α-(1→4)-linked D-galacturonic Acid Based Linear Homopolymer as Drug Release Modulator in HPMC Based Hydrodynamically Balanced System

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ABSTRACT

Objectives: Natural polysaccharides are increasingly being used for diverse applications in drug delivery such as controlled release of drugs owing to their wide availability, renewability, biodegradability and biosafety. Furthermore, polysaccharides from plants utilized as food may possibly mitigate regulatory requirement for approval as well. Mung bean (Vigna radiata L. seeds) is a key cereal in India composed of several non-starch polysaccharides. In the present work we have attempted to isolate, characterize and pharmaceutically evaluate the polysaccharide derived from dehusked Mung beans using water-acetone precipitation method. Methods: The obtained polysaccharide was characterized using Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry, Powder-X-ray diffraction and 13C Nuclear Magnetic Resonance spectroscopy. Further, the polysaccharide derived from Mung beans was carboxymethylated using Williamson Ether Synthesis. The degree of substitution of carboxymethylated Mung bean polysaccharide was determined to be 0.76. The carboxymethylated polysaccharide was then used in combination with hydroxypropyl methylcellulose K4M to fabricate hydrodynamically balanced sustained release capsule dosage form taking moxifloxacin HCl as model drug. Results: The system emerged as a hydrodynamically balanced system that remains floated for 12 hr. The drug release mechanisms involved swelling, dissolution and erosion of polymer matrices. The erosion operated at later times and was sensitive to the osmotic stress exerted by ionic polymer and the drug present in the matrix. Conclusion: The findings of the study suggest that polymer matrices composed of chemically modified Mung bean polysaccharide and hydroxypropyl methylcellulose K4M may form a promising carrier for sustained stomach specific delivery of model drug moxifloxacin Hydrochloride.

Key words: Mung bean polysaccharide, Williamson ether synthesis, Carboxymethylated polysaccharide, Moxifloxacin Hydrochloride, Hydrodynamically balanced system.

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INTRODUCTION

Recently, the evolution and utilization of polymers derived from plant sources have garnered considerable interest due to their wide availability, renewability, biodegradability and biosafety. Moreover, derivation of polysaccharides from natural sources such as pulses/cereals may possibly mitigate regulatory requirement for approval as well. Because of this, plant derived polysaccharides are increasingly being used for distinct applications in drug delivery. The seeds of Vigna radiate L. are an important pulse consumed predominantly in India and other Asian countries including China, Burma, Korea, Pakistan, Japan, Thailand and other parts of Southeast Asia. Mung bean is composed of several non-starch polysaccharides. Most of the reported polysaccharides from Vigna radiate L. seeds were derived from water extracts. The derived polysaccharides have been evaluated for antioxidant and immunoregulatory activities but not for pharmaceutical drug delivery applications. In the present work the authors have made an attempt to isolate, characterize and pharmaceutically evaluate the polysaccharide derived from dehusked Mung beans using water-acetone precipitation method. The obtained polysaccharide was studied utilizing techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Powder X-ray diffraction (PXRD) and 13C NMR spectroscopy. Further, the polysaccharide derived from Mung bean was chemically modified using Williamson Ether Synthesis. The carboxymethylated polysaccharide was then used to fabricate single unit hydrodynamically balanced (HBS) system taking moxifloxacin HCl as hydrophilic model drug.

Among the reasons for selecting moxifloxacin HCl (MX) is its utility in the treatment and prevention of upper gastrointestinal tract infections. MX has been reported to present in high tissue concentration in the stomach mucosa (10.90 ± 5.10 mg/kg); which was up to 9.7 times superior to the serum concentrations. This increased concentration of MX in the stomach mucosa was another reason for potential use of MX in the treatment of bacterial infections in the upper gastrointestinal tract. Therefore, in the present investigation, MX was considered as a model drug for the stomach specific drug delivery through HBS capsules. Another reason for selecting the MX as model drug was its hydrophilicity (24 mg/ml). It has been reported that drugs with high aqueous solubility created high diffusional driving force coupled with osmotic stress in hydrophilic polymer matrices leading to burst release of drugs.

