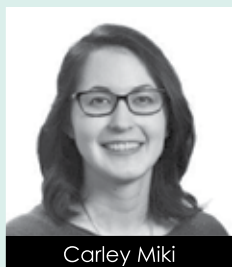




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# Phytoglycogen Nanoparticles:

## 1. Key properties relevant to its use as a natural moisturizing ingredient

**KEYWORDS:** Phytoglycogen, polysaccharides, moisturizer additive, water retention, rheology.

**Abstract** Phytoglycogen is a natural polysaccharide nanoparticle that can be extracted in a monodisperse form from some varieties of non-GMO sweet corn. Because it is environmentally friendly and sustainable, it is an ideal natural botanical extract. Phytoglycogen has a simple chemical composition – it is a polymer of glucose – but has a complex highly branched dendrimeric physical structure. This structure, combined with its hydrophilic nature, contributes to its many desirable properties including high water retention, high dispersion stability, unique rheological behaviour, and the capacity to form films. Together, these properties make phytoglycogen an attractive, natural, multifunctional additive for incorporation into moisturizer formulations. This paper, the first in a series of papers on uses of phytoglycogen, will review key properties of the material. Where possible, these properties will be compared to hyaluronic acid, a leading natural moisturizer in common use today.

### INTRODUCTION

Proper skin hydration is essential for normal healthy skin function. For example, appropriate skin water content is essential for the enzymatic functions required for normal desquamation, and water content strongly influences the skin's mechanical properties such as plasticity, softness, flexibility, and elasticity (1-5). The outer layer of the skin, the stratum corneum (SC), is primarily responsible for its water holding properties, and regulates water loss (1, 2). SC water retention depends on its structure and composition, which consists of corneocytes containing a variety of hydrophilic molecules (termed natural moisturizing factors) and hydrophobic intercellular lipids (1, 2, 5, 6). The hydrophilic components hold water and the hydrophobic lipid matrix serves as a barrier to water loss. These complementary properties of the skin's biology give rise to its natural water holding properties, and this biology influences the development of skin moisturizers. Generally, moisturizers seek to incorporate both humectant and occlusive ingredients in their formulations, mimicking and augmenting the skin's natural water retention mechanisms (1).

Moisturizer development is also driven by the diverse skin hydration needs of consumers (1). These needs depend on numerous factors including the consumer's age, ethnicity, and their motivation for using a moisturizer, as well as climate considerations (1). These factors make the development of effective moisturizer formulations an ongoing challenge in the cosmetics industry. Largely for these reasons, skin moisturization remains the top unmet consumer skin need (1), creating strong

demand for improved moisturizer formulations. At the same time, consumer trends have moved towards a preference for natural, sustainably sourced, and environmentally friendly ingredients. The demand for improved moisturizing formulations coupled with consumer preferences for natural ingredients creates a strong need for new, natural moisturizing agents.

Natural polysaccharides are an important class of biomolecules that find many uses as ingredients in moisturizer formulations and cosmetics in general (7-9). Some polysaccharides, such as hyaluronic acid, are used as active moisturizing ingredients (7-10). Others, such as xanthan gum, are primarily used as viscosity modifiers and psychosensorial agents (9). Ultimately, the desirable properties of a polysaccharide that determine its utility in a moisturizer formulation arise from its molecular structure and chemical composition. The purpose of this short review is to introduce the reader to the natural polysaccharide phytoglycogen, and provide a basic description of its structure and hydration properties – in direct comparison to hyaluronic acid. An emphasis will be placed on those properties that make it a promising new ingredient for moisturizing applications.

### WHAT IS PHYTOGLYCOGEN?

Phytoglycogen is a natural polysaccharide produced in high quantities by some varieties of non-GMO sweet corn, as well as other plants (11-14). It has a similar structure and energy storage function to that of the well-known polysaccharide

glycogen (or “animal glycogen”) that is found in humans and other animals. In sweet corn, phytoglycogen is naturally produced as a monodisperse nanoparticle with a diameter of ~35 nm, as measured by neutron scattering (12); dynamic light scattering yields a larger hydrodynamic diameter of ~70 nm. Phytoglycogen has a simple chemical composition – it is composed solely of glucose monosaccharide units, as are many common natural polysaccharides such as cellulose, dextran, and starches. These glucose monosaccharide units are connected by  $\alpha$  (1, 4) glycosidic bonds to form chains that undergo  $\alpha$ (1,6) branching every 10-12 glucose monomers (15-17). This high degree of branching gives the phytoglycogen nanoparticles a tree-like or dendrimeric structure. It is this special structure and monodispersity that leads to many of phytoglycogen’s useful properties (12, 18).

