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Polysaccharides: Structure and Solubility

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Abstract

Understanding the solubility of polysaccharides is extremely important for their food applications as most functions of polysaccharides including stability, emulsifying property, drug delivery, membrane forming properties, etc., are all achieved in aqueous solution. This chapter aims specifically at the mechanism of solubility of polysaccharides from the molecular level. General understandings of the solubility including definition, testing methods, and the solution behaviors were provided; the relationships between polysaccharide solubility and the structural features in terms of molecular weight, degree of branching, charging properties, chain flexibility, and the special groups were all discussed. With all the information provided, the molecular modification and further applications of polysaccharides in both food and nonfood areas could be promoted.

Keywords: polysaccharides, structure, solubility, conformation, solution behavior

1. Introduction

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic bonds. Natural occurring polysaccharides exhibit distinct structural features in terms of molecular weight, monosaccharide composition, glycosidic linkage patterns, configuration (α or β), charging properties, degree of branching, etc. These diversified structural properties determine the functional properties of polysaccharides, such as solubility and rheological properties, which in turn benefit their extensive applications in both food and nonfood areas. As most polysaccharides perform their function in aqueous solution, understanding the solubility of polysaccharides, therefore, becomes critically important [1]. This chapter specifically addressed the mechanisms of polysaccharide solubility from molecular level. The relationships between polysaccharide solubility and molecular structures were established. It also should be noted that the current chapter only focused on the polysaccharides in aqueous solution; the solubility of polysaccharides in other organic solvents was not covered.

2. Solubility of polysaccharides

Polysaccharides display a wide range of solubility; some are water insoluble, e.g., cellulose; some are only hot water soluble, e.g., starch; and some are readily dissolved in cold water, such as pullulan and gum arabic. The dissolution of polysaccharides is different from that of the small crystalline molecules. The dissolution of most crystalline small molecules involved the disintegration of the crystalline structure and release of the separate atoms, ions, polysaccharides dissolution is a continuous hydration process with the conversion of inter-polysaccharide binding to polysaccharide-water binding, and most of the non-starch polysaccharides are in amorphous state. The dissolution process is more or less assisted by entropy as the molecules assume lower-energy conformations.

Polysaccharides have strong affinity to water molecules due to the presence of multi-OH groups. However, this also leads to a strong interaction among polysaccharide molecules via hydrogen bonding. Therefore, the balance between molecule-molecule interaction and molecule-water interaction is the key to understand the polysaccharide solubility. For soluble polysaccharides, the interactions between polysaccharide molecules and water molecules are energetically favorable, and the solvent creates a solvating envelope around the polymer chain, which keeps the polysaccharide molecules away from each other. For polysaccharides with poor water solubility, the intramolecular interactions between polymer segments dominate, leading to aggregation and eventually precipitation or gelation when ordered molecular structure, e.g., junction zone, is formed. In between, under specific conditions, the polymer-polymer interaction can precisely compensate the polymer-water interaction, which referred as *theta* condition. Under *theta* condition, the chain conformation is defined solely by bond angles and short-range interactions given by the hindrances to rotation about bonds and polymer coil dimensions [2].

Second virial coefficient (A_2), which describes the contribution of the pair-wise potential to the pressure of the gas, could reflect the polymer-water interaction. Good solvent, poor solvent, and *theta* condition can be indicated when the second virial coefficient is above, below, or equal to zero, respectively. In a real experiment, the second virial coefficient of the polysaccharides in aqueous solution can be determined by static light scattering using Zimm plot (**Figure 1**) based on the Eqs. (1–2) [3]:

$$Kc/R_\theta = 1/M_w + 1/3 \left(R_g^2/M_w \right) q^2 + 2A_2c \quad (1)$$

where K is an optical contrast factor, c is the polymer concentration, R_θ is the Rayleigh ratio (normalized scattering intensity), and the scattering vector q is defined as

$$q = 4\pi n_0 \sin(\theta/2)/\lambda_0 \quad (2)$$

with n_0 as the refractive index and λ_0 as the wavelength in a vacuum.

For example, A_2 of xyloglucans from flaxseed kernel cell wall was reported as 3.9, 0.9, and $-0.54 \times 10^{-4} \text{ cm}^3 \text{ mol/g}^2$ in 0.5 M NaOH solution, H_2O , and 0.1 M NaNO_3 solution, respectively, indicating xyloglucan prone to dissolve in 0.5 M NaOH than 0.1 M NaNO_3 [4]. 0.5 M NaOH

