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Potential applications of *Abelmoschus moschatus* polysaccharide as colon release tablets-Rheology and gamma scintigraphic study

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ABSTRACT

In the present scenario of pharmaceutical development, researchers are focusing on the herbal excipients for different delivery systems. This work describes diverse characteristics of novel polysaccharide extracted from the stem of *Abelmoschus moschatus* (AM). Further, polysaccharide was explored for its potential role as polymer in controlling colon-specific release of metronidazole (MNZ). Cell toxicity study confirmed its safety aspects in biological applications. The aqueous solution of polysaccharide exhibited non-Newtonian pseudoplastic flow property. The consistency coefficient (k) was found to increase with increase in concentration and particle size of AM polysaccharide. Viscosity was found to increase by increasing pH of the aqueous solution of AM. *In-vivo* gamma scintigraphy study of radiolabeled MNZ tablet in human volunteers confirmed small intestinal transit time of 300–480 min and colon arrival time of 360–480 min. The present study revealed that AM polysaccharide may be a choice of pharmaceutical excipients as an alternative to synthetic polymers.

1. Introduction

Over the years, excipients have evolved from being simply inert and cheap vehicles for the active pharmaceutical ingredient to being the indispensable components of drug formulations which are of paramount importance in formulation design. They play an imperious role in influencing the dose, stability, functioning as well as bioavailability of a drug dosage form. Therefore, careful selection of pharmaceutical excipients is crucial during preparation of conventional or specialized dosage forms as their inherent characteristics could severely impact the final product. Despite these roles, literature reveals their potential toxic nature. Some of the excipients induced toxicities include fatal renal failure from diethylene glycol, osmotic diarrhea caused by mannitol, cardiotoxicity induced by propylene glycol and the hypersensitivity reactions caused by lanolin, acacia, and parabens [1]. Consequently, toxicities induced by exposure to excipients need to be suitably accounted for in the risk benefit analysis of every designed/prepared formulation.

Growing concern towards excipients induced toxicities has led researchers to look expectantly towards the excipients of natural origin viz.-a-viz. Plants, animals, fungi and seaweeds. Of these, polysaccharides derived from plant sources comply with many requirements of pharmaceutical excipients such as non-toxicity, biocompatibility, easy availability, low cost, renewability and better patient tolerance [2]. In addition, they also present flexibility for chemical modifications for fine-tuning the desired properties and are offer excipients of choice [3]. Extensive research is underway to explore polysaccharides from unconventional natural resources/plant sources for their potential use in pharmaceutical applications. Several plant polysaccharides have found application/use as pharmaceutical excipients till now. Literature suggests use of plant polysaccharides such as chitosan [4], gaur gum [5], Katira gum [6] and Fenugreek [7] for targeted delivery of active ingredients specifically to the colonic region. Similarly, alginate [8] and gelatin [9] have been investigated as excipient choice for controlled release drug delivery systems. Apart from conventional drug delivery, natural polysaccharides have also proven their potential application in novel drug delivery systems including nanocapsules, nanoparticles, conjugates, and micellar systems [10].

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One such plant is *Abelmoschus moschatus* (AM, commonly called musk mallow, syn. *Hibiscus abelmoschus*) a perennial aromatic and medicinal plant native to tropical Asia. It has been applied in Chinese traditional medicine for the treatment of depression, anxiety, and antispasmodic activity [11]. It is also used in Ayurveda, Siddha and Unani system of medicine for treatment of a variety of ailments due to its diuretic, demulcent and antiseptic properties [12]. Researchers have extended the pharmacological profile of AM by evaluating its hepatoprotective activity, its ability to improve insulin sensitivity, ability to protect skin (favours FGF-2 activity in skin) and antilithiatic activity [13,14]. Liu et al., found myricetin as the active ingredient in *Abelmoschus moschatus* to lower plasma glucose in streptozotocin induced diabetic rats. They also demonstrated improvement of insulin sensitivity in obese Zucker rats by the myricetin from AM [15]. Gul et al., demonstrated antioxidant, free radical scavenging, antimicrobial, antiproliferative activities of AM extract and AM has also been demonstrated for use as an adjuvant therapy in neurological and psychiatric conditions owing to its antidepressant, anxiolytic, antiepileptic and sedative properties studied in animal models [16].

