Acacia gum: History of the future
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Highlights

- Acacia Senegal gums are made of three molecular fractions and minor components
- Molecular fractions structures are extensively reviewed
- Hydration, rheological and interfacial properties of Acacia gum are discussed
- Interfacial properties of Acacia gums are related to high $M_w$ components content
- Future research areas are identified including major challenges and bottlenecks
Acacia gum: History of the Future

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Abstract

On behalf of the 90th birthday of Professor Glyn O. Phillips, it is a great honor for authors of this publication to make a review on Acacia gum, one of the favorite polysaccharides extensively studied by Glyn and his collaborators all around the world during these last five decades. After remembering a synthetic historical perspective, the present critical review summarizes the main updated data of this complex polysaccharide from the chemical composition to the functional properties with a particular attention toward structure and bulk and interfacial properties. Biological properties of Acacia gums were not considered. Some of the main challenges in a near future for a better understanding of the functional properties of this polysaccharide concerns the detailed study of the gum maturation mechanism upon exudation, the structure and conformation of different molecular fractions, the role of minor components (minerals, polyphenols, lipids) on the structure and functionality of gums, the physicochemical properties of purified molecular fractions and the ways to modified them upon enzymatic modifications. In our opinion, the main challenges for a better understanding of the interfacial function of this polysaccharide (adhesion and stabilization at liquid and solid interfaces) will be to probe the interfacial induced conformational changes. This area of research seems to have been quite neglected during these last past years and fundamental questions arising from the adhesive and stabilizing properties of Acacia gum are still without answer today.

In addition, the amino-acid sequence contained in this complex polysaccharide are totally unknown today and future developments based on enzyme/chemical modifications and liquid chromatography coupled to on line mass spectrometry could unravel the sequence and decipher between the existence of one or more amino-acid sequences in Acacia senegal gum.

We sincerely hope Glyn, one of the “father of Arabic gum”, will find some positive echo in this review.

Keywords: Acacia gum; hydration; rheology; aggregation; coacervation; interfaces and emulsions
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6. Literature
1. General Overview: What is Acacia gum?

A significant number of studies have been done on the composition, polydispersity, structure, physico-chemical and functional properties of Acacia *senegal* gum. Unfortunately, Acacia *seyal* gum has attracted much less attention over years. Then most of the information reported in the following concerns Acacia *senegal* gum. When available, comparison between both gums is provided.

1.1. Definition and production

Acacia gum (AG, E414), also called gum arabic, is an edible dried gummy exudate obtained from the trunk and branches of *Acacia senegal* and *Acacia seyal* trees, which is rich in soluble fibers of low viscosity (Williams & Phillips, 2000). The gum production is a protection mechanism of tree against insects and molds invasion and of healing of wounds. Gum is found in arid regions (areas) of the sub-saharian belt, from Senegal to East Africa, and beyond to Pakistan and India (Cecil, 2005). According to the JEFCA ("Joint Expert Committee for Food Additives") of FAO/WHO, it is defined like "a dried exudation obtained from the branches of *A. Senegal* (L) Willdenow or close species from Acacia (leguminosae family)" (FAO, 1999). It includes therefore both *Acacia Senegal* and *Acacia Seyal* species.

Although harvested in Arabia, Egypt and Asia since Antiquity, sub-saharian AG has a long export history. Gum is harvested from *Acacia Senegal* or *Acacia Seyal* found in Sahel region all along a belt covering arid and semi-arid areas of Mauritania, Senegal, Mali, Burkina Faso, Niger, Nigeria, Chad, Cameroon, Sudan, Eritrea, Somalia, Ethiopia, Kenya and Tanzania. Total world exports of AG were of about 60 000 tons in 2009 ("Commodity Trade Statistics Database (COMTRADE/DBS)," 2011) but can reach 100 000 tons. Sudan is the biggest producer followed by Chad and Nigeria. In 2007 they produced together 90 to 95% of world exports. Gum is mainly exported to Europe from which it is re-exported worldwide.

1.2. Historical aspects

AG is the oldest and, apparently, best known of all natural gums (Verbeken, Dierckx, & Dewettinck, 2003). AG was already used in the Stone Age as food in Sahara (Chevalier, 1924) and as ingredient in adhesive and bone technologies in South (at least 70 000 years ago).
(d'Errico & Henshilwood, 2007; Lombard, 2008; Wadley, Hodgskiss, & Grant, 2009) and North East Africa (Olszewski, et al., 2010; Rots, Van Peer, & Vermeersch, 2011). It is very likely than in these arid lands where Acacia grows, human used AG for food and non-food applications since the more ancient times. Well before 4000 B.C. Chinese and Japanese used AG for painting. Its use can be also traced back to the third or fifth millennium B.C., the time of ancient Egyptians. Early Egyptian fleets shipped AG as a trade good. It was collected in Nubia and exported north to Egypt for use in the preparation of inks, watercolors and dyes. It was used as a pigment binder and adhesive in paints for making hieroglyphs, and ancient inscription refers to it as kami. Furthermore, it was used as a binder in cosmetics and inks and as an adhering agent to make flaxen wrappings for embalming mummies. Herodotus, writing in the fifth century B.C., mentions its use in embalming in Egypt. Cardboard (?), a specific type of Egyptian material for cases enclosing or elements placed on, mummified bodies, were painted with gum-containing pigments (Scott, et al., 2004; Scott, Warmlander, Mazurek, & Quirke, 2009). Ancient Greeks also mentioned the use of gum (Diderot & d'Alembert, 1777). Since the first century of the Christian era, the soluble gum provided by Sudan has been an article of commerce shipped to Arabian ports and hence to Europe (Caius & Radha, 1942; Parry, 1918). It was called gum arabic after its place of origin (Pomet, 1735). From Sudanese sources, AG was an article of commerce as early as the 12th century B.C (Cecil, 2005).

The therapeutic use of AG was already mentioned in Pliny, Discoridis and Theophrastus writings (Amy, 1934; Merat & de Lens, 1831). However, the Ebers manuscript (a medicinal papyrus written in 1550 B.C.) already suggested to use AG as a contraceptive in association with dates. The famous queen Cleopatra requested the preparation of curative recipes based on AG (Gramatica & Zanardelli, 2003). In the ninth century of our era, the Arab physician Abu Zayd Hunayn ibn Ishaq al-Ibadi, writing in his Ten Treatises on the Eye, described AG as an ingredient in poultices or eye compresses. It was also used to relieve topical irritation and to protect in cases of superficial excoriation, ulcers, burns, sore nipples, etc. (Caius, et al., 1942). The powdered gum is used in checking hemorrhage from leech bites. When blown up the nostrils, it stops severe nosebleed. The gum is also employed in catarrhal affections and irritation of the faces, by being held in the mouth and allowed to dissolve slowly. In Transvaal, a plaster made from capsicum fruit, Cape gum and strong
vinegar, is applied in acute inflammation of the bone marrow, and a mucilage of Cape gum to the mouth in thrush and sprue (Caius, et al., 1942).

By the Middle Age, AG was valued in Europe among scribes and illustrators. Following the gilding of letters in illuminated manuscripts, the application of color was the final stage. For this, illustrators mixed pigment in a binding medium. Between the 12th and the 19th centuries, AG was used in the composition of the metallo-gallic ink, the most used ink in Europe, for instance by painters like Rembrandt. The ink manufacturing was known at least two centuries before our era (Flieder & Duchein, 1983). During the same period, AG trade was controlled by the Turkish Empire, giving rise to the name turkey gum. An export trade was also developed for a time around Bombay, hence the names "East Indian" or "Indian Gum".

In 1445, Prince Henry the Navigator set up a trading post on Arguin island (off the coast of modern Mauritania), which acquired AG and slaves for Portugal. Between the 14th and the 19th centuries, AG was an important trade associated to slave economy (Cultru, 1910). From 1580, Portugal became the dominant influence along the coast. In 1638, however, they were replaced by the Dutch of the Occidental India Dutch Company, who were the first to begin exploiting the AG trade. Produced by the Acacia trees of Trarza and Brakna and used in textile pattern printing, this gum was considered superior to those previously obtained in Arabia. By 1678 the French had driven out the Dutch and established a permanent settlement at Saint Louis at the mouth of the Senegal River, where the French Company of the Senegal River (Compagnie Française du Sénégal) had been trading for more than fifty years (Raffenel, 1846). In 1685, Frederick William of Brandeburg replaced the French and created a colonial domain. Successors left the trading post to the Dutch in 1717. Since the trading of gum was important for the European industry, France occupied Arguin after the 1721, 1723 and 1724 campaigns. Arguin was definitely left for good in 1728 because maintain a garrison was too expansive and the trade of the gum moved to the south.

During most of the 19th century, AG was the major export product from the French and British trading colonies in modern Senegal and Mauritania. France in particular first started a conflict with inland African states over the supply of gum, providing an early spur for the conquest of French West Africa. As the Atlantic slave trade weakened in the early 19th
century, the Emirate of Trarza and its neighbors in what is today southern Mauritania collected taxes on trade, especially AG that the French were purchasing in ever-increasing quantities for its use in industrial fabric production. West Africa had become the sole supplier of world AG by the 18th century, and its export from the French colony of Saint-Louis doubled in the decade of 1830 alone. Taxes, and a threat to bypass Saint-Louis by sending gum to the British traders at Portendick, eventually resulted in a direct conflict between the Emirate of Trarza and the French. In the 1820s, the French launched the Franco-Trarzan War of 1825 in order to avoid Arabs controlling the gum trade. The war incited the French to expand to the north of the Senegal River for the first time, heralding French direct involvement in the interior of West Africa. AG continued to be exported in large quantities from the Sahel areas of French West Africa (modern Senegal, Mauritania, Mali, Burkina Faso, and Niger) and French Equatorial Africa (modern Chad) until these nations gained their independence in 1959-61. In the beginning of the 20th century, Europe consumed about 20,000 tons per year of AG (Chevalier, 1924).

1.3. Uses of Acacia gum

*Acacia senegal*/*seyal* trees are important for the ecology of arid and semi-arid areas where they naturally grow. They prevent soil degradation, fix atmospheric nitrogen and maintain soil moisture. Trees are resistant in period of drought. They act as wind barrier and are important for dune fixation. In addition, trees participate to soil fertilization and decomposition of dead leaves reinforces anti-erosive roots of trees (Wickens, Seif El Din, Sita, & Nahal, 1995).

The tree has wide usage: the foliage and seed pods make excellent fodder for livestock, ropes can be made from the bark fibers of the roots, and the thorny branches are often used to make hedges to enclose cattle or protect agricultural farms. The tree can also be used for small-scale carpentry or for making agricultural tools. When it passes its gum-productive age, between 15 and 25 years old, its wood is used for both fuel and charcoal production (Touré, 2008; Wickens, et al., 1995).

AG is unique among the natural gums because of its properties, including high solubility and it is widely used as a stabilizer, emulsifier, flavoring agent, thickener, or surface-finishing agent. It also activates turbidity or retards sugar crystallization. These properties make it a
very interesting additive in the food industry, including for the production of beverages (including Coca-Cola®), confectionery, emulsions, flavor encapsulations, bakery products and brewing (Touré, 2008; Verbeken, et al., 2003; Wickens, et al., 1995). In wine production, AG prevents color pigment and protein precipitations, confers body and stabilizes the color. As mentioned above, AG has been also used for ages in non-food industries including pharmaceutical, printing, textile, and cosmetic industries (Verbeken, et al., 2003).

The modern industrial era has produced an explosion of manufacturing uses for AG. According to Cecil (2005), gum was important in the 19th century in early photography as an ingredient in gum bi-chromate prints. It is now used in lithography, where its ability to emulsify highly uniform thin liquid films makes it desirable as an antioxidant coating for photosensitive plates. The same quality also makes gum useful in sprayed glazes and high-tech ceramics and as a flocculating agent. It is used as a binder for color pigments in crayons, a coating for papers and a key ingredient in the micro-encapsulating process for the production of carbonless copy paper, laundry detergents etc. It is used in textile sizing and finishing and for metal corrosion inhibition. Moisture-sensitive postage-stamp adhesives and matchsticks are also made with gum. Touré (2008) adds that AG is used in the cosmetic industry as an adhesive when making face powders and masks and to render also creams and lotions smoother. New uses begin to emerge such as for instance the stabilization of carbon nanotubes (Bandyopadhyaya, Nativ-Roth, Regev, & Yerushalmi-Rozen, 2002).

2. Chemical composition and structure of Acacia senegal gum

2.1. Chemical composition

AG is a complex polysaccharide, either neutral or slightly acidic, found as a mixed calcium salt of a polysaccharide acid (Arabic acid). The backbone is composed of 1,3-linked β-D-galactopyranosyl units. The side chains are composed of two or five 1,3-linked β-D-galactopyranosyl units, joined to the main chain by 1,6-linkages. Both the main and the side chains contain units of α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuropyranosyl, the last two mostly as end units (D. M. W. Anderson & Stoddart, 1996; Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Verbeken, et al., 2003). Idris et al. (1998) reported AG to be comprised of 39-42% galactose, 24-27% arabinose, 12-16% rhamnose, 15-16% glucuronic acid, 1.5-2.6% protein, 0.22-0.39% nitrogen, and 12.5-
16.0% moisture. The chemical composition and physical chemical properties of AG can vary with its origin (Acacia *senegal* or Acacia *seyal*), the age of the trees from which it was obtained, climatic conditions and soil environment, and the process submitted after its harvest (D. M. Anderson, Dea, Karamall.Ka, & Smith, 1968; D. M. W. Anderson, Douglas, Morrison, & Wang, 1990; Idris, Williams, & Phillips, 1998; K. A. Karamalla, Siddig, & Osman, 1998; Verbeken, et al., 2003) (Al-Assaf, Phillips, & Williams, 2005; Islam, et al., 1997).