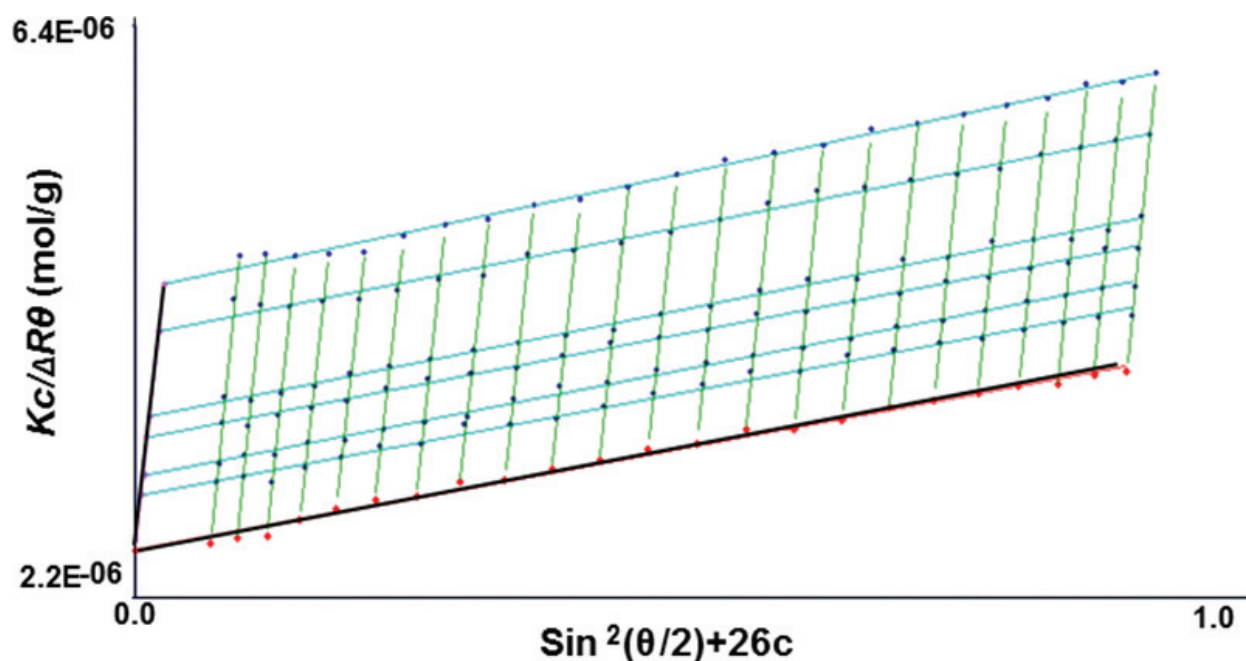


Figure 1. Zimm plot of polysaccharide from seed of *Artemisia sphaerocephala* Krasch determined by SLS (solvent, 0.5 M NaOH; temperature, 25°C). Adopted from Guo et al. [3].

has been reported as a good solvent for several different polysaccharides, such as β -glucan [5], arabino-glucuronoxylan isolated from seeds of *Artemisia sphaerocephala* Krasch [3], and gum ghatti [6]. This is because mild alkaline could break down the intermolecular hydrogen bonding, thus eliminating the aggregates in aqueous solution.

3. Structure and solubility relationships

The solubility of polysaccharides is determined by their molecular structures. Any structural feature that hinders the intermolecular association leads to higher solubility, such as branching structure, charged group (carboxylate group, sulfate, or phosphate groups); on the opposite, structural characters that promote the intermolecular association result in a poor solubility, such as linear chain, large molecular weight, and other regular structural characters.

3.1. Molecular weight and molecular weight distribution

Polysaccharides are polydisperse in molecular weight. Therefore the molecular weight of polysaccharides is mostly described in a statistic way, such as number average molecular weight (M_n), weight average molecular weight (M_w), and zeta average molecular weight (M_z), as shown in the below equations (Eqs. (3–5)). Here C_i refers to the concentration of molecules that having molecular weight of M_i . The molecular distribution of polysaccharides can be described by the polydispersity index (Eq. (6)). For monodispersed polymers, $PDI = 1$, while PDI less than 1.2 and above 2 are generally regarded as narrow disperse and wide disperse, respectively. Most natural occurring polysaccharides demonstrated high PDI value (above 2):

$$Mn = \frac{\sum C_i}{\sum C_i/M_i} \quad (3)$$

$$Mw = \frac{\sum M_i C_i}{\sum C_i} \quad (4)$$

$$Mz = \frac{\sum M_i^2 C_i}{\sum M_i C_i} \quad (5)$$

$$PDI = \frac{Mw}{Mn} \quad (6)$$

The molecular weight and molecular weight distribution play a critical role for the solubility of polysaccharides. High molecular weight molecules normally have a large excluded volume (Eq. (7)) that promotes intermolecular interaction of polysaccharide, and suppress its solubility. Almost all carbohydrate polymers with degrees of polymerization (DP) less than 20 are soluble in water [7]. Solubility decreases with the increase of molecular weight. For example, the amylose and amylopectin in starch are reluctant to dissolve in cold water due to high molecular weight, while maltodextrin (starch after chain cleavage by acid or enzyme) with the DP value less than 20 demonstrates very good solubility in cold water. The dissolution rate of polysaccharide samples is also highly affected by the molecular weight and molecular weight distribution. Higher molecular weight usually leads to lower dissolution rate, as disentanglement from the particle surface and subsequent diffusion to the bulk solution of large molecules take a longer time compared to that of small molecules. It has also been reported that samples with high polydispersity dissolved about twice as fast as monodisperse ones of the same Mn [1]:

$$V_h = \frac{[\eta]M}{\gamma N_A} \quad (7)$$

where V_h is the hydrodynamic volume, M is molar mass, N_A is Avogadro's number, $[\eta]$ is intrinsic viscosity, and γ is Simha's parameter related to the shape of a particle.

3.2. Charged polysaccharides vs. neutral polysaccharide

Charged polysaccharides are referred to polysaccharides that carry charged groups in the molecules, which include both negatively (acidic polysaccharides) and positively charged polysaccharides. The charged groups help with the solubility of polysaccharides, which is achieved by (1) increasing the molecular affinity to water and (2) preventing the intermolecular association due to the electrostatic effects posed by the charged group.

Acidic polysaccharides are polysaccharides containing carboxyl groups (e.g., pectin) and/or sulfuric ester groups (e.g., carrageenan). As shown in **Figure 2**, pectin is a polysaccharide containing the majority of α -(1-4)-linked D-galacturonic acid unit in the backbone and a small percentage of branching portion (rhamnogalacturonan I and II). The acidic group may be free (or as a simple salt with sodium, potassium, calcium, or ammonium) or naturally esterified with methanol. Therefore, most of the natural occurring pectin is readily soluble in water due

