NMR CHARACTERIZATION AND APPLICATION OF OXIDIZED *Sweitenia mycrophylla* GUM. A POTENTIAL EXCIPIENT

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ABSTRACT

Oxidized *S.mycrophylla* gum was prepared, characterized using NMR spectroscopy and evaluated for its binding capacity in paracetamol tablet formulation. All the granules possessed good flow properties with Hausner ratio of 1.00 - 1.20 and Carr's index of 9.0 – 13.0%. Tablets prepared with 4-8% w/w oxidized gum had hardness ≥ 4kg while tablets prepared with 6-8% w/w oxidized gum had friability of less than 1% comparable to that prepared with 4% w/w gelatin Bp which was used as a standard binder. The disintegration time of the tablets was <15 minutes and increased with increase in oxidized gum concentration. The tablets had fast dissolution in aqueous media’ with > 94% drug dissolution in 45 minutes, the study shows that oxidized gum can be used as a binder in conventional release tablet formulation.

Key Words: Carboxyl group, *Sweitenia mycrophylla* gum, excipient, NMR.

INTRODUCTION

Natural gums have been modified to overcome certain drawbacks, like uncontrolled rate of hydration, thickening, drop in viscosity, storage and microbial contamination[1]. Chemical modifications of polysaccharides allow the preparation of new polymers with specific properties. Chemical processes such as oxidation, acetylation, carboxymethylation, hydroxypropylation and cross-linking are widely used methods to prepare modified gums[2]. Chemical modification provides an efficient route not only for reducing such drawbacks but also for improving the physicochemical properties for better efficacy in utilization [3]. Oxidation of gum can result in formation of water soluble derivative by using reactive groups, to substitute free hydroxyl groups along the macromolecule backbone [4]. The oxidation of gums generally increases their hydrophilicity and solution clarity and make them more soluble in aqueous system[3]. Oxidation was selected as a chemical means of attaching pendant carboxyl groups (-COOH-) due to its technical simplicity, low cost of chemical; reagents and wide range application to produce oxidized gum which can perform as a better binder in drug formulation.

Binding agents are used to impact the structural strength required during the processing, handling and packaging of tablets. A number of plant gums have been used as binding agents in tablet formulations. Example includes Albizia gum [5,6] and guar gum[7]. They have been found useful in production of tablets with different mechanical strength and drug release properties for different pharmaceutical purposes. *Sweitenia mycrophylla* gum is non-starch polysaccharides obtained from the bark of *Sweitenia mycrophylla* tree (meliaceae), a large tree reaching a height of 30-40m and a girth of 3-4m, in favourable condition, it can reach 60m high and 9m girth.[8,9]. The physicochemical, toxicological, structural characterization and application of purified S. *mycrophylla* gum excipient had been investigated in our previous studies. [10, 11, 12, 13]. Evidently, there are no sufficient studies that confirms oxidation of this gum, characterization and its application as excipient in
drug formulation. Hence, this research aims at investigating the oxidation of this new gum in order to improve its physicochemical characteristics for better efficacy in drug formulation. The results of this research is likely to highlight the effect of oxidation on the binding capacity of the gum in tablet formulation.

MATERIALS AND METHODS

Reagents: All reagents used in this study are of analytical grade

Collection and Preparation of Samples

Gum was collected from the bark of *S. mycrophylla* tree in Owena Forestry, Ondo State, Nigeria. The plant was identified and authenticated at the herbarium of the forestry department, Federal University of Technology, Akure. Gum was tapped from the bark of the tree. The dried, cleaned gum sample was milled with a Kenwood blender (UK) and later sieved using a bin (mesh size-250microns) so as to obtain a fine and uniform sample, kept in labeled plastic container for subsequent analysis.

Purification of gum samples

Dried crude gum (10g) was stirred in cold distilled water (250ml) for 2 hours at room temperature. The supernatant was obtained by centrifugation and made up to 500ml and ethanol solution was added (1:4 v/v) to precipitate all the carbohydrate. The precipitated material was washed again with ethanol, followed by distilled water and dried at room temperature milled with Kenwood blender (UK) and later sieved using a bin (Mesh size-250microns) kept in labeled plastic container for subsequent analysis.