AG is a highly heterogeneous material that can be separated into three main fractions by hydrophobic interaction chromatography (HIC) (Randall, Phillips, & Williams, 1989). As an example, we showed on one sample that most of the gum (88.3% of total), an arabinogalactan-peptide (AGp, Fraction 1 or F1), had a very low protein content (1.1%) and a molecular weight of 2.9×10^5 g.mol^-1 (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). The second fraction (10.4% of total), an arabinogalactan-protein complex (AGP, Fraction 2 or F2), contained 9% protein and had a molecular weight of 1.9×10^6 g.mol^-1. The third minor fraction (1.3% of total gum), referred as glycoproteins (GP, fraction 3 or F3), will consist of at least three glycoprotein populations with molecular weight ranging from 2.5×10^5 to 2.6×10^6 g.mol^-1. One of the GP had a molecular weight of 2.95 x 10^5 g.mol^-1 and the highest protein content 24.6%, (Renard, et al., 2006). These different values may change depending on gum origin, age, storage conditions, etc... (Al-Assaf, Andres-Brull, Cirre, & Phillips, 2012).

Ray et al. (1995) fractionated AG by both HIC and gel permeation chromatography (GPC); their results were in broad agreement with those of Randall et al. (1989) and Renard et al. (2006). The main amino acids present in the proteinaceous component of AG and AGP were hydroxyproline, serine and proline, whereas in GP, aspartic acid was the most abundant (Islam, et al., 1997). Osman et al. (1993) fractionated AG by HIC to yield four fractions, all of which had a similar carbohydrate composition, but differed in their content of protein, amino acid composition and molecular weight distribution. All four fractions reacted with an array of anti-arabinogalactan-protein monoclonal antibodies via anti-carbohydrate epitopes and were precipitated by Yariv’s reagent, which indicated that all four fractions belonged to AGP’s family.

AGP-type macromolecules represent about 94-96% of total compounds found in Acacia *senegal* or *seyal* gums. Minor components are mainly minerals (~3-5%) including Na, K, Ca,
Mg, and trace metals such as Zn, Fe, Pb and Cu ions (D. M. W. Anderson, Bridgeman, Earquhar, & Mcnab, 1983; Debon & Tester, 2001; Kunkel, Seo, & Minten, 1997; Mhinzi, 2003). Small concentrations of tannins, around 0.4% (Mhinzi, 2003), can be found giving more or less colored gums, which is especially remarkable with Acacia seyal. Variability in tannins content was reported both for A. senegal (0.3-0.6%) or A. seyal (0.6-1.2%) gums but only on five samples (Minzhi, 2003). Other emphasized that tannins can be found in AGs but not on Acacia senegal var. senegal gums (K.A. Karamalla, 2000) which is clearly in contradiction with data from Minzhi (2002, 2003). Renard et al. (2006) identified traces of trans ferulic acid, ferulic acid and 8-5’ non cyclic diferulic acid in AG and AGP and GP fractions. AGs also contain traces of lipids (M. P. Yadav, Igartuburu, Yan, & Nothnagel, 2007; M.P. Yadav, Moreau, Johnston, & Hicks, 2012) and enzymes such as oxidases and peroxidases, diastases and pectinases (Billaud, Lecornu, & Nicolas, 1996; Fowler & Malandkar, 1925; Glicksman & Sand, 1973; Leo, Taylor, & Lindsey, 1945; Reinitzer, 1909).

2.2. Structure of Acacia senegal gum

AG is a highly branched polyanionic polysaccharide (Fincher, Stone, & Clarke, 1983; Keentok, 1984; Swenson, Kaustinen, Kaustinen, & Thompson, 1968; Yomota, Okada, Mochida, & Nakagaki, 1984) with a low charge density (Vandevelde & Fenyo, 1987). Recently, titration of AG and its fractions gave 223, 1259 and 1605 charges for AGp, AGP and GP, respectively (Renard, et al., 2006). A maximum persistence length of about 3 nm was estimated for the AGp main fraction of AG (Sanchez, et al., 2008).

Regarding the structure in solution, globular and close-packed shape of Acacia senegal gum molecules was suggested previously based on the low viscosity of gum solutions (D. M. W. Anderson & Dea, 1971; Swenson, et al., 1968). The globular or not-extended shape of AG molecules was also deduced from the relationships between the intrinsic viscosity [η] or the radius of gyration Rg or the ratio value between Rg and Rh and the molecular weight Mw, (Idris, et al., 1998). For instance, the Mark-Houwink-Sakurada exponent α, i.e. the slope of the log-log plot of [η] vs Mw, produced a slope of 0.54 (D. M. W. Anderson & Rahman, 1967) or 0.47 (Idris, et al., 1998). These values are mainly due to the AGp fraction as we found for this fraction a slope of 0.49 (Sanchez, et al., 2008). Based on a large number of structural data from literature, it appears that the [η] vs Mw relationship is not linear over the entire
M_w range, indicating that the conformation is molecular weight dependent (Al-Assaf, Phillips, Aoki, & Sasaki, 2007; Al-Assaf, et al., 2005; Al-Assaf, Sakata, McKenna, Aoki, & Phillips, 2009; D. M. W. Anderson, et al., 1983; D. M. W. Anderson, Douglas, et al., 1990; D. M. W. Anderson & Stoddart, 1966; D. M. W. Anderson & Weiping, 1990; Chikamai & Banks, 1993; Chikamai, Osman, Menzies, & Banks, 1995; Deeble, et al., 1990; Duvallet, Fenyo, & Vandevelde, 1993; Y. Fang, et al., 2007; Y. P. Fang, Al-Assaf, Phillips, Nishinari, & Williams, 2010; Idris, et al., 1998; Jurasek, Kosik, & Phillips, 1993; K. A. Karamalla, et al., 1998; Kateyama, et al., 2006; Kuan, Bhat, Senan, Williams, & Karim, 2009; Li, et al., 2009; Li, et al., 2011; Mahendran, Williams, Phillips, Al-Assaf, & Baldwin, 2008; Mukherjee & Deb, 1962; Osman, Menzies, Williams, Phillips, & Baldwin, 1993; Picton, Bataille, & Muller, 2000; Randall, et al., 1989; Renard, et al., 2006; Swenson, et al., 1968; Veis & Eggenberger, 1954; Q. Wang, Burchard, Cui, Huang, & Phillips, 2008). AG macromolecules with M_w below 1×10^6 g.mol\(^{-1}\) are thus more spheroidal than macromolecules with M_w above 1×10^6 g.mol\(^{-1}\) that appear more extended. These differences could be due only to differences in structures and/or alternatively to differences in the affinity for the aqueous solvent. Little information is available regarding the structure of Acacia seyal molecules. However, the few studies discussing about Acacia seyal gum structure evidenced that these molecules are more compact than those of Acacia senegal gum (Al-Assaf, et al., 2005; Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015). For both A. gums, the anisotropy increases with the increase of molecular weight, with however more anisotropic conformations for A. senegal molecules. The conformation varies from spheres to oblate ellipsoids for A. seyal molecules, while it varies from oblate ellipsoids to more anisotropic conformations, such as oblate and prolate ellipsoids, for A. senegal molecules (Lopez-Torrez, et al., 2015). The ellipsoidal conformation of A. senegal molecules is also confirmed using hydrodynamic technics as sedimentation velocity analytical ultracentrifugation and size exclusion chromatography coupled to multi-angle light scattering (SEC MALS) and differential viscometry (Gillis, Adams, Alzahrani, & Harding, 2016).

Few studies focused on the structural properties of Acacia gums according to the origin of gums (i.e. the country). This missing information could raise some questions about the influence of the geographical area of harvest and the post-harvested treatment, especially from raw to spray-dried gums, on the conformations of molecules. Our group analysed the
R_h conformation plots (log-log plot of R_h vs. M_w) after SEC MALS experiments of raw and spray-dried Acacia senegal and seyal gums harvested in several countries as Sudan, Chad, Senegal, Eritrea, Mali, Mauritania and Burkina Faso (Figure 1, unpublished data). These analyses concerned 202 spray-dried and 100 raw A. senegal gums, 28 spray-dried and 6 raw A. seyal gums. The hydrodynamic coefficient, ν_h, is constant for each specie with values ranging from 0.53 to 0.55 for spray-dried and raw A. senegal gum and 0.43 to 0.44 for spary-dried and raw A. seyal gum. Hence, the conformations of Acacia gum molecules depend on the Acacia gum specie, but not on the geographical area of harvest or the post harvested treatment (from raw to spray-dried gums). These ν_h values confirm again the more compact structure of Acacia seyal molecules.

To summarize AG is composed of a continuum of molecular species differing by their protein to sugar ratio, molecular weight and charges (Renard, et al., 2006), but also by different mesoscopic structures in solutions. The precise conformations of different molecular fractions remain uncertain from studies on total AG. The following section reports on the actual knowledge on the structures of the three molecular fractions of A. senegal gum isolated from HIC, i.e. the arabinogalactan-peptide (AGp), the arabinogalactan-protein (AGP) and glycoproteins (GP) fractions.

2.2.1. Structure of the arabinogalactan-peptide fraction (AGp, fraction 1 or F1)

The first structural model for AGp was recently proposed (Sanchez, et al., 2008). From small angle neutron scattering (SANS) experiments in charge screening conditions and dynamic light scattering, AGp appeared to be a dispersion of two-dimensional structures with a R_g of 6.5 nm, a R_h of 9.1 nm and an inner dense branched structure (Renard, et al., 2006; Sanchez, et al., 2008). Data analysis and modeling of SANS experiments revealed a disk-like morphology with a diameter of 20 nm, a thickness of less than 2 nm and a central intricated “network”. The structure of AGp could explain the low viscosity of AG solutions, and its ability to self-assemble and to interact with proteins. At the molecular level, no specific secondary structures could be detected using circular dichroism, which could be explained by the low amino-acid composition of the AGp fraction (Renard, et al., 2006). However, Fourier transform infrared spectroscopy suggested the presence of extended β-sheet and β-
turn structures but not $\alpha$-helix (Renard, et al., 2006). The absence of $\alpha$-helix could explain partly the absence of secondary structures as revealed by circular dichroism.

### 2.2.2. Structure of the arabinogalactan-protein fraction (AGP, fraction 2 or F2)

The arabinogalactan-protein fraction from AG represents about 10-15% of total molecules and about 9% of the total protein concentration. Its $M_w$ is variable but generally comprised between 1 and $4 \times 10^6$ g.mol$^{-1}$ (Al-Assaf, et al., 2007; Al-Assaf, et al., 2005; Al-Assaf, et al., 2009; Castellani, Guibert, et al., 2010; Elmanan, Al-Assaf, Phillips, & Williams, 2008; Idris, et al., 1998; Picton, et al., 2000; Randall, et al., 1989; Ray, Bird, Iacobucci, & Clark, 1995; Renard, et al., 2006; Vandevelde, et al., 1987).

At the molecular level, AGP contains various secondary structures, including about 27% of polyproline II structures, $\beta$-sheets, $\beta$-turns and unordered structures, but not $\alpha$-helices (Renard, et al., 2006). A wattle-blossom model was proposed to describe the structure of the AGP complex. It was postulated that the high molecular weight fraction of the gum is composed of large carbohydrate blocks with a molecular weight of approximately $2 \times 10^5$ g.mol$^{-1}$, these blocks being covalently linked to a polypeptide backbone (Connolly, Fenyo, & Vandevelde, 1987, 1988; Fincher, et al., 1983). An alternative model was suggested by Qi et al. (1991) in the form of a hairy twisted rope. This model would be comprised of a core rod-like protein (150 nm long) with a highly repetitive amino-acid sequence and the carbohydrate blocks (30 sugar residues) attached to hydroxyproline residues. However, as indicated previously, most studies strongly suggested that the molecules of the AGP complex have a spheroidal structure, which better supports the wattle-blossom model (Connolly, et al., 1988; Idris, et al., 1998; Picton, et al., 2000; Vandevelde, et al., 1987).

