

# A novel xyloglucan gum from seeds of *Afzelia africana* Se. Pers.: Some functional and physicochemical properties

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Afzelia gum (AzG) is a nonstarch polysaccharide extracted from the seeds of *Afzelia africana* Se. Pers. Some physicochemical and thermal properties of the polysaccharide have been determined and compared with those of hydroxypropylmethyl cellulose (HPMC). The isothermal swelling characteristics show that AzG and HPMC are hydrogels. The swelling of AzG unlike that of HPMC is pH responsive as characterized by its swelling profile and mean swelling time (MST) in different pH buffer solutions. The thermograph of AzG shows four characteristic peaks. Three endothermic peaks and an exothermic peak, while that of HPMC is characterized by two peaks, an endothermic peak and an exothermic peak. The moisture sorption characteristic of HPMC and AzG show that both polymers are moderately hygroscopic. The equilibrium moisture uptake of AzG was greater than that of HPMC at low (21-47) relative humidity (RH); at higher (75-100) RH, the equilibrium moisture uptake was considerably higher for HPMC. The gel clarity of AzG and HPMC were pH responsive. AzG produced similarly lower gel clarity in pH 4 and pH 9 solutions its highest clarity was in pH 7 solution. The reverse was the case for HPMC dispersions. The results of the various properties evaluated indicate that AzG has potential as pharmaceutical.

**Key words:** Afzelia gum, gel clarity, hydroxypropylmethyl cellulose, moisture sorption, swelling isotherm, thermal properties

## INTRODUCTION

Hydrogels used for foods and pharmaceuticals can be obtained from both synthetic and natural sources.<sup>[1,2]</sup> Non-starch polysaccharides (NSPs) constitute an important source of natural hydrogels essentially due to their relative safety, biocompatibility, and biodegradability as well as their ready availability.<sup>[3]</sup> The ability to swell by both natural and synthetic hydrogels is one of the main properties that make them useful as matrix system for the controlled delivery of bioactive agents. The process of water transport into and out of a hydrophilic polymer and the corresponding structural changes that occur are important and determine their use as matrix systems for the delivery of agents.<sup>[4]</sup>

*Afzelia africana* Se. Pers. is a tree belonging to the subdivision Caesalpiniodea, Fam. Leguminosae. This large tree is found growing wild in the dense evergreen forests, savannah and the coastal forests of east of East Africa and West Africa.<sup>[5,6]</sup> In the eastern part of Nigeria, *A. africana* is widely distributed where the seed is commonly used as a soup and beverage thickener. Afzelia gum (AzG) is the gum extracted from the seeds of *A. africana*. Unlike guar gum and other well-known natural gums, little is known of the physicochemical and

functional properties of AzG. However, because of the traditional uses of the gum material contained in the powdered seeds a lot of interest has been stimulated.<sup>[6-8]</sup> AzG has been characterized as a xyloglycan,<sup>[6]</sup> these are hemicelluloses or cell wall polysaccharides that are not soluble in water but can be solubilized by aqueous alkali. These groups of compounds are structurally related to cellulose. They consist of a cellulose backbone of  $\beta$ -glucopyranosyl residues, which are substituted at C-6.<sup>[8]</sup> AzG has also been characterized in terms of its monosaccharide and oligosaccharide compositions.<sup>[6]</sup> The gas liquid chromatography (GLC) analyses evaluated AzG as having glucose, xylose, and galactose, which are in the ratio 1.3:1:0.63 as the main monosaccharide with small amounts of arabinose, mannose, and uronic acid. This suggests either a complex mixed structure or the presence of more than one polysaccharide.<sup>[6]</sup>

AzG shares a close chemical resemblance to guar gum; both are xyloglycans thus making it like gua gum a potential pharmaceutical. There is a wide distribution of the plant especially in eastern Nigeria, extensive multi-scale chemical characterization of the gum has being carried out.<sup>[6]</sup> The extraction and chemical characterization of the gum coupled with its folkloric use as a soup and beverage thickener make it a material

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of interest as these indicates its potential as a food thickener or pharmaceutical excipient.

The objective of this study therefore, is to determine some physicochemical and thermal properties of AzG as a potential food additive or pharmaceutical excipient in comparison with HPMC, a well characterized hydrophilic polymer with cellulose backbone.

