SYNTHESIS AND CHARACTERIZATION OF GUAR GUM DERIVATIVES WITH ANTIOXIDANT MOIETIES

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ABSTRACT

The purpose of this research is to introduce new green, nontoxic, cost effective derivatives of guar gum with antioxidant moieties covalently bounded to guar backbone. Guar gum is one of the significant naturally occurring non-ionic polysaccharide which has wide applications due to its rheological modifying properties in medicinal industrial and other commercial sectors. The amendment of rheological properties of guar gum can improve and diversify its commercial applications. A novel and efficient synthesis of five different new derivatives of guar gum (3a-3e) were prepared by insitu activation of caffeic, cinnamic, coumaric ferulic, and hydrocinamic acid with dicyclohexylcarbodiimide (DCC) and N,N-dimethylaminopyridine (DMAP). Temperature Effect, reactants concentration and time interval played imperative role for determining the degree of substitution (DS) in all synthetic guar esters. The DS value was estimated quantitatively by titration method. Guar derivatives with variable DS were prepared and confirmed by FT-IR and $^1$H-NMR spectroscopy. Surface morphological study of new eco-friendly guar esters were achieved by scanning electron microscopy (SEM).

KEY WORDS: Guar gum, antioxidant moieties, DS, $^1$H-NMR, SEM

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INTRODUCTION

Despite the vast importance of green chemistry there is a need to modify naturally occurring biopolymers to have the advantages over synthetic polymers by eliminating the danger to health and environment \(^1\). Among these biopolymers, Guar gum (GG) is a promising candidate which has extensive applications due to its rheological modifying properties\(^2\). This green biopolymer represents galactomannan family of polysaccharides and derived from seeds of *Cymopsis tetragonolobus*\(^3\) a leguminous plant cultivated from centuries in Pakistan and India. Guar gum is a potential aspirant of natural biopolymer due to its diverse structure, properties, non-toxicity, biodegradability, and stability over wide pH range, solubility in hot and cold water and economical processing. Due to these fascinating properties and physiological effects it undergoes many chemical and physical changes to form new derivatives of desirable characteristics and functionalities. Due to its high molecular weight 2-3 kDa, it acts as a rheological modifier in many aqueous systems\(^4\). Chemically, it has long, straight chains of α-D-mannopyranosyl units linked by β-D-(1-4)-glycosidic linkage (Figure 1) in the ratio of 2:1. The GG backbone has three, free reactive hydroxyl groups present in each anhydrous glucose units which can be replaced by various desirable functional groups with different degree of substitution. Maximum, three degrees of substitution can be obtained in a sugar moiety\(^5-7\). However, substitution of guar gum is a difficult task due to its insolubility to afford uniform dispersion without degradation in any organic solvents. Many researchers attempted modification of guar gum like carboxymethylation,\(^8-11\) methylation,\(^12\) acetylation,\(^13,14\) hydroxypropylation,\(^15,16\) benzoylation,\(^17\) sulfonation,\(^18\) cationic guar gum\(^19\) etc., but no work has so far been carried out to link antioxidant moieties to guar backbone by covalent bond. With this aim we converted hydroxyl group of guar gum into different esters by introducing phenolic acids as antioxidant moieties to guar backbone which could enhance its application in pharmaceutical industry as a binder, disintegrators and potential carriers for targeted drug delivery\(^20-23\). For this purpose, Cinnamic acid and its derivatives (caffeic, cinnamic coumaric, ferulic, hydrocinnamic acids) were selected which exhibit strong antioxidant activity due to the presence of -CH=CH-COOH moiety as compared to other phenolic acids which have been reported as good free radical scavengers\(^24-27\). Synthetic antioxidants have much concern due to their toxicity but naturally occurring dietary fibres bounded with antioxidant have wide applications in medicines due to desirable drug release profile, cost-effectiveness and no toxicity. Many researchers fabricated antioxidant groups to different dietary fibers like starch\(^28\) and cellulose\(^29\). But no work has done on guar gum due to its structural complexity, high molecular weight and insolubility in any organic media. Taking into account the above procedures done on starch and cellulose we are reporting novel derivatives of guar gum with antioxidant groups bounded by reacting guar gum with cinnamic acid and its analogues e.g. ferulic acid, p-coumaric acid, caffeic acid and hydrocinnamic acid by in situ activation of acid with dicyclohexylcarbodiimide (DCC) and N,N-dimethylaminopyridine (DMAP) as a catalyst. Structure conformation of all synthesized guar esters were furnished by FT-IR, \(^1\)HNMR and SEM. Degree of substitution was estimated quantitatively by titration method.
EXPERIMENTAL