From a study on total Acacia gum, a more detailed picture of the wattle-blossom structure of AGP was proposed recently (Mahendran, et al., 2008). Mild alkaline hydrolysis of the gum followed by GPC analysis indicated that AG consists of carbohydrate blocks of $\sim 4.5 \times 10^4$ g.mol$^{-1}$, blocks much lower in mass than those previously reported, covalently linked to serine and hydroxyproline residues. Two folded polypeptide chains would be present in AG, one with a $M_w$ around $3 \times 10^4$ g.mol$^{-1}$ corresponding to about 250 amino acids and the second one with a $M_w$ of about $5 \times 10^3$ g.mol$^{-1}$, corresponding to about 45 amino acids. A number of around 400 amino acids was calculated previously for the AGP fraction (Qi, Fong, & Lamport,
1991). These values are much lower than the 2250 amino acid residues determined by Renard et al. (2006) for the AGP fraction. Such a large discrepancy is difficult to explain solely based on different experimental approaches. Rather we think that it is probably symptomatic of different assembly states of the AGP fraction, due to different origins and maturation history. The 45 amino acids peptide is probably associated with sugars in the AGP fraction, as already demonstrated by Renard et al. (2006). It was then assumed that carbohydrate blocks may have a thin oblate ellipsoid structure (Mahendran, et al., 2008; Sanchez, et al., 2008). The model is interesting since it gives a clearer view of the possible spatial configuration of AGP. The steric arrangement of carbohydrate blocks in such a configuration is questionable and merits much more investigation, as well as the fine structure of macromolecules. One can imagine that AGP is a two-dimensional object with a folded protein network and interacting massive sugar blocks or an assembly of sugar blocks linked (covalently or not) between them by several polypeptide backbones.

Regarding the possible morphology of AGP in solution, HPSEC-MALLS provided some informative insights. AGP in solution has a weight average molecular weight of $1.86 \times 10^6$ g.mol$^{-1}$ and a radius of gyration of 30 nm (Renard, et al., 2006). In addition, two exponent values are identified in the $R_g$, $[\eta]$, $R_h$ vs $M_w$ relationships highlighting two types of conformations depending on the molecular weight range considered (Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012). AGP would behave in solution as a branched or hyper-branched polymer with conformations ranging from globular to elongated shape depending on the size of the carbohydrate branches. SANS form factor revealed an elongated average conformation corresponding to a triaxial ellipsoid while inverse Fourier transform of the scattering form factor gave a maximum dimension for AGP of 64 nm (Renard, et al., 2012). TEM highlighted the existence of isolated spheroidal particles (diameters ranging from about 10 to 40 nm) or more anisotropic morphologies (lengths from 20 up to about 60 nm) (Renard, Garnier, Lapp, Schmitt, & Sanchez, 2013). Remarkably, all the particles were porous supramolecular assemblies of smaller structural subunits with dimensions of about 2-10 nm. These building structural subunits were mainly branched chains and ring-like structures with diameters of about 1-5 nm.

It was recently suggested that AGP would be in fact a molecular association resulting from an aggregated fraction of Agp units stabilized by low molecular weight proteinaceous
components found in the GP fraction of AG (Al-Assaf, et al., 2009). More accurately, maturation process would promote interactions between AGP, AGp and GP, inducing a molecular reorganization of the gum and the appearance of composite AGP architectures. In addition, spray-drying was found to increase the molecular weight of AGP due to self-aggregation (Al-Assaf, et al., 2009). In summary, the so called AGP is in fact a heterogeneous population varying in anisotropy, chain density and porosity, and of all possible molecular combinations between AGP, AGp and GP. This structural heterogeneity likely depends on chemical composition of gum sample and its maturation process, natural or induced by processing.

Very recently, AGP and AGp molecular fractions were degraded enzymatically using acidic and alkaline proteases in order to probe the conformation and structure of the two main fractions of Acacia senegal gum. While AGp fraction kept intact whatever the enzymes and conditions used, AGP was found to be degraded only in alkaline conditions. The absence of degradation in acidic conditions questioned about the potential modification of the structure of AGP molecular fraction at low pHs. The decrease in molecular weight of AGP after enzymatic treatment confirmed the accessibility of enzymes toward polypeptide cleavages and papain was found to be the most efficient protease with a decrease of $M_w$ from $1.79 \times 10^6$ to $1.68 \times 10^5 \text{ g mol}^{-1}$. The molecular structure of control and enzyme-treated AGPs surprisingly predicted similar secondary structures content. The similar conformations adopted by control and enzyme-cleaved AGPs probed at the molecular and mesoscopic scale (by SANS) would be in favor of a high flexibility of the polypeptide backbone before and after enzymatic treatment in accordance with the repetitive and palindromic nature of peptide sequence and the overall symmetry of the carbohydrate moieties along the protein backbone (Goodrum, Patel, Leykam, & Kieliszewski, 2000; Kieliszewski, 2001; Kieliszewski & Lamport, 1994). It was finally suggested that a self-similarity driven-process would be at the origin of the assembly of AGP from a consensus glycopeptide building block with a symmetrical distribution of arabinosides and polysaccharide substituents (Figure 2) (Renard, Lavenant-Gourgeon, Lapp, Nigen, & Sanchez, 2014).

2.2.3. Structure of the glycoprotein fraction (GP, fraction 3 or F3)
The glycoprotein fraction is a minor component of AG (< 2%) but is rich in proteins (25-50%). Regarding the amino acid composition, GP fraction is less rich in hydroxyproline and serine but richer in asparagine and aspartic acid, but also in tyrosine and phenylalanine residues (Renard, et al., 2006). Using HIC, at least three different fractions were identified in GP with M\text{w} ranging from 3 \times 10^5 to 3 \times 10^6 g.mol^{-1} (Renard, et al., 2006). Following HIC and SEC, the three fractions were purified and displayed each three molecular populations, with a clear continuum of species. It appears obvious that a deeper study of GP is needed to better understand the complexity of this minor fraction. Like AGP, the different glycoproteins are characterized by the presence of polyproline II conformation (9%), \alpha-helix (9%), \beta-sheet (38%), \beta-turns (23%) and unordered structures (18%) (Renard, Lepvrier, et al., 2014). However, no mesoscopic models have been proposed to date for these glycoproteins. As well, physical chemical properties of GP fraction are almost unknown, except its very active surface properties (Castellani, Gaillard, et al., 2010).

Very recently, Renard et al. (2014) studied the structure of one glycoprotein (GP) fraction of AG isolated from HIC and SEC, which revealed a mixture of spheroidal monomers and more anisotropic oligomers in GP solution as suggested by the two exponent values found in the R_g vs. M_w relationship and TEM observations (Renard, Lepvrier, et al., 2014). The GP conformation probed by SAXS was ascribed to a thin object with a triaxial ellipsoid morphology, certainly attributed to GP oligomers. A 9 nm diameter particle was also identified by SAXS in agreement with the dimensions (diameters of 8 to 11 nm) identified by TEM on single isolated ring-like structures. All the identified isolated particles had a spheroidal shape while slight anisotropy appeared when ring-like structures self-associated. Contrary to what was previously observed on AGp and AGP, no outer structure combined to an inner porous network of interspersed chains was observed in the spheroidal particles morphology. These spheroidal particles were structurally made of an inhomogeneous outer thick shell and a central hole giving rise to the particles a typical ring-like morphology with a 8 to 11 nm diameters (Renard, Lepvrier, et al., 2014).

In summary, the GP fraction from AG would be an assembly of ring-like glycoproteins modules. These ring-like structures were certainly due to hydroxyproline (Hyp) – arabinogalactan (AG) subunits, as suggested by the secondary structures content of GP (Renard, et al., 2013). GP monomer, with a rather globular shape and homogeneous long-
chain branches, would be able to self-associate giving rise to small oligomers with a rather compact conformation and bigger oligomers with a more extended conformation closely related to the self-association mode.

3. Physico-chemical properties of Acacia senegal gum

3.1. Solubility in polar and non polar solvents

The ability of AG to easily dissolve in water is not new (Diderot, et al., 1777; Pomet, 1735), and it is said that AG is soluble in cold and hot water up to concentrations of about 50-55% and insoluble in alcohol (Erni, et al., 2007; Ewart & Chapman, 1952; Izudorczyk, Cui, & Wang, 2005; Turner, 1832; Verbekeen, et al., 2003; Waters & Tuttle, 1916). However it is not so easy to prepare a dispersion of gum at 50% concentration and for instance a gum solubility of 37% was experimentally determined at 25°C (Taft & Malm, 1929). Despite its high solubility in water, the mineral composition of the bulk can induce the precipitation of AG. The aqueous solution of AG forms a white jelly with basic acetate while it is soluble with neutral lead acetate. The AG solution also precipitates using potassium or sodium silicate, borax, ammonium oxalate, mercuric chloride and ferric salts (Parry, 1918). In addition, it was actually demonstrated using a great number of solvents that AG is poorly soluble in solvents other than water (Taft, et al., 1929; Taft & Malm, 1931).

3.2. Solubility of Acacia gum in alcohol solutions

AG is soluble in dilute alcohol solution and precipitates when the alcohol concentration in water is of about 50%, with a complete precipitation of AG macromolecules with 60% alcohol (Norman, 1929; Parry, 1918). With 30% alcohol, no precipitate occurs, and with 40% alcohol an opalescent/turbid ("faint") precipitate is obtained (Waters & Tuttle, 1916). These results clearly depend on gum concentration. Alcohol precipitation has been long used to purify AG (Mukherjee, et al., 1962; Mukherjee & Ghosh, 1949; Nelson & Ander, 1972; Oakley, 1935; Taft, et al., 1931; Thomas & Murray, 1928; Veis, et al., 1954). However, it has been reported that prolonged contact with alcohol decreases the solubility of AG (van Beek, 1958).

The nature of interactions and mechanism of demixing in AG-ethanol- water ternary system have not been studied in great details. However the ability of alcohols (and some salts) to induce phase separation/precipitation in polysaccharides, and more generally biopolymers...
dispersions is not new and is called simple coacervation (Bamford & Tompa, 1950; H.B. Bohidar, 2008; Bungenberg de Jong, 1949a; Gupta, Reena, & Bohidar, 2006; Jamieson, Simicglavaski, Tansey, & Walton, 1976; Koets, 1944; Mohanty & Bohidar, 2003; Nixon, Khalil, & Carless, 1966; van Oss, 1988; Veis, 2011). Simple coacervation is basically a liquid-liquid phase separation of biopolymers experiencing a change in the solvent quality. This demixing mechanism will be discussed in the section « Assembly properties of AG ».

It would be useful to determine phase diagrams of AG-alcohol-water systems and to characterize in each phase the macromolecular composition, i.e. the relative compositions of AG, AGP and GP. As these three fractions display or are supposed to display different physical chemical properties, it is likely that simple ways to obtain AG enriched in one or another of the three fractions could be identified.

3.3. Hydration properties of Acacia gum macromolecules

The observed hydrophilic nature of AG has probably not motivated many studies on the hydration properties. Few papers can be found in literature. In one example, it was shown that AG is not hydrated to a great extent as has been claimed by many authors (Grollman, 1931). Using vapor pressure measurements, no hydration of the gum was measured with NaCl (0.05M) or KCl (0.07M), which would indicate that ions were preferentially hydrated. With 0.18M sucrose, 0.7g water/g of gum was measured. In a subsequent study, a hydration of 0.9g water/g for Ca gum and 1.1g water/g for Na gum was measured using a membrane equilibrium method (Oakley, 1937). A minimal value of 0.6-0.7 g water/g of gum was found by a cryoscopic method and no hydration of the gum in presence of KCl or KBr was found, which seems to confirm previous results (Gortner & Gortner, 1934). An interesting point was that when AG concentration in studied dispersions increased from 3% to 10%, the amount of bound water decreased from 1.2g to 0.6g/g of gum (Newton & Gortner, 1922). It is possible that some self-association (i.e. aggregation) of AG macromolecules occurred with increasing concentration, leading to the decrease of water accessibility towards AG macromolecules and to the release of bound water from AG macromolecules during self-association process. When analyzing interactions of biopolymers with water, one can thus distinguish free and bound water (Chandler, 1941; Gortner, et al., 1934). Free or freezing water is the water where melting/crystallization temperature and enthalpy of melting/crystallization are not significantly different from those of bulk water (Hatakeyama & Hatakeyama, 1998). Bound
water gathers non-freezing water that is very closely associated with the macromolecules and freezing bound water that is less closely associated but displays different physical properties than free water (Hatakeyama & Hatakeyama, 1998). Non-freezing water is made of a monolayer, possibly multilayers, of water molecules in very close interaction with the biopolymer. The monolayer is generally calculated from sorption isotherms (gravimetric method) following the use of an appropriate model, the most often used being the BET (Brunauer-Emmet-Teller) or the GAB (Guggenheim-Anderson-de Boer) model (Blahovec, 2004). This type of hydration water has to be considered as an integrating part of the native biopolymer structures (Luschermattli & Ruegg, 1982). The total amount of water ($W_c$) interacting with a biopolymer is then the sum of free and bound water. Another parameter dealing with the interaction of biopolymers and especially fibers with water is the water-holding capacity (WHC). The WHC is a measure of the ability of a fiber source to immobilize water within its matrix (Robertson & Eastwood, 1981). WHC encompasses both adsorbed water and possibly water trapped within macromolecules.