## MATERIALS AND METHODS

### Materials

*A. africana* seeds were obtained from a local market in Abuja Nigeria, diethyl ether and hydroxypropylmethyl cellulose (HPMC) were obtained from Sigma (Sigma-Aldrich, Germany), acetone and buffer tablets were obtained from Fisher (Fisher, USA),

### Methods

#### Extraction of AzG

The method by Ross-Murphy *et al.* was adopted for the extraction with slight modification<sup>6</sup>. The seeds of *A. africana* was washed to remove any adhering dirt and soaked in cold water for 1 hr to soften and remove the aril. It was then boiled for 3 hr until the seed coat was tender and soft enough to remove. The hydrated tegmen obtained after removal of the seed coat was rinsed with distilled water and dried at 50°C 24 hr. The dry tegmen was then pulverized with a blender to obtain multi particulate coarse granules. A sieve shaker (Retsch As200, Germany) with a 1 mm mesh sieve was used to separate and collect granules with sizes above 1 mm for further treatment. The granules collected were boiled in ethanol for 1 hr to denature proteins and inactivate enzymes. The granules were then treated with several portions of diethyl ether to remove lipids. The defatted granules were air dried for 6 hr to remove residual diethyl ether. A 25 g quantity of the defatted granules was then soaked in 500 ml of distilled water with continues agitation for 18 hr. The viscous supernatant was collected by filtration using a muslin cloth of 150 µm mesh size, centrifuged at 4500 rpm for 1 hr and then precipitated with acetone. The precipitate was air dried and placed in a desiccator for 72 hr. The dry powder was then transferred into an airtight container and stored at 25°C until used.

#### Determination of pH of gum dispersion

The pH of 1 and 2.5% dispersions of AzG were determined with a pH meter (Canon 126, England).

#### Isothermal swelling characteristics

Compacts of 300 mg weight ( $W_d$ ) of HPMC and AzG powders were prepared by compressing the powder with a compression machine (Shanghai Tiixiang and Chenta. Pharmaceutical Machinery Co. Ltd) fitted with an 8 mm flat faced punch and die.

The compacts were stored in a desiccator for 24 hr and then place on tarred glass plates (2 × 4 cm). The plates were transferred into Petri dishes containing 60 ml of distilled water, buffer solutions of pH 4, 7, and 9, respectively maintained at 27°C. At 15 min intervals, the glass plates with the hydrated compacts were removed, dried by blotting with tissue paper and then weighed ( $W_t$ ). The weight-swelling ratio (Q) was determined using equation 1. When the hydrated compacts reached a constant weight the swelling ratio at this point is considered to be equilibrium swelling ratio ( $Q_e$ ) and was determined using equation 2.<sup>[9]</sup>

$$Q = W_t/W_d \quad (1)$$

$$Q_e = W_e/W_d \quad (2)$$

The mean swelling time (MST) of the polymer compacts were evaluated using equation 3.

$$MST = (n/n+1) K^{-1/n} \quad (3)$$

To determine the parameters (n and K) in equation 3, equation 4 the power equation was used<sup>10</sup>.

$$W_t/W_d = Kt^n \quad (4)$$

$$\text{Log} \frac{w_t}{W_d} = \text{Log}K + n\text{Log}t \quad (5)$$

$W_t$  and  $W_d$  in equations 4 and 5 are same as in equation 1; n is a kinetic constant which depends on the solvent type and is used to characterize the transport mechanism of solvent into the polymer; and K is a kinetic constant incorporating the geometric characteristics of the polymeric system. The values of n and K were obtained from the plot of  $\text{Log} W_t/W_d$  vs  $\text{Log} t$  in equation 5, where n and K are the slope and intercept at the y axis, respectively.<sup>[9]</sup> The equation was applied only to the initial stages of swelling (1 to 4 hr).

#### Moisture Sorption Characteristics

AzG and HPMC powders were placed in Petri dishes and stored in a desiccator containing activated silica gel as the desiccant for one week at 25°C to remove residual moisture. The moisture sorption isotherms were determined by the gravimetric method.<sup>[11]</sup> A 1 g quantity of AzG was placed in an aluminum foil and put in a sealed glass chamber with a gauze holding tray containing water or saturated solution of different salts to provide the required relative humidity (RH) (water 100%, potassium chloride 84%, sodium chloride 75%, potassium thiocyanate 47% and calcium chloride 31%). The powders were then weighed at 12 hr intervals until equilibrium was attained. The percentage equilibrium moisture uptake was determined using equation 6.

$$\text{Moisture uptake} = M_e/M_d \times 100\% \quad (6)$$

Where  $M_e$  is the amount of moisture absorbed at equilibrium and  $M_d$  is the dry weight of the material.<sup>[12]</sup> The moisture sorption profile of percentage weight gain vs RH was then determined.