Preparation of oxidised gum

Standard method by [14] was used for the oxidation process. 10g gum was dispersed in 50cm$^3$ distilled water. The pH of the slurry was adjusted to 9.0 using 3% NaOH. NaOCl was added slowly for a period of 90 minutes and constantly monitoring the pH at 9.0 and simultaneously cooling was done with crushed ice and NaOCl. The reaction proceeded for four hours after NaOCl addition was completed. The pH of the mixture was then adjusted to 7.0 with what man No 1 filtered paper. The residue was then washed four times with distilled water and air dried at room temperature.

Determination of Degree of Oxidation and Carboxyl Percentage

The percent oxidation (%carboxyl) and degree of substitution (DS) were determined titrimetrically following the method of [15] with slight modification. One gram of the oxidized gum sample was suspended in 50ml of 75% ethanol solution. The slurry was then kept in a water bath at 50°C for 30min with constant stirring. Thereafter the slurry was then cooled at room temperature and 40ml of 0.05m potassium hydroxide added. The slurry was allowed to stand for 24h at room temperature with occasional swirling. The excess alkali was then titrated with 0.5M hydrochloric acid using phenolphthalein as an indicator. The solution was allowed to stand for another 2h. any additional alkali that might leach from the sample was titrated. Blanks with raw gum was analyzed concurrently. The sample volume, the hydrochloric acid normality and the volume of hydrochloric acid required to titrate the blank and sample was recorded and calculated according to equation 1 below. This measurement was done in triplicate and the mean and standard deviation recorded.

\[
\text{Carboxyl}\% = \frac{(\text{Vol.Blank} - \text{Vol.Sample}) \times \text{Molarity of HCl} \times 0.043 \times 100}{\text{Sample weight}}
\]

(1)

Degree of substitution which is the average number of sites per saccharide unit that posses a substituent group was calculated using equation 2;

\[
DS = \frac{(162 \times \text{Carboxyl}\%)}{(4300 - [42 \times \text{Carboxyl}\%])}
\]

(2)
NMR Spectroscopy

$^1$H and $^{13}$C NMR, $^{13}$C DEPT and Solid State NMR of *Sweitenia mycrophylla* gum were recorded in an NMR (600 MHz) spectrometer (Agilent technologies, America). Chemical shifts were reported in ppm relative to an internal standard TMSP (Tetramethyilsilanepropionic acid). Peak integra were performed using Agilent software, America.

TABLET PREPARATION AND EVALUATION

Different batch of granules of paracetamol (25.7% w/w), maize starch (8.2% w/w), talc (2.0% w/w) and magnesium stearate (0.3% w/w) were prepared using the wet granulation technique. The powdered were dry-mixed for 5 minutes in a planetary mixture (model A120, Hobart Company, UK) and massed with the appropriate amount of oxidized gum (binder) solution (1% w/v to 40% w/v and gelating 20% w/v) equivalent to 2% w/w – 8w/w and 4%w/w for the standard binder, gelatin BP.

Granules properties were evaluated and different batch of granules produced were compressed into tablets using a lubricated single punch tableting machine (DP 30 tablet press, Pharmao Industries co. Ltd, China). Tablet properties such as weight, hardness, friability disintegrates and dissolution time were determined in triplicate.

RESULTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration of Oxidized gum (% w/w)</th>
<th>Gelatin Bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Bulk density (g/cm$^3$)</td>
<td>0.481±0.03</td>
<td>0.481±0.03</td>
</tr>
<tr>
<td>Tapped density (g/cm$^3$)</td>
<td>0.655±0.02</td>
<td>0.655±0.02</td>
</tr>
<tr>
<td>Hausner ratio</td>
<td>1.299±0.01</td>
<td>1.299±0.03</td>
</tr>
<tr>
<td>Carr’s index (%)</td>
<td>13.0±0.25</td>
<td>11.0±0.30</td>
</tr>
</tbody>
</table>

Table 1: $^1$H and $^{13}$C NMR assignments of *S. mycrophylla* gum (10mg in 700μL, D$_2$O, 60°C) Referenced to TMSP in PPM