MATERIALS

All commercially available compounds were used without further purification except where stated. All moisture or air-sensitive reactions were carried out in flame-dried glassware under positive pressure of nitrogen using standard syringe/septum techniques. Anhydrous solvents were obtained by passing through a modified Grubbs system of alumina columns, manufactured by Anhydrous Engineering. When stated DMF and DMSO were dried over 4 Å MS beads for 24 h three times and stored under nitrogen. Routine reactions monitoring was performed using precoated Merck-Keiselgel 60 F254 aluminium backed TLC plates. The spots were visualised by UV254 light and 10% ethanolic-sulfuric acid. Partially hydrolyzed guar gum pharmaceutical grade (PHGG) purchased from Pakistan Gum Industries Karachi, trans-cinnamic acid (CA), hydrocinnamic acid (HCA), ferulic acid (FA), p-coumaric acid (PCA), caffeic acid (CaA), dicyclohexylcarbodiimide (DCC), N,N-dimethylaminopropylidine (DMAP) and dimethyl sulfoxide (DMSO) were supplied by (Sigma-Aldrich® UK). Analytical grade reagents and solvents were used throughout the experimental.

Instrumentations

Infrared spectra were analysed e on a Perkin Elmer Spectrum One FT-IR spectrometer in the solid or liquid state. 1H NMR spectra were recorded using either a Jeol lambda 300 MHz, Jeol Eclipse 400 MHz or Varian 400-MR 400 MHz spectrometer using DCl/D2O, DMSO-6 and D2O as a solvent. 10 mg/mL Sample were used for analysis. The chemical shifts (δ) were reported in parts per million (ppm) and the coupling constants (J) in Hertz (Hz). Jeol 5600 LV SEM was used for surface morphological studies of all synthetic polymers.

Purification of guar gum

Guar gum was stirred with ethanol overnight to dissolve impurities. After that guar solution was centrifuged (Sorvall RC 6Plus) at 7500 rpm for an hour to remove insoluble impurities. Clear supernatant liquid was decanted and filtered by glass fibre filter paper. Precipitates were dried under vacuum at 80°C for 24 hours.

Dissolution of guar gum in DMSO

Guar gum (2g) was dissolved in 50 mL of DMSO for an hour at 80°C under nitrogen to obtain transparent solution of Guar (GG).(Scheme.1)

Appendix

Scheme 1

Schematic approach to substitution of guar gum

\[
\text{GG (1) + PAs (2a-2e) } \rightarrow \text{ GG esters (3a-3e)}
\]
Synthesis of guar gum cinnamate (GGC)- 3a

Cinnamic acid (2.66 g, 18 mmol), dicyclohexylcarbodiimide DCC (3.7 g, 18 mmol) were added at 0°C to the solution of guar (GG) with continuous stirring under nitrogen followed by 0.4g DMAP. Temperature of flask was maintained at 50°C for 72hr and reaction performance was monitored by IR (ester peak observed) and TLC (disappearance of DCC). The precipitates of product were extracted with ethanol and washed with hot methanol, chloroform and ether to remove unreacted dicyclohexyl urea. Solvent was removed in vacuo to give the product GGC. DS: (calculated by titration) 0.35 and yield =1.63 g. FTIR (KBr)=3349 (OH stretch), 2920 (C-H stretch), 1668 (C=O ester), 1213(C-O-C ester) ¹HNMR (400 MHz, DMSO-d₆): ppm = 3.5-5 (sugar Protons), 6.31(1H, d ,8-H), 7.02(1H, d,7-H), 6.56-6.94(5H, m, Ar-H);