MATERIALS AND METHODS

Materials
Dehusked and dehulled Mung beans (Tata Sampann Mung Dal Split) were purchased from local market. Chloroacetic acid, Sodium...
hydroxide, Ethanol (95%) and concentrated Hydrochloric acid (36.5-38%) were of laboratory reagent grade (Central Drug House., India). Hydrochloric acid (HCl) reagent ACS (30%, ultrapure) was obtained from Spectrum Chemical, India. Acetone was purchased from Qualigens, India. All the reagents were used procured as such. Deionized water used was of Milli-Q grade, generated in our lab.

Isolation of polysaccharide

Dehusked Vigna radiata L. seeds were washed with distilled water until the washing was completely free from any haziness. Thereafter, the seeds were soaked in deionized water (1:3, seed: water ratio) for 24 h. With the help of a muslin cloth, the seeds were removed and filtrate was collected in a glass borosilicate glass beaker. The filtrate was then treated with acetone (1:4, filtrate: acetone ratio) as precipitating agent for polysaccharide.5 6 7 The extracted polysaccharide was subjected to three washings of 20 ml each with Milli-Q grade Deionized water. The obtained product was dried in oven at 50°C for 12 h. The dried product was finally powdered and passed through sieve no. 20 and stored in a desiccator until further use.

Characterization of Mung Bean Polysaccharide

Solubility Analysis

The solubility of Mung Bean Polysaccharide (MBP) was determined in distilled water, 0.1 M HCl, mixed phosphate buffer, pH 6.8, Dimethylsulfoxide (DMSO) and Tetrahydrofuran. For this, 0.1 g of MBP was dispersed in 10 ml of individual solvents in a glass beaker (25 ml capacity) at 25°C and 37°C. The contents of the beaker were then stirred using a magnetic stirrer for 1 hr and then kept overnight. The contents were filtered and the drug was assayed at 290 nm in the filtrate.

Spectroscopic analysis

Fourier Transform Infrared characterization

The Fourier Transform infrared spectrum (FTIR) of MBP was generated using Fourier Transform Infrared Spectrophotometer (Perkin Elmer IR Version 10.6.0). The discs made up of MBP and KBr (2 mg MBP in 200 mg KBr) were scanned in the range of 400–4000 cm⁻¹. The resolution was 2 cm⁻¹.

¹³C Nuclear Magnetic Resonance (NMR) characterization

The high resolution solid-state ¹³C CP/MAS NMR spectrum was recorded on the 400 MHz Varian solid-state NMR spectrometer at IIT, Roorkee, India. Briefly, the spectrum was recorded at the resonance frequency of about 100 MHz The spectrum was obtained at room temperature averaging over 5000–33000 scans. The chemical shifts were referenced to the Tetramethylsilane (TMS) using adamantane as an external standard.

Thermal Analysis

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis of MBP (5 mg) was carried out over 50-250°C at an interval of 5°C/minute using duplicate samples in crimped aluminum pans. Indium was used for the calibration of the instrument.

X-Ray Diffraction Studies

Powder X-ray diffraction characterization

The Powder X-ray diffraction (PXRD) pattern of the MBP was recorded using copper radiation (40 kV, 30 mA), on Philips Analytical X-RD (Model: PW 3710, Holland), in the range five to 1250 at the scanning rate of 0.020 /min of 2θ.

Carboxymethylation of Mung Bean Polysaccharide

The MBP was carboxymethylated following Williamson ether synthesis. Carboxymethylation was affected by Chloroacetic acid as reported by Kumar et al. with some modification. For Carboxymethylation, 2 g of MBP was dispersed separately in Milli-Q grade Deionized water (100 ml) in a 250 ml jacketed thermostated glass reactor equipped with a magnetic stirrer. After the MBP was dispersed in the deionized water, a known volume of sodium hydroxide solution (45%, w/w) was added, at about 1 ml per 15 min, with uninterrupted stirring room temperature. An aliquot part of 15 ml Chloroacetic acid was then added to the reaction mixture, over a period of 10 min. The chemical reaction mixture was then heated to 70°C for 30 min with uninterrupted stirring. The reaction mixture was then cooled and suspended into 80 % v/v ethanol. The precipitated material was filtered and washed three times with 60 ml of 80 % v/v ethanol, dried in an oven at 60°C overnight. The carboxymethyl Mung bean polysaccharide (CMBP) was characterized by FTIR spectroscopy as described in previous sections, and subjected to other studies described below.