### Gel Property

This was determined by measuring the transmittance of a 0.5% dispersions of AzG and HPMC made in buffer solutions of pH 4, 7, and 9 at 580 nm using a UV spectrophotometer (UV160A, Shimadzu Corporation, Japan).<sup>[13]</sup> Effect of pH on gel clarity was determined by evaluating a plot of transmittance against pH.

### Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) studies were carried out on a differential scanning calorimeter (DSC 204 F1, Phoenix NETZSCH, Germany) equipped with a thermal analysis system. Indium (156.8°C) was used as the internal standard. Samples of approximately 1 mg of AzG and HPMC were placed in an aluminum pan (25  $\mu$ l) and covered with a perforated lid, respectively. Dry nitrogen was used as the purge gas (purge 20 ml/min). The probes were heated from a start temperature of 25°C to 500°C at a rate of 10°C/min. The glass transition ( $T_g$ ), melting point ( $T_m$ ), and cold crystallization ( $T_c$ ) temperatures were evaluated with the Proteus analysis software.<sup>[14]</sup>

## RESULTS AND DISCUSSION

### Gum Yield

AzG is an off-white smooth powder. The yield of AzG was 34%w/w of the defatted dry granules of *A. africana* seeds using the extraction method discussed. During the extraction process seed granules above 1 mm size were used, this was to ensure that fine particles of the seed that could pass through the mesh of the muslin cloth were excluded.

### pH of Gum Dispersions

The pH of AzG gum dispersion in distilled water at 1.0%w/v and 2.5%w/v dispersions were  $6.5 \pm 0.3$  and  $6.1 \pm 0.1$ , respectively while that of HPMC were  $5.8 \pm 0.1$  and  $5.1 \pm 0.1$ , respectively. This shows that HPMC is comparatively more acidic than AzG. This is due to the presence of a higher number of ionizable groups that could protonate when dispersed in water to form acid.<sup>[15]</sup>

### Isothermal Swelling Characteristics

The characteristics of water transport in and out of hydrogels and the corresponding dimensional changes that occur when they are exposed to aqueous media of different pH are important in predicting or assessing their suitability as food additive or excipients for controlled drug

delivery. The swollen state of hydrogels is a consequence of the balance between cohesive and hydration forces on the hydrated chains. The hydrophilic polymers swell when water molecules interact with the polar groups mainly -OH and -COOH, on the polymer backbone or side chains.<sup>[16]</sup> The swelling properties of AzG and HPMC in distilled water (pH 7), buffer solutions; pH 7 (neutral), pH 9 (alkaline) and pH 4 (acidic) were studied as this is representative of the environment to which the hydrogels could be exposed during use. The isothermal swelling properties of AzG and HPMC in the different aqueous media were compared by plotting their respective swelling ratios against time. Figure 1a shows a continuous increase in swelling ratios for compacts prepared with AzG and HPMC for 21 hr without loss of the compact's integrity, an indication that they are true hydrogels.<sup>[16]</sup> When compared with HPMC, AzG showed a more pH responsive swelling profile [Figure 1a]. The swelling profile of AzG in the first 7 hr was similar in water, pH 7 and pH 4, however at pH 9 swelling was higher. The results shows that HPMC is non-pH responsive and the higher swelling of the compacts in distilled water relative to the pH 7 buffer solution may be due to the effect of electrolyte in the fluids.<sup>[14]</sup> The difference in the swelling characteristics of AzG and

