Nanoparticles Synthesis from Corn Cob (Xylan) and Their Potential Application as Colon-Specific Drug Carrier

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Summary: Corn Cob based Xylan, a natural polysaccharide extracted from agro-waste may be used as a tool to deliver drugs especially to the colon because of their timely retention in the physiological environment of stomach and small intestine and can only be degraded in colon by vast anaerobic microflora like *bifidobacterium*. The objective of present research study is to incorporate the drug namely 5-aminosalicylic acid (5-ASA) into xylan macromolecular backbone, either by surface adsorption or by intermolecular covalent bond formation so that absorption of drugs is prevented in upper gastrointestinal tract (GIT). To achieve the above objective, xylan prodrug of 5-ASA was synthesized via activation of carboxylic acid with N,N-carbonyldiimidazole. The structure of obtained xylan prodrug was evaluated by means FT-IR spectroscopy. The ester carbonyl absorption band was observed at 1690 cm^{-1} in addition to the bands originated from 5-ASA and xylan. The resulting prodrug and xylan itself assembled into spherical nanoparticles were analyzed by scanning electron microscopy. The prodrug of 5-ASA was synthesized which might be active against inflammatory bowel diseases, a novel thought towards advanced drug delivery from xylan based nanoparticles will be presented.

Keywords: corn cob; nanoparticles; xylan; xylan-5-ASA prodrug derivatives

Introduction

Delivery of drug specifically to colon has been developed for treating inflammatory bowel diseases (IBD) including Crohn's disease, ulcerative colitis, colon cancer and infectious diseases where it is necessary to attain a high concentration level of active ingredient such as 5-ASA. The 5-ASA is unstable in the gastric environment and prone to be absorbed in the upper gastrointestinal tract (GIT), which causes low drug bioavailability and low effectiveness for IBD. Thus successful delivery of 5-ASA to colon via the GIT requires the protection from being released in the stomach and upper GIT when administrated orally. Therefore, a system must be developed that can protect the 5-ASA during its transfer to the lower GIT.[1] To overcome these problems several approaches such as pH-dependent systems, positioned-release systems, low molecular weight prodrugs, macromolecular prodrugs, and biodegradable polymers have been developed for targeted delivery of drug.

Biodegradable polymer such as xylan (2nd most abundant polysaccharide in plant kingdom) is the most common hemicelluloses; represent more than 60% of the polysaccharides existing in the cell wall of corn cob, [2] an alternative option for biomass utilization. Its main chain is constituted of D-xylopyranose units in the backbone linked through β-1, 4-glycosidic bonds. The majority of D-xylans have other sugars in side chain, such as 4-O-methyl-D-glucuronic acid, O-acetyl-L-arabinose, L-arabinose,

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and D-glucuronic acid whereas; corn cob xylan presents a chemical composition of 4-O-methyl-D-glucuronic acid, L-arabinose and D-xylose in the proportion of 2:7:19 respectively. [2–5]

Xylan extracted from corn cob has poor flow ability[5,6] also known as corn fiber gum with a sticky behavior, used as an adhesive, thickener, and additive to plastics,[7] as biofuel production,[5,8] as an emulsifier and protein foam stabilizer during heating in the food industry,[9] as an additives in paper-making[10] and textile printing,[11] as a carrier for magnetite particles to protect from gastric dissolution when administrated orally,[12] as a prodrug carrier for ibuprofen release in the pharmaceutical industry.[13] Regarding pharmaceutical applications, it has timely retention in the physiological environment of stomach and small intestine and can only be degraded in colon by vast anaerobic microflora like *bifidobacterium* that's why this biopolymer may be considered as a suitable raw material for the biomedical applications, principally as a colon-specific drug carrier.[5]

Thus a novel approach to synthesize polymeric drug derivatives (prodrugs) was initiated, where the drug molecules are covalently linked to the polymeric backbone through ester linkages, with limited stability in the physiological environment. This approach should modify the pharmacokinetics properties of the drug and also obtain preferential localization, leading to a good sustained release system.[14] Thus, the objective of the present study is to synthesize and evaluate novel polymeric prodrug containing 5-ASA for sustained and site specific delivery.

Experimental Part

Raw Material

The corn cob sample was collected from agricultural field locally (Dehradun, Uttarakhand, India) and it was milled into powder in a laboratory Wiley Mill, and fractions passing through 20 mesh (841 μm) screens but retained on 80 mesh (177 μm) screens was collected. Sample was air-dried, homogenized in a single lot to avoid compositional differences among aliquots, and stored for compositional analysis and further treatment.