Synthesis of guar gum ferulate GGF- 3b

In situ activation of ferulic acid (3.5g, 18 mmol) was done by coupling with DCC (3.7g, 18 mmol) and DMAP (0.1 g, 0.5mmol) following the same procedure the as above. DS: 0.98 (calculated by titration) and yield =1.53 g. FTIR (KBr) =3415 (OH stretch), 2943 (C-H stretch), 1726 (C=O ester), 1225 (C-O-C ester) ¹H-NMR (400MHz, DMSO-d₆ /D₂O):ppm 3.8-5.4 (sugar protons), 3.69 (3H, s, OCH₃), 4.19 (1H,br s, OH), 6.17 (1H, d, 8-H), 6.74 (1H, d, 5-H), 7.02 (2H, m, 2-H and 6-H),7.40 ( 1H, d, 7-H);

Synthesis of guar gum caffeate (GGCa)-3c

A solution of guar gum (GG) and caffeic acid (3.24g, 18 mmol) in dry DMF (50mL) was stirred at room temperature then dicyclohexylcarbodiimide (3.7g, 18 mmol) was added, the mixture temperature was maintained at 70°C for 48h under inert atmosphere. Ester produced was precipitated in ethanol (100mL) and washed with hot methanol, ether and chloroform to dissolve dicyclohexyl urea formed as a by-product. Product dried under vacuum with continuous stirring of reaction mixture. DS = 0.69 (calculated by titration) and yield: 1.9 g. FTIR (KBr) = 3335 (OH stretch), 2932 (C-H stretch), 1729 (C=O ester), 1219 (C-O-C ester) ¹HNMR (400 MHz, DMSO-d₆ /D₂O): δ ppm = 3.2-5.0 (sugar Protons), 5.35 (2H, br s, 3-OH, 4-OH), 6.91(1H, d, 8-H), 7.60 (1H, d, 7-H), 6.93-7.17 (3H, m, 2-H,5-H and 6-H)
Synthesis of guar gum coumarate (GGCo)-3d

Coumaric acid (6.608 g, 36 mmol) was dissolved in dry DMSO followed by DCC (7.6g, 0.037 moles) with stirring for an hour. This mixture was added in 3 g guar solution suspended in DMSO at room temperature. The contents of flask were stirred for 72 hrs under nitrogen at 50°C. The precipitates were extracted with ethanol and washed with chloroform and ether to remove unreacted by products. Product was dried in vacuo. DS: 1.09 (calculated by titration) and yield: 2.63 g. FTIR (KBr) = 3335 (OH stretch), 2932 (C-H stretch), 1729 (C=O ester), 1219 (C-O-C ester) \( ^1 \)HNMR (400 MHz, DMSO-\( d_6 \)/D\( _2 \)O): \( \delta \) ppm = 3.2-5 (sugar Protons), 5.35 (1H, br s, 4-\( \text{OH} \)), 6.13(1H, d, 8-H), 6.65 (2H, d, 3-H and 5-H), 7.32 (2H, d, 2-H and 6-H), 7.41 (1H, d, 7-H)

Synthesis of guar gum hydrocinnamate GGH-3e

Hydrocinnamic acid (1.5 g, 10 mmol) and DCC (2.06 g, 0.01 mole) were mixed at 0°C in a solution of GG in DMSO with continuous stirring. Reaction mixture was heated at 50°C for 24 hrs under nitrogen. TLC indicated the consumption of all starting material. The precipitates were washed several time with ethanol and chloroform and dried in vacuo to give the product. DS: 0.54 and yield: 1.87 g. FTIR (KBr) = 3366 (OH stretch), 2946 (C-H stretch), 1723(C=O ester), 1300(C-O-C ester) \( ^1 \)HNMR (400 MHz, D\( _2 \)O): \( \delta \) ppm =5.0-6.9 (sugar Protons), 2.53 (2H, t, 7-H\( _2 \)), 2.89 (2H, t, 8-H\( _2 \)), 7.29(5H, m, Ar-H)

**Determination of degree of substitution (DS) by titration.**

Method of Wurzburg (1964)\(^{30}\) was modified to determine the degree of substitution of guar esters, quantitatively. For each experiment (0.1g) dry ester was dispersed in 10 mL of 0.5N ethanolic KOH solution and refluxed for 24hours. The excess soda was back-titrated against 0.1N HCL, using phenolphthalein as an indicator. A blank sample was also titrated for reference.

\[ \text{DS} = \frac{V_1 \times V_2 \times \text{HCl normality} \times \text{MM ester} \times 10^3 \times 100}{\text{Sample weight in g}} \]

\[ V_1 = \text{volume in mL for blank} \]
\[ V_2 = \text{volume in mL for sample} \]
\[ \text{MM ester} = \text{molecular mass of ester group} \]
\[ 162 = \text{molecular mass for glucose unit} \]

**Antioxidant potential**

Antioxidant potential of Novel guar derivatives 3a-3e was done by simple calorimetric method\(^{31}\). DPPH (2, 2-diphenyl-1-picryl hydrazyl) assay was used to determine the antioxidant potential, by observing the decrease in DPPH radical absorbance capacity at 517 nm by lee et al spectroscopic method.