Generally, water ($W_c$ or WHC) in AG amounts to about 3-6 g water/g gum, which is a range previously mentioned (Takigami, Takigami, & Phillips, 1995). The saturation $W_c$ value is in the range of values found for other polysaccharides such as xanthan and hyaluronan (Phillips, Takigami, & Takigami, 1996). The bound water ($W_b$) is around 1 g water/g gum and the non-freezing water ($W_{nf}$) is within 0.4-0.7 g water/g gum. In addition, the second virial coefficient $A_2$ (mL.mol.g⁻², also called B2 or B22), an indirect way to define the affinity for solvent, can be calculated from light scattering measurements. Negative $A_2$ values indicate a bad affinity for the solvent and attractive interactions between biopolymers while positive $A_2$ values indicate preferential interactions with the solvent and repulsions between macromolecules. Values of $5.10^{-5}$ (Picton, et al., 2000) or $4.2.10^{-5}$ mL·mol·g⁻² (Veis, et al., 1954) were found for AG. The values were low but positive, under the experimental conditions used, indicating a preferential interaction with water. An interesting result concerns the effect of temperature on the water monolayer ($X_m$). It was found that increasing the temperature from 25 to 45°C resulted to an increase of $X_m$ from 0.08 to 0.11 g water/g gum. As an increase in temperature lowers the energy of hydrogen bonding, and as hydrogen bonding is at the basis of polysaccharide hydration (Q. Wang & Cui, 2005), one could expect a concomitant decrease of the structural water, as determined for instance for starch (Al-Muhtaseb, McMinn, & Magee, 2004) or chitosan (Rosa, Moraes, & Pinto, 2010),
but also for soy proteins (Cassini, Marczak, & Norena, 2006). It is also well known that by increasing temperatures there is an increase of energy of adsorbed water molecules, which allow the leaving of some adsorbed water molecules from the active centers of the adsorbent. As a result, the amount of adsorbed moisture decreases. The results reported here for AG are somewhat counterintuitive. However, these results are not unique as similar trends were demonstrated for microcrystalline cellulose (Cadden, 1988) cited by Vernon-Carter et al., 2006) or myosin (Das & Das, 2002) cited by Vernon-Carter et al., 2006).

All these results converge on the same conclusion. The affinity of AG for water provides an extremely favorable environment for binding water, which is probably mainly due to the carbohydrate component of AG and its highly branched characteristic (Phillips, et al., 1996). The polypeptide component also interacts with water since it contains a significant number of hydrophilic aminoacids (Renard, et al., 2006). The sugars units would first bind water at the hydroxyl (OH) groups associated with the uronic acids, forming the non-freezing water (Phillips, et al., 1996). Freezing-bound water is also tightly associated with carbohydrate chains, which could form intra-molecular hydrogen bonds within the highly cross-linked gum structure (Phillips, et al., 1996). Thereafter, there would be large intra-molecular and inter-molecular voids which could be occupied by water in a variety of metastable states, preventing the formation of the ideal ice structure.

It is interesting to note that when AG in solution is dried, either with alcohol or by heating (vacuum distillation), it becomes practically insoluble (Thomas, et al., 1928). When it is thus dried, the gum swells in water to a jelly-like mass which does not dissolve except on long standing. AG in powder form heated above 100°C, and especially at 170 °C, when immersed in water, also swells up to a great extent but does not dissolve and the gel thus formed is non-sticky (Moorjani & Narwani, 1948). This insolubility is explained by the complete dehydration of the gum. However, it is likely that aggregation of AG macromolecules occurs as heating from 100 to 170 °C results in an increase of the viscosity of solutions. Protein degradation also occurs at temperatures above 100 °C that may affect the gum solubility (Cozic, 2007). The insolubilization of the gum by heating at high temperature (150 °C) is known for a long time (Fremy, 1860). Boiling the gum or adding alkali at cold temperature dissolves the gum again (Fremy, 1860). The ability of AG to re-bind water which has been
released by increasing the temperature is of great value in confectionary and jellies applications (Phillips, et al., 1996).

3.4. Rheological properties of Acacia gum

**Viscosity (at zero shear rate) of Acacia gum dispersions**

The molecular interactions of AG with the solvent determine in part the viscosity of dispersions. Viscosity is also governed by the shape, molecular size and concentration of molecules and is affected by temperature and pressure. Since AG macromolecules are weak polyelectrolytes, it is expected that pH, ionic strength and type of ions (according to the Hofmeister serie) must have a significant effect on the viscosity (Stephen & Churms, 1995). This can be clearly observed with arabic acid where a maximum of viscosity was reached at a pH in the range 5.5-6.3 (Thomas, et al., 1928). The lower viscosity at acidic pH can be explained by the fact that, due to neutralization of carboxyl groups at low pH, electrostatic repulsions decrease. This leads to a decrease in the hydrodynamic volume of the carboxyl-bearing polysaccharides, and hence, in the viscosity of the polysaccharide solution (Vanderreijden, Veerman, & Amerongen, 1994). Acid-induced hydrolysis of polysaccharide can also contribute to the lower measured viscosity. The decrease in viscosity observed at pH larger than 8-10 could be explained by a strong weakening of hydrogen bonds, resulting in less efficient interactions with water or, alternatively, to conformational changes resulting in a reduction of charged groups-induced electrostatic repulsions. It can also be noticed that the viscosity was almost steady between pH 5 and 9. A steady viscosity of AG dispersions was also measured between pH 6.2 and 8.5 by Riddell & Davies (1931).

The increase in NaCl concentration induced a decrease in viscosity. For a salt-free solution, electrostatic repulsions due to the charges on the macromolecule favor a stretched chain conformation as a result of long-range electrostatic effects. This behavior results in higher viscosity. Addition of a simple electrolyte screens these intermolecular electrostatic repulsions and allows the molecules to compact towards the volume of an uncharged polymer with the same number of residues linked in the same way (Giannouli, Richardson, & Morris, 2004), resulting in a lower viscosity (Smidsrod & Haug, 1971; Tinland & Rinaudo, 1989). Similar results were obtained by other authors (Amy, 1934; Williams, Phillips, & Randall, 1990).
Like for other common biopolymers, viscosity increased with AG concentration (Williams et al., 1990) and decreased with the increase in temperature (Stephen, et al., 1995). Regarding the effect of AG concentration, we collected a great number of data from literature and the relationship between the relative viscosity \( \eta/\eta_0 \) and the AG concentration was exponential over a 0.13-56 wt% concentration range. However, what is important to notice, irrespective of the geographical origin of AG, various chemical compositions and physical chemical conditions of sample preparation for viscosity measurements, practically all data are described by a single exponential (Figure 3). Such an exponential delimitates two regions, one region where the viscosity gently increases with concentration and the other region where viscosity sharply increases with concentration.

**3.5. Flow and viscoelastic properties of Acacia gum dispersions**

Unlike most polysaccharides, used for their thickening or gelling properties (Wand & Cui, 2005), AG dispersions display low viscosity even at quite high concentration and do not gel (except when AG powder is thermally treated at high T then rehydrated, as noted above). The observed low viscosity of AG dispersions probably can explain the belief that their flow behavior is newtonian, *i.e.* the relationship between the shear stress \( \tau \) (N.m\(^{-2}\)) and the shear rate \( \dot{\gamma} \) (s\(^{-1}\)) is linear (D. M. W. Anderson, et al., 1967; BeMiller, 2001; Izydorczyk, et al., 2005). Some experimental results seem to confirm this behavior (Salazar-Montoya, Jimenez-Avalos, & Ramos-Ramirez, 2012). This depends on gum concentration. Shear-thinning flow of AG dispersions is observed at high AG concentrations, typically 15-30% and above (Araujo, 1966; Dunstan, Chai, Lee, & Boger, 1995; Gomez-Diaz, Navaza, & Quintans-Riveiro, 2008; Nussinovitch, 1997; Williams, et al., 1990).

More recently, it has been shown that AG dispersions also display shear-thinning flow behavior, even at AG concentrations as low as 1-4% (Li, et al., 2009; Mothe & Rao, 1999; Sanchez, Renard, Robert, Schmitt, & Lefebvre, 2002; Weinbreck & Wientjes, 2004). It was hypothesized that the presence of AG aggregates could explain such an unusual flow behavior (Li, et al., 2009; Mothe, et al., 1999). Hydrogen bonding could partly explain the formation of these hypothetical aggregates (Li, et al., 2009). In addition, time-dependent or thixotropic flow behavior was also observed (Li, et al., 2009; Sanchez, Renard, et al., 2002). It was suggested that the aggregation of AGP component was at the origin of this behavior (Li,
et al., 2011). Time-dependent thickening flow behavior at low shear rates and low biopolymer concentrations have been previously reported for colloidal globular protein dispersions (Giordano, Grasso, Teixeira, Wanderlingh, & Wanderlingh, 1992; Lefebvre & Riot, 1997; Matsumoto & Chiba, 1990; Renard, Axelos, Boué, & Lefebvre, 1996; Renard, Robert, Faucheron, & Sanchez, 1999). It is generally supposed that bulk aggregation between globular proteins or protein aggregates is responsible for the observed rheological properties (Lefebvre, et al., 1997; Renard, et al., 1999). However, time-dependent rheological properties of diluted biopolymer dispersions can also be caused by surface properties of macromolecules during rheological measurements. In this case, an equilibrium between surface and bulk rheological properties may occur. These features were clearly demonstrated with AG dispersions (Sanchez, Renard, et al., 2002).

Viscoelastic properties of AG dispersions were also characterized and revealed, as expected, a predominant liquid-like behavior (Goycoolea, Morris, Richardson, & Bell, 1995; Matsumura, Satake, Egami, & Mori, 2000; Sanchez, Renard, et al., 2002). Indeed, mechanical spectra obtained at 6wt% AG concentration by oscillatory testing revealed that the viscous or loss modulus (G'', N.m^{-2}) was higher than the elastic or storage modulus (G', N.m^{-2}) throughout a wide frequency range but G' became larger than G'' at the highest frequencies (Sanchez, Renard, et al., 2002). Similar behavior was recorded at 18wt% (Matsumura, et al., 2000) or 50wt% (Goycoolea, et al., 1995) AG concentration. Interestingly, the evolution of G' and G'' as a function of frequency followed a power law behavior with exponents of 1.4 and 0.8, respectively, smaller than the exponents 2 and 1 classically found for viscoelastic liquids (Sanchez, Renard, et al., 2002). It was then concluded that AG dispersions were structured liquids. Surface effects also have an impact on measured viscoelastic properties. Dynamic mechanical spectra after 120 min rest of AG samples at 6wt% gum concentration in the rheometer therefore showed a typical gel-like behavior with G' larger than G'' over the entire range of selected frequencies (Sanchez, Renard, et al., 2002). The building-up with time of a predominantly elastic interfacial structure was demonstrated.

In summary, AG dispersions display newtonian flow behavior at gum concentrations below about 20wt% and shear-thinning above. However, in practical industrial situations where applied shear rates are usually above 100 s^{-1}, flow behavior of AG can be considered as newtonian. Sometimes, the measured flow behavior is non-newtonian and even thixotropic.
at low AG concentrations. This unusual behavior seems to be mainly due to surface effects and reversible shear-induced aggregation of AG macromolecules. In this case, further studies are needed to clarify the situation, by using for instance rheology coupled to small angle x-ray or neutron scattering.

3.6. Assembly properties of Acacia gum

Molecular associations and assembly of biopolymers depend strongly on the extent of macromolecule-solvent and macromolecule-macromolecule interactions. Several factors such as solvent chemical properties, physical chemical treatments and macromolecules physical chemical properties can influence the assembly pathway and of course the functional properties of the assemblies. It is well known that AG can associate with itself or other biopolymers, such as proteins. Its association and assembly properties are often used in several areas (food, pharmaceutical, medicine, etc.) to elaborate AG-based assemblies with specific functional properties. According to physical chemical conditions and the presence or not of other macromolecules as partner of the assembly, AG can assemble following different mechanisms such as aggregation or coacervation (simple or complex).

3.6.1. Self-association and aggregation properties of Acacia gum

Aggregation of AG is apparently a natural mechanism depending on the physiology of trees, and particularly the ageing (Idris, et al., 1998). The characterization of AG harvested on trees of different ages, from 5 to 15 years old, showed both the increase in the mean radius of gyration of AG and the proportion of aggregates in solution. This aggregation mechanism occurs when the trees grow older up to about 15 years. Aggregation of AG in aqueous solution was also evidenced in several studies using size exclusion chromatography (SEC) that showed an elution peak in the void volume of the column, in addition to the peaks corresponding to the three main fractions of AG (Idris, et al., 1998; Mukherjee, et al., 1949; Ray, et al., 1995; Sanchez, Renard, et al., 2002). This peak, which was removed from SEC chromatograms after filtration of AG solution on 0.45 µm filters, was attributed to the elution of aggregates (Al-Assaf, et al., 2009). Association and aggregation properties were also highlighted over a large concentration range in AG using scattering and microscopy experiments. Using static light scattering measurements, Wang et al. showed the increase of $R_g$ from 20 to 50 nm on filtered AG samples as the concentration
of AG increased from 0.0413 to 5.21 g·L$^{-1}$, respectively (Q. Wang, et al., 2008). Aggregates were also evidenced in more concentrated AG solution, ranging from 5 to 300 g·L$^{-1}$, by SAXS and SANS measurements, and observed using cryo-TEM (Dror, Cohen, & Yerushalmi-Rozen, 2006). All these experiments confirm the self-association behavior of AG in aqueous solution over a large range of concentration.