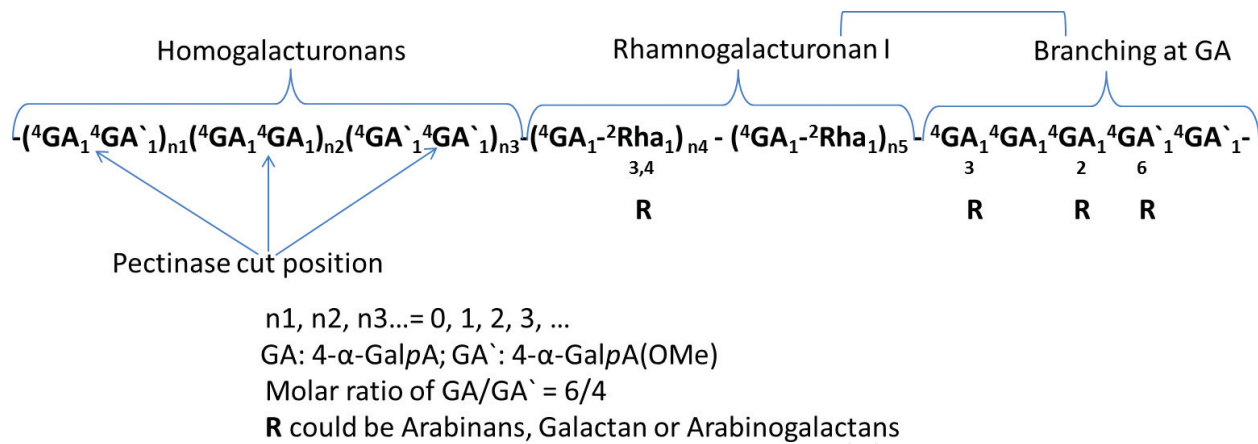


Figure 2. Pectic polysaccharides from American ginseng. Adpated from Guo, et al. [9].

to the charged group, although high in molecular weight. It also should be noticed that adding salt or reducing pH value could shield the charged effect, which leads to gelation under some circumstances. For example, high methyl ester pectin gel at pH 3.4 (rapid set pectin) or 3.2 (slow set pectin) with the presence of sucrose. Low methyl ester pectin can react with calcium ions to form gel, even under relative high pH environment. Therefore, when dissolving the pectin into water, it is essential to avoid the gelling condition; similar to other hydrocolloids, the dissolution usually needs high shearing mixing [8].

Carrageenans are a family of linear sulfated polysaccharides that are extracted from red edible seaweeds with the repeating unit of sulfate esters of 3- β -D-GalP and 3,6-anhydro- α -D-GalP. There are three types of commercial carrageenans including κ -carrageenan, λ -carrageenan, and ι -carrageenan, which showed different structural features regarding the number and position of sulfate groups in the repeating unit as shown in **Figure 3**. The higher levels of ester sulfate in λ carrageenan (three sulfate groups per repeating unit) contribute to gel inhibition and result in good water solubility. With the decrease of sulfate groups in the repeating unit, carrageenan start to form soft gel (ι -carrageenan, two sulfate groups per repeating unit) and rigid gel (κ -carrageenan, one sulfate group per repeating unit).

As one typical positively charged polysaccharide (**Figure 4**), chitosan is derived from the deacetylation of chitin. The positively charged groups come from protonation of its free amino groups, which is the key to its water solubility. Chitosan is insoluble in neutral and basic environments due to the lacking of a positive charge. However, in acidic environments, protonation of the amino groups increases the degree of water solubility. Following this property, chitosan has been widely used for drug delivery, e.g., the transport of insulin [11].

3.3. Branching polysaccharides vs. linear polysaccharides

The linear polysaccharides with highly regular conformation that can form crystalline or partial crystalline structures are mostly insoluble in water, while branching structure could increase the solubility for two reasons: (1) the branching structure could weaken the intramolecular interaction due to the steric effects, which prevent the intermolecular association, and

