Synthesis and Evaluation of p-Aminobenzoylhydroxypropyl Cellulose

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\textbf{Abstract:} Hydroxypropyl cellulose (HPC) was selected as a macromolecular carrier for the attachment of para-aminobenzoic acid (PABA) for different pharmaceutical, biomedical and nutritional applications. HPC-PABA ester conjugate was synthesized in two easy steps that were performed homogeneously. In the first step, the primary amino group of PABA was protected by 98\% formic acid and during the second step the protected PABA was coupled with the HPC polymer in DMF by using 4-(dimethylamino)pyridine as a base and N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC.HCl) as a coupling reagent at room temperature. The optimum conditions for deprotection process were also determined. Novel macromolecular conjugate has solubility in both water and organic solvents. HPC-PABA ester conjugate was synthesized with good percentage yield and purity and well identified by FT-IR and 'H-NMR techniques. According to this new synthesis plan HPC-PABA conjugate can be prepared easily and has potential for pharmaceutical, textile and biomedical sectors.

\textbf{Key words:} HPC, PABA, coupling reaction, macromolecular conjugate

\section*{INTRODUCTION}
Hydroxypropyl cellulose (HPC) is one of the commercially important cellulose ether due to its applications in food, coating, cosmetics, paper and pharmaceutical sectors (Hussain \textit{et al.}, 2013). HPC has valuable properties like good solubility in water and polar organic solvents, physiologically inert, pH insensitivity, thermoplasticity and maintenance of flavor intensity (Khan \textit{et al.}, 2008). HPC has potential to be used with food preservatives like sodium propionate, sodium benzoate, sorbic acid and methyl and propyl hydroxybenzoate in different food formulations (Wustenberg, 2014). HPC is normally used as an excipient in different pharmaceutical dosage forms as a thickening agent and binder (Hussain, 2008). HPC has three hydroxyl groups per anhydroglucose unit that can be employed in different organic synthesis reactions and this property is valuable for the designing of macromolecular conjugates (Khan \textit{et al.}, 2008). Carbohydrate polymers such as HPC can be used as a carrier for the attachment of the therapeutic agents (Zou \textit{et al.}, 2005) and p-aminobenzoic acid (PABA) as a linker for different applications (Pasut and Veronesi, 2007). PABA is a chemical that is classified as a non-protein amino acid. PABA is secure and its metabolism rate is fast by the liver (Furuya, 1995). PABA structurally consisted of a benzene ring that is substituted with a carboxyl group and an amino group. Both the amino and carboxyl groups of PABA can be used for different valuable chemical reactions. For the treatment of inflammatory bowel disease polyethylene glycol-5-aminosalicylic acid (Canevari \textit{et al.}, 2009) and dendrimer-5-aminosalicylic acid azo conjugates (Wiwattanapatapee \textit{et al.}, 2003) were synthesized and PABA was employed as a spacer in these synthesis schemes (Friend, 2005). PABA was also reported to use as a sunscreen against the development of sunburn and skin cancer from excess ultraviolet light exposure. It is known particularly for its nourishment to hair and supportive of blood cells, especially the red blood cells. PABA supports folic acid production by the intestinal bacteria and it is important for intestinal health. In instances of PABA deficiency, it is accessible as a nutritional supplement and also can be prescribed in the potassium salt form called Potaba. PABA can also be found in small amounts in some B-complex vitamins and multivitamin formulas (Sharon, 1998).

The aim of the present study is to synthesize and characterize HPC-PABA ester conjugate in a two simple and homogeneous steps. During the first step, PABA was protected by formic acid to avoid from self-coupling due to bifunctional nature. In the second step, first protected PABA was esterified with HPC and in the last protecting group was removed to get HPC-PABA conjugate. This approach will be helpful for the synthesis of a novel polyaromatic polyanime macromolecular conjugate having a pendant primary amine functionality which can be employed for different biomedical, nutritional and pharmaceutical applications (Khan and Ovatiarporn, 2015).

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MATERIALS AND METHODS
HPC powder (Mw=80,000) was obtained from Sigma Aldrich (USA) and dried at 50°C under vacuum for 72 h before use. PABA was analytical grade and purchased from Merck (Darmstadt, Germany). The rest of the chemicals and solvents were of analytical grade. FT-IR spectra were recorded on a Perkin-Elmer FT-IR model spectrum one spectrophotometer. 1H-NMR spectra were recorded by the Varian Nuclear Magnetic Resonance Spectrometer (500 MHZ). 1H NMR spectra of the ester (10 mg sample/ml) was measured in deuterated dimethylsulfoxide (DMSO-d6) at 60°C.

Protection of amino group of p-aminobenzoic acid: In the first step of the synthesis, PABA 3 g in 98% formic acid (30 ml) was refluxed for ten minutes and after that 30 ml of cold distilled water was added in the reaction flask for precipitate the product (Jung et al., 1998). The precipitates were filtered, washed several times with cold water and dried in a vacuum. 4-Formylaminobenzoic acid (4-f-PABA) was obtained with 98.56% yield. The product was then characterized by FT-IR.