Metronidazole (MNZ) is a widely used choice of drug for intestinal and colonic amoebiasis. MNZ is completely absorbed from upper GIT and biologically available for its therapeutic action within 1 h after a single dose (500 mg). Conventional dosage form of MNZ is unable to provide desired amount into colonic region for its local action [17]. Despite the reported efficacy of MNZ in the treatment of amoebiasis, there are associated side effects due to its rapid absorption in the upper GIT, which is thought to result in its high plasma concentrations. Several reports have indicated the carcinogenic and genotoxic effects of MNZ on rodent and human cell lines when used for longer duration of time [18]. Therefore, formulating a delivery system which can restrict release of MNZ in the upper GIT and allow complete release in colonic region could be a better strategy to achieve high therapeutic efficacy along with minimal side effects. It would be prudent to use natural polysaccharides for such a delivery system that will not only limit the adverse effects of synthetic excipients but also be low on toxicity.

With this objective, the present research work on AM polysaccharide is being explored for colon specific delivery of metronidazole for application in potential treatment and management of ulcerative colitis. To the best of our knowledge, this is the first study exploring the use of AM polysaccharide as a pharmaceutical excipient for colon delivery. This AM polysaccharide has been used as a release controlling excipient to prepare colon release tablets of MNZ which have been evaluated by *in-vitro* and *in-vivo* analysis. In addition to this, the complete characterization of AM polysaccharide such as molecular weight, cell toxicity along with rheological studies are being presented in a comprehensive manner as part of this work. Gamma scintigraphy study of these MNZ tablets has been carried out in healthy human volunteers which confirm the targeted delivery in colon.

2. Materials and methods

2.1. Materials

The AM stems were obtained from local farms in and around Meerut (India). All other chemicals used in the study were of analytical grade and either procured from CDH Laboratory (New Delhi, India) or Rankem Laboratory (New Delhi, India) and de-ionised water was used throughout the work.

2.2. Extraction of polysaccharide

The dry polysaccharide was extracted from the AM stems as per protocol elaborated in our previous publication [7]. The complete extraction method is given in supplementary file. Briefly, AM stems (100 g) were soaked in double distilled water (1 L) at room temperature, and boiled with continuous stirring in a water bath for close to 2 h till a slurry was obtained. The slurry was cooled down to room temperature and kept in the refrigerator overnight to allow settling of undissolved material. The supernatant was then separated by decantation after centrifuging at 1000 rpm for 30 min. The supernatant was then concentrated to 1/3rd its volume using a rotary evaporator (RE301, Yamato, Japan).This concentrate was precipitated in acetone keeping the polysaccharide:acetone ratio of 1:3. A white precipitate was obtained after repeated washing with acetone which was further purified by extensive dialysis against distilled water (molecular weight cut-off 1200). Purified polysaccharide was dried in a hot air oven at 60 °C till it was completely dry and constant weight was obtained. The AM polysaccharide powder obtained by this method gave a yield of ~8.2% w/w. The molecular weight of AM polysaccharide was determined by the light-scattering equation as explained in details in supplementary section.

2.3. Cell toxicity study of AM polysaccharide

MTT assay was performed to assess the cellular toxicity of AM polysaccharide. HT-29 were incubated in media consisting of 10% Fetal Bovine Serum (FBS) and Penicillin (100 U/mL) under a humidified atmosphere of CO_2 at constant temperature (37 ± 0.5 °C). Cells were exposed to AM polysaccharide aqueous solution of different concentration (10, 20 and 30 μg/100 μL) in a 24-well plate with 100 μL of media at a plating density of 1×10^5 cells/well for 2 h. Cells were carefully washed using saline to remove samples solution. Finally, a 0.5% solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) was added (5 mg/mL). Absorbance of test and control samples was recorded at 570 nm. Percentage cell viability was calculated using equation (1).

$$\% \text{ Cell Viability} = \text{absorbance of test sample/absorbance of control} \times 100 \quad (1)$$

2.4. Rheological properties of polysaccharide

Brookfield Rheometer (R/S-CPS-Plus, Brookfield Engineering, Massachusetts, USA) was used to determine the flow behaviour and effect of various parameters on the apparent viscosity of AM polysaccharide. The rheometer was equipped with cone and plate geometry (4°/40 mm). The temperature was regulated by using Peltier thermoregulator (Brookfield PTR-I) and the obtained rheological data were recorded with the software RHEO 2000 version 2.5. Sample (1 mL) was placed in measuring the gap between the rotating cone and the stationary lower plate, allowed to equilibrate for 5–10 min (depending upon the sample concentration) at the desired temperature (25 °C). All the rheological measurements were made in triplicate and the average readings were recorded.