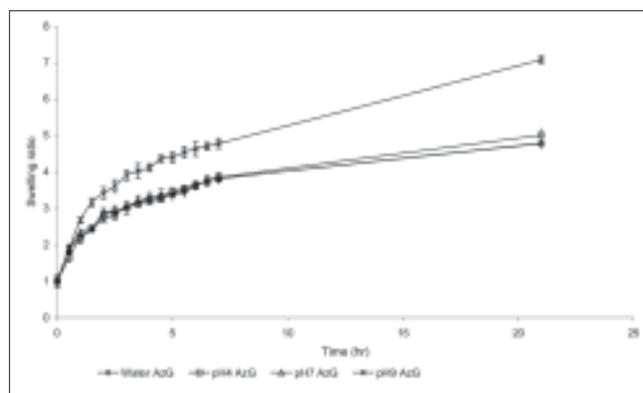


Figure 1a: Swelling profiles of AzG in water and different pH solutions

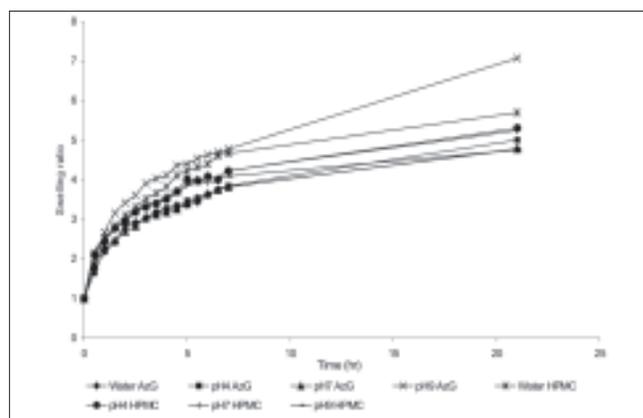


Figure 1b: Swelling profiles of AzG and HPMC in water and different pH buffer solutions

HPMC in the various fluids became pronounced after 24 hr [Figure 1a]. The higher swelling of AzG at pH 9 is because of the increased ease of ionization of the pendant OH groups of the linear and branched galactose chains of the xyloglucan in alkaline and acidic media leading to intermolecular repulsion between linear and branched polymer chains. This results in increased diffusion of fluid into the AzG matrix resulting from the increased void space and flexibility in the polymer chains. In the different pH media the equilibrium swelling ratio of AzG [Figure 1b] can generally be presented thus, pH 9 > pH 4 > pH 7 = water. AzG show a higher swelling in both acidic (pH 4) and alkaline media (pH 9) compared to neutral pH (pH 7). This indicates that the swelling of AzG at different pH is dependent on the ionization of the various pendant acidic or basic functional groups that either protonate or deprotonate and control the diffusion of water into the polymer chain network.<sup>[17]</sup> Glucose, xylose, and galactose residues make up the xyloglucan of AzG<sup>[6]</sup> and this contains numerous bonded and unbonded OH groups that may ionize by protonation or deprotonation to induce intermolecular chain repulsion that cause increased water uptake.

The swelling ratio of AzG in acidic media is considerably less than that in alkaline media could indicate that the extent of chain relaxation due to ionization in the acidic media is less than in alkaline media [Figure 1a]. AzG compacts in pH 7 and distilled water produced super-impossible swelling profile. This characteristic swelling indicates that the swelling of AzG may not be affected by the presence of electrolytes. However, HPMC did not show any pH responsiveness, its swelling ratio was however higher than that of AzG in the different media except in pH 9 solution where AzG was considerably higher [Figure 1b]. The equilibrium swelling of HPMC in distilled water was higher than in buffer solution with pH 7. This indicates that swelling of HPMC is affected by dissolved electrolytes in the swelling media.

Figure 2 shows the bar chart representation of the rate of

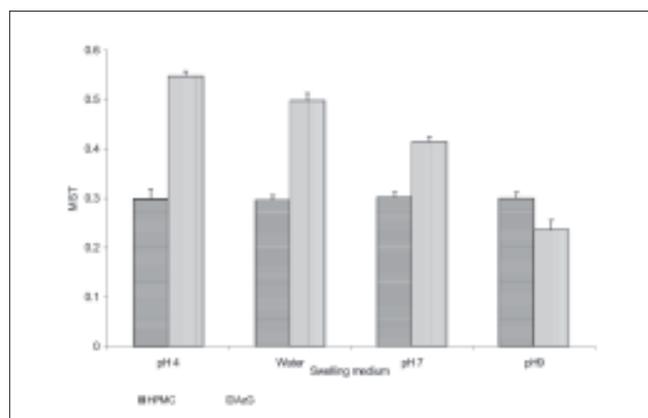


Figure 2: Comparative MST for HPMC and AzG in distilled water and different pH buffer solutions

swelling of AzG and HPMC as determined by their relative MST in the various media. The results show that AzG in the pH 9 medium had the highest rate of swelling, followed by those of HPMC in the various fluids.