Degree of substitution of Carboxymethyl Mung Bean Polysaccharide

The extent of carboxymethylation of carbohydrate polymers is expressed in terms of degree of substitution. It is the average number of substituted carboxymethyl groups per anhydro sugar unit present on the carbohydrate polymers. In the present study, the degree of substitution (DS) was determined by potentiometric back titration and was calculated using the equation 1:

\[
DS = \frac{0.162 A}{1.162 A\text{at}} \quad \text{Eq. 1}
\]

Here, A is the milli equivalents of NaOH required per gram of sample.

Equilibrium swelling

Equilibrium swelling of both MBP and CMBP were determined in pH 1.2 and 6.8 buffers (HCl-KCl and mixed Phosphate buffer). A carefully weighed amount (100 mg) of the MBP or CMBP (W₅), packed in bags made up of muslin cloth, was submerged in 50 mL each of the buffer solution maintained at 37°C and left to swell for 2 h. The swollen mass was removed from the muslin cloth and the excess water was removed with the help of a tissue paper. The swollen mass was then reweighed (W₆) on an electronic balance. The swelling index was calculated by the following equation:

\[
\text{Swelling index} = \frac{W_6 - W_5}{W_5} \times 100 \quad \text{Eq. 2}
\]

Carboxymethyl Mung Bean Polysaccharide as drug release modulator

To study the effect of CMBP on drug release, single unit HBS capsules were fabricated by physically mixing moxifloxacin HCl with the required quantity of HPMC K4M (non-ionic polymer) and CMBP (anionic polymer) using laboratory double cone mixer for 15 min (Table 1). The blended mass was then encapsulated into hard gelatin capsules (size 0) using hand operated capsule filling machine.

The prepared formulations were tested for in vitro drug release and drug release kinetics using a USP XXXI dissolution apparatus (paddle type, Electro Lab, Mumbai, India) at 50 rpm in 900 ml HCI-KCl buffer, pH 1.2 at 37 ± 0.5°C. At predetermined intervals, a 1 ml aliquot part was withdrawn and replenished with an equal volume of fresh HCI-KCl buffer. The withdrawn samples were analyzed UV spectrophotometrically at 290 nm.
RESULTS
Isolation of Mung Bean Polysaccharide
The polysaccharide was extracted by a water extraction process with no involvement of heat treatment during the extraction process. The yield of MBP was found to be 7g/100g of dehusked Mung bean. The extracted MBP was slight yellowish white free flowing powder.

Solubility of Mung Bean Polysaccharide
The solubility of MBP was determined in various solvents and buffers, the results are given in Table 2.

Fourier Transform Infrared spectrum of Mung Bean Polysaccharide
FTIR spectra of MBP (Figure 1) exhibited a broad stretching peak at 3404 cm\(^{-1}\) for OH stretch, intermolecular H-bridge between the OH groups and a weak band at 2930 cm\(^{-1}\) due to CH\(_2\) antisymmetric stretch, conforming carbohydrate nature of the polymer. The bands at 1153 and 1018 cm\(^{-1}\) may be assigned to C-O-C and C-O stretching vibrations of pyranose ring common to all polysaccharides. A weak peak at 1119 cm\(^{-1}\) could be due to ring antisymmetric stretch. FTIR band at 1244 cm\(^{-1}\) could be attributed to OH in-plane deformation in COOH groups. The peaks at wave number 1407 and 1454 cm\(^{-1}\) could be attributed to CH\(_2\) scissors vibration. The FTIR peak at 1642 cm\(^{-1}\) could be attributed to free carboxylate group in the MBP. The broad spectral region between 1800–700 cm\(^{-1}\) serves as fingerprint region for monosaccharide types, substituents and epimers of different sugars.\(^{14}\) In this broad spectral region, the deformation peak of C–H occurred between 1500–1200 cm\(^{-1}\) [1244 and 1407 cm\(^{-1}\)] and that of C–O–C occurred in the range of 1150–1000 cm\(^{-1}\) [1018, 1153 cm\(^{-1}\)].