2.4.1. Steady shear flow behaviour

In order to determine effect of particle size and concentration on flow behaviour, dried polysaccharide was passed respectively through mesh no 80 and 60 (corresponding to 180 μm and 250 μm respectively) and collected. Aqueous solutions of five different concentrations (4, 5, 6, 7 and 8% w/v) were prepared for each particle size by dispersing the required amount of AM polysaccharide powder in deionised distilled water under slow stirring for 4 h using a magnetic stirrer (5MLH Plus, Remi, Mumbai). The resultant solution was stored overnight at room temperature for complete hydration. Flow behaviour of AM polysaccharide was examined under a range of shear rates from 10 to 100 (s^{-1}) (as per instrument specification) in 100 s followed by immediate decrease in shear rate from 100 to 10 (s^{-1}) in 100 s. Flow behaviour index (n) and consistency coefficient (k) were calculated for the curve by fitting the data obtained into the power law model equation (2)

$$\tau = k\gamma^n \quad (2)$$

Where τ is the shear stress (Pa), γ is shear rate (s^{-1}), k is consistency coefficient (Pas^n) and n is flow behaviour index (dimensionless).

In order to predict the effect of particle size and concentration more precisely, the apparent viscosity of samples was also determined at varying shear rates from 10 to 300s^{-1} in 300s.

2.4.2. Effect of salt concentration

In the present work, an attempt was also made to determine the possible interactions of monovalent and divalent salt with an aqueous solution of the extracted polysaccharide. Effect of monovalent salts (NaCl and KCl) and divalent salts ($CaCl_2$ and $MgCl_2$) at different concentrations (0.2, 0.4, 0.6, 0.8 and 1% w/v) on the apparent viscosity of AM hydrated polysaccharide solution (5% w/v) was determined at constant shear rate (60 s^{-1}) for 100 s. Samples were prepared by dissolving required amount of salt into solvent followed by polysaccharide dispersion into the salt solution.

2.4.3. Effect of pH

Changes in apparent viscosity of 5% (w/v) hydrated polysaccharide solution by the influence of pH (varying from 3 to 11) were measured at a constant shear rate (60 s^{-1}) for 100 s. 0.1 mol/L NaOH and HCl were used to adjust the pH of polysaccharide solution.

2.5. Preparation of colon release MNZ tablets

MNZ tablets were prepared by using AM polysaccharide as release control material. Five batches of tablets were prepared as shown in Table 1. Granules were prepared by wet granulation method with interchange amount of AM polysaccharide and polyvinyl pyrrolidone (PVP) into aqueous media. The wet mass was passed through a sieve (No 22) and the obtained granules were allowed to dry. Dry granules were lubricated with magnesium stearate and talc (1%w/v each). Granules equivalent to 100 mg of drug was compressed on 10 mm flat round punch on 8 station tableting machine (Cadmach, India) by applying 10–20 KN force.

Prepared matrix tablets were coated with Eudragit S-100 (2.5%w/v solution in isopropyl alcohol: acetone in ratio 1:1). The coating was performed by dipping the AM matrix tablet into the Eudragit coating solution for 30 s followed by complete drying (at 80 °C).

2.6. In-vitro drug release

Dissolution study of all batches was performed by using USP type II (paddle type) apparatus (TDT-08L, Elecrolab, India). Tablets were analyzed for their drug release behaviour under various physiological conditions of GIT to simulate mouth to colon transit. Initially the dissolution media was 0.1 N hydrochloric acid (900 mL, pH 1.2) for 2 h, then pH of the dissolution media was adjusted to 6.8 using potassium dihydrogen phosphate and sodium hydroxide for the next 4 h and the final pH was kept at 7.4 with 4% (w/v) of rat cecal material. Rat cecal material was added into dissolution media to simulate colonic condition. The fresh cecal material was collected approximately 30 min before the commencement of dissolution study.

Dissolution media (mL) was withdrawn at 1 h interval and an equal quantity of phosphate buffer saline of certain pH was added into the media to maintain the volume of dissolution media. The sample was processed by filtration and drug concentration was determined using UV–visible spectrophotometer (UV1700, Simadzu, Japan) at 277 nm for quantitative estimation of MNZ.