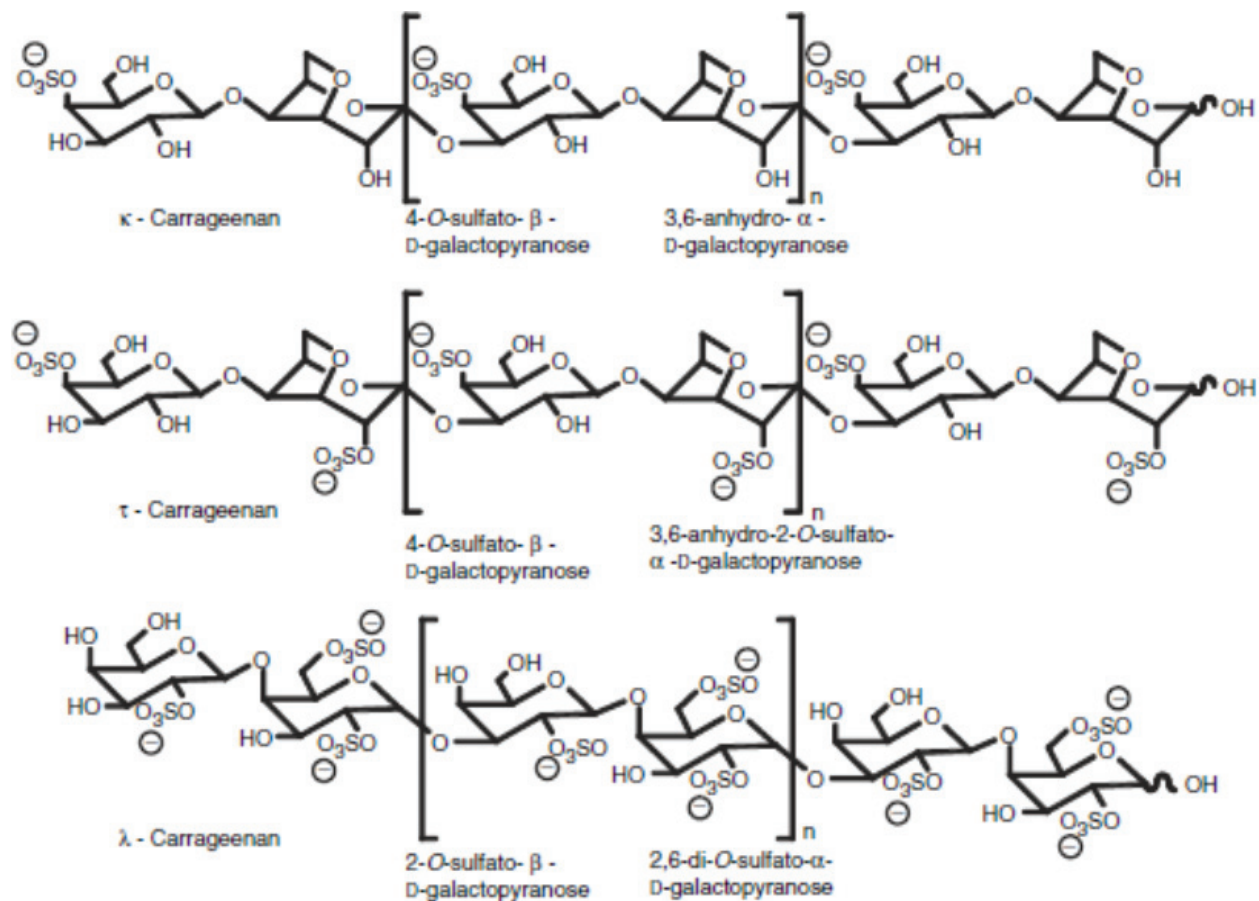


Figure 3. Comparison of structural features of κ-, λ-, and ι-carrageenan. Adopted from Cui et al. [10].

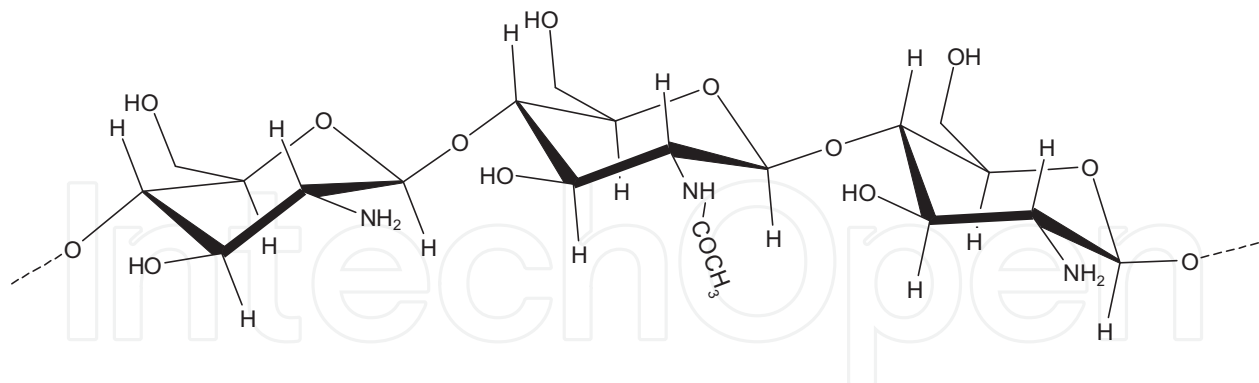


Figure 4. Structural feature of chitosan.

(2) the highly branched structure could also decrease the excluded volume when compared to polysaccharides with same molecular weight, which potentially increases the critical concentration and therefore improves the water solubility.

Taking cellulose as an example, cellulose with a regular (1 → 4)-β-D-glucan chain is essentially insoluble in aqueous medium. However, it can be modified by decreasing the Mw and introducing either charged (sodium carboxymethyl cellulose (CMC)) or branching groups (methyl cellulose (MC), hydroxylpropyl cellulose (HPC), hydroxylpropyl methyl cellulose (HPMC)) to

increase the solubility (**Figure 5**). MC, HPMC, and HPC all can dissolve in cold water and are insoluble in hot water. MC and HPMC form gel when the temperature increases (52°C for MC and 63–80°C for HPMC), while HPC cannot form gel when heating, instead, it precipitates when temperature is above 45°C. Unlike MC and HPMC, CMC is well dissolved in both cold and hot water [12].

Starch contains both amylose and amylopectin. Amylopectin exhibits better solubility than amylose due to the highly branched structure, although the latter has relative low molecular weight (amylose, 10^5 ; amylopectin, 10^7 – 10^9). According to the structure and solubility difference, amylose and amylopectin can be separated from each other in starch granules according to the following procedure: firstly, starch granules are completely dispersed in hot water or aqueous dimethyl sulfoxide; amylose then can be precipitated by the addition of butanol (as a crystalline complex due to the linear structure) after cooling. Afterward, amylopectin can be recovered from the supernatant by lyophilization [13].

Guar gum and locust bean gum both belong to the galactomannan family (**Figure 6**), while the degree of branching for guar gum (galactose to mannose 2:1) is higher than locust bean gum (galactose to mannose about 4:1), which could easily prevent strong cohesion of the main backbones of different neighboring molecules, so that no extensive crystalline regions of guar gum can be formed, while locust bean gum is easy to form gel due to the naked region of the molecules, which favors the formation of junction zone [14].

Xylans of all higher plants possess (1–4) linked D-xylP residues as the backbone, substituted by various degrees with sugar units including arabinose, xylose, and glucuronic acid (4-O-methyl). (1–4) linked D-xylP as a linear chain showed the least solubility as it can be regarded as cellulose

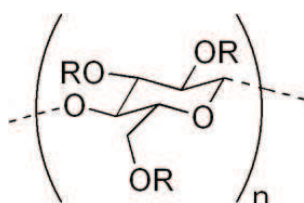


Figure 5. Schematic chart for cellulose derivatives. R=H or CH₃ for methyl cellulose; R=H or CH₂COOH for CMC; R=H or CH₃ or CH₂CH(OH)CH₃ for HPMC; and R=H or CH₂CH(OH)CH₃ for HPC.

