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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 ^{13}C 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.[1] Because of this, plant derived polysaccharides are increasingly being used for distinct applications in drug delivery.[2] 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[3-5] 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[6,7] 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 ^{13}C 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.[8] 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.[9,10]

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.[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 than 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}.

^{13}C Nuclear Magnetic Resonance (NMR) characterization

The high resolution solid-state ^{13}C 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.[11-12,12a] 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.[12] 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)[13,14] was determined by potentiometric back titration and was calculated using the equation 1:

$$DS = \frac{0.162\ A}{1.162\ Aat} \qquad \text{--------- (Eq.1)}$$

Here, A is the mill 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 pre weighed amount (100 mg) of the MBP or CMPB (W_1), 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 (W_2) on an electronic balance. The swelling index was calculated by the following equation:[15]

$$\text{Swelling index} = \frac{W_2 - W_1}{W_1} \times 100 \qquad \text{--------- (Eq.2)}$$

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 HCl-KCl buffer, pH 1.2 at 37 ± 0.5°C.[16,17] At predetermined intervals, a 1 ml aliquot part was withdrawn and replenished with an equal volume of fresh HCl-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.[24] 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 α-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.

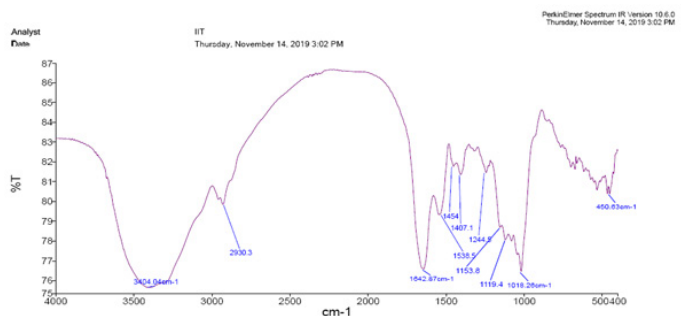

Figure 1: FTIR Spectra of Mung Bean Polysaccharide.

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 MPB. 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 $-CH_2$ 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 α-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%).

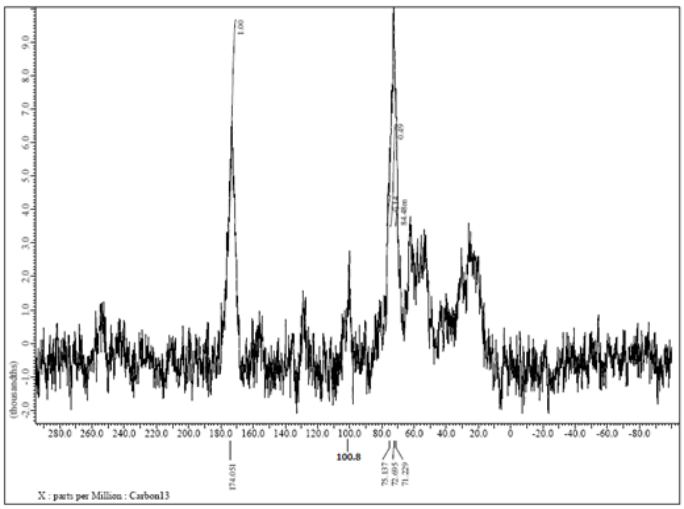

Figure 2: ^{13}C NMR Spectra of Mung Bean Polysaccharide.

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.[18,19] 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.[20] 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

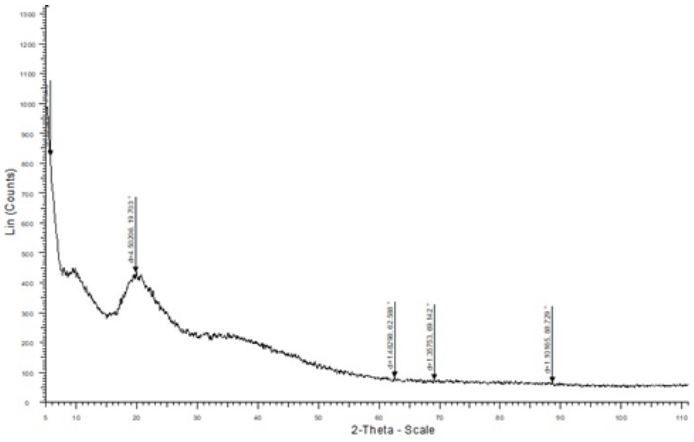

Figure 3: PXRD pattern of Mung Bean Polysaccharide.