2.7. Colon targeting by gamma scintigraphy

In order to determine targeting efficiency of an optimized batch of AM polysaccharide based MNZ tablet, gamma scintigraphy technique was used. This is a validated modality that predicts the *in-vivo* performance of dosage form through a non-invasive mode of operation. By labeling radioactive material into the delivery system, it is possible to visualize GI transit pattern of the developed delivery system.

2.7.1. Radiolabeling of MNZ

Radiolabeling of MNZ was performed with ^{99m}Tc-pertechnetate as per protocol of previous publication with slight modification [19,31]. Briefly, MNZ was transferred into an Eppendorf tube containing mixture of $SnCl_2{\cdot}H_2O$ solution in 1 M HCl (15 mg/mL), 2.5 mL NaCl (0.9% w/v), 2.5 mL CH_3COONa (100 mg/mL) and $NaTcO_4$ (of about 20 MBq radioactivity). The drug suspension was mixed well and centrifuged at 3000 rpm for 10 min. The supernatant was removed and the labeled drug was stored after freeze drying.

2.7.2. In-vivo colon targeting efficiency

Targeting efficiency of prepared AM polysaccharide based MNZ tablets was determined in healthy human volunteers. The ethical protocol was approved by Human Ethical Committee (No: IEC/INM/15-16/1-13) and informed consent was obtained for experimentation with human subjects before the commencement of the study. During the study period, all volunteers were restricted for any other medication or therapy.

Radiolabeled MNZ tablet (containing 300 μCi of technetium pertechnetate complexed to MNZ) was orally administered in volunteers along with filtered potable water. The volunteers were asked to be moderately active during the study period. Static images (60 s/image) were captured using gamma camera (Siemens gamma camera) at 15, 60, 180, 360, 480 and 720 min.

2.8. Statistical analysis

All obtained data were expressed as Mean value ± Standard deviation. One way analysis of variance (ANOVA) was applied to determine the significant difference between the data by using Sigmastat 2.03 software (SPSS, Inc, Chicago, IL).

3. Result and discussion

This work was taken up to explore AM polysaccharide as a pharmaceutical excipient in order to minimize dependency over synthetic ones which are associated with several toxicities. In our previous work, we thoroughly characterized the AM polysaccharide by physical and chemical methods. Physicochemical data of AM polysaccharide led us to believe that this could be used as a potential excipient for colonic delivery [20]. Bearing this in mind, the rheological characteristics of

Table 1
Batch details of Metronidazole tablet containing AM polysaccharide as drug release controlling polymer.

Formulations	AM mucilage	PVP	Thickness (mm)	% weight variation	Hardness (kg/cm^3)	Friability (%)
AM1	300	20	4.15 ± 0.11	4.87 ± 0.21	10.29 ± 0.38	< 1
AM2	275	45	4.12 ± 0.18	5.38 ± 0.37	11.18 ± 0.84	< 1
AM3	250	70	4.11 ± 0.17	4.99 ± 0.82	10.87 ± 0.63	< 1
AM4	225	95	4.17 ± 0.11	6.48 ± 0.73	10.73 ± 0.89	< 1
AM5	200	120	4.09 ± 0.16	6.11 ± 0.37	11.05 ± 0.74	< 1

* Each tablet contains 100 mg of MNZ, 2.5 mg magnesium stearate and 2.5 mg talc.

AM polysaccharide were studied in detail from an excipient view point before evaluating its *in-vivo* efficacy for colon targeting with the help of nuclear medicine techniques. An average molecular weight of extracted polysaccharide was found to be 1.78 × 10^6 kg/mol (data is presented in supplementary file, table TS2).

3.1. Cell toxicity study

It is always recommended to assess the safety profile of any novel material before its intended biological use and evaluation of its cellular interaction taken it a step closer to its eventual *in-vivo* use. Since the AM polysaccharide is being explored for the delivery of MNZ to the colonic region, its cytotoxicity towards HT-29, a human colorectal adenocarcinoma cell line with epithelial morphology was evaluated. A plot of percentage cell viability as a function of AM concentration revealed (data is presented in supplementary file, Fig. S2) negligible cytotoxicity towards HT-29 cells, and it may therefore be considered as a safe excipient for colonic delivery.