\(^{13}\)C NMR of Mung Bean Polysaccharide
The \(^{13}\)C Nuclear Magnetic Resonance spectrum of MBP is exhibited in the Figure 2. A resonance at ~174 ppm corresponds to the C6 region (COO\(^{-}\)) of the polysaccharide. The resonance peak at 100.8 ppm can be assigned to C-1 carbon of the polysaccharide. The peak at 100.8 could be assigned to the resonance of glycosidic \(\alpha\)-linkage. The resonance peak at 75.13 ppm could be attributed to the C-4 carbons. The resonance peak at 72.69 ppm could be attributed to C-5 carbons of the MBP, whereas, the resonance peak at 71.29 ppm may be due to C-2 and C-3 carbon. The anomeric C1 resonance numbers in a \(^{13}\)C NMR spectrum tells us about the number of different sugar residues.

Powder X-ray diffractogram of Mung Bean Polysaccharide
The PXRD pattern of MBP is exhibited in Figure 3. The carbohydrate polymer sample showed peaks at approximately 19.7, 62.5, 69.14 and 88.7°. However, other peaks are very weak and could not be resolved.

Differential Scanning Thermogram of Mung Bean Polysaccharide
Figure 4 shows the DSC curve of the MBP. The glass transition temperature was 127.7°C and there was a melting peak but unfortunately we could only see its initiation in the thermogram at around 300°C.

Carboxymethylation of Mung Bean Polysaccharide
Carboxymethylation of MBP was done by Williamson ether synthesis. The yield of CMBP was found to be 71.88%. The solubility of CPMB (1% w/v) when tested in distilled water (37°C), found to be partially soluble, compared to completely insoluble behavior of MBP. The pH of the dispersion was found to be 6.9. It was also observed that the solution was slightly viscous; therefore, its viscosity was determined using Brookfield rheometer (LVDV-III, spindle cp 40). The viscosity was found to be 69.8 cps. The extent of carboxymethyl substitution, also called degree of substitution, of CMBP was determined to be 0.76 carboxymethyl groups/g of MBP. The CMBP was characterized by FTIR to confirm the chemical changes in CMBP.

Fourier Transform Infrared Spectrum of Carboxymethyl Mung Bean Polysaccharide
The spectra of CMBP (Figure 5) exhibited a peak due to O-H stretching of alcoholic group at 3439 cm\(^{-1}\). This peak is comparatively less broad than the corresponding peak in the FTIR spectrum of MBP (3439 cm\(^{-1}\)). This could be attributed to the carboxymethylation of some of the OH groups.

FTIR bands at 2927 cm\(^{-1}\), 1641 cm\(^{-1}\), 1413 cm\(^{-1}\), 1217 cm\(^{-1}\) and 1018 cm\(^{-1}\) correspond to, C-H stretching of the –CH\(_3\) groups, C=O stretch of COO\(^{-}\) of carboxylate anion, asymmetric and symmetric stretching of COO\(^{-}\) group, stretching vibrations of C-O in ethers and esters and stretching vibration of C-O \(\alpha\)-glycosidic bond respectively. The bands at 814, 642, 564 and 525 cm\(^{-1}\) in the infrared spectra are attributed to the skeletal modes of the pyranose ring.

Swelling behavior of Carboxymethyl Mung Bean Polysaccharide
MBP exhibited low swellability in both acidic (pH 1.2) and basic media (pH 6.8) [150.65 and 179.45 %]. CMBP exhibited higher swellability in pH 6.8 than in pH 1.2 (203.43 and 297.67%).