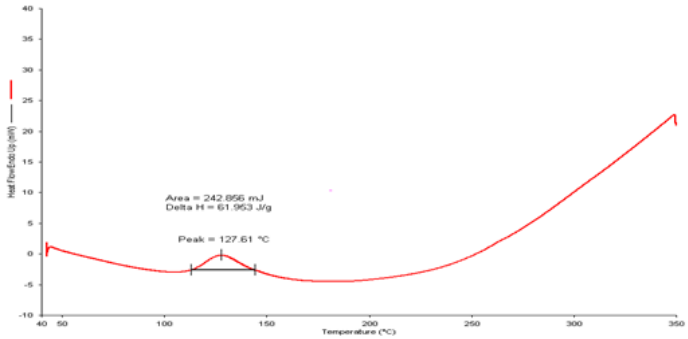

Figure 4: DSC thermogram of Mung Bean Polysaccharide.

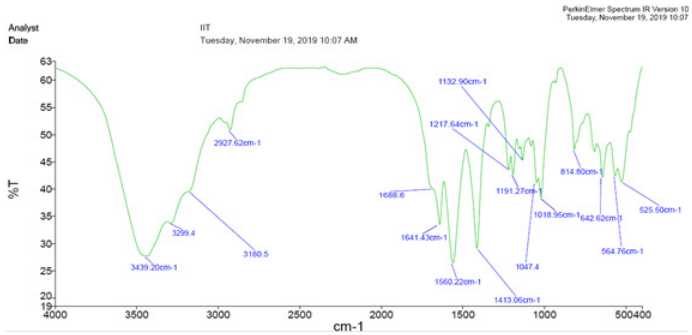

Figure 5: FTIR Spectrum of Carboxymethyl Mung Bean Polysaccharide.

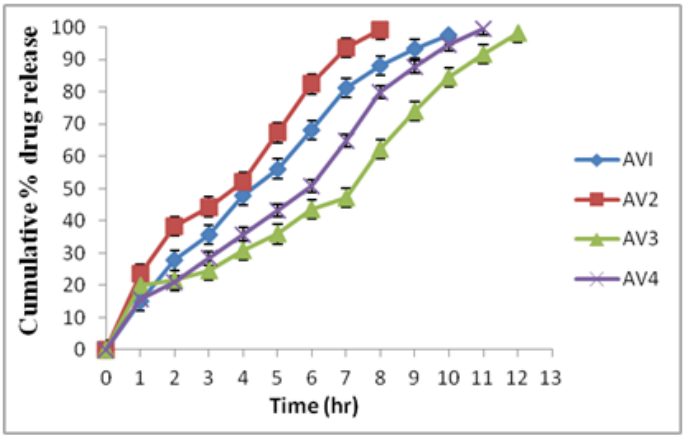

Figure 6: Cumulative % drug release from various HBS capsule formulations [(Mean ± SD (*n*=3)]

Table 1: Composition of formulations containing moxifloxacin HCl.

Formulation code	HPMC K4M (mg)	MBP (mg)	CMBP (mg)	MX (mg)
AV1	250	----	---	218
AV2	225	25	---	218
AV3	225	---	25	218
AV4	200	---	50	218

Table 2: Solubility of Mung Bean Polysaccharide in various solvents.

Solvents	Solubility at 25°C
Distilled water	Practically insoluble
Acetonitrile	Practically insoluble
Tetrahydrofuran	Practically insoluble
Dimethyl Sulfoxide	Practically insoluble
0.1 M HCl**	Practically insoluble (both at 25°C and 37°C)
pH 6.8 phosphate buffer*	Practically insoluble(both at 25°C and 37°C)

*Although insoluble but swelled in pH 6.8 phosphate buffer

** Slightly swelled in 0.1 M HCl

ribbon like structure are the ones which most easily associate and form crystals. Generally, reinforcing, cell-wall carbohydrate polymers are least soluble. Linear polysaccharides of uniform structure can develop crystalline regions which firmly tie molecular segments together in effective cross-links. Such molecules are very difficult to dissolve in water.[21] It has also been reported that carbohydrate polymers composed of one type of sugar unit uniformly linked in linear chains are generally water insoluble even if the carbohydrate polymers molecules have low molecular weight with low degrees of polymerization[16] (20-30). Examples of polysaccharides with least solubility include cellulose, pectic acid, alginic acid, xylan, chitin, amylose etc. All these polysaccharides have either α or β (1→4)-glycosidic linkage.[21]