3.2. Steady shear flow behaviour as function of concentration and molecular size

Apparent viscosity of various concentration of AM polysaccharide solution was evaluated by subjecting them to variable shear rate from 10 to 300 (s^{-1}). The AM solutions were prepared from two different particle size of the extracted polysaccharide viz-a-viz 180 μm and 250 μm. Fig. 1a and b shows the respective correlation of different concentrations of polysaccharides between their apparent viscosities and shear rate. From the figure it is evident that AM polysaccharide shows high viscosity at low shear rate which decreases exponentially with increase in shear rate. It can be well explained by the theory given in previous reports [21], that at low shear rates, the particle of a polymer are partially aligned in the direction of flow which is reflected in their high viscosities values, but subsequently either as the shear rate increases, these particles become completely oriented and fully aligned to the direction of flow which results in a reduction in their apparent viscosities owing to a decrease in their intramolecular frictional forces. Higher apparent viscosity was recorded at higher concentration of polysaccharide which might be due to the restricted molecular movement and interfacial film formation at higher solid content.

Similar exponential decrease in apparent viscosity with increasing shear rate was seen when the polymer's particle size was increased from 180 μm to 250 μm. Earlier studies have also confirmed the effect of particle size on apparent viscosity in a similar manner which may be due to increase in the molecular weight and conformity of higher molecular size particle. It was also reported that large size particle of polysaccharide may contribute to absorbing high amount of water which ultimately improve hydration capacity of polysaccharide thus improving viscosity of the resultant solution [22].

Obtained rheological data of AM polysaccharide was fit into the power law model to determine the flow behaviour index (n) and consistency coefficient (k) and the influence of polysaccharide concentration and particle size on these properties (Rheogram and log shear rate *vs* log shear stress curves are presented in supplementary file Figs. S3a and S3b and Figs. S4a and S4b). The power law model suitably explains the flow property of AM polysaccharide with high regression coefficient (r^2). The polysaccharide solution showed a pseudoplastic (shear thinning) flow behaviour, since the flow behaviour index (n) was found to be less than 1 in all solutions prepared by changing the concentration and particle size (Table 2) [23]. Rheological data also indicate that an increase in the polysaccharide concentration from 4 to 8% (w/v) decreased the flow behaviour index (n) and increased the consistency coefficient (k). The decrease in the flow behaviour index (n) as a function of concentration indicates an increase in the shear thinning behaviour of the polysaccharide. The relation between the consistency coefficient (k) values and the polysaccharide concentration might be due to an increased water binding capacity of the polysaccharide due to intermolecular interaction or entanglement [24]. This data also indicated that higher particle size exhibited higher flow behaviour index (n) and consistency coefficient.

Table 2
Steady shear flow behaviour data of AM polysaccharide at different concentrations and particle sizes.

Concentration (%w/v)and particle size(μm)	Flow behaviour index(n)	Consistency Coefficient(k)	Correlation coefficient (r^2)	Flow behaviour
(180 μm)				
4	0.3737	1.1235	0.9697	Pseudoplastic
5	0.3297	1.3222	0.9863	Pseudoplastic
6	0.1913	1.6945	0.9715	Pseudoplastic
7	0.2759	1.7072	0.9952	Pseudoplastic
8	0.2128	1.8498	0.9964	Pseudoplastic
(250 μm)				
4	0.3482	1.1881	0.9817	Pseudoplastic
5	0.3513	1.3743	0.9876	Pseudoplastic
6	0.2007	2.0771	0.9926	Pseudoplastic
7	0.2142	2.1139	0.9912	Pseudoplastic
8	0.184	2.1841	0.9964	Pseudoplastic

3.3. Effect of salt type and concentration

The effect of salt concentration is an important parameter while predicting the behaviour of pharmaceutical products. Previous literature reports are in support that physiological availability of different salts at different area in varying concentration plays a crucial role in pharmacokinetics such as drug dissolution, polymer chain relaxation and even ionisation mediated drug absorption thus influencing the performance of drug products [25]. Therefore, this experimental approach was planned so as to determine the effect of polyelectrolyte on apparent viscosity of AM polysaccharide thus predicting the physiological performance of the pharmaceutical product comprising the AM polysaccharide as excipient. The polysaccharide solution showed a

