ABSTRACT

Objective: The objective of the present study was to chemical modification, characterization and evaluation of mucoadhesive potentiality of Assam bora rice starch as potential excipients in the sustained release drug delivery system.

Methods: The starch was isolated from Assam bora rice and esterified using thioglycollic acid and characterized by Fourier transform infrared spectroscopy (FT-IR), Differential scanning calorimetry (DSC) and Nuclear magnetic resonance (NMR). The 10% w/v gel formulation based on modified bora rice starch loaded with irinotecan (0.6%) was prepared and evaluated for various rheological properties, in-vitro mucoadhesion using goat intestine and in-vitro drug release study in phosphate buffer pH 6.8.

Results: The chemical modification was confirmed by FT-IR and NMR studies with the presence of the peak at 2626.74 cm⁻¹ and a singlet at 2.51 respectively due to–SH group. Ex-vivo mucoadhesion studies showed 6.6 fold increases in mucoadhesion of the modified starch with compared to native starch (463.3±6.79g for native starch; 308.7±95.31g for modified starch). In vitro study showed 89.12±0.84 % of drug release after 6 h in phosphate buffer pH 6.8 and the release kinetics followed Non-Fickian diffusion.

Conclusion: The modified Assam bora rice starch enhanced a mucoadhesive property of the native starch and thus, can be explored in future as a potential excipient for the sustained release mucoadhesive drug delivery system.

Keywords: Assam bora rice starch, Ex-vivo mucoadhesion, Modified starch, In vitro drug release

INTRODUCTION

The bioadhesion is the term that refers to any kind of bond formed either between two biological surfaces or between a biological and a synthetic surface. Since the concept of bioadhesion is coming into light a lot of attempts were taken to improve the adhesive property of the polymer. The Mucoadhesive polymers which form noncovalent bonds such as hydrogen bonds, van der Waal’s forces, and ionic interactions have weak bioadhesion [1]. The mucoadhesive delivery systems gain the superiority by having some advantages over conventional systems such as—mucoadhesive delivery systems can be eagerly delivered on the site of action, this kind of systems allow for intimate contact of the formulation with the mucosal surface that enhance the permeability for macromolecules, also these delivery systems can lengthen the residence period at the employed site of the dosage form [2, 3]. Thioldated polymers or thimers covalently anchor with mucus by disulphide bonds and can mimic the natural mechanism of secreted mucus glycoprotein. The cystine rich sub domain of mucus glycoprotein undergoes thiol/disulphide exchange reaction and forms disulphide bonds between polymer and mucus layer [4, 5].

Thiolated polymers can be prepared by interacting the thiol moieties, with the established polymers (hydrophilic) like chitosan [4], poly[(acrylates)] [7]. Apart from having a strong mucoadhesive property also have advantages as prolonged residential time of delivery, prevention to adhesive bond failure, reduction in side effects as well as a cost; also thimers has permutation enhancing and enzyme inhibitory properties. Thiolation has been done in several polymers and polysaccharides like chitosan.

Rice is widely grown, consumed and important cereal crop in India. Rice generally contains two types of starch components—Amylose and Amylopectin. The amount of each starch component determines the texture of the cooked rice and whether it will be fluffy, creamy or sticky. Chemically starches are polysaccharide and composed of monosaccharide or sugar molecules linked together with a-D-(1–4) and/or a-D-(1–6) linkages. The Bora rice chemically can be characterized by the lack of amylase content and the highest amount of amylpectin. Bora rice is also known as sticky rice or glutinous rice is a short-grain, Asian varietal that is used in many cuisines for its characteristic sticky texture and also possess a good gelling property [8, 9]. It’s only when the conditions are right that the rice starch molecules can break apart, causing the structure of the grain to become soft, mushy and sticky [10]. During the Ahom reign in Assam Bora rice with egg was used in constructing buildings only because of its sticky quality.

As this selective polysaccharide has its bioadhesive nature, it can be used as a drug delivery system for targeted and optimal drug delivery due to the intimacy and duration of contact [11, 12]. Still, Native starches cannot achieve enough stability under shear, acidic condition, retrogradation tendency, thermal decomposition; also cannot provide enough adhesiveness due to the weak bond formation. Sometimes solubility (in cold-water) and mechanical properties (lower flexibility and tensile strength) also limits its applications [13]. To overcome these limitations or to utilize these drawbacks as our advantage starch modification is necessary [14]. Generally, starch modification involves alteration of physicochemical characteristics of the starch. Chemical modification leads to increase in hydrophobic or hydrophilic or mucoadhesive property or may be some other changes through esterification, etherification, thiolation, cross linking, acid modification etc. Also starch could be modified.