Upon literature survey, it was observed that the infrared spectra of carbohydrate polymers are divided into three specific Infrared regions viz. 1200–900 cm^{-1}, 3000–2700 cm^{-1} and 900–600 cm^{-1}. The intensive peaks in the Infrared region between 1200 - 900 cm^{-1} are attributed to strongly coupled C–C, C–O stretching and C–O–H, C–O–C deformation modes of various oligo and polysaccharides.[22] In the Infrared region 3000–2700 cm^{-1} carbohydrate polymers show few sharp absorption bands, for example, band at 2930 cm^{-1} assigned to asymmetric C–H stretching of CH_2; band at 2870 cm^{-1} assigned to symmetric C–H stretching of CH_3 and C–H stretching bands in the range of 2700–3100 cm^{-1}.The region between 900 and 600 cm^{-1} exhibits a number of characteristic weak bands superimposed on an underlying broad spectral contour.[23] The carbohydrate polymers share a common functional group, that is, hydroxyl. These polymers self-assemble and form hydrogen-bonded network with porous hierarchy unique to respective monosaccharide building blocks.[20] The FTIR peaks in the Infrared region (3600 to 3000 cm^{-1}) are due to O–H stretching modes in alcoholic groups. The stronger the hydrogen bond, the greater the intensity of the corresponding ν O–H band and the greater its shift towards lower wave numbers. The FTIR spectrum of MBP, suggested that the polysaccharide was a pyranose form of sugars.

Carbohydrate polymers generally have two NMR-active nuclei, ^{13}C and ^{1}H. Chemical shifts in the ^{13}C NMR spectrum of a carbohydrate polymer follow the same general trends as for ^{1}H NMR spectroscopy, although the probability of "accidental overlap" is less because the dispersion of ^{13}C shifts (δC 60–110 ppm) is approximately 20 times wider in the ^{13}C NMR spectrum. Compared with ^{1}H-NMR spectrum; ^{13}C-NMR has a wide range of displacement values and only a few signals overlap.[25,26]The FTIR and ^{13}C NMR spectra of MBP, indicated it to be a pectic polysaccharide, most probably, a carbohydrate polymer molecule consisting of α-(1→4)-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 carboxymethylation 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 groups are substituted by carboxymethyl group by an S_N2 reaction.[36-38]

$$MBP\text{–}OH + NaOH \longrightarrow MBP - ONa + H_2O \quad \text{(Eq. 3)}$$
(MBP- alkoxide)

$$MBP\text{–}ONa + ClCH_2COONa \longrightarrow MBP\text{–}OCH_2COONa + NaCl \quad \text{(Eq. 4)}$$
(Carboxymethylated MBP; CMBP)

CMBP exhibited pH dependent swelling characteristic. The pH dependent swellability of CMBP is attributed to the presence of carboxymethyl groups. The carboxymethyl groups ionize when the pH of the environment is higher than the pKa of the carboxylic group (3.4–3.7). Therefore, at pH 1.2, the carboxyl groups are protonated to promote the formation of intramolecular hydrogen bonds, thereby reducing the overall swellability. At pH 6.8, the carboxyl groups get deprotonated and higher swellability is observed due to collective electrostatic repulsion forces between the ionized acid groups.[39] Polymer matrices composed of hydroxypropyl methyl cellulose (HPMC) with ionic/non-ionic polymers have been utilized as hydrophilic matrices to modulate the drug release and overcome most of the challenges associated with hydrophilic polymeric matrices. Hydrophilic polymer matrices made up of HPMC with anionic polymers have been reported to exhibit pH-independent release profiles for weakly basic drugs.[40] The addition

of anionic polymers in the polymer matrix can effect drug release in alkaline dissolution media by decreasing the micro-environmental pH and also extend the drug release in acidic dissolution media by forming an insoluble mass, which acts as a barrier to drug diffusion.[41]

There was extended release of MX from formulation AV1. this could be attributed to the swelling of HPMC based polymeric matrices when exposed to acidic gelation medium, due to hydrogen bond disruption between the polymeric-chains to form a thick gelatinous layer at the boundary of the matrix, with simultaneous erosion. Further, the solubility of the drug in dissolution medium is an important factor determining the mechanism of drug release from. HPMC based hydrophilic matrices.[42-45] For drugs, such as MX, that have high water solubility (24 mg/ml in water at 25°C), the drug dissolves within the gel layer and diffuses out into the dissolution media. Therefore, it is of utmost importance to ensure the integrity of the gel layer after the MX has been dissolved and released from the gel layer.[46] This suggests that it is critical to have a strong gel layer through which diffusion can occur;[47] therefore, the extended MX release could be attributed to the high molecular weight HPMC K4M polymeric-matrices.