**DPPH radical scavenging activity**

0.005mL of Test compounds (120-124) were added into 0.095mL ethanolic solution of
DPPH dissolved in DMSO for making total volume upto 0.1mL (final concentration of 100µM and 20 µM for DPPH and derivatives respectively). The mixture was mixed vigorously on a vortex mixer and incubation was done for half an hour in a water bath for 30 min in a water bath at 37 °C. Control was 0.005mL of DMSO instead of test compound and it was also placed in incubator with sample 96-well tubes covered with parafilm to avoid evaporation for 30 minutes. UV-Visible Spectrophotometer (UV-2800 Hitachi) was used to calculate decrease in absorbance of the samples at 517 nm. All assays were done in triplicate. The activity was explained as inhibition percentage. It was considered by means of the formula:

Radical scavenging activity (%) = \[\frac{(A_x - A_y)}{A_x} \times 100\]

Where
A\(_x\) = Absorbance of blank,
A\(_y\) = Absorbance of sample.

RESULTS AND DISCUSSION

Guar gum esters were synthesised by introducing antioxidant moieties by the action of phenolic acids with free hydroxyl groups. We developed a novel route for synthesis of these esters via \textit{inisitu} activation of phenolic acids by using coupling reagents; N,N'-dicyclohexylcarbodiimide (DCC) and DMAP. This approach was better replacement of conventional method via acid chloride formation. N, N'-dicyclohexylcarbodiimide (DCC) served as dehydrating agent. Beside this it also activate phenolic acids towards ester formation. The reaction mechanism involves the formation of intermediate O-acyl isourea by reacting acid with DCC. Free hydroxyl group of GG attack the reactive intermediate and gave rise to corresponding ester and DCU (dicyclohexyl urea) as a by-product (Stagelish esterification) as shown in (scheme 2 and 3)

\begin{scheme}
\textbf{Synthetic route for guar esters}

\includegraphics[width=\textwidth]{synthetic_route_guar_esters.png}

\textbf{REACTION SCHEME}
\end{scheme}
Effect of temperature, concentration of reactants and time duration were explored for each experiment. Optimum reaction parameters were established for each derivative. It was observed that efficiency of reaction was increased by elevating temperature and amount of reactant. At room temperature no esterification was observed for 72h probably due to requirement of high temperature for effective collision between reactants to form the reactive intermediate. Reaction was supervised by FTIR and Thin-layer chromatography TLC. Disappearance of DCC during TLC analysis directed the completion of reaction, using n-hexane:ethylacetate (3:1) as a solvent system. The spots were developed by spraying 10% ethanolic-sulfuric acid. Purification of guar gum was furnished to remove insoluble impurities. The degree of substitution was determined by acid-base titration method. The FTIR spectrum for native guar gum (Figure 2) showed broad band in a region of 3324 cm\(^{-1}\) were attributed to stretching vibration of O–H bond that exhibited the presence of large number of free hydroxyl groups in the guar backbone. The intensity of O–H band is reduced in modified esters (Figure 3) of guar that ultimately confirmed the conversion of hydroxyl groups in to ester moieties. The finger printing region of guar gum consists of characteristic peaks from 862 to 1145 cm\(^{-1}\), designated to the C–O bond stretch. The bands at 1636 cm\(^{-1}\) and 1376 cm\(^{-1}\) are due to bending vibration of OH and CH\(_2\), respectively. The sharp band at 2926 cm\(^{-1}\) was due to CH\(_2\) group stretching, observed in spectrum of HCA ester.
Guar gum derivatives featured sharp band between 1720-1730 cm$^{-1}$ due to C=O stretch typical for esters appear which can be easily differentiate from C=O bond stretch in unsaturated acids between 1650-1690 cm$^{-1}$ except for guar cinnamate where C=O ester bond stretch observed at 1668 cm$^{-1}$ which can be justified that due to the presence of aromatic ring and unsaturation reduced the bond intensity for C=O stretch. Another characteristic absorption band for C-O-C stretch for esters appeared in the region of 1200-1300 cm$^{-1}$ for guar derivatives. The C=C aromatic ring stretch observed at 1518, 1504, 1501, 1490, 1539 cm$^{-1}$ for GGC, GGF, GGCa, GGCa, GGHc, respectively. Two characteristic bands due to bending motion caused by CH bond appear at 1550-1600 cm$^{-1}$. There was restriction for characterization of guar gum and its derivatives in NMR spectroscopy due to high viscosity and polymeric structure of guar solution even at low concentrations. In order to get better resolved spectra careful acidic degradation or $insitu$ hydrolysis of guar gum in DCL or D$_2$O is recommended because poor resolution of native gum due to high molecular weight. Guar gum and its derivatives were characterized by $^1$HNMR spectroscopy. Native gum exhibited peak at 3.90, 4.15 ppm due to anomeric protons of mannose and galactose backbone. The galactose showed sharp peak than mannose. (Figure.4). A multiple signal resonated between 2.5-3.5 ppm due to other sugar protons. These signals were downfield in modified guar derivatives due to replacement of hydroxyl groups by more electronegative moieties. The additional signals of phenolic ester groups grafted in guar gum chain were authenticated in NMR spectrum there by confirming the modification in guar backbone.
Surface morphological studies of native guar and its esters were carried out by scanning electron microscopy. Significant changes in shape and size of native gum and derivatives designated modification in guar backbone. Guar esters shows porous structure with networking which enhanced as degree of substitution increased. (Figure.5)
**DPPH Assay**

