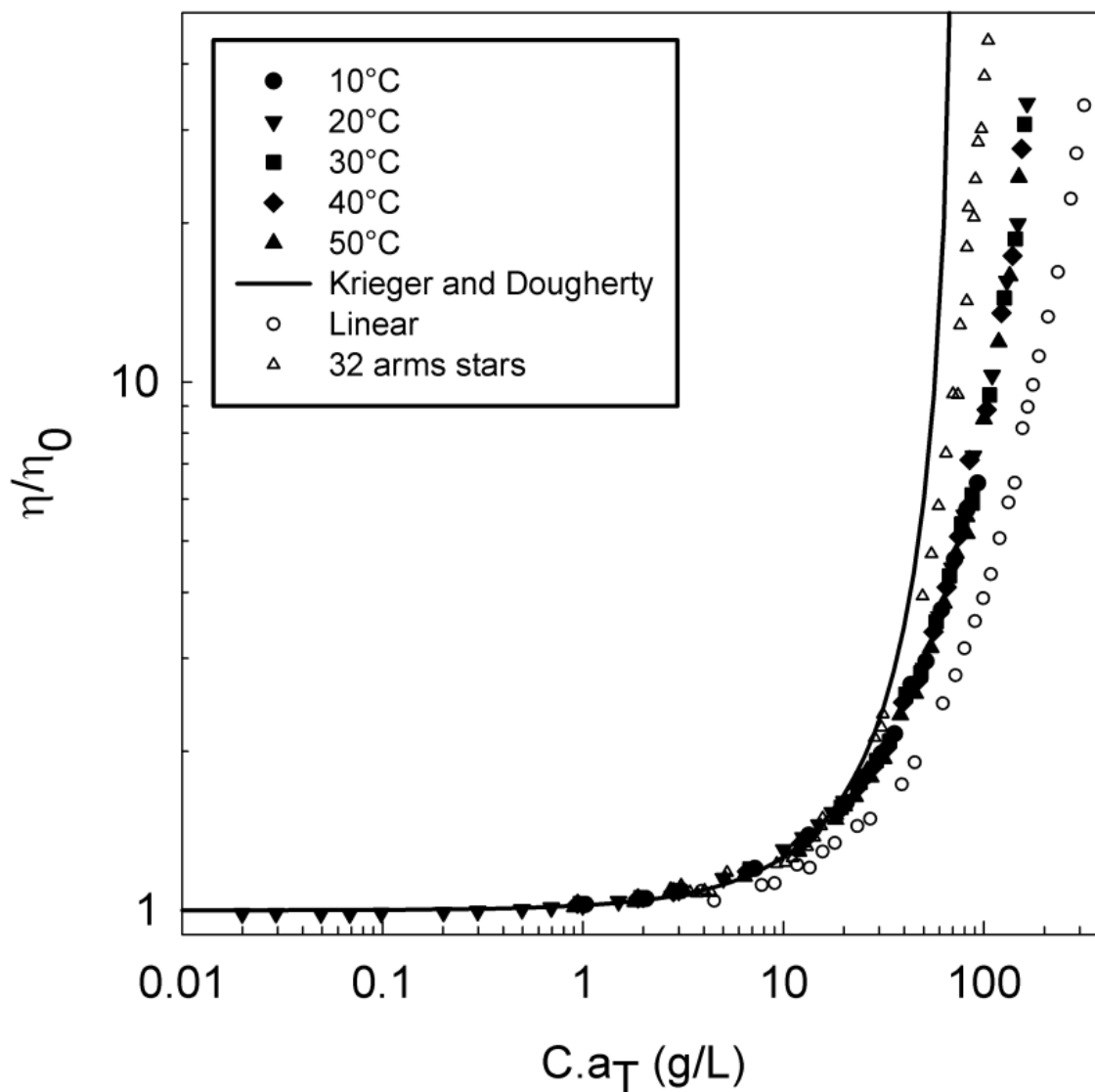


Figure 4. Relative viscosity as a function of concentration of the CA gum solutions (filled symbols). Comparison with Hard Sphere model (line) linear polymer (open circles) and 32 arms starlike polymer (open triangles).