The present study was designed with the aim to improve the mucoadhesive property of the Bora rice starch by thiol treatment and to characterize by Differential Scanning Calorimetry (DSC), Fourier Transform Infra-Red Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR). The thiolated starch was further explored for mucoadhesive application by formulating a gelling system with irinotecan as a model drug and evaluated for various parameters such as ex-vivo bio-adhesion and rheological properties.
MATERIALS AND METHODS

Materials

Assam Bora Rice was collected from the local market of Dibrugarh district of upper Assam, India. Irinotecan was obtained as a gift sample from Emcure Pharmaceuticals Limited (Pune, India). Thioglycolic acid and Triethanolamine was procured from Sisco Research Laboratory Pvt. Ltd. (New Mumbai), Carbopol 934P were purchased from Yarrow Chem. Products (Mumbai, India) respectively. Freshly excised goat intestine was obtained from the local butcher shop (Azara, Guwahati). All other chemicals used were of analytical grade.

Isolation of starch from Assam bora rice

Rice was fully milled in a laboratory miller and then passed through a screen with 125 μm openings to filter the flour particles after being milled. The flour (66g) was then suspended in 240 ml NaOH (pH 11) solution with continuous stirring and allowed to soak for 30 min. The rice flour was then blended at 4,000 rpm for 10 min and the upper grayish layer was discarded. Sediment containing white starch layer was collected and washed with distilled water over muslin cloth. Washings were then again centrifuged at 4,000 rpm for another 10 min and the upper grayish protein layer was removed and the starch layer was collected and washed with distilled water. Washed starch was then milled. The flour (66g) was then suspended in a 0.25% 240 ml NaOH (pH 11) solution with continuous stirring and allowed to soak for 30 min and neutralized with 0.2M NaOH solution. The solution was then filtered through a Buchner funnel and oven dried at 50°C for 120 min and starch powder was collected and allowed to pass through 120 mesh sieve and stored at room temperature [15-16].

Chemical modification of bora rice starch

The isolated Assam bora rice starch was modified by esterification of rice starch with thioglycolic acid. The reaction was carried out with 2 moles of thioglycolic acid for every 1 mole of the hydroxyl group in starch. Starch (5g) was dissolved in 40 ml of hot water. 1.8 ml of 80% thioglycolic acid and 2 ml of 7N HCl was added to this above solution. The mixture was allowed to react for 150 min at 80°C. The reaction mixture was poured into 500 ml of methanol. Off white precipitates of thiolated starch so obtained were washed twice with methanol and dried at room temperature. As thioglycolic acid is soluble in water and methanol, precipitation with methanol from an aqueous solution and subsequent washings by keeping precipitates over night was found to be a sufficient purification method for modified starch.

Characterization of thiolated starch

Differential scanning calorimetry (DSC)

DSC thermograms of native starch and modified starch were recorded using a differential scanning calorimeter (Perkin Elmer 4000). About 5 mg of the sample were crimped in a standard aluminium pan and heated in a temperature range of 30°C to 400°C at a heating rate of 10°C per minute in a nitrogen atmosphere.

Fourier transform infra-red spectroscopy (FT-IR)

Starch and modified starch were subjected to FT-IR spectroscopy in a Fourier-transform infrared spectrophotometer (Bruker, Alpha) in the range of 4000–400 cm⁻¹.

Ex-vivo mucoadhesive potential

A comparative mucoadhesive potential evaluation of native starch and modified starch was done by carrying out tensile tests employing texture analyzer (TA. XT Express, Stable Micro System). The analyser was equipped with different weight load cell. At room temperature, fixed amount (1g) of samples of each formulation was placed on the upper probe. Probe with freshly excised goat mucosal tissue was attached to the lower probe as a model membrane. During the measurement, the probe was lowered at a rate of 0.5 mm/s until a contact with the model membrane at a constant force of 30g obtained. The force of 30g was maintained for 5 min and the upper probe was moved upwards at a rate of 5 mm/s. The force required to detach the sample from tissue was considered as an index of mucoadhesive potential [22].

Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance spectroscopy of the Starch and modified Starch was carried out by using an NMR spectrometer (Bruker Avance II, 400 MHz). The Starch and modified Starch in powder form were scanned from 1 to 10 ppm range under the following measurement conditions: Magnet 9.4 T superconducting Magnet; Probe-BBO 400 MHz, with Z-gradient, 2H lock; for observation of nuclei like 1H, 13C, 31P, 15N, etc. with 1H decoupling. Any of these nuclei can be fully automatically selected and optimally tuned and matched.

Particle size distribution

Modified Starch was dispersed in distilled water to get a stock solution of 1% (w/w). The solution was then homogenized at 15,000 rpm and filtered twice. This dispersion was then swiftly transferred in the sample cell and particle size distribution was determined using Zeta Sizer (Nano ZS 90, Malvern Instruments, UK) with the help of software.

In vitro degradation study

The degree of degradation of modified starch was evaluated in different simulated fluids such as simulated body fluid (SBF), simulated nasal fluid (SNF) and in simulated gastric fluid (SGF). Thin films of modified starch prepared by a casting method and were placed in Petri dishes which contain simulated fluids. Then the Petri dishes were incubated for 15 min at 37°C.

After a predetermined time interval, the films were withdrawn and weighed before and after drying and were studied for degradation characteristics such as swelling degree and weight loss [23, 24].

Swelling degree—The test was done by comparing the weight (w) gained due immersion in simulated fluid with the original weight (w₀) of the sample and was calculated by the equation below,

\[ SD(\%) = \frac{w - w_0}{w_0} \times 100 \]

Weight loss—The weight loss was measured in terms of percentage by calculating the difference between the dry weight (w) of the sample after degradation and original weight (w₀) of the sample using the following equation,

\[ WL(\%) = \frac{w - w_0}{w_0} \times 100 \]

Formulation of oral mucoadhesive gel system

Irinotecan loaded oral mucoadhesive gel was prepared using modified bora rice starch. Mucoadhesive property of native starch and modified starch was evaluated by formulating gels using irinotecan as the model drug. In brief, Carbopol 934P (0.5%, w/v), as a gelling agent was dispersed in aqueous solution of irinotecan (0.6%, w/v) that contain starch or modified starch (10%, w/v) as mucoadhesive agent and allowed to hydrate overnight followed by addition of triethanolamine.

Characterization of prepared gel

Determination of viscosity

The viscosity of irinotecan loaded gels containing native starch (NS) and modified starch (TS) was measured using a Brookfield Viscometer (Brookfield DV-E Viscometer) at various speeds using spindle 25 at a range of 25-30°C.

Adhesive strength analysis

The gel adhesiveness was performed by using a texture analyzer (TA. XT Express, Stable Micro System) in adhesive mode. The formulation was transferred into a beaker and a cylindrical probe was forced down into sample at a defined rate (0.5 mm/s) of and to a defined depth (10 mm). At least three replicate analyses of the sample were taken at temperatures at 25°C±0.5°C and from the force-time plot the adhesiveness (the work necessary to overcome...
the attractive forces between the surface of the sample and the surface of the probe) was derived.

**Ex-vivo bioadhesive strength**

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from the mucosal tissue. Briefly, the freshly excised goat intestinal tissue was collected from a local slaughterhouse and used immediately after the collection. At the time of testing a section of the tissue was secured to lower probe having a surface area of 3.14 cm$^2$. At the room temperature, fixed amount of sample of each formulation was placed on the upper probe and the probe was lowered down until the sample contacted the surface of the tissue. Immediately, a slight force was applied for 30 Sec to ensure the intimate contact between the tissue and the sample and the mucoadhesive force was expressed as the minimal weights that detached the sample from the tissue surface [22].

**In vitro permeation study**

For in vitro permeation study a dialysis membrane was being mounted on the Keshary-Chien diffusion cell displaying a permeation area of 3.15 cm$^2$. The receptor compartment was filled with Phosphate buffer (pH 6.8) and the receptor medium was maintained at 37±0.5˚C. 1 g of gel formulation was placed in the donor compartment. At defined time interval, 1 ml of sample was withdrawn from the acceptor compartment, replacing with the same volume of the pre-warmed receiver solution to maintain the sink condition for a period of 6 h. The samples withdrawn were filtered and used for analysis using UV-visible spectrophotometer at 254 nm.

**RESULTS AND DISCUSSION**

**Synthesis of the modified starch**

Thiolation of bora rice starch was achieved by the ester bond formation between the hydroxyl groups of starch with carboxyl groups of thioglycolic acid. The collected product was found to be off-white in colour and soluble in water with some heat. The average yield of this synthesis amounted to 72±3.78% of the utilized amount of the rice starch.