The apparent contradiction between the high solubility of AG in water and its propensity to self-associate could origin from the chemical composition of the molecular fractions isolated from AG. Indeed, as described above, AG is composed of arabinogalactan-protein type macromolecules (Akiyama, Eda, & Kato, 1984). In plant kingdom, the association property of AGPs is well established: these macromolecules have specific functions in interaction and recognition cellular mechanism (Showalter, 2001). It is also well known that AGPs can self-assemble and aggregate both in vitro and in vivo (Baldwin, McCann, & Roberts, 1993; Capataz-Tafur, Trejo-Tapia, Rodriguez-Monroy, & Sepulveda-Jimenez, 2011). Hence, the self-association property of AG in solution is consistent with the adhesive nature of AGPs. Very recently, it was proved that the capacity of adventitious roots of English ivy (Hedera helix) to climb vertical surfaces was due to AGP assembled in nanospheres that were the key component of the high-strength adhesive secreted by this plant (Y. J. Huang, et al., 2016).

Recently, studies devoted to the characterization of the three dimensional structure of isolated AG, AGP and GP fractions, also evidenced the self-assembly behavior of these isolated macromolecules (Renard, et al., 2012, 2013; Renard, Lavenant-Gourgeon, et al., 2014; Sanchez, et al., 2008). These studies highlighted some differences between the three main fractions towards their affinity for aqueous solvent and of course their ability to self-assemble and aggregate in aqueous solution. The study focusing on GP fraction showed first of all that it was not easy to rehydrate GP powder. A significant proportion of GP macromolecules aggregated with the formation of a substantial undissolved material after centrifugation (Ray, et al., 1995; Renard, et al., 2013). On the contrary, the rehydration of AGp and AGP powders were complete in aqueous solution without the formation of undissolved material (Renard, et al., 2012; Sanchez, et al., 2008). Hence, GP fraction contains some macromolecules with a lowest affinity towards aqueous solvent compared to those included in AGp and AGP fractions, in agreement with the delayed elution of this fraction by HIC. The self-aggregation behavior of GP fraction during its rehydration was attributed to the
more pronounced hydrophobic nature of the GP macromolecules (Renard, et al., 2013; Renard, et al., 2006). Transmission Electron Microscopy (TEM) experiments performed on each fraction also revealed that the self-aggregation behavior and the morphology of formed aggregates depend clearly on the fraction studied. It is likely that drying of samples for TEM experiments could impact the observed morphologies. Self-assembled or aggregated macromolecules were observed with AGP and GP fractions (Renard, et al., 2012, 2013), but not with AGp fraction (Sanchez, et al., 2008). The aggregates evidenced with AGP and GP macromolecules in aqueous solution could be promoted by several intermolecular attractive weak forces such as hydrogen bond between saccharidic residues as suggested by Mahendran et al. (2008) and/or hydrophobic interaction between polypeptide backbones as suggested by Renard et al. (2012).

Differences between AGp, AGP and GP fractions towards aggregation in aqueous solution could be explained by their differences in chemical composition and particularly their protein content and amino-acids composition. The protein content in AGp, AGP and GP fractions is therefore around 1.1%, 9% and 24.6%, respectively (Renard, et al., 2006) and GP fraction contains more hydrophobic amino-acids such as glycine, valine, isoleucine, leucine, phenylalanine than AGp and AGP fractions (Renard, et al., 2006). Hence, both the high protein content and the presence of hydrophobic amino-acids in higher proportion in GP fractions could explain the highest sensitivity of GP macromolecules towards self-assembly and aggregation in aqueous solution.

After harvesting, AG can be submitted to several physical treatments such as heating, spray-drying or irradiation before to be used. These treatments can also influence the extent of the natural aggregation process of AG. Aggregation behavior is enhanced when AG is heated or irradiated. Al Assaf et al. showed that $R_g$ increased from 33 to 73 nm when dried AG was stored at 110°C during two days under control humidity (Al-Assaf, et al., 2007). Aggregation was also revealed in spray-dried AG samples, displaying a large proportion of aggregates in solution compared to raw material. Aggregation in spray-dried AG has been attributed to the pasteurization step included in the process (Al-Assaf, et al., 2009). During heat treatment, aggregates are formed between AGp and GP fractions involving hydrophobic association of the proteinaceous components. Irradiation of AG in the presence of acetylene also enhances aggregation with an increase of $R_g$ from 25 to 67 nm for AG irradiated at 6 kGy (Al-Assaf, et
al., 2009). In this case, aggregates were formed through C-C covalent bonds between the carbohydrate moieties.

Interestingly, aggregating AG through controlled Maillard reaction can improve its functional properties (Al-Assaf, et al., 2007). Consequently, the natural functional properties of AG could be improved by its self-assembly properties.

3.6.2. Coacervation of Acacia gum

Coacervation has generally been defined as a liquid-liquid phase separation occurring in (bio)-polymers solutions under suitable conditions (Bungenberg de Jong, 1949b). It is well accepted that coacervation corresponds to a dehydration process of (bio)-polymers. The phase separation gives rise to two incompatible and immiscible liquid phases: a (bio)polymer dense phase, called the “coacervate” phase, coexisting with a very dilute colloidal phase, the supernatant (Bamford, et al., 1950; Bungenberg de Jong, 1949a; Menger & Sykes, 1998). The dilute liquid phase remains in equilibrium with the coacervate phase. Depending on the number of (bio)-polymers involved in the phase separation, coacervation can be classified as simple or complex (Bungenberg de Jong, 1949a). In the following sections, we will discuss about the assembly of AG according to simple and complex coacervation mechanism.

3.6.2.1. Simple coacervation

Simple coacervation is a liquid-liquid phase separation where only one (bio)-polymer is involved (H.B. Bohidar, 2008; Bungenberg de Jong, 1949a). It occurs as a result of a decrease in the solubility of (bio)-polymers through water competition caused by modifying the physical chemical properties of the solvent. In aqueous solutions, simple coacervation can be promoted by the action of salts (sodium sulphate, sodium chloride), the addition of a water-miscible non-solvent (ethanol, methanol, acetone, etc...), by modifying pH or increasing/decreasing temperature, thus turning the aqueous solvent medium, good for the (bio)-polymer, into a marginal one (H.B. Bohidar, 2008; Bungenberg de Jong, 1949a; Ezpeleta, et al., 1996; Mauguet, et al., 2002; Mohanty, et al., 2003).

The addition of ethanol to AG/water dispersion led to the formation of a new dense phase containing liquid droplets, called coacervates, dispersed in a continuous phase. AG macromolecules will tend to spontaneously self-associate according to a coacervation mechanism once a critical ethanol percentage has been reached. Whatever the AG
concentration, the coacervation mechanism occurs for ethanol concentration below the precipitation point (unpublished data). Simple coacervation induces the formation of coacervated and diluted phases that both contain concentrated and diluted AG molecules, respectively. The binodal curve, delimiting the one-phase and two-phases regions, was determined by our group for A. senegal gum in water/ethanol solution (Figure 4, unpublished data). Upon the increase in ethanol percentage and AG concentration, the solubility/affinity of AG molecules for water/ethanol solution decreases.

In this ternary water/ethanol/AG system, ethanol acts as a suitable dehydrating agent which shifts the energetic balance in favor of the attraction between AG macromolecules (Koets, 1944). When ethanol is added, the quality of the solvent decreases, becoming a poor solvent for the solubility of AG macromolecules, by both modifying the hydrogen bond network and the polarity of the medium (H. B. Bohidar & Mohanty, 2004; Mohanty, et al., 2003). In addition to the role of ethanol in the disturbance of hydrogen bonds network, ethanol also decreases the dielectric constant and consequently the polarity of the solvent that could favor the self-association of macromolecules (Mohanty, et al., 2003). Consequently, induced coacervation of AG macromolecules by the addition of a non-solvent such as ethanol corresponds to a dehydration process due to the modifications of solvent physical chemical properties.

3.6.2.2. Complex coacervation

Complex coacervation is based on the ability of two oppositely charged (bio)-polymers to interact and associate involving a liquid-liquid phase separation with the formation of liquid droplets called coacervates (Bungenberg de Jong, 1949b; Doublier, Garnier, Renard, & Sanchez, 2000; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Complex coacervation was first evidenced in 1911 by Tiebackx by mixing Arabic gum with gelatin (Tiebackx, 1911). AG is a weak polyelectrolyte negatively charged extensively used for the elaboration of complex coacervates by mixing it with positively charged proteins extracted from both animal (gelatin, bovine serum albumin, β-lactoglobulin, sodium caseinate, etc) and plant (wheat, pea, soybean and lentil proteins) kingdoms (Aryee & Nickerson, 2012; Bungenberg de Jong, 1949a, 1949b; Burgess & Singh, 1993; Dong, et al., 2013; Ducel, Richard, Saulnier, Popineau, & BOURY, 2004; Liu, Low, & Nickerson, 2009; Niu, et al., 2015; Schmitt, et al., 1998; Schmitt, Sanchez, Thomas, & Hardy, 1999; Weinbreck, de Vries, Schrooyen, & de Kruif, 2003;
Complex coacervation between AG and proteins occurs by charge neutralization with the involvement of non-specific electrostatic interactions between de-protonated carboxyl groups of AG macromolecules and the protonated amino groups of proteins. As complex coacervation mechanism mainly occurs by the involvement of weak electrostatic interactions, this assembly mechanism is substantially influenced by the physical chemical properties of the solvent (pH, ionic strength, nature of salts and temperature) and the structural and physical chemical properties (global charge, charge distribution, flexibility and concentration) of biopolymers (polysaccharides and proteins) (Schmitt, et al., 1998; Ye, 2008). The identification of the specific conditions resulting in a two phase system, named phase diagram and where binodal curve is determined, is a tedious work that requires time and large quantities of raw material. Trying to overcome these major drawbacks, phase diagram of a β-lactoglobulin - AG mixture has been recently determined through an innovative miniaturized approach based on millifluidic (Amine et al., submitted). In this work authors proved that by using turbidity measurements based on image analysis within only 2 µl biopolymers droplets, binodal curve was able to be determined with a good agreement with those obtained in bulk. This method should find applications for the screening of numerous protein-polysaccharide mixtures for industrial issues.

Indeed, the formation of complex coacervates between AG and proteins is of industrial interest to value them, by enhancing their functional properties and developing novel biopolymer assemblies. One of the first and the most important industrial applications of complex coacervation is microencapsulation. Polysaccharide/protein microcapsules are used in many industries (food, pharmaceutical, cosmetics, agricultural, etc...) to entrap and protect sensitive molecules (aroma compounds, bioactives, drugs, enzymes) against processing (heat, shear, redox potential, etc...) and storage conditions (oxygen, temperature and moisture). The use of microcapsules is also a mean to deliver the encapsulated interest...
molecules to the specific target with the optimal kinetic by changing the physical chemical conditions (pH), mechanical process (chewing) or enzymatic action (Schmitt, et al., 2011). Several AG/protein (gelatin, whey proteins, gliadins, pea globulin) microcapsules were developed and elaborated to encapsulate different molecules such as lemon and orange flavors, oil, pesticides and flavonoid compounds (Aberkane, et al., 2012; Bosnea, Moschakis, & Biliaderis, 2014; Ducel, et al., 2004; Eratte, Wang, Dowling, Barrow, & Adhikari, 2014; Gao, et al., 1984; Hedayati, Jahnshahi, & Attar, 2012; Jain, Thakur, Ghoshal, Katare, & Shivhare, 2015; Leclercq, Harlander, & Reineccius, 2009; Lv, Yang, Li, Zhang, & Abbas, 2014; Omi, Umeki, Mohri, & Iso, 1991; Palmieri, Martell, Lauri, & Wehrle, 1996; Weinbreck, et al., 2004). The preparation of microparticles by complex coacervation was similarly performed on AG/polysaccharide mixtures such as AG and chitosan (Butstraen & Salaün, 2014).

The rheological properties of solutions could also be modified by the formation of complex coacervates between AG and proteins. The rheological properties of protein and polysaccharide in associative conditions result in different behaviors compared to each individual biopolymer. It is expected that the bulk viscosity of the system is improved with the formation of microstructures as complex coacervates (Schmitt, et al., 2011). The characterization of the rheological properties of AG/whey protein coacervates evidenced that these assemblies displayed a viscous character (Weinbreck, et al., 2004). As electrostatic interactions mainly stabilize complex coacervates, protein/polysaccharide molar ratio, pH and ionic strength are key parameters for the rheological properties of complex coacervates. The maximum of viscosity is obtained for mixing conditions leading to the complete charge neutralization of the two biopolymers. This viscous behavior was similarly evidenced on AG/Chitosan complex coacervates (Espinosa-Andrews, et al., 2013). In their work, they highlighted an interrelationship between the biopolymers mass ratio, the charge density and the viscoelastic properties of the coacervated phase.

The complex coacervates formed between AG and proteins gather the surface properties of each biopolymer. Hence, complex coacervates can also be used to stabilize air/water or oil/water interfaces in a variety of foamed and emulsified products (Dickinson, 2008). Schmitt et al. reported that the surface activity of AG/β-lactoglobulin complexes formed at
charge neutralization ratio (pH 4.2) was as high as the pure adsorbed protein in the same condition (Schmitt, et al., 2005). However, the viscoelastic properties of the film formed by AG/β-lactoglobulin complexes were stronger than those of the pure protein and the gas permeability of the film was significantly reduced compared to pure β-lactoglobulin. Similar results were obtained in chilled dairy foams using whey protein isolate/AG complexes (Schmitt & Kolodziejczyk, 2010). Consequently, the stability of the foam can be improved by the adsorption of AG/protein complexes at the air bubble interfaces. Emulsions can also be stabilized by AG/protein complexes or coacervates. Ducel et al. evidenced that AG/pea globulin and AG/α-gliadin complexes or coacervates tend to decrease more strongly the oil/water interfacial tension than the pure protein (Ducel, Saulnier, Richard, & Boury, 2005). The coacervates films were characterized by a long relaxation time and a high surface elasticity. In addition, the authors reported that charged complex coacervates were more efficient to stabilize oil droplets.