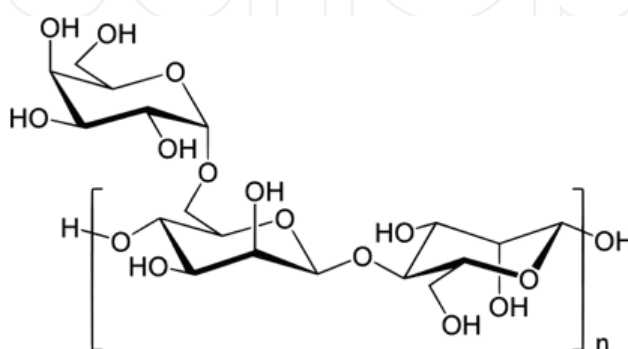


Figure 6. Schematic chart for galactomannan structure.

with hydroxymethyl group replaced with H. However, with the increase of degree of substitution such as arabinose (arabinoxylan) (as shown in **Figure 7**), its solubility dramatically increased [15].

Gum arabic has a highly branched structure (**Figure 8**). Although with a relatively high molecular weight, gum arabic showed excellent water solubility (up to 30% at room temperature). It has been reported that even 30% gum arabic solutions have a lower viscosity than 1% xanthan gum and sodium carboxyl methylcellulose at low shear rates. Gum arabic has been commercially used as emulsifiers due to the covalent bond with protein, in which protein functioned as hydrophobic group attached to the oil droplet and keeps the whole emulsion system stable. Similar to gum arabic, gum ghatti (**Figure 9**) also contains highly branched structure and demonstrated excellent water solubility (up to 20%). According to methylation analysis [16], the

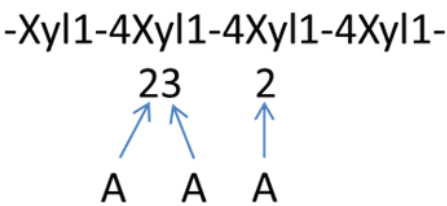


Figure 7. Schematic chart of arabinoxylan. A: T-AraP.

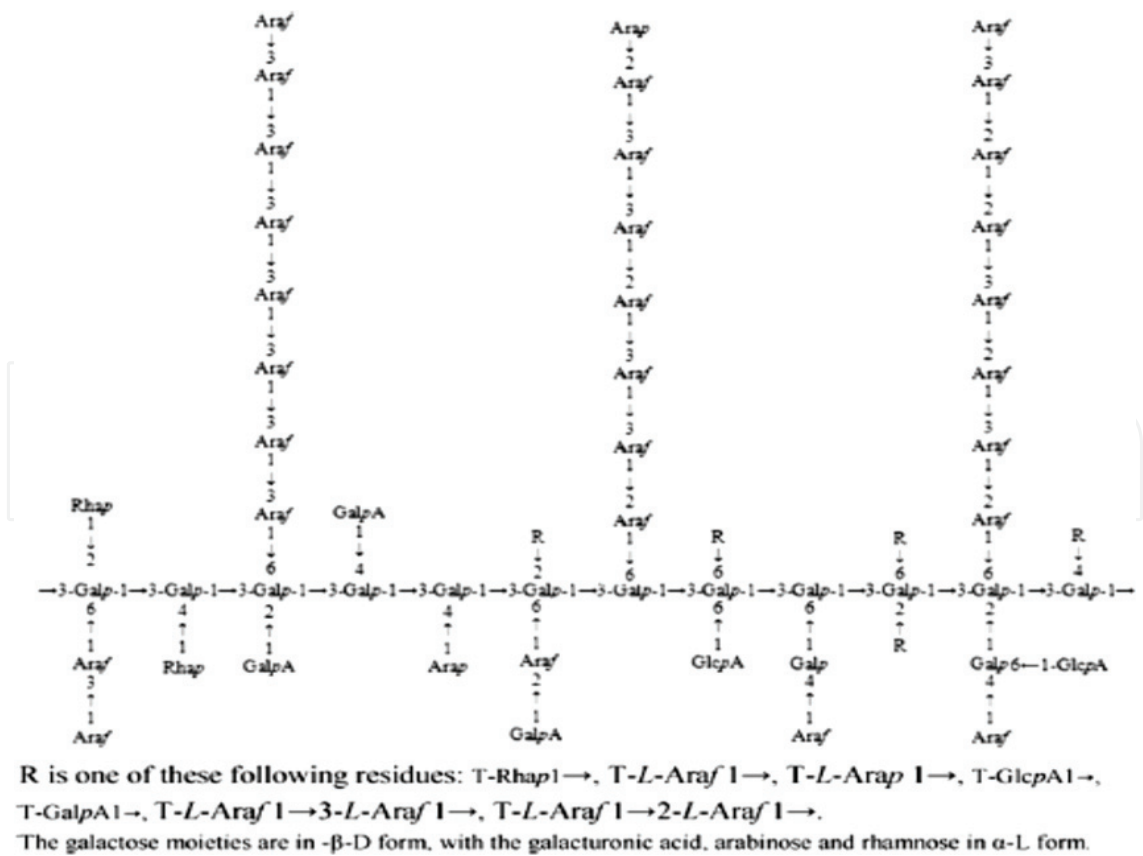


Figure 8. Proposed structure of gum arabic (Acacia senegal), adopted from Nie et al. [17].