<table>
<thead>
<tr>
<th>Sugar Residue (α-D-Galactose)</th>
<th>$^1$H</th>
<th>$^{13}$C</th>
<th>Sugar Residue (β-D-Mannose)</th>
<th>$^1$H</th>
<th>$^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>5.40</td>
<td>98.87</td>
<td>B1</td>
<td>4.70</td>
<td>102.1</td>
</tr>
<tr>
<td>A2</td>
<td>3.58</td>
<td>71.90</td>
<td>B2</td>
<td>4.11</td>
<td>77.2</td>
</tr>
<tr>
<td>A3</td>
<td>3.75</td>
<td>73.00</td>
<td>B3</td>
<td>3.63</td>
<td>73.5</td>
</tr>
<tr>
<td>A4</td>
<td>4.00</td>
<td>74.80</td>
<td>B4</td>
<td>3.85</td>
<td>77.1</td>
</tr>
<tr>
<td>A5</td>
<td>3.95</td>
<td>76.00</td>
<td>B5</td>
<td>3.90</td>
<td>75.2</td>
</tr>
<tr>
<td>A6</td>
<td>3.25</td>
<td>63.50</td>
<td>B6</td>
<td>3.50</td>
<td>62.5</td>
</tr>
</tbody>
</table>

Data are mean ± sem of triplicate results.

Table 2: Flow properties of Paracetamol granules prepared with oxidized *S. mycrophylla* gum compared with gelatin
Data are mean±sem of triplicate results

Table 3: Physical properties of paracetamol tablets formulated with oxidized *S. mycrophylla* gum compared with gelatin Bp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration of Oxidized gum (% w/w)</th>
<th>Gelatin Bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total weight (mg)</td>
<td>417±4.2</td>
<td>412±3.9</td>
</tr>
<tr>
<td>Hardness (kg)</td>
<td>2.4±0.4</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>Firability (%)</td>
<td>1.2±7.5</td>
<td>1.43±0.10</td>
</tr>
<tr>
<td>Disintegration time (min)</td>
<td>1.0±0.1</td>
<td>1.3±0.11</td>
</tr>
<tr>
<td>Dissolution time, d45 (Min)</td>
<td>102.8±4.4</td>
<td>97.6±2.4</td>
</tr>
</tbody>
</table>

Data are mean±sem of triplicate results

**PLATE. 1:** *Swietenia mycrophylla* crude exudates Gum

**Fig 2:** $^1$H NMR Spectrum (600MHz) of oxidized *Swietenia mycrophylla* gum (10mg in 700 µL D$_2$O, 60°C) Referenced to TMSP

**Fig 3:** $^{13}$C NMR Spectrum (600MHz) of unmodified *Swietenia mycrophylla* gum (10mg in 700µL D$_2$O, 60°C) Referenced to TMSP

**Fig 4:** $^{13}$C NMR Spectrum (600MHz) of Oxidized *Swietenia mycrophylla* gum (10mg in 700 µL D$_2$O, 60°C) Referenced to TMSP

**Fig 1:** $^1$H NMR Spectrum (600MHz) of unmodified *Swietenia mycrophylla* gum (10mg in 700 µL D$_2$O, 60°C) Referenced to TMSP
DISCUSSION

In the $^1$H-NMR studies, the resonance for H-2 to H-6 (Fig 1) can be found at 3.2 to 4.2 ppm. $^1$H NMR spectra tend to have overlapping signals in the 3.2 to 4.2 ppm region and coupling information is difficult to assign. However, it should be noted that for pyranose rings for proton, 1H signals are generally downfield from the axial 1H, thus for a monosaccharide, an anomeric OH in the α- position (which has an equitoria H) has the $^1$H resonance at 5.3 to 5.8 ppm, whereas the β- counterpart (having an axial H) resonate at 4.5 to 4.8 ppm.\[13\]. The anomic proton of residue A (α-D-galactose) (Fig 1) had the chemical shift larger than 5.0 ppm and very small J-coupling constants of $^3$J$_{H_1-H_2}$, suggesting that this residue was α-linked, meanwhile the anomic proton of residue B had the chemical shift smaller than 5.0 ppm and relative large J-coupling constant of $^3$J$_{H_1-H_2}$ suggesting residue B (β-D- Mannose) was β—linked [16]. The proton assignment of residue A (from H-1 to H-5; 5.42, 3.58, 3.75, 4.00 and 3.95 ppm) and residue B (4.71, 4.10, 3.62, 3.80 and 3.75 ppm) were obtained. [13,17]. Any substituent on oxygen atom shifts the neighboring protons. This shift usually moves downfield, depending on the substituent in question. [18]. The proton chemical shifts are sensitive to the attachment of a non-carbohydrate group such as carboxyl, acetyl, methyl or carboxymethyl groups. Attachment of such groups affect the proton and carbon resonances where the group is located. This place these resonances in a less crowded area of the spectra and helps in the identification of functional groups introduced during modification of polysaccharides. Such appended group may also contain NMR-active nuclei which may give rise to additional splitting due to couplings. [19].