3.7. Surface properties: adsorption at solid-liquid and liquid-liquid interfaces

Surface properties of AG, and of a number of plant gum exudates, are unique in the polysaccharide world. By surface properties, we mean both the ability of AG to decrease interfacial tension between gas-water, liquid-liquid or solid-liquid interfaces, and to stabilize these interfaces through steric and electrostatic interactions and hydration forces (Adamson & Gast, 1997). These properties can be used to form and stabilize foams (Redgwell, Schmitt, Beaulieu, & Curti, 2005), emulsions and to stabilize solid nanoparticles. It can be noticed that studies on foaming properties of AG are rare as compared to studies on stabilization of liquid or solid particles, especially nanoparticles.

3.7.1. Surface properties: adsorption at solid-liquid interfaces

Surface properties of Acacia senegal gum can be used to stabilize solid nanoparticles such as carbon nanotubes (Amiri, Shanbedi, Eshghi, Heris, & Baniadam, 2012; Bandyopadhyaya, et al., 2002; Dror, Pyckhout-Hintzen, & Cohen, 2005; Edri & Regev, 2010; M. T. Kim, Park, Hui, & Rhee, 2011; Kumar, Reddy, & Ramaprabhu, 2008; Najeeb, Lee, Chang, & Kim, 2010) , gold, silver, magnetic or bioceramic nanoparticles (Batalha, Hussain, & Roque, 2010; Gils, Ray, & Sahoo, 2010; Kannan, et al., 2012; Kattumuri, et al., 2007; Ma, et al., 2012; Roque & Wilson, 2008; J. E. Song, et al., 2011) ; and flavonoids nanoparticles(Aberkane, et al., 2012)).
Obviously, the ability of AG to stabilize solid interfaces was at the basis of ink and paint manufacturing (Balantrapu & Goia, 2009; J. K. Song, Choi, & Chin, 2007; D. W. Wang & Zhao, 2009).

AG was also used to stabilize latex nanoparticles as a model system of interface with a surface coverage of about 0.5 – 5 mg/m² depending on solvent conditions and initial AG concentration (Gashua, Williams, & Baldwin, 2016; M. L. Snowden, Phillips, & Williams, 1987). The surface coverage was found to be similar at liquid-liquid interfaces in O/W emulsions (Randall, Phillips, & Williams, 1988). The high M_w fraction of AG, AGP, was found to be the most effective to be adsorbed at the interface after only 15 min while AGp fraction was ineffective in the stabilization of the latex dispersions (M. L. Snowden, et al., 1987). Electronic spin resonance data indicated that AG adsorbed at the solid-liquid interface with approximately half of its segments close to the surface in trains and the other half in loops and tails extending away from the surface into solution (M. L. Snowden, et al., 1987). An alternative model of end-on or multilayer adsorption was recently proposed to explain the high layer thickness after adsorption of AG on latex particles (Gashua, et al., 2016). As noted above, AG has also been explored as coating agent of nanomaterials for biomedical applications, namely magnetic nanoparticles (Ali, Ziada, & Blunden, 2009; Banerjee & Chen, 2008, 2010; Palma, et al., 2015). AG coupled magnetic nanosystem could therefore find applications as a MRI contrast agent for cell-labeling applications. Other applications concerned the use of AG as a nontoxic material in the production of readily administrable biocompatible gold nanoparticles for diagnostic and therapeutic applications in nanomedicine (Kattumuri, et al., 2007)(Kattumuri, Katti, Bhaskaran, Boote, Casteel, Fent, Roberton, Chandrasekhar, Kannan & Katti, 2007) or to improve antioxidiant properties of nanoparticles (Kong, et al., 2014). Surprisingly, studies of the adsorption of AG on 2D solid surfaces and their related interfacial properties have never been performed to the best of our knowledge while novel and efficient techniques such as ellipsometry coupled or not to quartz crystal microbalance exist today. In addition, there are no information in the literature on the testing of AG adhesion or any appropriate standard adhesion method used for a similar material. Future prospects in this important area of adhesion properties on solid surfaces could be very interesting and useful to unravel the role and function of AG at solid-liquid and liquid-interfaces.
3.7.2. Surface properties: adsorption at liquid-liquid interfaces

A great number of studies dealt with emulsification properties of AG (Bai, Huan, Gu, & McClements, 2016; Briggs & Schmidt, 1915; R.A. Buffo & Reineccius, 2000; R. A. Buffo, Reineccius, & Oehlert, 2001; Castel, Rubiolo, & Carrara, 2017; Castellani, Guibert, et al., 2010; Charoen, et al., 2012; Charoen, et al., 2011; Chivero, Gohtani, Yoshii, & Nakamura, 2016; Desplanques, Renou, Grisel, & Maliaci, 2012; Dickinson, 1988, 2003; Dickinson, Elserson, & Murray, 1989; Dickinson, Galazka, & Anderson, 1991a, 1991b; Dickinson, Murray, Stainsby, & Anderson, 1988; Djordjevic, Cercaci, Alamed, McClements, & Decker, 2008; Dluzewska & Leszczyński, 2005; Gashua, et al., 2016; Gharibzahedi, Mousavi, Khodaiyan, & Hamedi, 2012; X. Huang, Kakuda, & Cui, 2001; Jayme, Dunstan, & Gee, 1999; Jin, Cai, Li, Yadav, & Zhang, 2017; Y. D. Kim, Morr, & Schenz, 1996; Klein, Aserin, Svitov, & Garti, 2010; Ma, et al., 2012; Mahfoudhi, et al., 2014; Matsumura, et al., 2000; McNamee, O'Riordan, & O'Sullivan, 1998; Mirhosseini & Tan, 2010; Nakamura, 1986; Nakauma, et al., 2008; Ozturk, Argin, Ozilgen, & McClements, 2015; Padala, Williams, & Phillips, 2009; Piorkowski & McClements, 2014; Prakash, Joseph, & Mangino, 1990; Ray, et al., 1995; Reiner, Reineccius, & Peppard, 2010; Seifriz, 1925; Shi, et al., 2017; Shotton & Davis, 1968; Shotton & Wibberley, 1960; M. J. Snowden, Phillips, & Williams, 1988; M. L. Snowden, et al., 1987; Vasile, Martinez, Pizones Ruiz-Henestrosa, Judis, & Mazzobre, 2016; Vernon-Carter, et al., 1996; Vernon-Carter, Pedroza-Islas, & Beristain, 1998; Xiang, et al., 2015; M. P. Yadav, et al., 2007; X. Yao, et al., 2016; X. L. Yao, et al., 2013; Zhang & Liu, 2011). The listing is far from being exhaustive but shows clearly the abundant literature on the subject. We know that physical chemical parameters (pH, ionic strength, type of ions, temperature, homogenization pressure, etc.) all influence the structure of biopolymer-stabilized emulsions and their stability. Here we wish to focus on the composition and structural aspects at the expected origin of surface properties of the gum.

It is today widely accepted that it is the protein-rich high-molecular weight fraction of AG, the AGP complex, which mainly provides the surface properties of gum. A fair non-linear relationship between AGP concentration and emulsion stability was recently shown with a seemingly AGP critical concentration of about 10% (Nishino, Katayama, Sakata, Al-Assaf, & Phillips, 2012). Here emulsion stability was checked following heating at 60°C for 3 weeks and we may wonder whether surface properties of AGP were highlighted or heat-induced.
aggregation of AGP at the interfaces, in line with the process of “Super Gum” formation described below (Al-Assaf, et al., 2007; Aoki, Al-Assaf, Katayama, & Phillips, 2007; Aoki, Katayama, et al., 2007). We remember here that “AGP” molecular fraction both encompasses the so-called AGP and the high M<sub>w</sub> GP fractions as well as minor concentrations of the AGp fraction. One direct consequence is that companies using gum in their products, for instance to make stable oil-in-water (o/w) emulsions, want to obtain gum samples with high proportions of AGP, e.g. above 12%. This explains also why modified gum with higher content in high-molecular weight fractions was developed recently through controlled Maillard reaction (Al-Assaf, et al., 2007). The modified gum contains about 20% of AGP and displays better o/w emulsion stabilizing properties than unmodified gum, reinforcing the current opinion on the role of AGP on gum surface properties. However, the reality seems a bit more complex since AG samples containing small amounts of AGP, e.g. 8%, can produce sometimes o/w emulsions with better stability than gums with higher levels of AGP. A detailed analysis of experimental facts reported in literature can then be useful to clarify the effect of gum molecular composition on its surface properties. The discussion will benefit from what we know on the surface properties of individual molecular fractions (AGp, AGP and GP).

Despite its good surface properties, AG is far from being as efficient as proteins to form and stabilize oil-in-water emulsions. An oil to emulsifier ratio of about 1:1 is therefore needed for AG while a lower ratio of 1:10 is common for proteins. It is then not surprising that protein-rich macromolecules play an important role in the emulsifying/stabilizing properties of AGs (Randall, et al., 1988; Ray, et al., 1995). It was then reported with samples of various AG species, having nitrogen contents in the range from 0.1% to 7.5%, a good correlation between the nitrogen content of the gum and its limiting long-time interfacial tension and between the emulsifying capacity and the initial rate of change of tension with time (Dickinson, et al., 1988). It was also shown by SEC on supernatants after removing oil droplets that protein-rich fractions adsorb strongly at the oil-water interface (Randall, et al., 1988). Similar results using the same methodological approach were obtained by others (Alftren, Penarrieta, Bergenstahl, & Nilsson, 2012; Flindt, Al-Assaf, Phillips, & Williams, 2005; Padala, et al., 2009). However, a careful examination of results reveal that while a preferential adsorption onto oil-water interfaces of protein-rich fractions occurred, all
molecular fractions seems to be present at the interfaces with however different adsorption kinetics. In the frame of the wattle-blossom model, it is supposed that, in analogy with block co-polymers, the more hydrophobic protein chain anchors at the interface while the protruding hydrophilic carbohydrate blocks attached to this chain provide a strong steric barrier towards flocculation and coalescence (Islam, et al., 1997; Randall, et al., 1989). However, numerous hydrophilic hydroxyamino acid residues are present in the polypeptide chain, and no structural data today exist about the spatial position (buried or in periphery) of the polypeptide chain, casting some doubt on the model proposed by Islam et al. and Randall et al.

In fact, the efficiency of AG is better related to the way it adsorbs onto interfaces and the mechanical properties provided by interfacial films rather than to a low interfacial tension (Shotton & Wibberley, 1961). Surface charged groups provide the basis for some electrostatic contribution to the colloidal stabilization, however the relative low value of the (negative) zeta potential, 10-20 mV under beverage emulsion conditions (Jayme, et al., 1999; Ray, et al., 1995), and the pH-independent destabilization mechanism (coalescence), indicates that the primary mechanism is steric stabilization (Dickinson, 2003; Trindade, et al., 2008). It is better to say electro-steric stabilization mechanism as suggested by Jayme et al. (1999) as both minerals (R. A. Buffo, et al., 2001) and low pH (R. A. Buffo, et al., 2001; Djordjevic, et al., 2008; Vernon-Carter, et al., 1998) are unfavorable to emulsion stability. Another very important parameter defining emulsion stability is interfacial rheology. The ability of AG to form highly cohesive viscoelastic interfacial multilayer (gel-like) films is not new (Briggs, et al., 1915; Serrallach, Jones, & Owen, 1933; Shotton, 1955; Shotton & Wibberley, 1959; Shotton, et al., 1961; Wibberley, 1962). AG interfaces are jammed, very strongly shear-elastic and exhibit non-linear interfacial shear rheology indicative of 2D soft solid behavior (Erni, Jerri, Wong, & Parker, 2012; Erni & Parker, 2012; Erni, et al., 2007). Electro-steric forces, probably including hydration forces, and elastic interfaces are at the origin of emulsion stabilization by AG, especially against coalescence (Dickinson, et al., 1988).