Despite being composed solely of nano-sized particles, phytoglycogen is not classified as a “nanomaterial” by major regulatory and certification organizations such as the EU, COSMOS, ACO, and Health Canada. This is because of a number of important factors:

- Phytoglycogen is produced by nature as a nano-sized particle. It is not intentionally manufactured, but rather it is simply extracted in its natural state from a plant (normally sweet corn) (19).
- Phytoglycogen is water soluble.
- Phytoglycogen is not biopersistent; the polysaccharide readily degrades into simple sugars in both the body and the environment.

Phytoglycogen is a safe and natural botanical extract. These qualities, combined with its unique physical and chemical properties, make phytoglycogen a promising material for applications in moisturizing formulations.

### SPECIAL STRUCTURE YIELDS SPECIAL PROPERTIES

The unique structure of phytoglycogen is at the core of its special properties. Its high degree of chain branching gives rise to a tree-like or dendrimeric architecture, resulting in densely packed nanoparticles. Neutron scattering experiments have shown that the nanoparticles have a uniform density, implying a backfolding of the higher generation polysaccharide chains towards the centre of the structure (12). This creates a tightly packed and highly-branched internal environment of hydrophilic polysaccharide chains and associated interstitial water molecules (hydration water). The dendrimeric structure of the phytoglycogen nanoparticles has been shown to give rise to a number of interesting properties including high water retention, low viscosity and high stability in water, and rheological modifying and film forming capabilities (12, 19, 20). This review will discuss the relationship between phytoglycogen’s special structure and its hydration properties. We will compare some of the key properties of phytoglycogen to those of hyaluronic acid (Table 1), a linear chain polysaccharide commonly used in cosmetic formulations.

### STRUCTURE, SIZE, AND DENSITY

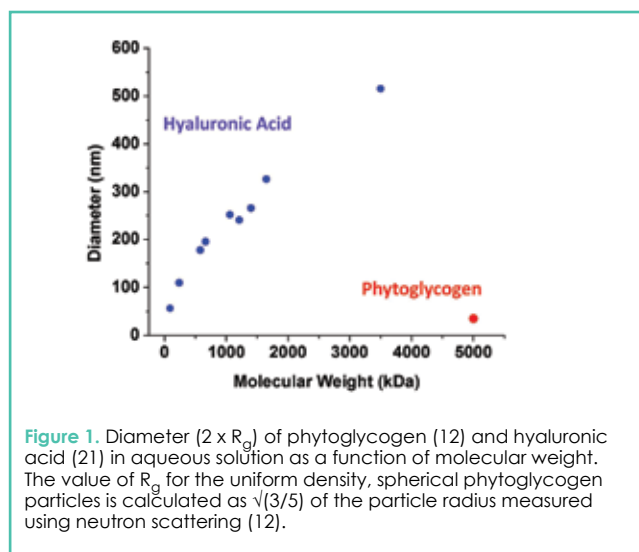
Polysaccharide chain structure influences both the size and density of polysaccharide particles in aqueous solutions. In an

	Phytoglycogen	Hyaluronic acid (HA)
Sources	Plants (corn)	Bacteria, animals
Structure	Dendrimeric nanoparticle	Linear chain
Polysaccharide Repeat Unit	D-Glucose	D-glucuronic acid & D-N-acetylglucosamine
Molecular Weight (cosmetics)	5,000 kDa	800 - 1,200 kDa
Chemical Charge	Non ionic	Anionic
Viscosity in Water	Low	High
Particle Density in Water*	~500 Da/nm <sup>3</sup>	~0.12 Da/nm <sup>3</sup>

**Table 1.** A comparison of some key properties of phytoglycogen and hyaluronic acid (HA).

\*Calculated for phytoglycogen [MW = 5000 kDa,  $R_g$  = 13.4 nm] (12) and HA [MW = 1000 kDa,  $R_g$  = 125 nm] (21).

aqueous environment, phytoglycogen’s dendrimeric structure results in a much more compact particle relative to linear HA. Figure 1 shows a plot of particle diameter, defined as twice the radius of gyration ( $R_g$ ), as a function of molecular weight for phytoglycogen and HA. As is evident, phytoglycogen (MW ~5000 kDa) has a diameter roughly equivalent to only ~100 kDa HA. The particle density (molecular weight divided by the spherical volume) of phytoglycogen is therefore several orders of magnitude greater (Table 1) than that of HA, in solution. Therefore, at equivalent molecular weights, phytoglycogen may be able to more readily penetrate the skin.