### Moisture Sorption Characteristics

The moisture sorption profiles of HPMC and AzG [Figure 3] show that both polymers are moderately hygroscopic as the equilibrium moisture uptake was greater than 15% at simulated tropical isothermal humidity (25°C; 75% RH).<sup>[18,19]</sup> There was a general increase in the equilibrium moisture uptake by AzG and HPMC with increase in RH. The sorption profile shows an initial significant increase at lower RH (22-47% RH), followed by a slight increase between 47 and 75% RH and finally a considerable increase between 75 and 100% RH. The general initial increase in moisture uptake with increasing RH at the lower humidity (22-47%) is an indication that both HPMC and AzG possess polar groups in their molecular chain.<sup>[20]</sup> Between 47 and 75% RH increase in moisture sorption was comparatively slight when compared to that at 22-47% RH, this indicates saturation of the polar groups by moisture. This thus, shows that the available sites for moisture interaction became saturated at low RH. The apparently high moisture sorption at higher humidity is characteristic of condensation of water molecules over the already moisture saturated particles thereby forming layers that have decreased interaction with the polymer molecular surface.<sup>[21]</sup>

Moisture sorption has been reported to be one of the most sensitive technique for assessing variation in the amorphous or crystalline content of polymers,<sup>[20]</sup> as well as predicting some physicochemical<sup>[13,22]</sup> and functional<sup>[23]</sup> properties of polymers. This is because the moisture uptake is predominantly due to the interaction of the water molecules with the amorphous part of the polymer network. Moisture is taken up by the polymer powder bed as a result of the water molecules interacting with the polar groups of the polymer chains. In the amorphous part of the

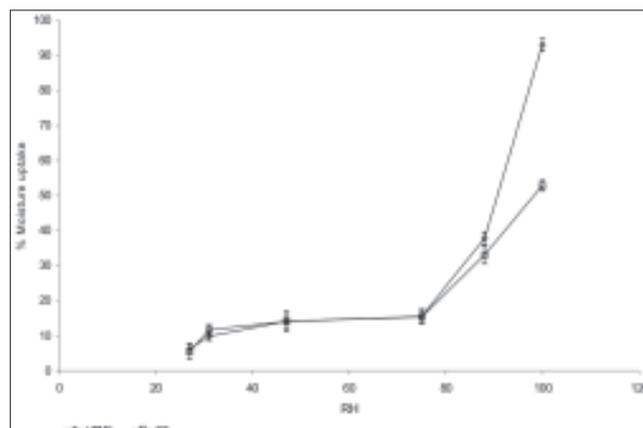


Figure 3: Moisture sorption profiles for HPMC and AzG at various RH

polymer network, there is a larger surface area available for interaction due to the numerous chain entanglements.<sup>[22]</sup> HPMC and AzG have similar isothermic moisture uptake profiles [Figure 3]. Between the 22 and 47% RH ranges the sensitivity of the moisture uptake of the polymers is attributed to the polar groups on the polymer chain<sup>[24]</sup> [Figure 3]. The equilibrium moisture uptake of AzG was higher than that of HPMC at the low RH (22–47% RH). It has been reported that for similar polymeric materials the moisture uptake profile for the amorphous form exhibits a higher shift when compared to the more ordered crystalline form.<sup>[24,25]</sup> Thus, AzG is more amorphous than HPMC. At 75–100%, RH there was considerable difference in moisture sorption between AzG and HPMC. This shows that moisture condensation in HPMC is higher than in AzG even though AzG has been characterized to be extensively branched with numerous unbonded OH groups.<sup>[6]</sup> The number of OH groups within the AzG polymer chain network available for interaction with water molecules could have been reduced by pH induced inter and intra-molecular hydrogen bonding due to interaction within the AzG chain network that could have made the polymer more hydrophobic. The crystalline domain of polymers has been proposed to have extensive secondary intermolecular bonding. This secondary bonding causes the hydroxyl groups on adjacent glucose units to interact with each other and hence reduces the available sites for adsorption of water molecules.<sup>[26]</sup> Apart from the polymorphic nature of polymers, several other factors could affect their moisture or water uptake. These include physical properties like particle size, surface area, porosity, and surface vs. bulk sorption. Though efforts were made to harmonize these parameters, slight differences in particle size were however observed. The AzG powder was comparatively coarser in form than the HPMC thus, resulting in higher bulk porosity and reduced surface area for interaction with moisture. All these may have generally contributed to diminish the moisture uptake by AzG at the higher RH.