Since the presence of proteins and steric effects are important in the formation and stabilization of emulsions, structural modifications of Acacia senegal gum was proposed as a mean to convert it into a good emulsifier (Al-Assaf, et al., 2007). Structural modifications
were induced through controlled Maillard reaction incubating kibbled gum many weeks at 60°C under controlled moisture mimicking natural maturation process according to the authors (Al-Assaf, Phillips, Sasaki, & Katayama, 2003). This process resulted in an increase of $M_w$ of AG from about $4.2 \times 10^5$ g.mol$^{-1}$ up to about $20 \times 10^5$ g.mol$^{-1}$ (Al-Assaf, et al., 2007). Modified gums allow the production of oil-in-water emulsions with smaller droplets, improved interfacial viscoelasticity and emulsion stability as compared to the unmodified control gum. The effect of AG molecular weight ($M_w$) on emulsion stability (Dickinson, et al., 1991a) or surface rheological properties (Nakamura, 1986) was previously demonstrated. In this case, the initial size of emulsion droplets was not impacted by $M_w$ but emulsion stability was improved with high $M_w$. Similar results using matured gums were obtained in other studies (Aoki, Katayama, et al., 2007; Castellani, Al-Assaf, Axels, Phillips, & Anton, 2010; Castellani, Gaillard, et al., 2010; X. L. Yao, et al., 2013). In addition, it was shown that modified gums have the ability to decrease interfacial tension faster than unmodified gums, which is an important parameter in determining emulsifying capacity as noted above (Aoki, Katayama, et al., 2007; Castellani, Al-Assaf, et al., 2010; Castellani, Guibert, et al., 2010; X. L. Yao, et al., 2013). The improved stability of produced emulsions could be due partly to the higher viscoelasticity of interfaces formed from matured gums (Castellani, Al-Assaf, et al., 2010; Castellani, Guibert, et al., 2010). It is interesting to note that surface properties of matured gums are very close to that of the AGP fraction as obtained by HIC chromatography (Castellani, Gaillard, et al., 2010).

Although these results using matured AGs seem conclusive on the interfacial properties of high $M_w$ protein-rich macromolecules, some comments may be of interest. The first remark is that few studies have been done to definitely conclude on the benefit of matured gums regarding initial emulsion droplet size and emulsion stability. In addition, emulsions have never been prepared under the same experimental conditions (homogenizing conditions, gum concentration, oil to emulsifier ratio, dispersion pH, etc...), rendering the comparison of results difficult. For instance, it was shown that initial emulsion droplet size was significantly lower for oil to emulsifier ratios above 1 but was not different for ratios below 1 (Kateyama, et al., 2006). Another concern deals with the nature of supramolecular structures produced by Maillard reaction. Not only the AGP $M_w$ (and their concentration) increases but the composition of supramolecular structures change too with the incorporation of AGp and low
M₆ GP fractions (Aoki, Al-Assaf, et al., 2007). Obviously it can be anticipated that the global architecture of supramolecular structures is also modified. In these conditions, how to compare matured high M₆ macromolecules with a smaller AGP fraction of control gum?

Another approach to demonstrate the role of protein-rich high M₆ macromolecules in AG surface properties was to hydrolyze it with protease-type enzymes. In this case, loss of emulsification properties was observed (Chikamai, Banks, Anderson, & Weiping, 1996). Acacia senegal gum, hydrolyzed by pronase (a mixture of proteases) for 24h at 37 °C, did therefore form interfacial films less viscoelastic than with native AG (Elmanan, et al., 2008), in relation with the significant decrease of protein-rich high M₆ AGP concentration (Aoki, Al-Assaf, et al., 2007; Connolly, et al., 1987, 1988; Elmanan, et al., 2008; Randall, et al., 1988). However, by hydrolyzing AG while measuring interfacial viscoelasticity showed that, after 510 min hydrolysis, interfacial viscoelasticity decreased but remained high. It is possible that hydrolyzing the gum in the bulk or at an interface did not produce the same results. One can note that high M₆ macromolecules present in Acacia seyal gum are also hydrolyzed by pronase but to a lesser extent, which can be partly due to its more compact conformation (Elmanan, et al., 2008; Flindt, et al., 2005). As AGP architecture is modified after hydrolysis, one may wonder whether the loss in surface properties is due to a decrease in M₆ or to a change in macromolecular conformation. In fact, both chemical structure of the polysaccharide component (Connolly, et al., 1987; Mahendran, et al., 2008; Randall, et al., 1988) and AGP conformation remain largely unaffected by proteases, suggesting a self-similar structure for the AGP component (Renard, Lavenant-Gourgeon, et al., 2014).

Owing to the expected surface properties of protein-rich molecular fractions of AG, some scarce studies tried to unravel the specific properties of individual macromolecular components. Unlike one study where four fractions were obtained by SEC (Ray, et al., 1995), the remaining studies were classically concerned by HIC fractions, i.e. AGp, AGP and GP (Castellani, Al-Assaf, et al., 2010; Castellani, Gaillard, et al., 2010; Fauconnier, et al., 2000; Lopez-Franco, et al., 2004; Ray, et al., 1995). Among these studies, one of them studied the effect of fractions on oil-in-water emulsions characteristics (Ray, et al., 1995) and three studies focused on interfacial properties of fractions as determined by interfacial tension measurements and Langmuir-Blodgett films (Castellani, Al-Assaf, et al., 2010; Castellani, Gaillard, et al., 2010; Fauconnier, et al., 2000; Lopez-Franco, et al., 2004). It was clear from
these studies that GP fraction was the more efficient to decrease interfacial tension with values at equilibrium of about 23 mN.m\(^{-1}\) as compared to values of 30 mN.m\(^{-1}\) for AGP and about 45 mN.m\(^{-1}\) for AGp (Castellani, Al-Assaf, et al., 2010). It is important to note that experiments were done at 0.05wt% gum concentration and that similar experiments at higher gum concentration should be instructive. In terms of surface pressure and limiting area, GP fraction was again the more efficient and AGp fraction the less efficient (Castellani, Gaillard, et al., 2010; Fauconnier, et al., 2000; Lopez-Franco, et al., 2004). Results were unclear in terms of surface elasticity as determined from Langmuir-Blodgett film experiments. One study showed that the whole gum produced more elastic films than fractions which formed films with similar elasticity (Lopez-Franco, et al., 2004). Another study showed that films from AGp fractions were more elastic than films made from AGP or GP fractions, the latter being the less elastic (Fauconnier, et al., 2000). These results were obtained using the same Acacia \emph{senegal} gum concentration (10wt%) but different subphase composition, which may explain in part the observed differences.

Although there is no doubt about the important surface activity of AG protein-rich molecular fractions, and of the important role of high \(M_w\) fractions in the stabilization of emulsions, nitrogen content alone cannot be used to predict performance of AGs for emulsification. Gums with similar protein content may exhibit significant differences in emulsifying capacity and emulsion stability (Dickinson, et al., 1991b), confirming the view that it is the nature and distribution of the proteinaceous component of the gum which is important, not just its overall amount. \textit{Gums} with higher protein content do not \textit{also} necessarily produce more stable emulsions and some Acacia \emph{seyal} gum samples with a much lower protein content (0.8%) have been found to give better emulsion stability than Acacia \emph{senegal} gum (R. A. Buffo, et al., 2001). More surprisingly, it was shown recently that high \(M_w\) arabinogalactan macromolecules extracted from Peach exudate and not containing protein displayed better emulsifying and emulsion stabilizing properties than Acacia \emph{senegal} gum (Qian, Cui, Wang, Wang, & Zhou, 2011). It has been suggested that AGP surface properties depend considerably on the polysaccharide component (Goodrum, et al., 2000). Anderson (1978) suggested that the superior emulsifying power of gum Arabic may be related to the significant proportions (< 10 mol%) of terminal Rha groups, which possess hydrophobic centers (D. M. W. Anderson, 1978). In addition, the \(\beta-1,3\) galactan backbone should form an
amphipathic helix, and recently confirmed by Kitazawa et al. (Kitazawa, et al., 2013), the inside of the helix comprising a hydrophobic surface with the bulk of the galactan hydroxyl groups oriented toward the outer surface of the helix (Goodrum, et al., 2000). The “hydrophilic” carbohydrate blocks attached to the polypeptide chain could therefore have a substantial impact on the adsorption of AG onto hydrophobic surfaces.

The presence of surface active components in AG implies that the nature of dispersed phase is important to determine emulsion formation and stability. Based on the varying degree of stability against washing of emulsions made with different oils, it was suggested that the efficiency with which AG is adsorbed depends at least in part on the nature and polarity of oil (Dickinson, et al., 1991b; Shotton, et al., 1960). A non polar dispersed phase will therefore display a higher interfacial tension with water and will favor adsorption of the more tensioactive components. This is the basis of the extensive use of AG to stabilize orange oil emulsions, limonene, the main orange oil component, being a non polar molecule. This also may explain why the hydrophobic GP fraction adsors faster at interfaces than the AGP fraction when the non polar n-hexadecane is used (Castellani, Gaillard, et al., 2010). For instance, hexadecane-in-water emulsions made with AG are more stable than decanol-in-water emulsions (Chanamai & McClements, 2002). In addition, both polarity and water solubility of the dispersed phase influence the destabilization mechanism. With a high polarity and high water-soluble oil (e.g. decanol), oil-in-water emulsions are unstable to both Ostwald ripening and coalescence when stabilized by a weakly adsorbing biopolymer such as AG (Chanamai, et al., 2002). When a low polarity and high water-soluble oil is used (e.g. decane), emulsions are stable to coalescence, but unstable to Ostwald ripening. Finally, with low polarity and low water-soluble oil (e.g. hexadecane), emulsions are stable to both Ostwald ripening and coalescence (Chanamai, et al., 2002).

Finally, it is important to mention that trace levels of lipids, probably attached to the “AGP” macromolecules, would improve the surface properties of AG (M. P. Yadav, et al., 2007; M.P. Yadav, et al., 2012). This points out the potential role of minor components present in AGs on their surface properties, e.g. free proteins, peptides, oleoresin, feruclic acids. These low $M_w$ components could explain that some gums with low AGP concentrations, for instance lower than 10%, still display excellent ability to stabilize oil-in-water emulsions or that gums
with similar protein content and AGP concentration produce emulsions with significant differences in stability. This subject should deserve much more attention in the future.

4. Enzymatic modifications of Acacia gum

Modifications of AGs with enzymes first occurred during its maturation process. The gum from the earliest exudation, called “green gum”, is not entirely soluble yielding a glairy mucus-like fluid from which a perfect solution separates after a certain time. After storage during several months, a change is observed probably due to enzymes, so that gum becomes fluid and entirely soluble (Reinitzer, 1909). Neither the nature of these enzymes nor their origin is known. A recent Ph.D. student has studied these changes during gum maturation and it appeared that green gum displayed a peculiar rheological behaviour and was highly heterogeneous with part of kibbles containing very high M\textsubscript{w} AGP (Cozic, 2007). Upon storage, the gum recovered a classical distribution of molecular fractions.

Enzymatic modifications of AG, especially protein hydrolysis, were used primarily to demonstrate that most of proteins were associated with the higher molecular weight component of the gum and contribute to the elaboration of the wattle-blossom model (Connolly, et al., 1987, 1988; Randall, et al., 1988). It was then demonstrated that protease treatment of Acacia \textit{senegal} gum mostly degraded the protein-rich high M\textsubscript{w} component of the gum, i.e. both AGP and high M\textsubscript{w} GP fractions, but did not degrade or marginally AGp and low M\textsubscript{w} GP components (Aoki, Al-Assaf, et al., 2007; Connolly, et al., 1987, 1988; Flindt, et al., 2005; Mahendran, et al., 2008; Osman, et al., 1993; Randall, et al., 1988, 1989; Renard, Lavenant-Gourgeon, et al., 2014). The lower M\textsubscript{w} obtained after hydrolysis was in the range 1.7-2x10\textsuperscript{5} g.mol\textsuperscript{-1}, depending on the enzymes and conditions used with the lower values obtained with papain (Renard et al., 2014). This can be considered as the nominal building block of the gum as obtained by protein enzymatic hydrolysis. The limiting intrinsic viscosity values obtained were in the range 12-15 mL.g\textsuperscript{-1} (Chikamai, et al., 1996; Connolly, et al., 1987, 1988; Renard, Lavenant-Gourgeon, et al., 2014). The decrease of gum viscosity after enzyme hydrolysis has been suggested as a way to improve their processing (Chikamai, et al., 1996).

When using specific enzymes of polysaccharide such a \(\beta\)-galactosidase, a limited 8% decrease in galactose content was observed in parallel to a 27% increase in protein content (Chikamai, et al., 1996). Analysis by SEC showed a broadening of the AGP fraction and a
narrowing of the AGp fraction, GP fraction being unchanged. Interestingly, a recent patent reported the use of β-galactosidase to produce modified AGs with improved emulsifying properties (Heidebach, Sass, & de With, 2013). Very recently, a glucuronosyltransferase was isolated from Arabidopsis thaliana and used with AG. It was found that glucuronic acids were incorporated up to 1/3 of AG total weight. Oil-in-water emulsions made by the enzyme-modified gum arabic were slightly smaller in droplets size and remarkably more stable compare to emulsions made with native AG (Dilokpimol & Geshi, 2014).

5. Conclusions and future prospects

Acacia gum is a plant exudate mainly produced in sub-sahalian regions of Africa. It is a natural ingredient of geo-political and economic importance. Two gums are authorized for uses, Acacia senegal gum and Acacia seyal gum. The first one was the past the most used and studied whereas a growing demand of low cost natural gum can explain the growing part of Acacia seyal sales in recent years. Acacia gums are used by humans since prehistoric times and continue to be widely used today, the World demand having risen by 25% since the last ten years. Gums are mainly used in Food industry (confectionary, drinking industry) but also in non-food applications (pharmacy, cosmetics, materials).