**Differential scanning calorimetry**

The DSC thermograms of native Starch and modified starch were presented in fig. 2. The DSC curve of starch shows an endotherm at 76.18˚c with a heat of fusion 114.72 J/g. While the DSC thermograms of modified starch shows a sharp endotherm at 81.11˚c with a heat of fusion 150.20 J/g. The shifting of the endothermic peak as well as the heat of fusion in DSC thermogram revealed the crystal nature and gave a partial approval of a modification of the bora rice starch, which was further confirmed by FT-IR and NMR studies.

**Ex-vivo bioadhesive strength**

The bioadhesive strength of native starch and modified starch were displayed in fig. 4 (a) and (b) respectively. The maximum force of a detachment of starch and modified starch from the intestinal mucosa under the same target force was found to be 46.3±6.79 g and 307.3±95.31 g respectively. Thus, it was suggested that the modified starch exhibits almost 6.6 fold higher mucoadhesive strength than native starch. The Greater mucoadhesive potential of modified starch compared to native starch could be associated with the formation of a disulfide bond between the-SH groups of modified polymers and mucus membrane. This potentiality of the modified starch encouraged us to explore it for mucoadhesive applications. The mucoadhesive property of native and modified starch was further comparatively evaluated preparing the gelling systems of both.

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**Fig. 1: Chemical synthesis of modified starch**

**Fig. 2: DSC thermogram of native and modified starch**
To explore the possible modification of native starch, we studied the \(^1\)H NMR spectra of both native starch and modified starch. As shown in fig. 5 (a) and (b), the majority of the native starch and modified starch shifts were between 2 and 6 ppm. In NMR spectra of starch, a singlet has been observed at 3.6 (\(-\text{O} - \text{CH}_2\)). Another singlet at 4.59 for \(-\text{O} - \text{CH}_2\) and multiplets at 4.54 for \(-\text{O} - \text{CH}_2\) - \text{OH} also been found.
Multiplets at 3.47 and a singlet at 5.1 appeared due to –CH–OH and –O– respectively. In case of modified starch the spectra was observed almost similar to native starch, but appearance of a new singlet at 2.50 due to – SH was found which confirms the success of the thiolation.

**Particle size distribution**

The average particle size and polydispersity index (PDI) of modified starch were determined as it could be explored for nanoparticle applications. The PDI is a dimensionless number that indicates the width of the size distribution. The Z-average value for modified starch was found to be 312.4±10.01 nm and the PDI was reported to be 0.534±0.05. The particle size analysis provided a convincing approach for the modified starch to be used as other novel (nanoparticulate) carriers.

**In vitro degradation study**

In vitro swelling study represented the ability of the film to swell and revealed the sensitivity of both the native and modified starch in different simulated pH. The degree of swelling was found to be higher in SBF than SNF followed by SGF. While the weight loss in SGF found to be lower than other fluid. The film is swollen, less in SGF and the weight loss also lower in SGF.

According to the results, the higher degree of swelling in SBF indicates that the modified starch film is highly swellable in respective fluid and possesses faster weight loss that was mainly due to the breakdown of the starch molecules.

**Characterization of the gelling system**

**Determination of viscosity**

The results of the viscosity study were presented in table 1. The viscosity of the modified starch based gel (MSG) and native starch based gel (NSG) were found to be 9950±636.39 cP and 8899±799.03 cP respectively at a defined speed of 5 rpm. The particle size analysis provided a convincing approach for the modified starch to be used as other novel (nanoparticulate) carriers.

**Table 1: Viscosity profile of oral mucoadhesive gel**

| RPM | Viscosity(cP)* | NSG* | MSG** |
|-----|----------------|------|-------|
| 0.5 | 4358±0.211.42 | 4672±202.68 |  |
| 1   | 18720±7556.60 | 21300±49.49 |  |
| 3   | 13940±7184.24 | 17000±704.27 |  |
| 5   | 8899±799.03   | 9950±636.39 |  |

All the values are represented as mean±SD (n=3), * NSG–Native starch based gel, **MSG–Modified starch based gel

**Adhesive strength analysis**

An optimal gelling system must have the suitable adhesive strength (table 2) so that it can be retained at the mucosal surface for a longer period of time after administration. So during the development of such formulation, it is important to achieve the condition that allows in enhancing the residence time at the administration site. The adhesive strength for NSG and MSG was found to be 20.4±0.49 g and 13.5±8.9 g respectively. In the case of modified starch gel, a marked increase in gel adhesiveness was observed and was measured as the area of the peak of the force-time curve.