### Gel Property

Gel clarity is one of the important functional properties of hydrogels, with the highly clear gels being favored for use in food and drugs as thickeners or binders.<sup>[27]</sup>

The gel clarity of AzG and HPMC was assessed by measuring light transmission through their dispersions in aqueous media. The effect of pH on the gel clarity was also evaluated. The gel clarity of AzG and HPMC are shown in Figure 4. It is presented as a plot of average transmittance against concentrations. HPMC showed higher gel clarity relative to AzG in the various aqueous media. The lower gel clarity exhibited by AzG is essentially due to the formation of larger aggregates in its aqueous dispersions that reduced the transmittance of light through the dispersion.<sup>[28]</sup> This

could be due to higher molecular weight and degree of cross-linking in the AzG polymer chain. In water, the minimum gel clarity was 97% for HPMC and 88 for AzG [Figure 4]. The dispersions of AzG in the various aqueous media were translucent as compared to the transparent dispersions of HPMC this could be due to the extensive cross-linking the polymer chains of AzG.<sup>[29]</sup> Figure 4 shows the effect of pH on gel clarity of AzG and HPMC. The gel clarity of AzG and HPMC varied with the pH of the dispersion. The effect of pH on the clarity of the AzG hydrogel is probably because of the neutralization of acidic and basic groups in the polymer chains. The over neutralization would cause a reversion of the polymer dispersion to large aggregates resulting in the diminishing of light passage through the dispersion as shown by the dispersion of AzG in buffer solutions of pH 9 and 4. The reverse was the case with HPMC.<sup>[30]</sup>

### DSC Thermal Analysis

Thermal analysis using the DSC is a useful method of characterizing polymers based on evaluation of their exothermic and endothermic characteristics.<sup>[31]</sup> The thermographs and the thermal analysis for AzG and HPMC are shown in Figure 5 and Table 1 respectively.

The thermograph of HPMC is characterized by two endothermic peaks. The first peak (A1), which is a second order transition endotherm, corresponds to the glass transition while the second (A2) is a first order transition endotherm corresponding to the polymer melting. The glass transition does not involve heat transfer or change in heat capacity, it is a characteristic of the amorphous domain of the polymer,<sup>[32]</sup> while the melting transition is characteristic of the crystalline domain of the polymer.<sup>[14]</sup> The thermograph of AzG is different from that of HPMC [Figure 5]. The thermograph of AzG has four characteristic peaks. The first peak (B1) corresponds to its glass transition. While the second (B2) is a mild melting peak, the third (B3) is a prominent exothermic peak that corresponds to the polymer cold crystallization which merged with a mild but

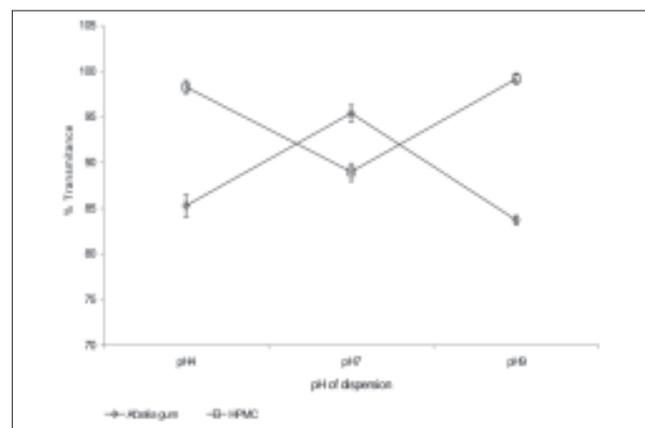
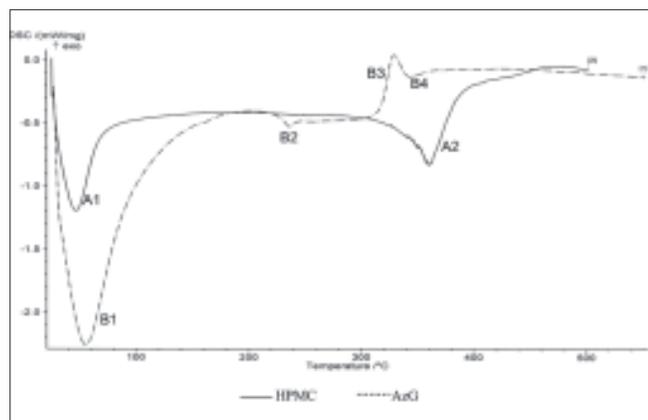