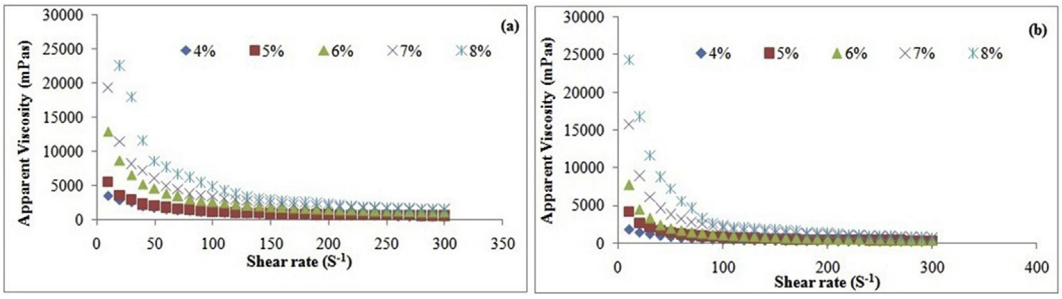

Fig. 1. Effect of concentration variation and particle size of AM polysaccharide on the apparent viscosity as a function of shear rate. 1(a) 80 μm and 2(b) 250 μm, at constant temperature of 25 °C.

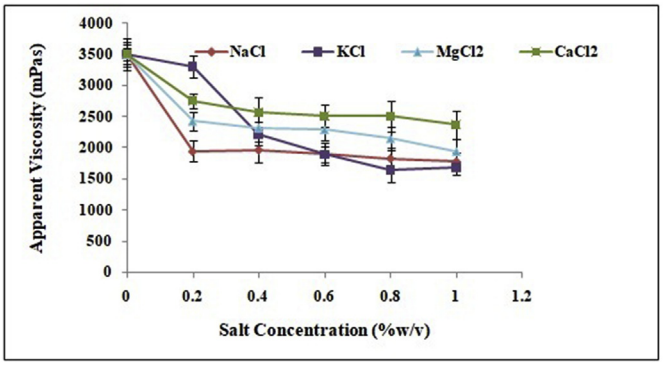

Fig. 2. Effect of variation of salt concentration on the apparent viscosity of 5% w/v AM polysaccharide at constant temperature (25 °C) and shear rate (60 s^{-1}).

rapid initial drop in viscosity when 0.2% w/v of NaCl, $MgCl_2$, and $CaCl_2$ was added (Fig. 2). The viscosity values decreased significantly ($p < 0.05$) from 3504.56 ± 62.75 to 1943.45 ± 49.34, 2422.46 ± 34.27 and 2754.26 ± 53.81 mPas after addition of 0.2 % w/v of NaCl, $MgCl_2$ and $CaCl_2$ respectively while a lesser decrease in value (3298.96 ± 29.76) was observed in addition of KCl. Further addition of increasing salt concentrations up to 1.0%w/v of salts did not change the viscosity. These results confirmed that monovalent as well as divalent salts affect the viscosity of AM in a similar manner. According to the theory by Torres et al. [26], reduction of viscosity after addition of salts may be attributed to inter-molecular repulsion force and expansion of negatively charged polyelectrolyte polysaccharide molecules. As a result the increased flexibility of polymeric chain initiates a conformational change, which causes a drop in viscosity.

3.4. Effect of pH

There are many reports in the literature [26], referring to the effect of media pH on the viscosity mediated behaviour of polymers such as solubility, swellability and polymer chain relaxation. Their polymeric behaviour finally decides the fate of the formulation in terms of drug release after its administration. Therefore, this study was performed to determine the effect of pH on the viscosity of AM polysaccharide and thus pH dependant drug release of the entrapped drug. It is clear that apparent viscosity of the polysaccharide augmented at a slow rate on increasing the pH of the solution from 3 to 6 but increased at higher on varying pH rate in the alkaline region from 8 to 12 (Fig. 3). The reason for slow increase in viscosity in acidic medium can be explained as follows. At acidic pH, a partial ionisation of the hydrophilic group of the polysaccharide occurs, which causes an electrostatic repulsion between the respective polymeric chain of low magnitude and tends to keep the molecule in an extended form; whereas, in alkaline medium a

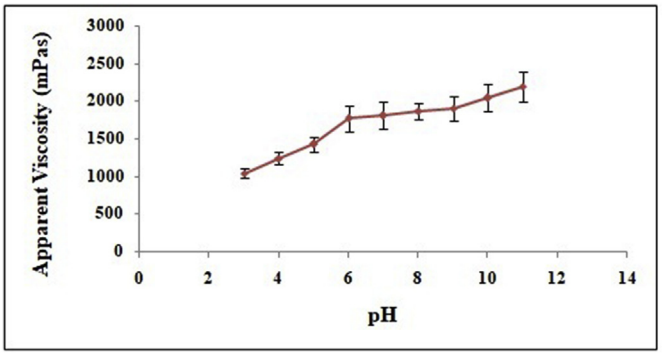

Fig. 3. Effect of pH variation on the apparent viscosity of 5% w/v AM polysaccharide at constant temperature (25 °C) and shear rate (60 s^{-1}).