Extraction of Xylan

The xylan was extracted from corn cobs following the technique described by Garcia et al. (2001) with some modifications in the process. After grinding, the dried corn cobs were dispersed in water under stirring for 24 h. Then, the sample was treated with acidified sodium chlorite (CH_3COOH/$NaClO_2$) in order to remove lignin and other extractives. The xylan was further extracted from the pulpy mass by using 4% (w/v) sodium hydroxide solution. During extraction, the system was maintained under moderate stirring at 60 °C for one hour. Afterward, the extract was neutralized with acetic acid, and xylan was separated by settling down after methanol addition subsequently, several washing steps were performed by using methanol. Finally, the sample was filtered, dried at 60 °C in vacuum oven up to a constant weight.

Preparation of Micro and Nanoparticles

In order to prepare micro and nanoparticles, the extracted xylan was dissolved in 1N NaOH with gentle stirring and it was precipitated by 1N HCl solution with an equal volume so that a neutral solution was achieved with concentration of xylan 1 mg/ml.

Synthesis of 5-N-formylaminosalicylic Acid (5-fASA)

In the present experiment, 8 g (52 mmol) of 5-ASA was taken and dissolved into 80 ml of 98% formic acid followed by refluxing for 30 minutes. After that 160 ml cold distilled water was added for precipitation. The precipitates were filtered, washed several times with cold water and dried in vacuum oven with 82% yield.

Preparation of Xylan-5-ASA (Xyl-5-ASA_1:1) Ester Conjugates

To the solution of 5-fASA (2 g, 11 mmol) in DMF (55 ml), CDI (1.95 g, 12 mmol) was

added slowly, reacted for 1 hour at room temperature and then xylan (1.44 g, 11 mmol) in DMSO (55 ml) was added drop wise with constant flow rate. To the reaction mixture, triethylamine (1 ml) was added and stirred for 24 hours at room temperature. To produce precipitates 1N HCl solution or acetone was added in excess. The obtained precipitates were hydrolyzed with 0.1N HCl for 10 minutes at 80 °C. The product was washed several times with acetone to remove acid, dried in vacuum oven and weighed till constant weight. The obtained ester prodrug of 5-ASA with 67% yield, was ground in mixture mill (Retsch, MM-400). The powder after grinding was simply dispersed in water with sonication for 10 minutes for SEM analysis.

Characterization

Fourier transform infrared (FT-IR) spectroscopy was performed using a Nicolet spectrophotometer. Samples were oven dried at 105 °C for 4 h, mixed with KBr in a ratio of 1:200 mg (xylan: KBr) and pressed under vacuum to form pellets.

Scanning Electron Microscopy (SEM) was performed, using a FEI Quanta 200F microscope. The samples were dispersed in water using sonication method. One to two drops of suspension was placed on glass slide for SEM analysis at 15–20 kV after evaporation of water and gold coating.

Results and Discussion

The de-waxed 10 g corn cob powder was delignified with acidified $NaClO_2$ solution at 70 °C without degrading the cellulose and hemicellulose.

Reaction scheme:

$$5\ NaClO_2 + 4\ CH_3COOH \rightarrow 4\ CH_3COONa + 4\ ClO_2 + NaCl + 2H_2O$$

The ClO_2 act as bleaching agent and most of the lignin was removed. The obtained product was treated with 250 ml of 1N NaOH solution. The alkali treatment cleaves the β-alkyl-aryl ether bonds (β-O-4 linkages) in lignin via the formation of an epoxide intermediate with an intra-molecular nucleophilic displacement of the β-phenoxide by either α- or γ- alkoxide formed from the alcohol in strong base.[15] Subsequent nucleophilic attack by hydroxide cleaves the epoxide ring and produces a trihydroxy propane structure. Thus the residual lignin was removed by NaOH treatment.