In the oxidized gum $^1$D$^1$H NMR spectrum (Fig 2) the spectrum for the oxidized gum revealed the occurrence of new peaks at 1.9 ppm and confirming the introduction of carboxyl group (-COOH-) substituents in position of C-6 of residue A and C-4 of residue B. Substitution at oxygen usually moves the $^1$H shift downfield. The extent of the shift depends on the nature of the substituent. [13,17]. Although $^{13}$C-NMR has a much weaker signal, it has significant advantages over $^1$H-NMR spectra in the analysis of polysaccharides, because the chemical shift in $^{13}$C-NMR are spread out over a broader range (0-200 ppm). This broad distribution of signals helps to
overcome the severe overlapping problems associated with the $^1$H-NMR spectra. [17]. In the $^{13}$C spectrum, signals from anomeric carbons appear in the 90 to 105ppm region while the nonanomeric carbons are between 60 and 85ppm for polysaccharide with de-oxygen sugars, the $\text{CH}_3$ signals appear in a much higher field (15 to 20ppm). The anomeric C-1 carbons are the most diagnostic; thus from C-1 alone one can often determine the different types of sequences present and their relative proportions. The resonance of C-2 to C-5 can be found around 65-78ppm. The primary OH (C-6 for pyranoside) resonate at 60-70ppm. [17].

Of the two types of sugar residues conformation, signals derived from $\alpha$ – anomeric carbons mostly appear in the region of 98 to 100ppm while most of the $\beta$ – anomeric carbons will appear between 101 and 105ppm. [20, 15].

The signal of carbon atoms having primary hydroxyl groups, such as C-6 appear at a higher field of 60 to 64ppm, while the signals of carbon atoms with secondary hydroxyl groups, the non-anomeric carbons for C-5 shifts by 10ppm to a lower field. [17, 20].

The carbon anomeric region of $^{13}$C NMR of the unmodified gum (Fig 3) showed two major signals at the anomeric region which may be attributed to two neutral sugar components of the polysaccharide which were assigned as C-1 of $\alpha$-D-sugar residue A at 98.87ppm and C-1 of $\beta$-D-sugar residue B at 102.1 ppm. The signals due to non-anomeric carbons C-2 to C-5 appear between 60 and 85ppm. The spectrum region of anomeric carbons (102.1 and 98.87ppm) and the methylene carbons (62.50 and 63.50ppm) are well depicted (Fig 3). The resonances of the carbon atoms were well resolved (Fig 3) and identified as the resonances of C-2, C-3, C-5 of residue B and C-2, C-3, C-4 and C-5 of residue A (Table 1).

These facts are almost identical with gums of other origins [17]. The small peak at around 94ppm is consistent with the chemical shift expected for C-1 (OH) in $\alpha$ – configuration of residue A ring [20]. The $^{13}$C NMR spectrum for modified gum derivatives (fig 4) shows some differences in relation to unmodified gum. The anomeric signals decrease considerably due to chain degradation. A new signal at 175.5ppm was observed for the carbonyl carbon of the carboxyl groups. [17]. Results of $^{13}$C-DEPT NMR 135° sub-spectra of the unmodified gum and its derivative are shown in (fig 5-6). The $^{13}$C-DEPT NMR experiment was used to identify the methylene groups signals of the carbon atoms bearing two protons which have opposite amplitude to the CH and $\text{CH}_3$ carbons.

The $^{13}$C-DEPT NMR 135° spectro (fig. 5 and 6) for the unmodified gum showed at a high field two inverted signals (62.45 and 63.65ppm) assigned to methylene carbons (C-6) of the sugar residues. Resonance were assigned with the aid of literature data. [17, 21, 18, 22, 16, 23]. The spectrum presented only two signal for C-6 of branched $\beta$-D-mannopyranosyl residue which may indicate according to [18]. The presence of residue B triad where the intermediate residue is substituted. The CH$_2$ peaks at 62.45 ppm and 63.65ppm arises from CH$_2$OH of the polysaccharide. This therefore indicate that 6-0 substitution of sugar residue A is present as also found for polysaccharides from other sources. [24, 25, 26].