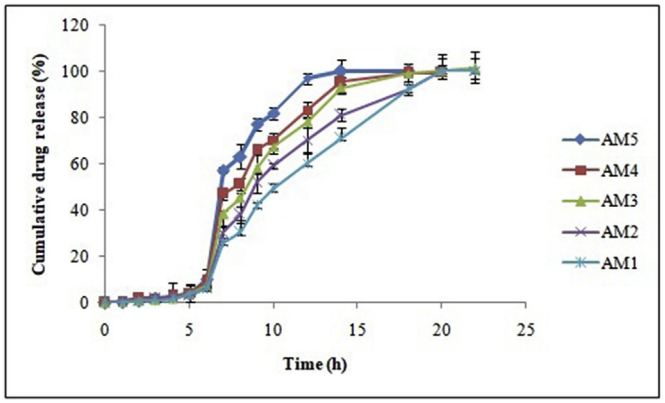

Fig. 4. Percentage cumulative release of metronidazole from AM polysaccharide based colon release tablets as a function of time.

complete ionisation of the hydrophilic groups of the polysaccharide leads to a higher rate of molecular extension, due to which, the viscosity increases rapidly [27]. According to the theory given by Feng et al., maximum viscosity will be obtained in any solution when molecular chains are in closed form to the rod conformation. The increase in apparent viscosity due to change in pH may be related to the change in conformation of the polysaccharide molecules [28]. From the above data we could conclude that pH variation affects the hydrodynamic behaviour and thus the flow properties of the AM polysaccharide solution. These results also indicates that the AM polysaccharide can be used as a pharmaceutical excipient in formulations where drug release is expected due to the pH dependent swelling property of polymer such as duodenal or colon release tablet in GI tract.

3.5. In-vitro drug dissolution study of tablet

The results of *in vitro* drug release from AM polysaccharide based colon release tablets are depicted in Fig. 4. The drug release from prepared tablets was found to be pH dependent. During the initial 6 h of study, a negligible amount of MNZ (5.83–10.29) was released from the prepared tablets due to an impermeable layer of eudragit at acidic media. However, at 6 h when rat cecal material was added, a significant change in drug release pattern was observed. At this point, eudragit was presumed to be dissolved completely in the dissolution media and drug release was governed by solely AM polysaccharide. The reason of increased release of MNZ in presence of rat cecal material may be due to the breakage of the extracted polysaccharide under the action of enzymes produced by polysaccharide-degrading bacteria present in the cecal material.

It was also observed that a relatively lesser amount of drug was release when a higher concentration of polysaccharide was used for tablet preparation. This can be attributed to the fact that the tablet swell by absorbing the media from their surroundings and form a semi-permeable three-dimensional gel structure through which drug has to be diffuse in order to get release from the tablet. It has been demonstrated that the swelling rate increases with increase in the polysaccharide concentration due to higher water uptake and holding capacity of the tablet [29]. At higher polysaccharide concentration, as the polysaccharide swells a thick layer of gel was formed around the tablet which increases the diffusion path length of the drug and leads to a reduction in drug release. The values of t_{50} (time required to release 50% of drug from the tablet) increased significantly ($p < 0.05$) from 411 to 477,503, 536 and 607 min in batches AM5, AM4, AM3, AM2 and AM1 respectively. Although, all prepared batches showed minimum release in acidic media and maximum in colonic media, only AM5 formulation was considered for further study as it showed complete drug release between 14 and 18 h of time course which is also reported