Figure 1: FTIR Spectra of Mung Bean Polysaccharide.

Figure 2: \(^{13}\)C NMR Spectra of Mung Bean Polysaccharide.
Drug release modulation by Carboxymethyl Mung Bean Polysaccharide

In the presented study, we have made an attempt to study the effect of combining CMBP with HPMC K4M on the release of a weakly basic drug MX in acidic dissolution medium (0.1 M HCl, pH 1.2). The drug release was determined in triplicate.

Various hydrophilic polymeric matrices were prepared as depicted in Table 1. After combining with MX, the drug-polymer mixtures were encapsulated in transparent size 0 capsules and subjected to *in vitro* drug release studies. During the entire study it was observed that all the formulations remained buoyant on the dissolution medium (no lag time) during the drug release studies and once the capsule shell completely disintegrated (30 min), the hydrated polymer matrices acquired the shape of a cylindrical plug. This cylindrical plug eroded with time but remained floated for entire duration of drug release studies. Thus the developed formulations were designated as hydrodynamically balanced systems (HBS).

In the presented work, the time required to release 25, 50, 75 and 100% of the drug was taken as the basis to express moxifloxacin HCl release from hydrodynamically balanced capsule dosage form (Figure 6). From formulation AV1 containing HPMCK4M about 27, 56, 81 and 97% of MX was released at the end of 2nd, 5th, 7th and 10th h. In formulation AV2, MBP was combined with HPMC K4M. Although MBP is insoluble in 0.1 M HCl attributed to the presence of crystalline portions in its polymer chain. MBP swells to a certain extent in the acidic medium. Being less swellable in acidic dissolution medium, MBP contribute minimally to the swelling of HPMC based hydrophilic polymer matrix, however, it might act as pore former in the swelled hydrogel matrix. From formulation AV2, about 24, 52, 82 and 99% MX was released at the end of 1st, 4th, 6th and 8th hour. In case of formulation AV3, anionic CMBP was mixed, in comparable amount as that of MBP, with non-ionic HPMC K4M. About 24, 47, 74 and 98% MX was released at the end of 3rd, 7th, 9th and 12th hour. Formulation AV4 composed of CMBP (50 mg), HPMC K4M (200 mg) and MX. Here about 28, 50, 80 and 99% MX was released at the end of 3rd, 6th, 8th and 11th hour. The release profiles were comparable to the formulation AV3.

**DISCUSSION**

The MBP from the water extract was precipitated by acetone (1:4, water: acetone ratio). Acetone is relatively hydrophobic water-miscible solvent. When added to the water extract, it changes the hydrophilic/hydrophobic nature of the original solvent, that is, water. As the solvent becomes less hydrophilic, the attractive hydrogen bonding forces within the polysaccharide chain become more prevalent and the portions of the chain tend to bond within and between the polysaccharide chains, rather than with the solvent. These intra- and intermolecular hydrogen bonds lead to agglomeration and precipitation as solvation decreases.

MBP was found to be practically insoluble in all the solvents tested for evaluation of the solubility. The poor solubility of MBP could be explained on the basis of molecular weight or degree of polymerization. Generally low molecular weight carbohydrate polymers having degree of polymerization <15-20 are water soluble. Highly branched polysaccharides are almost always exhibit high solubility in water. Solubility of carbohydrate polymers decreases with increase in the ease with which their molecules associate and with narrowness of molecular weight distribution. Carbohydrate polymers having regular, extended...
Carbohydrate polymers generally have two NMR-active nuclei, \(^{13}C\) and \(^1H\). Chemical shifts in the \(^{13}C\) NMR spectrum of a carbohydrate polymer follow the same general trends as for \(^1H\) NMR spectroscopy, although the probability of "accidental overlap" is less because the dispersion of \(^1C\) shifts (\(8C \text{ to } 100 \text{ ppm}\)) is approximately 20 times wider in the \(^{13}C\) NMR spectrum. Compared with \(^1H\)-NMR spectrum; \(^{13}C\)-NMR has a wide range of displacement values and only a few signals overlap. \(^{25}\) The FTIR and \(^{13}C\) NMR spectra of MBP, indicated it to be a pectic polysaccharide, most probably, a carbohydrate polymer molecule consisting of \(\alpha-(1\rightarrow4)\)-linked \(D\)-galacturonic acid (\(D\)-GalpA) residues.