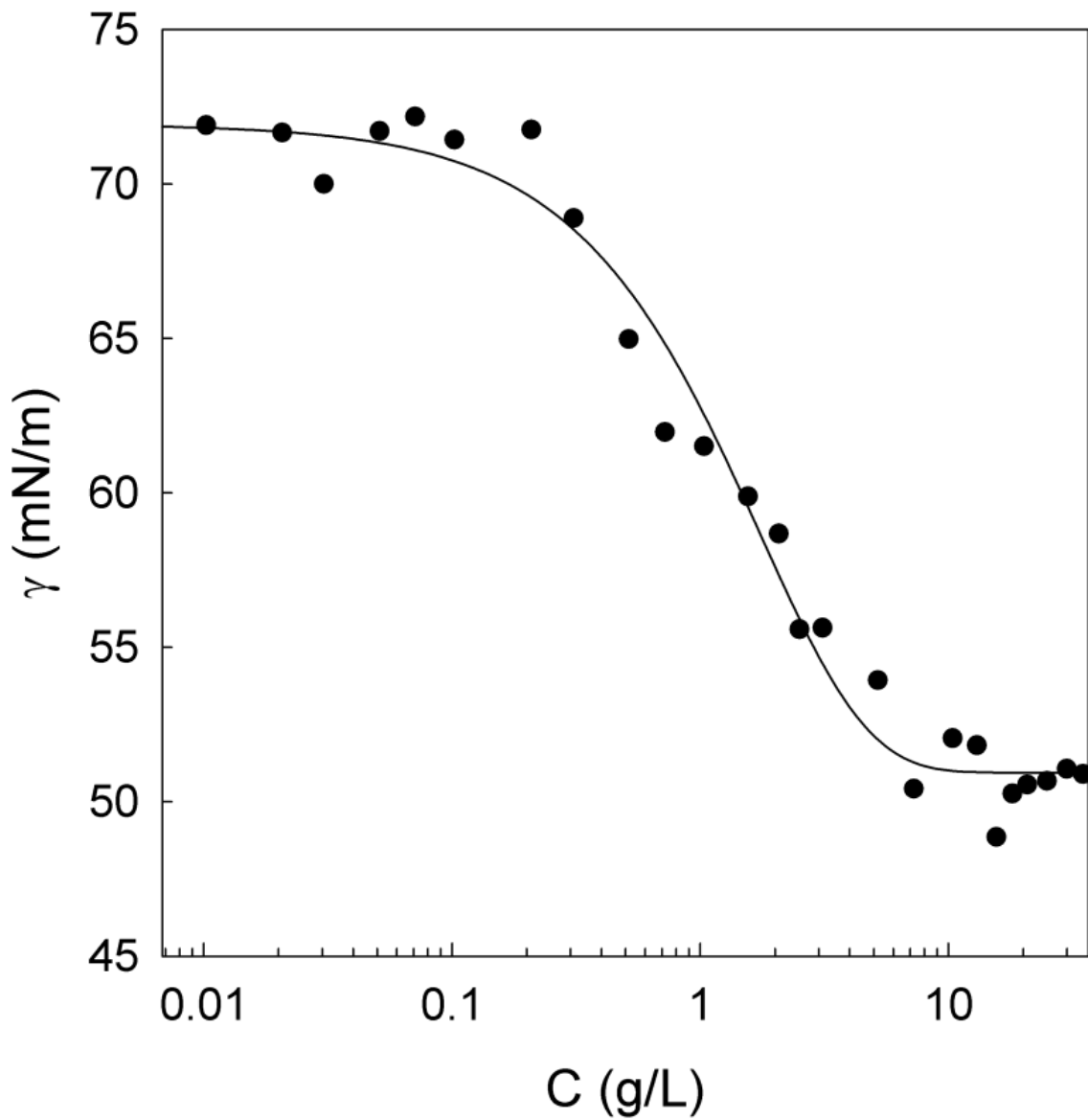


Figure 5. Water surface tension as a function of *CA* gum concentration. Line represents fit using a single exponential decay.

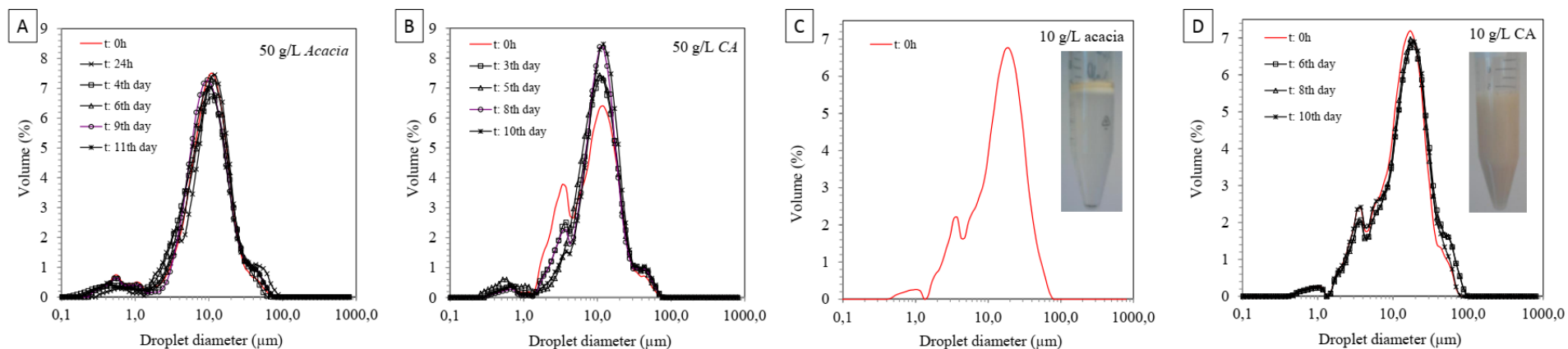


Figure 6: Size and size distribution of droplets in o/w emulsions (orange essential oil/water) prepared from 50 g/L of *Acacia Senegal* (A) or CA gum (B) and 10 g/L of *Acacia Senegal* (C) and CA gum (D). Captions: pictures of emulsion samples 1 hour after emulsification.