Acacia senegal gums are composed of arabinogalactan-proteins (AGP) type biopolymers. It contains a continuum of hyperbranched amphiphilic charged polysaccharide-protein complexes differing by the amount of protein, type of sugars, sugar to amino-acid ratios, degree of branching, conformation and physicochemical properties. It also contains minor components such as minerals, polyphenols and traces of lipids. Arabinogalactan-proteins in Acacia senegal gums have generally anisotropic shapes and can be described as highly porous ellipsoidal objects. In fact, these macromolecules are kinds of sponges, which can explain their ability to interact with different kinds of entities (e.g. proteins, minerals, polyphenols, etc...). Besides their known biological properties, Acacia gum biopolymers display interesting functional properties such as high affinity for water, low newtonian viscosity even at quite high gum concentrations and the ability to adsorb and stabilize gaz-liquid, liquid-liquid and solid-liquid interfaces. Surface properties of gums are strongly related to the presence of protein-rich high molecular weight species.
Based on the literature survey, socio-economic challenges and our own work, some important issues and bottlenecks can be identified both on scientific and technological point of views. Some of the most relevant points, in our opinion, are described in the following.

Maturation mechanisms of Acacia gums: once exuded, gums evolve with time through sun drying, oxidation, (enzymatic) hydrolysis and interactions between AGP and between AGP and minor components upon storage and processing. The consequences are a change in biopolymer composition and distribution, structure and functional properties. Probably one of the most challenging questions is the mechanism leading to the known gum from the “green gum”. Initial glairy gum is composed by very high $M_w$ AGP that imparts specific rheological behaviour. With time, even in the dry state, the $M_w$ of AGP decreases, which is may be caused by the action of some glycosidases and/or proteases. The presence of these enzymes has never been reported. Apart from hydrolysis, gum can also be oxidized which can have significant functional consequences, especially when it is rich in polyphenols.

Composition, structure and functional properties of Acacia *seyal* gum: for economic reasons, Acacia *seyal* gum represents actually 50-75% of sales. However, it has been much less studied than Acacia *senegal* gum. The differences between both gums in terms of sugars and amino-acid compositions are mostly known, as well as the major differences in $M_w$ distribution. However, Acacia *seyal* gum is richer in minerals and polyphenols, less rich in proteins, more compact, more unstable in solution, less charged, less surface active, less hydrolysable by enzymes. The structure and conformation of AGP from Acacia *seyal* are unknown and their functional properties badly known. We do not know the reasons for the molecule compactness and whether such a compactness can be modified changing the solvent polarity or temperature. We know that assemblies with proteins are possible but stabilization of assemblies appear difficult. We do not know the structure of oil-in-water interfaces covered by this gum. However, we know that stabilization of emulsions or suspensions can be achieved using high gum concentrations. The presence of polyphenol can be detrimental as far as solubility is concerned, however this could open the possibility to crosslink AGP molecules by oxidative enzymes.

Nature and concentration of minor components: Acacia gums contained 3-6% of minor components which deserve much more attention. Part of variability in gum functional
properties must originate from these minor compounds. Among these components, the major part is composed by minerals (3-5%). The concentration and nature of ions are important since they are supposed to impact the charge density of AGP, which in turn plays an important role in the hydration, solubility and compactness of AGP as well as in the stabilization of colloidal suspensions. Polyphenol type molecules are present in colored gum nodules depending on the gum type (Acacia \textit{senegal} vs Acacia \textit{seyal} gum) and origin. The nature of these polyphenols and their concentration need to be determined; they can result in oxidation phenomena and modify functional properties of AGP. Traces of lipids (the GPI anchor) linked to high M\textsubscript{w} AGP has been reported in Acacia \textit{senegal} gum. It is obvious that the presence of lipids impacts the gum surface properties. We need to know whether lipids are present, at which concentration and whether they are limited to GPI or other kinds of lipids. When we perform SEC-MALLS experiments on gums, UV profiles show invariably the presence of highly rich-protein molecules with low M\textsubscript{w} (long elution times) at very small concentrations. These molecules could be free proteins or oligosaccharide-proteins issued from AGP degradation. It cannot be ruled out that these molecules could be some of the enzymes mentioned in the literature, e.g. oxidases, or may be hydrolase-type enzymes responsible of the time-dependent maturation of gum. We know that these enzymes are present but we do not know their nature or origin.

**Structure of arabinogalactan-proteins (AGP):** all biopolymers in Acacia \textit{senegal} and \textit{seyal} gums are hyperbranched arabinogalactan-proteins with various M\textsubscript{w}, size, sugar or amino-acid composition, sugar to amino-acid molar ratio, charge density and hydrophobicity. We know that protein-rich high molecular weight fractions have important surface properties and the ability to stabilize colloidal suspensions. We know that these fractions display anisotropic conformations. However, results were obtained on mixtures containing fractions in various proportions including gum aggregates. Highly purified molecules are then needed to provide new structural insights through the use of combined approaches, for instance using Hydrophobic Interaction Chromatography (HIC) then Size Exclusion Chromatography (SEC). Other possibilities are to combine controlled alcohol or salt-induced precipitation and HIC, SEC or Ion-Exchange Chromatography. The architecture of fractions remains unclear despite recent advances. The organization of sugar blocks onto the polypeptide or polypeptides chains is unknown, e.g. nature of interactions between sugar chains and between sugar
chains and polypeptides, steric constraints, possible secondary structures of sugar blocks (e.g. helix of the β 1,3- galactan backbone). In addition, the global structural organization of the molecules is directly related to the amino-acid sequences of polypeptides that determine the linking of sugar blocks. These sequences are unknown for Acacia gum. Determine the amino-acid sequences is challenging because sugar blocks have to be degraded either by chemical or enzymatic treatments or both. Please note that the charge density of AGP should be determined as well, both to better understand the structure and the macromolecular functional properties.

Functional properties of Acacia gums and their arabinogalactan-proteins (AGP) fractions: functional properties (hydration, assembly, rheological and surface properties) of Acacia senegal gum have been characterized in more or less details. This is not the case for Acacia seyal gum. A number of issues remains (solvent affinity, formation of aggregates, unusual rheological behavior, etc.) but the most important one concerns the functional properties of the different AGP fractions that have been rarely studied so far. Only one paper reports emulsions made with the two major fractions purified from HIC (HIC-F1 and HIC-F2). One can find in the literature some conflicting data on interfacial properties of F1, F2 and F3 but fractions were not highly purified and no systematic investigation of experimental conditions was tried (gum concentration, pH, ionic strength, nature of oil, temperature). It is impossible to understand and control gum functional properties without a precise knowledge of fraction functional properties. Today, we have no idea about their hydration properties, their rheological properties, their solution properties (phase behavior, assembly) depending on the solvent polarity, their surface properties. In addition, the question of functional synergism between fractions deserves some attention.

Solution properties: solvent affinity, phase behavior, formation and dynamics of aggregates: we have noted recently aggregation-disaggregation phenomena involving protein-rich AGP of Acacia senegal gum depending on gum concentration. This suggests that some molecular fractions display different solvent affinities. This can be seen as well through the huge hydration properties of the soluble part of F3 fraction as compared to total gum, F1 or F2 fractions. The effect of solvent polarity on the dynamics of intra- and inter-molecular interactions between AGP should be studied in relation with the ionic strength, the chaotropic solvent and temperature, then varying the strength of hydrogen bonding,
electrostatic and hydrophobic interactions. Building phase diagrams should allow predicting stability of gum solutions depending on environmental conditions. The formation of a small concentration (1-2%) of AGP-based aggregates is mostly unavoidable and participates to the variability in gum functionality.

**Rheological properties: unusual flow behaviour and viscoelastic properties:** shear-thinning and time dependent flow of diluted Acacia *senegal* gum is probably due to surface effects and may be to AGP-induced aggregation. Performing rheo-SAXS experiments will help to clarify the situation. At high concentrations, gum solution display viscoelastic properties seemingly dominated by the viscous component. We do not know whether highly concentrated solutions can be characterized as soft colloidal gels or polymeric gels and what is the role of AGP fractions on viscoelastic properties.

**Emulsifying-stabilization properties of Acacia gum: processing conditions, role of AGP, structure and mechanical properties of interfaces:** a huge number of studies have reported the formation and stabilization of oil-in-water emulsions based on Acacia *senegal* gum. No clear picture emerged from such studies about the intricate relationships between gum concentration, nature of oil phase (hydrophobicity), protein-rich AGP concentration and homogenization energy. The important role of protein-rich fractions makes no doubt, however it seems to depend on gum concentration, hydrophobicity of dispersed phase and quantity of interfaces. The relative contributions of the different fractions during the full emulsification process need to be clarified. Solid-like viscoelastic properties of interfaces and electrosteric repulsion mechanisms stabilize emulsions. Depending on process conditions, the structure of interfaces remains unknown, especially in terms of thickness (monolayers vs multilayers), surface gum concentration and topology of fractions. These characteristics will affect emulsion stability and for instance permeability to hydrophobic molecules such as aroma.

**Nano/microparticles-based on AGP assembly with other biopolymers:** formation, stabilization, industrial scaling: the formation of nano/microparticles based on electrostatic interactions between Acacia *senegal* gum and proteins is not a difficult task at the lab scale. On the other hand, the stabilization of these assemblies against changes in pH or ionic strength is a bottleneck that has limited up to now their industrial applications. Since
assemblies are obtained through weak interactions, cross-linking stabilization without chemical treatments imply either the use of enzymes or physical treatments. Using enzymes to stabilize Acacia gum-protein microparticles has been reported, this is a possible but expensive way. Another way is protein denaturation by heating, which has been proven to be efficient at the lab scale. An important challenge is the formation and stabilization of Acacia gum-protein nano/microparticles at the industrial scale. Issues about mixing conditions and heating in large volumes need to be considered.

**Enzymatic modification of Acacia gum:** the possibility to modify the structure and functional properties of Acacia gums clearly represents the best future way of innovation. Actually, the only way to propose new ingredients based on Acacia gums is i) to enrich them with AGP, ii) to purify AGP, iii) to assemble them with proteins, iv) to graft lipids by chemical reaction. Enzymes can potentially expand the modification possibilities of gums. Two ways should be explored. The first one concerns hydrolysis of gums. This can be achieved either by the use of proteases or/and glycosidases. Proteases have demonstrated their ability to degrade Acacia gums and to significantly decrease their viscosity, which is of particular use in industry. However, protein-rich fractions are degraded, which impairs their surface properties. A major bottleneck is then to decrease the gum viscosity while maintaining high surface properties. The use of glycosidases may be a solution, however their efficiency is impaired by the hyperbranched characteristic of gums. Fortunately, a number of enzymes or enzymatic cocktails are actually available and must be screened. Enzymes with more specific activities could be found but the questions of their availability and costs should be questioned. In particular, the possibility to increase the concentration of charged carboxylate groups removing methyl groups appears interesting. The second way, probably the most promising, is to graft onto the gum specific molecules or chemical groups. Then one can imagine to graft lipids or oligopeptides to improve amphipathic properties of gums or to graft carboxylate groups to increase the gum charge density. It remains to find efficient enzymes compatible with an industrial use then to optimize grafting processes. Finally, one major bottleneck is to crosslink gum molecules by enzymes to form 100% gum nano/microparticles. This should open a lot of new applications as texturing agents and stabilized microcapsules for aroma protection.

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Figure captions

Figure 1: $R_h$ conformation plots of spray-dried (black) and raw (red) Acacia senegal (A) and seyal (B) gums. 202 spray-dried and 100 raw A. senegal gums, 28 spray-dried and 6 raw A. seyal gums were analysed (unpublished data).

Figure 2: (left) Elementary building blocks 1 and 2 used to build arabinogalactan-protein (AGP) macromolecular assembly from Acacia Senegal gum. Building block 2 was built by a linear arrangement of five building blocks 1. (right) Three-dimensional model of AGP made of the assembly of 6 building blocks 1 and 8 building blocks 2 by covalent and/or non-covalent interactions. The elementary building blocks were constructed taking into consideration the quasi-palindromic nature of polypeptide backbones and as a consequence the overall symmetry, or self-ordering, of carbohydrate moieties and arabinoside oligomers attached to it. The self-similarity approach was then used to build the AGP macromolecular assembly from the elementary building blocks 1 and 2 (adapted from Renard et al., 2014).

Figure 3: Effect of concentration on the relative newtonian viscosity ($\eta/\eta_0$) of Acacia senegal gum dispersions. Data were taken from Taft and Malm (1931), Riddell and Davies (1931), Briggs (1941), Schleif et al. (1951), Williams et al. (1990), Goycoolea et al. (1995), Mothe and Rao (1999), Sanchez et al. (2002) and Li et al. 2009. Gum samples were from different geographical origins. Measurements corresponded to different pH (4-8), temperature (20-30°C) and ionic strengths. Solid line (this paper), dashed lines (Gooycoolea et al., 1995) and dot lines (Gaïa et al., 1981) are exponential fits of data (unpublished data).

Figure 4: Phase diagram of Acacia senegal gum-ethanol-water system determined by the cloud point method (pH 5 citrate buffer, 25°C). Appearance of turbidity was checked by UV-Vis spectroscopy. The solubility curve appears in red and delineates the monophasic (I) from the biphasic (II) region. Inset: Micrographs of Acacia senegal gum-ethanol-water system with concentrations of 45% ethanol and 20 g.L$^{-1}$ gum. The arrow in the phase diagram indicates the composition of the mixture. Scale bar: 5 µm (unpublished data).
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