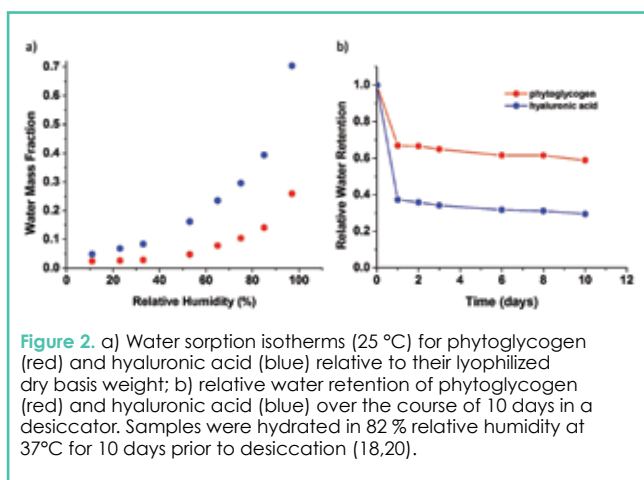


**Figure 1.** Diameter ( $2 \times R_g$ ) of phytoglycogen (12) and hyaluronic acid (21) in aqueous solution as a function of molecular weight. The value of  $R_g$  for the uniform density, spherical phytoglycogen particles is calculated as  $\sqrt{(3/5)}$  of the particle radius measured using neutron scattering (12).

### WATER RETENTION

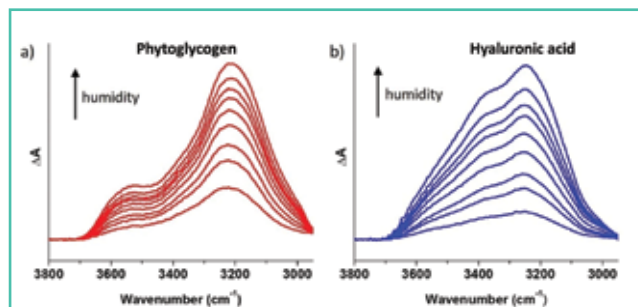
The mechanical properties of the stratum corneum (SC) have been well studied, and depend on the combined content of water and hydrophilic materials. Water is the principal plasticizer of the SC, but its effectiveness is limited by its volatility (1, 4, 5). Humectants have been shown to improve the mechanical properties of the skin by plasticizing the SC, and their effectiveness has been correlated to their ability to retain water (4). Figure 2 shows the water mass fraction and relative water retention of phytoglycogen as a function of time compared to hyaluronic acid (HA), a strong humectant and moisturizing agent. As is evident

in Figure 2, HA holds more water on a per mass basis than phytyglycogen at all examined relative humidities. This is consistent with the relative densities of their polysaccharide chains. However, phytyglycogen exhibits a stronger relative water retention capacity compared to HA, retaining >60 % of its initial water content versus ~30% for HA, after 10 days under desiccating conditions (20). The ability of humectants to plasticize the SC is correlated to their water retention and water holding capacity. The exceptional water retention properties of phytyglycogen suggest that it is a strong humectant, with the ability to hydrate and plasticize the SC, thereby improving skin softness, flexibility, and elasticity. Additionally, phytyglycogen's smaller particle size may allow for deeper skin penetration and thus provide greater water retention in the lower layers of the skin. Phytyglycogen's strong water retention capacity complements the exceptional water holding capacity of HA, suggesting the potential for complementary humectant action by the two polysaccharides in moisturizing applications.



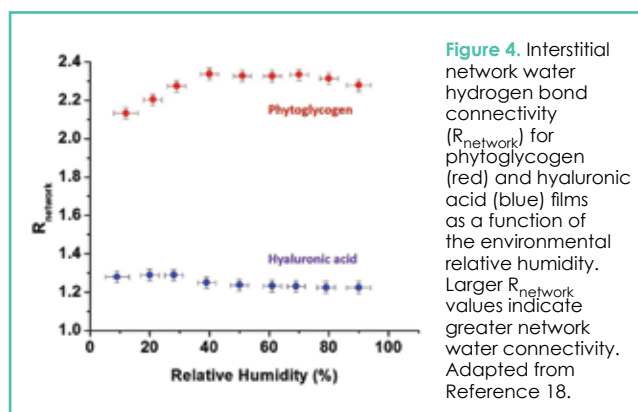
The physical origin of phytyglycogen's water retention capacity has been investigated using several analytical techniques. Infrared (IR) spectroscopy has been used to examine the hydrogen bonding structure of water molecules sorbed by films of phytyglycogen and other polysaccharides, including HA. The frequency at which water absorbs IR light depends on its degree of hydrogen bonding – the stronger the hydrogen bonding, the lower the IR frequency absorbed by the water molecules. Figure 3 shows IR spectra of water sorbed by phytyglycogen as a function of the environmental relative humidity. For comparison, IR spectra for water sorbed by hyaluronic acid (HA) are also shown. The shape of the IR absorbance bands depends on the relative amounts of water sub-populations in different hydrogen bonding environments (18,22-24), with absorption at lower wavenumbers indicating a stronger interaction with water. By comparing the absorbance intensity at wavenumbers corresponding to these different water sub-populations, semi-quantitative data describing the strength of the water hydrogen bond network can be obtained.