Esterification of HPC with protected p-aminobenzoic acid and deprotection process: A 100-ml one-necked flask was equipped with a stopper and a magnetic stirring bar. HPC (Mw=80,000) (2 g, 5.96 mmol) was added into the flask and dissolved in DMF (40 ml) at room temperature. 4-(dimethylamino) pyridine (1.46 g, 11.92 mmol) was introduced followed by the addition of 4-formylaminobenzoic acid (2 g, 12 mmol) and EDC.HCl (2.285 g, 11.92 mmol), respectively and stirring was continued for 48 h at room temperature under nitrogen atmosphere (Khan et al., 2008). After 48 h, the protecting group i.e., 98% formic acid which was condensed with the amino group of PABA was removed by adding 0.5 M HCl solution in the flask. The product was stirred vigorously for 60 minutes at 80°C (Zou et al., 2005). The final product (HPC-PABA) was obtained from hydrolysis resulting in HPC-4-f-PABA. The resulting reaction mixture was purified by dialysis method using dialysis bag (MWCO = 12,000-14,000 Da) against distilled water overnight and pure HPC-PABA ester conjugate was achieved by freeze drying and characterized by FT-IR and 1H-NMR. The product yield was calculated as 72.34%.

RESULTS AND DISCUSSION
HPC-PABA ester conjugate was attempted to synthesize for a variety of applications in pharmaceutical, textile, biomedical and food industries. p-Aminobenzoic acid was selected as the linker because of its appropriate functionalities and non-toxic to human body (Sharon, 1998). It contains carboxylic groups for ester formation with the hydroxyl groups of HPC and in addition its aromatic amino group after deprotection can facilitate many derivatization reactions. In the first step, amino group of PABA was protected by formylation which proceeded easily in formic acid in good yield (Fig. 1). In the second step, which was ester bond formation N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC.HCl) was used as a coupling reagent and optimum molar ratio of HPC to 4-f-PABA was used 1:2. After 48 h, the protecting group i.e., 98% formic acid which was condensed with the amino group of PABA was tried to remove by stirring the product in 0.5 M HCl for 20, 40 and 60 minutes at 60 and 80°C (Fig. 2). It was observed that the product was precipitated on addition of 0.5 M HCl and the deprotection of HPC-4-f-PABA was accomplished in one hour by hydrolysis of the precipitates at 80°C. It is worth to note that the ester bond between PABA and HPC was found broken at 80°C.

Figure 3A shows the FT-IR spectra of PABA, 3B shows protected PABA by formic acid and HPC-PABA ester conjugate was shown by 3C. FT-IR of PABA displayed the characteristic peaks of primary amine which shows two N-H stretching vibration at 3481 and 3363 cm⁻¹, C-N stretching at 1291 cm⁻¹, N-H bending at 1624 cm⁻¹ and -C = C and C-H in the benzene ring at 1600 and 698 cm⁻¹, respectively. After protection process of PABA, 4-f-PABA did not show the two N-H stretching vibrations at 3461 and 3363 cm⁻¹. It indicated that the protection process was successful and PABA was now completely converted to 4-f-PABA before coupling to HPC. In addition, the FT-IR spectrum of HPC-PABA ester conjugate (Fig. 3C) demonstrated the absorption bands of -C = O stretching vibration at 1748 cm⁻¹ (HPC-O-C = O). Moreover, since the final product of HPC-PABA ester conjugate contained 4-aminobenzoate moiety, therefore the characteristic peaks of primary amine which shows two N-H stretching vibration at 3337 and 3224 cm⁻¹, C-N stretching at 1268 cm⁻¹ and -C = C and C-H in the benzene ring at 1601 and 765 cm⁻¹ were also observed. The FT-IR results confirmed that the desired product was successfully achieved by this method.

The spectrum of HPC-PABA ester conjugate was also confirmed by 1H-NMR spectra with a 500 MHZ Unity Inova, Varian Nuclear Magnetic Resonance Spectrometer in DMSO-d6 (not shown). The spectrum of HPC-PABA ester conjugate shows broad peaks at 1.0-1.25 ppm from

![Fig. 1: Reaction scheme for the protection of amino group of PABA](image-url)
the methyl groups in the hydroxypropyl moieties. Peaks in a range of 3.2-4.1 ppm belong to protons of cyclic glucose units. The characteristics resonance peaks from 6.75 to 7.88 ppm are attributed to the protons of aromatic moiety of PABA. A tiny peak detected at 4.95 ppm belongs to the resonance of the methine protons of modified HPC. FT-IR and 1H-NMR characterizations demonstrated that HPC-PABA ester conjugate was developed successfully.

**Conclusions:** Synthesis of HPC-PABA ester conjugate was accomplished in a two easy steps that were performed homogeneously. Novel macromolecular conjugate has solubility in both water and organic solvents. HPC-PABA ester conjugate with primary aromatic amine functionality were synthesized and characterized effectively with excellent percentage yield and purity. HPC-PABA ester conjugate has potential to be used in different biomedical and pharmaceutical applications.

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