FT-IR Spectroscopy of Holocellulose and Xylan Extracted from Corn Cob

FT-IR spectroscopy is a powerful technique used for studying the physico-chemical and conformational properties of polysaccharides. The main absorption band of FT-IR spectra of the holocellulose and extracted xylan were shown in Figure 1a. The analysis of FT-IR data shows that the holocellulose has most significant absorption band at 1734 cm^{-1} relates to C-O stretching of carbonyl group[16] which was disappeared in xylan may be due to the removal of acetyl group of acetylated xylan (i.e. hemicelluloses) by alkali treatment at elevated temperature. A broad absorption band in the range of 3415–3420 cm^{-1} that can be attributed to the -OH stretching associated to polar groups linked through intra- and intermolecular hydrogen bonding.[17] Bands at 2913 cm^{-1} and 2923 cm^{-1} indicate symmetric C–H stretching vibration due to CH_2 and CH_3 groups.[18] A sharp band at 1633–1642 cm^{-1} was also detected and attributed to H-O-H stretching, which occurs mainly in the amorphous state, and crystalline spectra measured in KBr which belongs to the absorbed water molecules.[5,19] The band at 1430 cm^{-1} assigned to H-CH and -OCH in-plane bending vibrations in both holocellulose and xylan component.[20] In addition, an absorption band near 1383 cm^{-1} was detected owing to the C-H bending vibration present in holocellulose and hemicellulose chemical structures.[20] Nevertheless, the presence of the arabinosyl side chains is

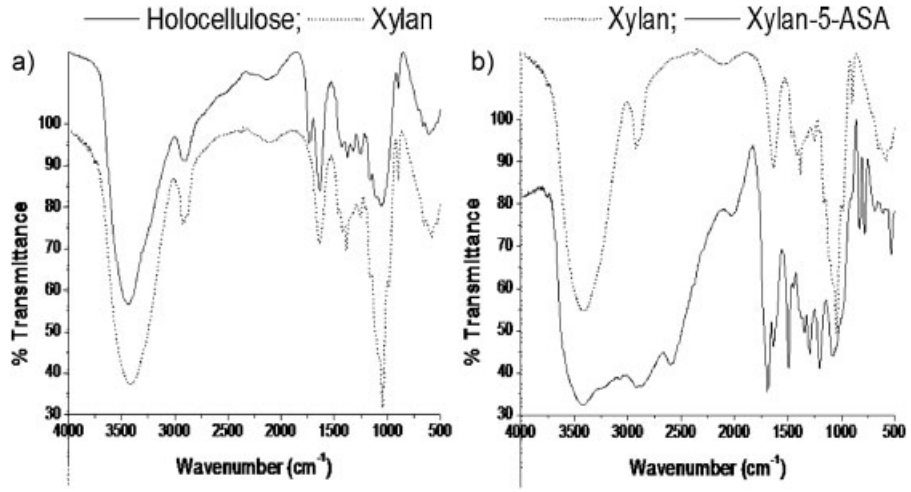

Figure 1.
FT-IR spectrum of (a) holocellulose and xylan, (b) xylan and xylan-5-ASA ester conjugate.

characterized by a low intensity shoulder at 1156–1160 cm^{-1} corresponding to the C–O–C vibrations in the anomeric region of hemicelluloses.[20] The bands in the region of 1125–1000 cm^{-1} are typical of xylans. The prominent band at 1043–1046 cm^{-1} is attributed to the C-C, C-O stretching vibration in hemicellulose (xylan) and C–OH bending vibration [19] in both holocellulose and xylan. Finally, a sharp band at 895–899 cm^{-1}, which is a typical of β-glycosidic linkages between the sugar units in hemicelluloses, was detected in the anomeric region.[21]

FT-IR Spectroscopy of Xylan-5-ASA Ester Derivative

The FT-IR spectrum of xylan-5-ASA ester conjugate is shown in Figure 1b, the main characteristic absorption bands were observed at 1690 cm^{-1} (carbonyl stretching of xylan-5-ASA ester), 1450–1600 cm^{-1} (C-C multiple bond stretching of aromatic ring), 700–835 cm^{-1} (aromatic substitution that is substituted at o- and m- position), 1634 cm^{-1} (N-H bending of primary amino group), 3417 cm^{-1} (N-H stretching of primary amino group), 1297 cm^{-1} and 1346 cm^{-1} (C-N stretching of primary amine) in addition to the bands originated from 5-ASA and xylan. On the basis of these bands the reaction scheme may be illustrated as in Figure 2. Whereas, when ester prepared by physical addition simply then there was no characteristic or additional bands were observed (spectra not shown).

Field Emission Scanning Electron Microscopy (FE-SEM)

Field scanning electron microscopy gives the morphological evidence of the xylan nanoparticles with variable structures having an average diameter of 103 ± 21 nm (Figure 3a) whereas prodrug of 5-ASA has spherical structure with an average diameter of 258 ± 87 mn (Figure 3b), as analyzed by Image-J software is also an indication of incorporation of drug molecule on to the bio-macromolecular backbone of xylan.