The most important method for the structure determination of crystalline polysaccharides is powder X-ray diffraction (PXRD). The result of the XRD shows that MBP has both crystalline and amorphous portions. Structural and functional group differences in polysaccharides influence the thermal behavior and affect the transition temperature. DSC is one of the frequently used thermal techniques that estimate the exothermic or endothermic changes as temperature is increased. Most of carbohydrate polymers are composed of carboxylic acid functional groups. Thus, thermal cleavage of the carboxylate groups and generation of carbon dioxide gas from the carbohydrate backbone probably a mechanism for the thermal transitions. \(^{27}\) The thermal treatment in DSC resulted in physical and chemical changes in carbohydrate polymers that yield curves that are unique for a given carbohydrate polymer. \(^{28}\)

While the evolution and application of polymers derived from plant sources have attracted increased attention due to their sustainability, biodegradability and biosafety; they evidence certain drawbacks, such as solubility, swelling characteristics, variable chemical composition, pH-dependent solubility, poor mechanical strength. \(^{29,31}\) The above mentioned drawbacks of natural polymers can be overcome via chemical modification. \(^{32}\) The chemical modification of naturally derived polymers is the most important route to modify their properties and to use this renewable resource in the context of pharmaceutical product development. Modifications which are most frequently done to the natural polymers include carboxymethylation and grafting. Among the chemical modifications, carboxymethylation is a widely studied conversion as it increases the aqueous solubility and solution clarity of modified polymers. Site specific targeted drug release can be also achieved by attaching pH-sensitive carboxylic acid groups to the polymeric structure, via carboxyethylation process. \(^{33-35}\) In the present investigation, carboxymethylation of MBP was done by Williamson ether synthesis. In this synthesis, the polysaccharide alkoxide is reacted with monocholoroacetic acid and the primary and secondary alcohol portions. Structural and functional group differences in polysaccharides result of the XRD shows that MBP has both crystalline and amorphous portions. Structural and functional group differences in polysaccharides influence the thermal behavior and affect the transition temperature. DSC is one of the frequently used thermal techniques that estimate the exothermic or endothermic changes as temperature is increased. Most of carbohydrate polymers are composed of carboxylic acid functional groups. Thus, thermal cleavage of the carboxylate groups and generation of carbon dioxide gas from the carbohydrate backbone probably a mechanism for the thermal transitions. \(^{27}\) The thermal treatment in DSC resulted in physical and chemical changes in carbohydrate polymers that yield curves that are unique for a given carbohydrate polymer. \(^{28}\)

Table 1: Composition of formulations containing moxifloxacin HCl.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>HPMC K4M (mg)</th>
<th>MBP (mg)</th>
<th>CMBP (mg)</th>
<th>MX (mg)</th>
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<tr>
<td>AV1</td>
<td>250</td>
<td>----</td>
<td>---</td>
<td>218</td>
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<tr>
<td>AV4</td>
<td>200</td>
<td>---</td>
<td>50</td>
<td>218</td>
</tr>
</tbody>
</table>

*Although insoluble but swelled in pH 6.8 phosphate buffer

** Slightly swelled in 0.1 M HCl
The polysaccharide α-(1→4)-linked D-galacturonic acid (D-GalpA) based linear homopolymer was successfully isolated from dehusked mung bean by cold extraction using water-acetone precipitation method. The polysaccharide was chemically modified to its carboxymethylated derivative that was used to fabricate sustained release capsules using moxifloxacin HCl. The system emerged as a hydrodynamically balanced capsule formulation. Further studies are underway that would assess its in vivo performance and biosafety.
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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS


REFERENCES

Verma, et al.: D-GalpA Homopolymer for HBS


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