Figure 4 shows a plot of the absorbance ratio ( $R_{\text{network}}$ ) of the IR bands associated with the strongly hydrogen bonded water sub-population (centred at  $\sim 3250 \text{ cm}^{-1}$ ) relative to a more weakly hydrogen bonded water sub-population (centred at  $\sim 3400 \text{ cm}^{-1}$ ), as a function of environmental



**Figure 3.** Infrared (IR) absorbance spectra of O-H stretching vibrational mode of water sorbed by a) phytyglycogen and b) hyaluronic acid films at environmental relative humidities between 10 – 90 %. The shape (frequency distribution) of these IR absorbance bands contains information regarding the hydrogen bonding environment of the sorbed water molecules. Adapted from Reference 18.

relative humidity. This IR absorbance ratio serves as a measure of the hydrogen bond network connectivity of the sorbed water molecules (18, 22-24). For comparison, data for water sorbed by HA is also plotted. For bulk liquid water,  $R_{\text{network}}$  is equal to  $\sim 1$ . The larger  $R_{\text{network}}$  values of  $\sim 2.3$  for phytyglycogen indicate that the order and connectivity of the sorbed interstitial water hydrogen bond network is greater than for that of bulk water. Furthermore, the larger  $R_{\text{network}}$  values for phytyglycogen, compared to HA ( $R_{\text{network}} \approx 1.25$ ), shows that water held by phytyglycogen exhibits a greater degree of hydrogen bonding over the entire range of relative humidities. These results are consistent with the gravimetrically measured water retention data shown in Figure 2b, which showed that phytyglycogen retains a greater fraction of its sorbed water in a desiccating environment than HA.



More generally, this work revealed a correlation between the chain architecture of polysaccharides and the structure of the sorbed water hydrogen bond network. The highly-branched dendrimeric structure of phytyglycogen was found to enhance water connectivity via the promotion of highly hydrogen bonded water sub-populations at the expense of less hydrogen bonded sub-populations, compared to linear chain polysaccharides such as HA and chitosan (18). These results suggest that the dendrimeric structure of phytyglycogen is responsible for its exceptional water retention properties. However, this highly branched structure of phytyglycogen also limits the quantity of water that can be taken up by the particles. In contrast, hyaluronic acid's linear chain structure provides greater flexibility to the polysaccharide chains, leading to greater film swelling and

contributing to its greater water sorption capacity (25, 26). The complementarity of the water structure and sorption capacity of phytohyloglycogen and HA suggest the potential for synergistic effects when both products are used together. This phenomenon will be explored in the second paper in the series.

The hydration of phytohyloglycogen in aqueous dispersions has been examined using small angle neutron scattering (SANS) and quasielastic neutron scattering (QENS) (12). These neutron scattering techniques provide independent measures of the phytohyloglycogen hydration in an aqueous environment, as well as the dry and hydrated molecular weight of the nanoparticle. The dry molecular weight – only the polysaccharide, without any associated water – was found to be  $4.16 \times 10^6$  g/mol; the hydrated molecular weight – the polysaccharide plus the associated hydration water – was found to be  $14.7 \times 10^6$  g/mol (12), showing that each phytohyloglycogen nanoparticle contains ~250% its mass in water.

The exceptional nature of phytohyloglycogen hydration is further illustrated via its effects on water dynamics. The QENS measurements showed a dramatic slowing down of water diffusion inside the phytohyloglycogen particles relative to that of bulk water outside the particles, with perturbation of the dynamics of ~7 water molecules per constituent phytohyloglycogen hydroxyl group (12), compared with only ~3 water molecules per hydroxyl group for most polysaccharides (27, 28). The large number of water molecules perturbed by phytohyloglycogen is attributed to its highly branched, closely packed chain architecture (12). This strong interaction of phytohyloglycogen with water is consistent with the IR studies discussed above and further supports the relationship between phytohyloglycogen's dendrimeric structure and its water retention properties.