**Table 2: Gel adhesiveness and mucoadhesiveness profile of starch based gel**

| Formulation | Gel adhesion (g) | Mucoadhesion (g) |
|-------------|-----------------|-----------------|
| NSG*        | 20.4±0.49       | 18.3±1.32       |
| MSG**       | 43.5±8.90       | 37.0±1.69       |

All the values are represented as mean±SD (n=3). * NSG–Native starch based gel, **MSG–Modified starch based gel

**Ex-vivo bioadhesive strength**

The bioadhesive strength of MSG and NSG was found to be 18.8±1.82 g and 37±4.19 g respectively (table 2). The results of the evaluation showed that the modified starch exhibit almost 2 folds better mucoadhesive property than the native starch formulation. The bioadhesive property of native starch may be due to the secondary hydroxyl (OH) groups which could be considered as the main source of mucoadhesion while the better mucoadhesive strength of modified starch might be assigned with the covalent anchoring via disulfide bond formation of the modified starch with the mucus involving thiol exchange reaction.

**In vitro release study**

In vitro, drug release profiles were shown in fig. 5. The MSG and NSG formulation showed 89.12±0.04 % and 86.77±0.55 % of drug release after 6 h of study in phosphate buffer pH 6.8 respectively. The study revealed that no significant changes in the drug release pattern from modified starch and native starch, which indicate no change in gel network of both the starch which is supported by the viscosity study result showed in table 3.

Although having the greater viscosity the divergence has been produced after observing the drug release study of the gel containing modified starch, which provided little faster release than the native starch based gel. This could be illustrated by taking help of the ex-vivo mucoadhesive study where the modified starch based gel showed a higher performance due to the formation of cross linking exhibited by disulfide bonds of the modified starch.

**Table 3: Comparative release kinetics parameter of irinotecan from different gelling systems**

| Formulation | Zero | First | Higuchi’s square root | Korsmeyer-peppas |
|-------------|------|-------|----------------------|------------------|
|             | R² K | R² K  | R² K                 | R² K             |
| NSG*        | 0.908±0.281 | 0.950±0.005 | 0.962±4.360 | 0.992±0.430 0.922 |
| MSG**       | 0.982±0.294 | 0.956±0.005 | 0.964±4.523 | 0.991±0.657 0.854 |

n=3, * NSG–Native starch based gel, **MSG–Modified starch based gel

Fig. 6: In vitro release profile of both the gelling systems. All the values are represented as mean±SD (n=3)
The in vitro drug release data was applied in various kinetic models and the correlation coefficient (R) and the release rate constant were determined from the slope of the respective formulations that is shown in table 2. Both the formulations were found to be best fitted in the zero order model with R² value of 0.9822 and 0.9908 for MSG and NSG formulations respectively. Further, to confirm the drug release mechanism from the formulations, the in vitro drug release data at various time points were fitted into the Korsmayer–Peppas equation. The correlation coefficient (R² values) of 0.9910 and 0.9926 for MSG and NSG respectively, and the release exponents (n values) were found to be 0.854 and 0.922 respectively. The obtained n values of both the formulations were greater than 0.5 and less than 1. Hence, it is revealed that the mechanism of drug release was a non-Fickian or anomalous diffusion [25].

CONCLUSION

On account of this study, it can be concluded that the isolated starch from Assam bora rice was successfully modified by thiol esterification method. The presence of thiol group in the modified starch was confirmed by FT-IR and NMR studies with the presence of peaks at 2626.74 cm⁻¹ represents the –SH stretching and singlet appeared at 2.51 due to –SH group. DSC thermogram showed a sharp endothermic peak at 81.11°C that revealed the crystallinity of modified starch. The mucoadhesive strength of the gel was found to be 37±1.69 g and tensile strength found to be 43.5±8.90 g. In vitro study showed 89.12% of drug release after 6 h at phosphate buffer pH 6.8 and the release kinetics followed Non-Fickian diffusion. Thus, the modified bora rice starch can be used as a potential mucoadhesive excipient for the sustained release drug delivery system.

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AUTHOR CONTRIBUTION

The authors meet the following conditions:

• Authors made valuable contributions to design, and/or acquisition of data, and/or analysis and interpretation of data.
• Authors also participated in drafting the article and revised it extensionally for its appropriate formatting.

CONFLICT OF INTERESTS

The authors declared no conflict of interest

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