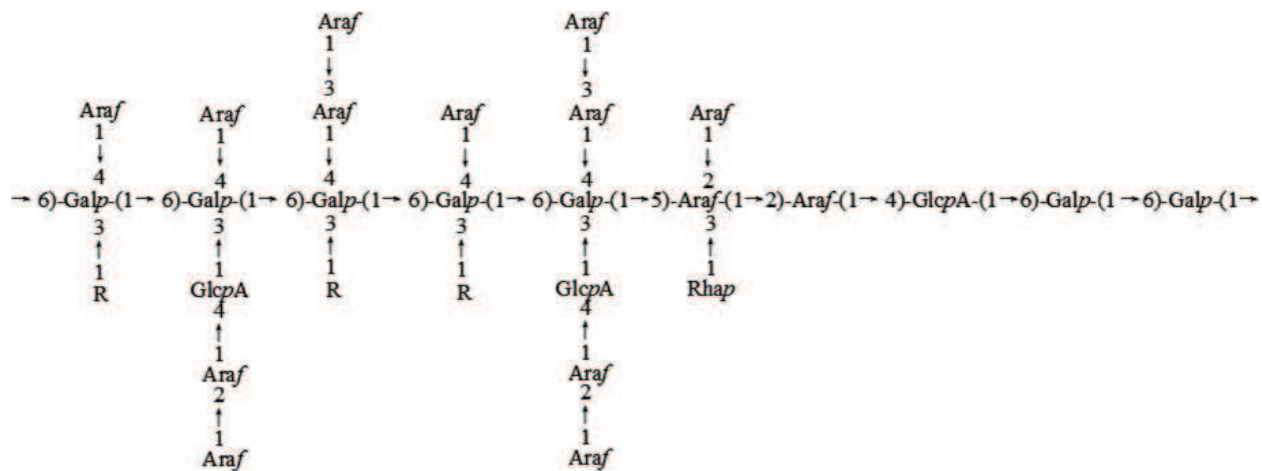


Figure 9. Proposed structure of gum ghatti, adopted from Kang et al. [18].

most branched sugar residues in gum ghatti were 3,4,6-Galp (14.20%), 3,6-Galp (5.10%), 2,3,6-Galp (4.87%), 2,3,5-Araf (4.09%), and 2,3-Manp (2.34%), respectively. The good solubility of gum ghatti could also be attributed to the 1–6 linked glycosidic bonds, which will be discussed in the later session.

3.4. Hydrophobic groups

Similar to branching effects, the presence of some hydrophobic groups, e.g., O-Ac and O-Me, could also affect the solubility of the polysaccharides.

O-acetyl substituents are present on many cell wall polymers including various hemicelluloses (**Figure 10**), the pectic polysaccharides and the polyphenol lignin, which have been previously summarized [19].

It has been reported that the totally acetylated xylan (DA 2.0) only dissolves in nonpolar solvents like chloroform or polar aprotic solvents like dimethyl sulfoxide, whereas the weakly acetylated xylan (DA ~0.5) is totally soluble in water, and the non-acetylated xylan (DA 0) is only partially soluble in hot water, due to spontaneous intramolecular hydrogen bonding [20].

Similar conclusion has also been drawn from acetylated glucomannan. One example is polysaccharides from *Dendrobium officinale* (traditional Chinese herbs), which belong to glucomannan family but highly acetylated, as shown in **Figure 11**. This polysaccharide is readily dissolved in water. The solubility, however, is significantly decreased if the acetyl group was either removed through alkaline treatment or increased through acetylation reaction. It has also been reported that the immunomodulating effect of this polysaccharide was also affected by the acetyl group [21].

3.5. Linkage pattern and conformation of sugar residues

The conformation can be simply regarded as the way that polymer chains align themselves in solution to adopt an orientation with lower energies. Two general types of conformation

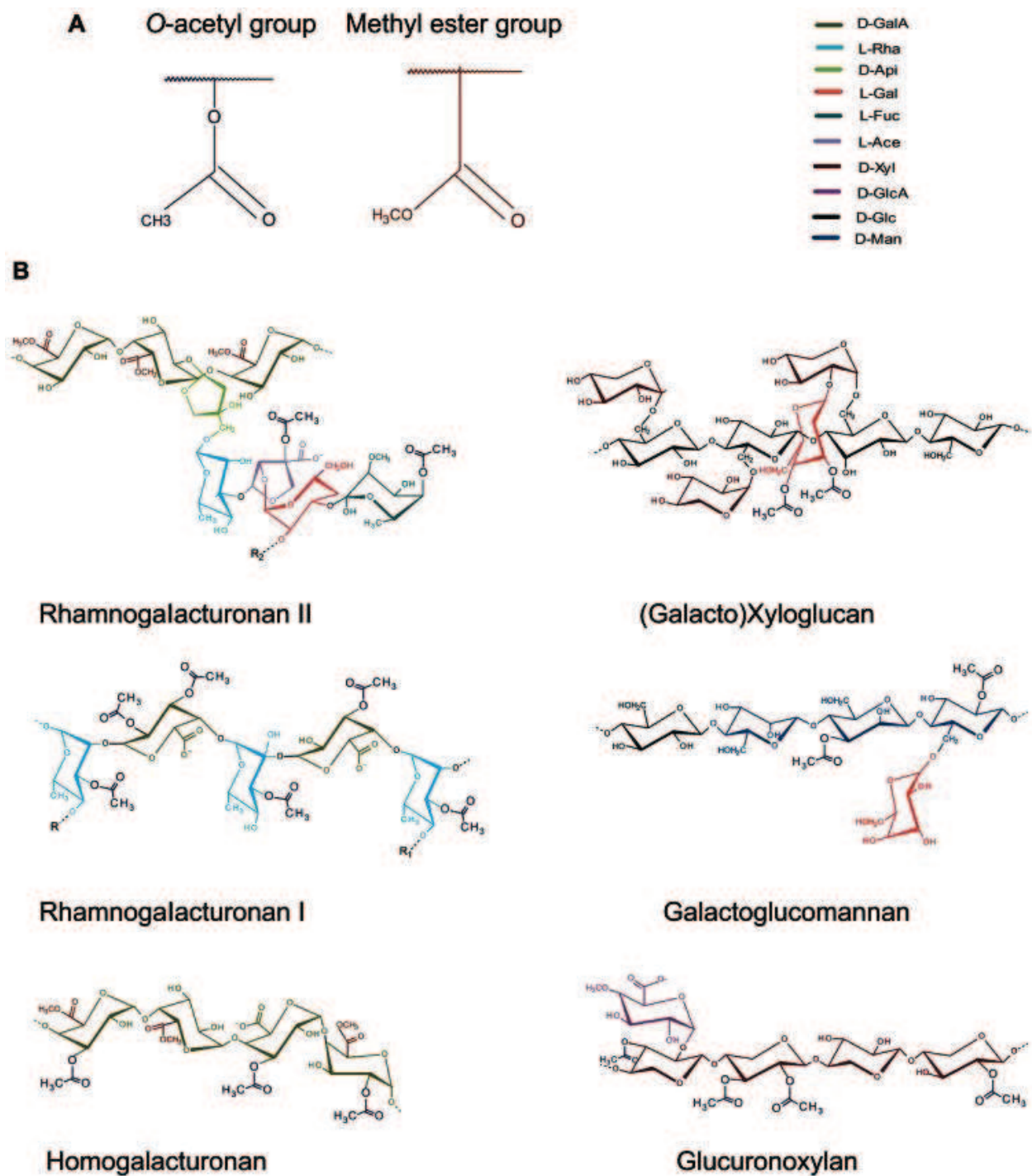
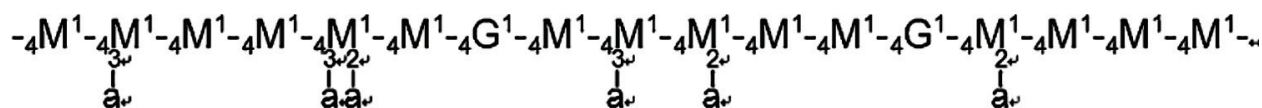