On the other hand there was rapid release of MX from formulation AV2. The significantly fast drug release (p<0.05 compared to AV1) could be attributed to the penetration of comparatively large amount of acidic dissolution medium into the hydrogel matrix due to pore formation by MBP. Consequent polymer swelling (HPMC K4M) and formation of micro cavities within the hydrated polymer matrix promoted the drug release.[48] In case of formulation AV3, anionic CMBP was mixed. This has resulted in significant retardation (p<0.05 compared to AV2) of MX release. This could be attributed to the synergistic increase in viscosity of the mix hydrogel, leading to increased resistance to drug diffusion.[49] There could be one more reason that there is interaction between anionic CMBP and cationic MX. The release of highly soluble cationic drugs have been reported to be extended from combinations of HPMC with anionic sodium carboxymethyl cellulose compared with HPMC alone, attributed to interection between drug and anionic polymer.[49]

The *in vitro* moxifloxacin HCl release pattern from hydrodynamically balanced capsule formulations was studied through fitting the dissolution data into different important kinetic models. In case of hydroxypropyl methylcellulose based hydrodynamically balanced capsules bearing model drug MX, r^2 value of formulation AV1 was found best fitted to Korsmeyer-Peppas kinetic model. This model provides an insight into the kind of drug release, for the first 60% of release regardless of the geometric shape of the delivery device, mechanism occurring from the swellable polymeric-matrices. The Korsmeyer-Peppas model (equation5) is useful for the study of drug release from polymeric matrices when the release mechanism is not known or when more than one type of phenomenon of drug release is involved.[50,51]

$$f_1 = \frac{M_i}{M_\infty} = Kt^n \qquad \text{--------- (Eq.5)}$$

where f_1 is quantity of the drug released from the system; M_∞ is the quantity of the drug at equilibrium state; M_i is amount of the drug released over time t; K is the constant of incorporation of structural modifications and geometrical characteristics of the system and n is the exponent of release (related to the drug release mechanism) in function of time t. Depending on the value of n, the diffusion of drug through polymeric matrices is either Fickian diffusion (Case I) or non-Fickian diffusion (Case II, anomalous case and super case II). Fickian diffusion defines the solute transport process in which the polymer relaxation time (t_r) is much greater than the characteristic solvent diffusion time (td). When tr ≈ td, the macroscopic drug release becomes anomalous or non-Fickian. In the Fickian diffusion, n=0.5 (planer film), 0.45 (cylinder) and 0.43 (sphere) and the drug release is governed by diffusion. When 0.5 < n < 1.0, the diffusion is non-Fickian or anomalous transport (Case II) and the mechanism of drug release is governed by diffusion and swelling. The Super Case II model is characterized when n >1. A good-fit to the Korsmeyer- Peppas model indicates the combined effect of diffusion and relaxation mechanisms for the release.[50-52]

In case of formulation AV1, the n value is 0.82. This suggests that drug transport is non-Fickian anomalous transport. The diffusion and swelling rates have similar magnitude. The rearrangement of polymeric chains occurring slowly and the diffusion process simultaneously cause the time-dependent anomalous effects. In formulation AV2, MBP was mixed with HPMC K4M. The addition of MBP enhanced the release of MX from the hydrogel matrix, which means that insoluble MBP (20 mesh particle size) facilitated the movement of dissolution medium into the HPMC based hydrogel matrix increasing the solubilization of MX, thereby increasing the movement of drug out of the matrix. The r^2 value was found best-fit when fitted to zero-order model. The n value is 0.61. This suggests that the velocity of the dissolution medium diffusion into the interior of the polymer matrix was high and the polymeric relaxation was low compared to formulation AV1.

In case of formulation AV3, the r^2 value was found best fitted to zero-order model. The n value is 0.54. In this case an increase in swelling of the hydrogel is expected due to addition of CMBP to the HPMC K4M matrix. The rate of polymer hydration depends on the structure of the polymer or polymer matrices used in formulations in addition to the degree of interaction of functional groups within the polymeric matrices with water. The better the interaction between a polymer surface and water, the faster the polymer hydrates because the first layer of adsorbed water molecules enables easier adsorption of additional water molecules.[53] The presence of the methyl substituent of HPMC and carboxymethyl substituent of CMBP permits interaction of the molecule with water, thereby promoting hydration and consequent swelling such as that observed.[53] The magnitude of swelling was further increased with decreased polymer relaxation compared to formulation AV2 and AV1. This has allowed the erosion of the swollen matrix to occur at a rate equating to the movement of the front between the glassy and the rubbery polymer.[49]

In case of formulation AV4, the concentration of CMBP in the polymer matrix was increased to 50 mg. Although the drug release pattern remained anomalous but there was increase in the value of n (0.72 (AV4) compared to 0.61 and 0.54 for AV2 and AV3 respectively. This suggests an increase in the polymer relaxation time compared to formulation AV1 and AV2. This observation revealed that swelling, dissolution and erosion of polymer matrices were all involved in the release process. The erosion operates at later times and is sensitive to the osmotic stress exerted by ionic polymer and the drug present in the matrix.

CONCLUSION

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 system that remained floated for 12 h. 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

MX: Moxifloxacin HCl; **MBP:** Mung Bean polysaccharide; **CMBP:** Carboxymethyl Mung Bean polysaccharide; **FTIR:** Fourier Transform Infrared Spectroscopy; **DSC:** Differential Scanning Calorimetry; **PXRD:** Powder X-Ray Diffraction.

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