Conclusion

The synthesized prodrug of 5-ASA has a significant absorption band at 1690 cm^{-1} (carbonyl stretching of ester bond) which confirm the formation of ester linkage with xylan bio-macromolecules. The incorporation of drug may also be confirmed indirectly by increasing the particle size

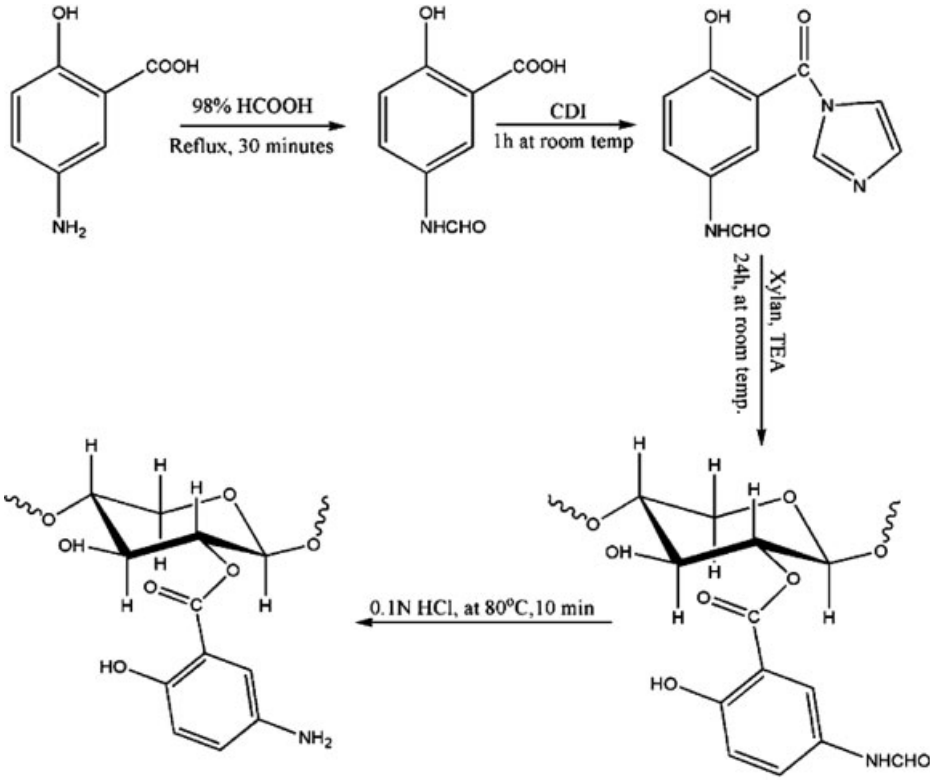

Figure 2.
Schematic representation of synthesis of xylan-5-ASA derivative (prodrug).

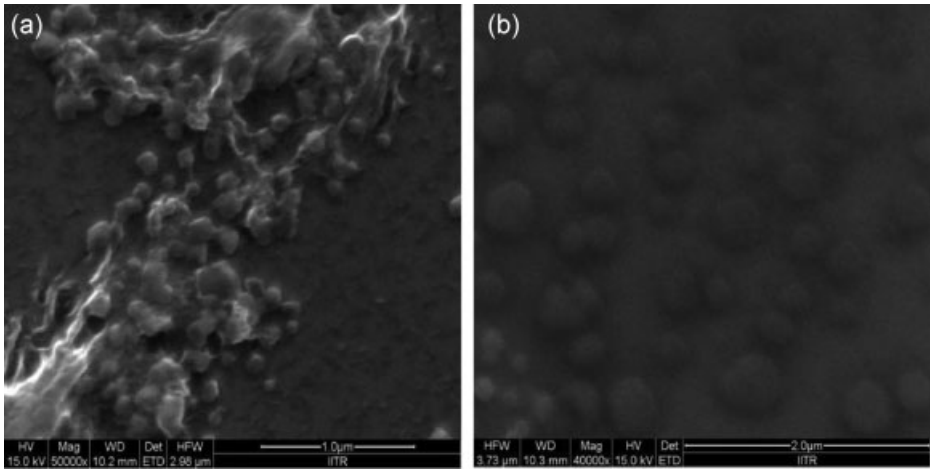

Figure 3.
SEM micrograph of (a) xylan particles (scale bar 1 μm) and (b) ester conjugate/prodrug of 5-ASA (scale bar 2 μm).

with spherical nature as analyzed by SEM micrograph. Thus the above synthesized prodrug of 5-ASA might be applicable for sustained and colon-specific release of 5-ASA to avoid side effect. Therefore, synthesis of xylan prodrug could be an important issue concerning to the environment because a green biopolymer from a renewable source was in fact a great challenger for the pharmaceutical industrial research and development sectors.

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