Figure 10. *O*-acetylation of cell wall polysaccharides. (A) Generic representation of *O*-acetyl group as found at different -OH positions in many cell wall polysaccharides. Note the structural similarity between *O*-acetyl- and methyl ester groups that decorate carboxylic acid residues in polygalacturonic acid. (B) Occurrence of *O*-acetyl groups in cell wall matrix polysaccharides. Adopted from Pawar et al. (2003) [20].

for polysaccharides can be simply divided—ordered conformation and disordered conformation—which is decided by the regularity of the molecular structure. In aqueous solution, most of non-starch polysaccharides with heterogeneous structure demonstrated disordered



M: β -D-pyranosyl-mannose; G: β -D-pyranosyl-glucose; a: O-acetyl group

Figure 11. Proposed structure of polysaccharides from *Dendrobium officinale*. Adopted from Xing et al. [22].

conformation, including random coil, rigid, and spherical conformation. High-performance size exclusion chromatography (HPSEC) could be used to study the conformational properties of polysaccharides in aqueous solution. Combined refractive index (RI) light scattering detectors (LALS and RALS) with an online viscometer, the relationship of M_w and the intrinsic viscosity $[\eta]$, and R_g , R_h and R_g/R_h (ρ) can be obtained. The double logarithmic plot of the molecular weight vs. intrinsic viscosity could be well described using the Mark-Houwink equation (8):

$$[\eta] = kM_v^\alpha \quad (8)$$

where k and α could reflect the corresponding conformation of these polysaccharides. It is well known that the exponent α normally lies in the range of 0.5 to 0.8 for linear random coil polysaccharides and increased with increasing chain stiffness. Polysaccharides with α below 0.5 and above 0.8 are generally regarded as spherical and rigid chain conformation. α is also affected by the interaction between polysaccharides and solvent. Low α value favors poor solvent, and high α indicates a good solvent. The logarithmic plot of the molecular weight versus intrinsic viscosity of heteropolysaccharide isolated from seeds of *Artemisia sphaerocephala* Krasch is shown in **Figure 12**, from which a random coil conformation of this polysaccharide was determined. However, the curve in **Figure 12** is not exact linear, a slightly decreased slope was observed with the increase of M_w , which was attributed to the increased percentage of side chains in the high M_w fraction by the authors [3].

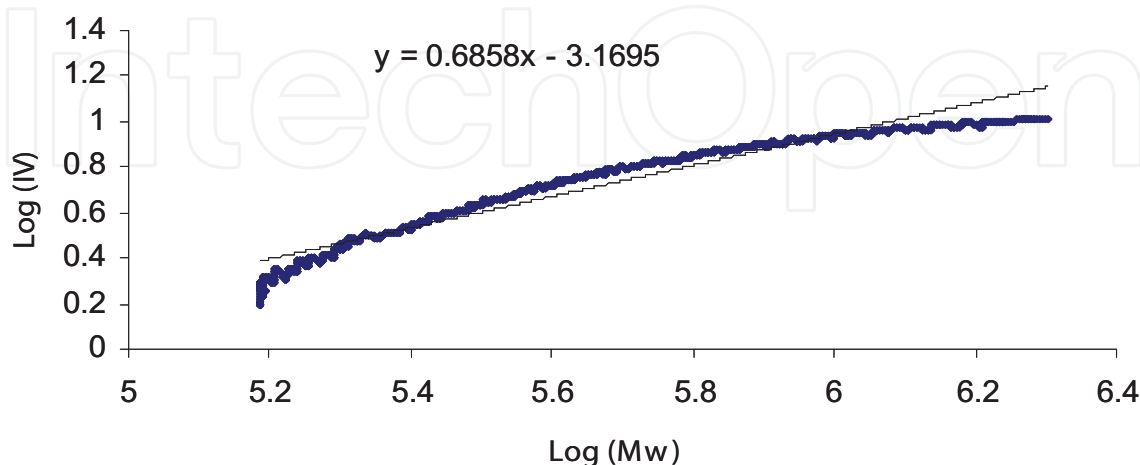


Figure 12. Logarithmic plot of the molecular weight vs. intrinsic viscosity of weight heteropolysaccharide isolated from seeds of *Artemisia sphaerocephala* Krasch. Adopted from Guo et al. (2013) [3].