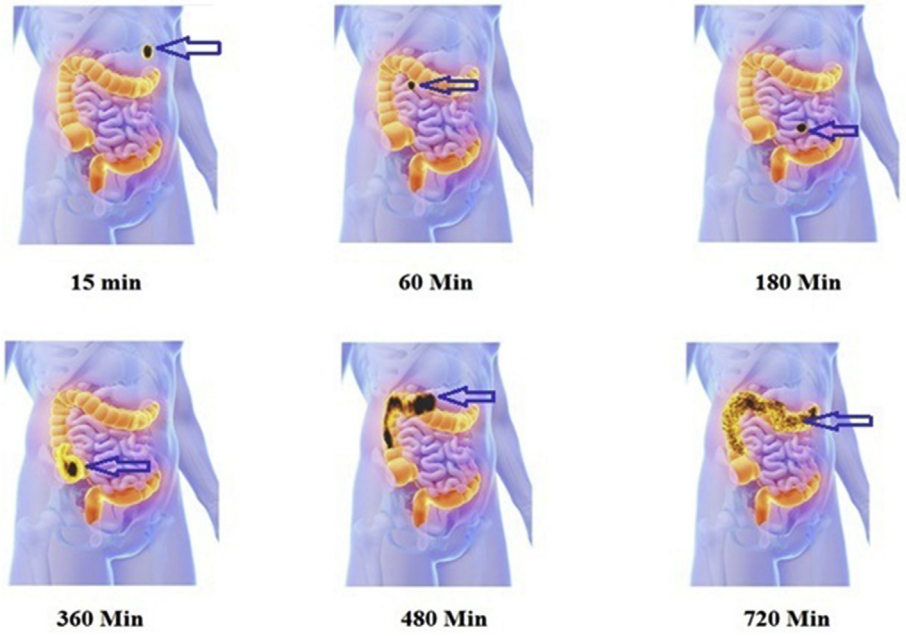

Fig. 5. Gamma scintigraphic images of AM polysaccharide based colon specific tablet in human volunteer, up to 12 h post ingestion.

to be the most appropriate transit time for colon delivery [6].

3.6. Colon targeting efficiency

In the past, planar scintigraphy based techniques using gamma radiotracers have proved helpful in imaging infection and inflammation [30]. Colon targeting efficiency of AM5 formulation was determined in human volunteers by gamma scintigraphy. Anterior abdomen images were captured to assess the position of the radiolabeled MNZ tablets. Gamma images (Fig. 5) indicate that the transit time of radiolabeled formulations from the stomach and small intestine were 90–150 (110 ± 15) min and 300–480 (390 ± 30) min respectively. Approximately 360–480 min were taken by the formulation to reach ileocecal junction. As predicted, the tablets maintained their structural integrity with no sign of radio labeled MNZ release in upper GIT. Prepared formulations started to release the radio-labeled MNZ after 360 min of oral administration and distributed and retain well in the colonic region for the next 720 min. Gamma scintigraphy images clearly indicated that Eudragit coated AM polysaccharide based tablets remained intact until it reached to the colon where AM polysaccharide gets metabolized by the colonic bacteria and pectinolytic enzymes and allows MNZ release from the tablets. Outcomes of gamma scintigraphy study was in accordance with the *in-vitro* drug release study and in favour of the statement that AM polysaccharide based eudragit coated tablet can be considered as a promising delivery system for colon-specific drug targeting.

4. Conclusion

A natural polysaccharide extracted from the *Abelmoschus moschatus* has been used to prepare tablets for colon specific release of MNZ. The Polysaccharide was evaluated for various physical and rheological parameters. The extracted polyscaccharide was found to be non-cytotoxic and a detailed study of the rheological parameters revealed that it exhibited different rheological behaviours under different conditions, which should be taken into consideration during the development of formulations comprising the selected polysaccharide. The AM Polysaccharide exhibited non-Newtonian pseudoplastic flow behaviour, which indicates that it may be used as an excipient for mucoadhesive and sustained drug delivery systems. Increase in the polysaccharide concentration decreased the flow behaviour index (n) and increased the consistency coefficient (k) while, a reciprocal relationship was observed between shear rate and apparent viscosity. *In vitro* drug release study confirmed the polysaccharidal cleavage in presence of colonic bacteria that allows complete drug release from the delivery system. The gamma scintigraphy study confirmed that prepared delivery system allowed the maximum release of radiolabbeled MNZ in the colonic region. Therefore, the present study concludes that AM polysaccharide may be used as a potential release controlling excipient in colon-specific drug delivery systems.

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CRediT authorship contribution statement

Nitin Sharma: Conceptualization, Investigation, Methodology. **Pradhi Srivastava:** Data curation, Methodology. **Anjana Sharma:** Project administration, Writing - original draft. **Dhruv K. Nishad:** Investigation. **Ritu Karwasra:** Writing - review & editing. **Kushagra Khanna:** Investigation. **Dipti Kakkar:** Conceptualization, Supervision, Formal analysis, Writing - review & editing. **Aseem Bhatnagar:** Writing - review & editing.

Declaration of competing interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

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