## RHEOLOGICAL PROPERTIES

The rheological properties of moisturizing creams are important for both their processing and sensorial attributes such as skin feel (29). Desirable properties include shear thinning, in which the viscosity decreases with increasing shear rate, and thixotropic flow, in which the viscosity decreases with time under an applied shear. These properties allow for creams that are viscous when at rest in containers, i.e. under low shear conditions, to be readily pumped or dispensed and easily and pleasantly applied to the skin, i.e. under high shear conditions. Polysaccharide gums are often incorporated into moisturizing formulations as rheological modifiers to impart desirable viscosity and viscoelastic properties to these products. Aqueous dispersions of phytohyloglycogen nanoparticles have rheological properties that make them attractive additives to moisturizing formulations.

Phytohyloglycogen is easily dispersed in water and the resulting dispersions are very stable (for at least several years). The rheological properties of the nanoparticles are insensitive to environmental conditions such as ionic strength and pH (3-9), making them versatile additives that are easy to incorporate into different formulations (20). Figure 5 shows some key rheological properties of phytohyloglycogen dispersions, with comparisons to HA. At concentrations below 20% w/w, the dispersions behave as Newtonian fluids, with their viscosity and shear stress independent of shear rate. At concentrations greater than 20% w/w, the dispersions exhibit shear thinning behaviour (20). Interestingly, as shown in Figure 5, the

nanoparticles can be dispersed at very high concentration (up to ~25% w/w) while maintaining very low viscosities (<3 Pa·s) (20). This behaviour is unique among natural polysaccharides. High molecular weight polysaccharides usually exhibit high viscosities even at low concentrations. For example, the linear polysaccharide chains of HA form expanded random coils in solution. These expanded coils have very large hydrodynamic diameters, as measured by dynamic light scattering, for a given

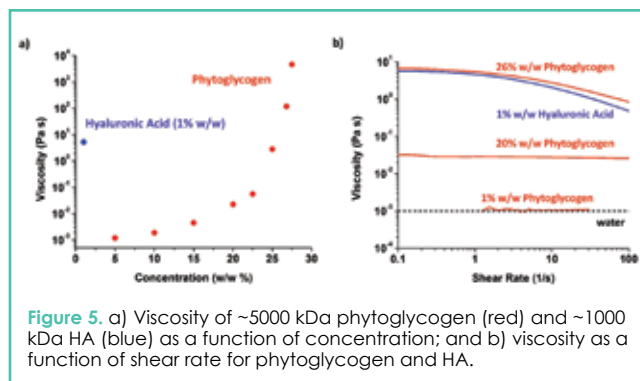


Figure 5. a) Viscosity of ~5000 kDa phytohyloglycogen (red) and ~1000 kDa HA (blue) as a function of concentration; and b) viscosity as a function of shear rate for phytohyloglycogen and HA.

molecular weight of the HA chain, particularly when compared to densely packed phytohyloglycogen particles. For example, a 100 kDa HA chain has a hydrodynamic diameter of ~70 nm, which is comparable to that of a 5,000 kDa phytohyloglycogen particle (30). These loose, expanded HA coils can interact with each other via chain entanglement, even at low concentrations, leading to high viscosities – a 1% w/w HA solution has a viscosity comparable that of a jelly (31, 32). In contrast, the high stability and low viscosity of phytohyloglycogen dispersions implies that there is very little interaction between the nanoparticles in water. It is likely that phytohyloglycogen's monodispersity, highly branched structure, and special interaction with water gives rise to this unique rheological behaviour.

## CONCLUSIONS

Phytohyloglycogen is an extraordinary natural polysaccharide with many promising technological applications. This review has highlighted key properties that make these nanoparticles an attractive, multifunctional ingredient for use in moisturizer formulations. These properties include exceptional water retention and rheological properties. These physical properties ultimately stem from the highly branched dendrimeric structure of the phytohyloglycogen particles, which differentiates it from other polysaccharides, such as HA, that are commonly used in cosmetic formulations. These structural differences lead to physical properties that can be complementary to those of existing ingredients like HA. The potential for synergistic moisturizing action between phytohyloglycogen and HA will be explored further in Part II of this series. As a sustainably sourced botanical extract, phytohyloglycogen is uniquely placed to meet the functional needs of moisturizing formulations and the demands of consumers.

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