In addition to the C-6 resonance observed in $^{13}$C-DEPT, CH$_2$ sub-spectra (Fig 5) as described above, other peaks attributed to residue A units in the region were observed at 98.87, 71.9, 73.0, 74.8, 76.0 and 63.5ppm. [13, 25].

In the $^{13}$C-DEPT NMR spectra of the modified gum (Fig 6), the signal at 63.45ppm appeared with opposite amplitude to those of CH$_3$ and CH which can be
attributed to the modification of CH$_2$ primary carbons (C-6). The presence of carboxyl groups during oxidation cause an increase in the $^{13}$C chemical shift, [13, 16].

$^{13}$C-solid state NMR spectra of the gum and its derivative are shown in (Fig 7 and 8). The spectra give line widths which are typical of an armophous substance. The C-4 carbon accounts for high frequent shoulder while C-1 anomeric carbons give the signals between 90 and 110ppm. The shape of this band suggest it is composed of multiple signals but the low resolution suggests the contrary. The peak at 62ppm is assigned to the C-6 of the monosaccaride repeating unit which is attributable to the –CH$_2$OH belonging to galactose and mannose repeating units. The cluster of resonances around the peak at 72.2ppm and 83.8ppm are assigned to C-2, C-3 and C-5. The peaks at 84.4ppm and 89.0ppm are attributed to C-4 and the absorption peak at 102.1ppm is assigned to C-1 of β–mannose in the gum. [12, 28]

The modified gum $^{13}$C solid state NMR spectrum (Fig 8) showed a decrease in signal intensities at both the C-6 and C-4 peaks of the armophous gum sample indicating that the polysaccharide underwent a preferred degradation of armophous region during modification reactions[29]. The signal at 175.5ppm (Fig 8) is attributed to the carboxyl group in the carboxyl gum. [13, 30, 31].

The flow characteristic of granules produced with different concentrations of oxidized S.mycrophyllya gum mucilage is shown in Table 2. The different batch of granules exhibited good flow properties with Husner ratio and Carr’s index values of 1.0 - 1.20 and 9.0- 13%, respectively. There was, however, no direct correlation between the flow properties of granule and the gum concentration used. The flow properties granules prepared with 4-8% w/w oxidized gum were comparable to that 4% w/w gelatin Bp. Table 3 provides details on the physical properties of paracetamol tablets prepared using oxidized gum as binder compared to gelatin Bp. All the tablets prepared had uniform tablet weight. Tablet hardness increased with increase in gum concentration. The hardness of tablets containing 2% w/w oxidized gum was < 4kg while that containing 4 - 8% w/w was > 4 kg. The friability of the tablets decreased with increase in gum concentration. Tablets prepared with 2-4% w/w oxidized cashew gum had friabiliy values > 1% while that of 6-8 % w/w had friability <1%.

Thus, tablets prepared with 4-8% w/w oxidized S.mycrophyllya gum passed the Bp tablet hardness test while that containing 6-8% w/w oxidized S.mycrophyllya gum passed the Bp tablet friability test. The disintegration time of the tablets was < 15min and increased with increase in gum concentration. All the tablets exhibited fast dissolution in aqueous media with > 94% of the drug released in 45min. The dissolution rate of the tablets decreased with increase in oxidized S. mycrophylla gum, concentration. The fast disintegration and dissolution by the tablets have shown that oxidized cashew gum is suitable for use as a binder- in conventional tablets intended for fast disintegration and release in the gastrointestinal tract. The results obtained in this study were better than the results obtained in our previous study when we utilized the purified S.mycrophyllya gum as an excipient in paracetamol tablet formulation.[12].

CONCLUSION

Oxidized S. myrophyllya gum was synthesized, characterized by NMR and utilized as an excipient in paracetamol tablet formulation. The carboxyl groups incoorporated was detected by NMR analysis. Oxidized
S. mycrophylla gum at concentration of 4-8% w/w was successfully employed as binder. A better tablet physiochemical properties of uniformity of weight, hardness, friability, disintegration and dissolution were obtained compared to the standard excipient used. The study confirms that oxidation improves the binding properties of the native gum. The gum is safe and may therefore be used as excipient in drug formulation. The relative abundance and easy availability of this gum may reduce cost and save foreign exchange in Nigeria.

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