Figure 4: Effect of pH on light transmittance through AzG and HPMC dispersions



**Figure 5:** DSC thermographs for HPMC and AzG

distinct fourth peak (B4). Polymer cold crystallization is an exothermic transition that is characteristic of the pseudo-amorphous domain of the polymer chain network. The further heating of the polymer after the glass transition resulted in the rearrangement of the pseudo-amorphous chain conformation to a more orderly laminar conformation, which then transit into a mild but distinct melting endotherm.

The thermographs of AzG and HPMC also reflect the changes that occurred in the mechanical conformation of the polymers during the heating process. The thermograph of AzG show changes, from a brittle solid to a rubbery material and finally degradation of the material. For accurate comparison, the thermal analysis of HPMC and AzG were carried out at similar heating rate since heating rate will affect the glass transition temperature and the residual enthalpy of the secondary transitions. The  $T_g$  of AzG and HPMC were determined at the same heating rate and similar material moisture content. The  $T_g$  of AzG was greater than that of HPMC [Table 1]. This difference can be accounted for by one or more of the inherent properties of AzG such as higher molecular weight and higher degree of cross-linking since it is made up of a complex polymer network characterized by (1-4)- $\beta$ -linked-D-glucan backbone substituted by D-galatopyronosyl residues linked (1-2)- $\beta^6$ . Other factors that could have contributed to AzG having a comparatively higher  $T_g$  include less rotational freedom in the polymer chain due to the presence of numerous cross-linking  $\beta$ -glucopyranosyl residues and increased intra-molecular hydrogen bonding due to the numerous OH groups. The difference in the  $T_g$  is a decisive factor in predicting the difference in their mechanical properties. The presence of more than one melting curve in the thermograph of AzG may indicate the presence of a mixed polymer or the presence chain branching in the AzG chain network.<sup>[6]</sup> In addition, the prominent exothermic peak in the thermograph of AzG is characteristic of a typical semi crystalline polymer. The exothermic shift represents

**Table 1: Comparative thermal properties of HPMC and AzG**

Parameters	HPMC	AzG
$T_g$ (°C)	Peak A1	Peak B1
Onset	37.8	47.7
End	38.1	51.2
$\Delta H$ J/(gK)	2.249	0.815
$T_m$ (°C)	Peak A2	Peak B2
Onset	333.7	232.1
End	364.5	278.4
$T_c$ (°C)		Peak B3
Onset		312.2
End		323.2
$T_m$ (°C)		Peak B4
Onset		323.2
End		347.7

the crystallization of the pseudo-amorphous portion of AzG's polymeric network.<sup>[14]</sup> The transition of AzG into a crystalline polymorph was then followed immediately by melting of the crystals. The small melting peak observed for AzG could be due to the presence of a high degree of the polymer material in the pseudo-amorphous form. For some highly branched polymers, it has been proposed that the bulk of the melting endotherm is associated with the pseudo-amorphous materials.<sup>[14]</sup>

Though both AzG and HPMC are structurally related to cellulose, the melting temperature of HPMC [Table 1] was considerably higher than that of AzG. This could be due to the crystallites of AzG having more chain flexibility and less laminal dipoles while HPMC crystallites are more closely packed with a high number of aromatic side chains or a combination of all of these. The comparatively higher melting temperature and amount of energy used to effect melting in HPMC indicate that it has more crystalline domain than AzG.

## CONCLUSION

Some important physicochemical, functional, and thermal properties have been determined for AzG in comparison with HPMC, both polymers being similarly hydrophilic with cellulose backbone. The results obtained from the various studies show various similarities and differences between HPMC a semi synthetic polysaccharide and AzG - a natural polysaccharide. Despite the various differences in their physicochemical and thermal properties, both show similar functional properties by being hydrogels. AzG also showed a high degree of pH responsiveness in its swelling characteristics.

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