DPPH is abbreviated as 1, 1-diphenyl-2-picrylhydrazyl is a stable free radical that has ability to readily accept an electron to convert into diamagnetic stable molecule. Its radical scavenger capacity was determined by decrease in absorbance at 517 nm due to the transformation of DPPH into DPPH+ with change in colour from dark violet to yellow. DPPH method is an easy calorimetric method used for measurement of antioxidant potential of variety of natural compounds. It is accurate, valid, economical sensitive methodology for evaluation of free radical scavenging activity of variety of naturally occurring compounds including cinnamic acids and its derivatives. Biological activities of cinnamic acid and derivatives are well proved by many researchers.

**4.1.8 Radical Scavenging studies of DPPH**

Radical Scavenging potential of DPPH was studies for test compounds 120-124. Faster reaction was observed for Phenolic acids (2a-2e) as compared to their derivatives. Guar esters showed significant radical scavenging potential at almost all concentration. The activity increased by increasing amount of test compound and by increasing DS value of sample. Maximum Scavenging was observed for compound 2c and compound 23 proved to be inactive. Minimum Scavenging was noticed for compound 2d and at lower DS it is also showed inactivity. Result is summarized in Table.1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radical Scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>28.45±0.45</td>
</tr>
<tr>
<td>2b</td>
<td>23.15±0.91</td>
</tr>
<tr>
<td>2c</td>
<td>45.6±0.67</td>
</tr>
<tr>
<td>2d</td>
<td>7.0±0.32</td>
</tr>
<tr>
<td>2e</td>
<td>2.4±0.8</td>
</tr>
<tr>
<td>3a</td>
<td>18.3±0.9</td>
</tr>
<tr>
<td>3b</td>
<td>18.43±0.5</td>
</tr>
<tr>
<td>3c</td>
<td>30.23±0.7</td>
</tr>
<tr>
<td>3d</td>
<td>2.32±0.5</td>
</tr>
<tr>
<td>3e</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The successful synthesis of guar gum esters with variable DS value and biological potential were achieved with good yield. First time guar gum esters by in situ activation of cinnamic acid, ferulic acid, p-coumaric acid, caffeic acid and hydrocinnamic acid was achieved by
Coupling with dicyclohexylcarbodiimide (DCC), N,N-dimethylaminopyridine (DMAP). These esters possess remarkable antioxidant potential which can enhance its application in pharmaceutical industry. We are in the hope of finding possibly even higher biological activity of new biopolymers by adapting same approach with different antioxidant moieties. This in turn can be helpful to understand which analogues might work better to improve the chances of success.

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