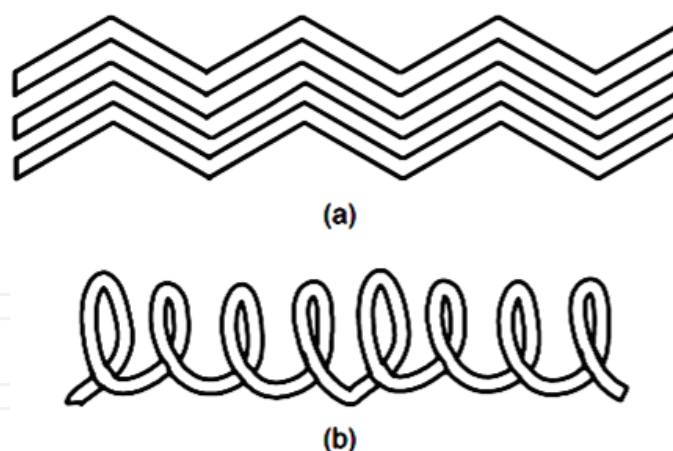


Figure 13. Linkage patterns and conformation types of polysaccharides: (a) ribbon-like and (b) hollow helix. Adopted from Cui and Wang [23].

If the values of the torsion angles are fixed by cooperative interactions between residues, such as in solid or gel states, an ordered conformation can be adopted. Two general ordered conformations are ribbon-like and helix conformations (**Figure 13**). Polysaccharide with ribbon-like conformation is most easily aligned and closely packed through numerous hydrogen bonds and van der Waals forces. The resultant compact structures essentially prevent solvent penetration and retain insolubility in water. Polysaccharides such as cellulose, xylan, and mannan, which contained the zig-zag type linkages, all belong to this type. Another ordered conformation is hollow helix conformation, in which the bonds adopt a U-turn form, as in 3- β -D-GlcP (curdlan) and 4- α -D-GlcP (amylose from starch). Compared to ribbon-like conformation, the hollow helix conformation showed a relative better solubility, but still not comparable with disordered conformation (random coil), e.g. amylose adopts the helix conformation and is only dissolvable in hot water [23] (**Figure 13**).

Polysaccharides with the same monosaccharides but different linkage patterns (solubility) also showed varied solubility. Compared to cellulose, the solubility of cereal β -glucan is increased with the insertion of 1 \rightarrow 3 linkage bond into the 1 \rightarrow 4 glycosidic bond. Generally speaking, any structures that contain especially flexible units such as (1 \rightarrow 6) linkages lead to easier solubility because of a large favorable entropy of the solution. For example, by introducing single β -D-GalP substituents (1 \rightarrow 6) linked to mannan backbone, the resulting galactomannans are fairly soluble in water [24]. Pullulan is another very typical example, which is also known as α -1,4- and α -1,6-glucan, and prone to dissolve in water.

4. Discussions

It can be seen that polysaccharides have very complex structural features regarding monosaccharide composition, glycosidic bonds (linkage patterns), the degree of branching,

molecular weight distribution, and the presence of special groups. These structural features highly affected the solubility of polysaccharides, in either positive or negative way. All in all, any structure that hinders the intermolecular association usually leads to a higher solubility, such as branching structure, charged group (carboxylate group, sulfate, or phosphate groups); on the opposite, structural characters that promote the intermolecular association result in the poor solubility, such as linear chain, large molecular weight, and other regular structural characters. It should be noted that these structural features are not isolated but intercorrelated with each other to decide the solubility of any specific polysaccharide. For example, the excellent solubility of gum arabic is not only contributed by its highly branched character but also affected by the charged group as well as the 1–6 linkage bonds. The good solubility of amylopectin is contributed by both highly branched structure and 1 → 6 linkage bonds. Therefore, it is wise to consider the relationships of solubility with overall polysaccharide structure features. Some examples of polysaccharides with different solubility groups have been previously summarized [7], while some modification has been made (**Table 1**).

Understanding the relationships between solubility and structural features of polysaccharides could better facilitate the food and nonfood applications. The solubility of some originally insoluble or poor soluble polysaccharides such as cellulose and starch can be increased through basic structural modification. Take starch for example; starch can be physically or chemically modified to improve water solubility. The physical modification methods involve the treatment of native starch granules under different temperature/moisture combinations, pressure, shear, and irradiation, while chemical modification involved with acid treatment, cross-linking, oxidation, and substitution (esterification and etherification) to either decrease the starch molecular weight, increasing the substitution or increase the charged groups.

Solubility groups	Polysaccharide	Monomer	Special/charged group	Branching situation	Conformation
Poor solubility	Cellulose	4-β-D-GlcP	No	Linear	Ribbon
	Xylan	4-β-D-XlyP	No	Linear	Ribbon
	Mannan	4-β-D-ManP	No	Linear	Ribbon
Intermedia	Cereal β-glucan	4-β-D-GlcP,3-β-D-GlcP	No	Linear	Random coil
	Konjac glucomannan	4-β-D-GlcP,4-β-D-ManP	Acetyl group	Linear	Random coil
	Locus bean gum (galactomannan)	4-β-D-ManP,T-α-D-GalP	No	Less and short branched	Random coil
Good solubility	Gum arabic/gum ghatti	3,4,6-, 3,6- Galp,-Araf, and so on	Glucuronic acid	Highly branched	Spherical
	Pullulan	4-α-D-GlcP,6- α-D-GlcP	No	Branched	Random coil
	Amylopectin	4-α-D-GlcP,6- α-D-GlcP	No	Highly branched	Spherical

Table 1. Examples of polysaccharides with different solubility.

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