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Research Article

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Synthesis and characterization of hydroxypropyl cellulose-*p*-aminobenzoic acid ester conjugate

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ABSTRACT

Hydroxypropyl cellulose (HPC) was modified to contain chloroacetate groups by the reaction of HPC with chloroacetyl chloride in dried dichloromethane by using pyridine as a catalyst. The coupling of para-aminobenzoic acid (PABA) to HPC functionalized with chloroacetate groups was carried out in a homogenous reaction between chloroacetylated HPC and sodium salt of PABA in dimethylsulfoxide (DMSO). The structures of chloroacetylated HPC and HPC-PABA conjugates were well characterized by FT-IR and ^{1}H-NMR techniques. The degree of substitution (DS) per anhydroglucose unit (AUG) of chloroacetylated HPC and HPC-PABA ester conjugate was calculated by using integrated area from^{1}H-NMR spectra. Synthesis and structural characterizations of HPC-PABA ester conjugate was successfully carried out and this strategy will contribute to the development of a potential macromolecular carrier for biomedical and pharmaceutical applications.

Keywords: *p*-Aminobenzoic acid, Esterification, Hydroxypropyl cellulose,^{1}H-NMR , FT-IR

INTRODUCTION

For many years pharmacists have been employing cellulose as the most abundant natural material in many aspects for drug development and research, but there is the disadvantage of its insolubility to limit its applications[1, 2]. Presently a number of water soluble cellulose derivatives are available in the market and that can be used in different pharmaceutical applications[3, 4]. HPC is one of the pharmaceutically valuable cellulose ether due to its suitable properties like safety, pH insensitivity and can be soluble both in water and polar organic solvents[5-8].HPC is routinely used as an excipient in a variety of pharmaceutical dosage forms as a binder, film for coating and thickening agent. Its applications in mucoadhesive delivery systems have also been reported for several different drugs[9-12].

HPC is cheap and biocompatible pharmaceutical excipient with three hydroxyl groups per anhydroglucose unit that can be used in organic synthesis reactions and which makes it a valuable tool for the fabrication of macromolecular prodrugs[13, 14].The bioactive compounds can be coupled to the polymeric backbone by the participation of hydroxyl and carboxyl groups of the polymers and compounds, respectively [15]. Carbohydrate polymers such as HPC can be modified into a useful reactive carrier for the attachment of the therapeutic agents and to introduce a spacer arm between the polymer and the therapeutic agents[16]. It was reported that therapeutic agents can be attached directly to the polymer chain are stable and could not split off easily due to the steric hindrance effect since enzyme could not reach and break down the covalent bond between drug and polymer[17]. PABA is an aromatic naturally occurring non-protein amino acid. It is safe and its absorption rate from the gastrointestinal tract is very fast and completely metabolized by the liver[18]. Structurally, PABA consists of a benzene ring substituted with an amino and a carboxyl group. Through substitution of its amino and carboxyl group, PABA has also been functioned as a structural moiety for many drugs with a wide range of therapeutic applications. PABA was used as a spacer in

the synthesis scheme of dendrimer-5-aminosalicylic acid (5-ASA)azo conjugates and polyethylene glycol-5-ASA prodrugs and it could facilitate 5-ASA release from the polymeric conjugates[19, 20].

The goal of the present study is to synthesize and characterize HPC-PABA ester conjugate in a two simple and homogeneous steps. During the first step, HPC was chloroacetylated with chloroacetyl chloride, while in the second step the chloroacetate groups were reacted with sodium-*p*-aminobenzoate to give the desired HPC-PABA ester conjugate. This strategy will contribute to the development of a novel polyaromatic polyamine macromolecular carrier containing a pendant primary amine functionality which can be used for different biomedical and pharmaceutical applications.

EXPERIMENTAL SECTION

Materials and instruments

HPC powder (Mw~100,000) was purchased from Sigma Aldrich (USA) and dried at 50°C under vacuum for 3 days before use. PABA and chloroacetyl chloride were analytical grade and obtained 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. ^{1}H-NMR spectra were recorded by the Varian Nuclear Magnetic Resonance Spectrometer (500 MHz). ^{1}H NMR spectra of the esters (10 mg sample/ml) were measured in deuterated dimethylsulfoxide (DMSO-d_6) at 60 °C.

Esterification of hydroxypropyl cellulose with chloroacetyl chloride

In the first step of the synthesis, 10.5 g of HPC(93.8 mmol eq. to-OH groups) was dissolved in 120 ml of dried dichloromethane in a two-necked round bottom flask with constant mechanical stirring at room temperature. Pyridine (7.5 ml, 93.8 mmol) was added to the flask as an acid acceptor. Dried dichloromethane solution (10 ml) containing 7.5 ml of chloroacetyl chloride (93.8 mmol) was then added drop wise at 0-5 °C with stirring. The reaction mixture was kept stirring at 25 °C for overnight under nitrogen atmosphere. The solution that was obtained after the required time gave clear and viscous with light yellow color. The solution was evaporated for removing dichloromethane and re-dissolved in DMSO in order to transfer into a dialysis bag(MWCO = 12,000-14,000 Da). The polymer product was purified by dialysis against distilled water for at least 3 days. The purified chloroacetylated HPC ester conjugate was finally obtained by lyophilization. The product was obtained in 78 % yield with 0.52 degree of substitution (DS).

Reaction of chloroacetylated HPC with sodium-*p*-aminobenzoate

In the second step of synthesis, the resulting chloroacetylated HPC (12.0 g, 31.58 mmoleq. to $ClCH_2CO$- groups) was dissolved in 120 ml DMSO at room temperature and then 6.0 g sodium salt of PABA (37.9 mmol) was added slowly while stirring. The reaction was performed at 30 °C and under intense stirring for about 8 h. The final product was purified by dialysis against distilled water for at least 2 days. The purified HPC-PABA adduct was obtained by lyophilization in 88 %yield, based on with 2.53 degree of substitution (DS).

Calculation process for the degree of substitution

The degree of substitution (DS) of chloroacetylated-HPC was calculated form the spectrum of ^{1}H-NMRby using the integration ratio of the peaks arising due to the methyl protons of hydroxypropyl pendants and protons of $ClCH_2CO$-group that incorporated into HPC through esterification [3]. On the other hand, the DS of HPC-PABA adduct was also calculated form the spectrum of ^{1}H-NMR by using the integration ratio of the peaks appearing due to protons of anhydroglucose unit of HPC and peaks appearing due to protons of aromatic moiety of PABA[21].

RESULTS AND DISCUSSION

Cellulose derivatives containing NH_2-functionalized aromatic groups has been of interest to great expectations concerning their suitability as support matrices for biocompounds. However, their synthesis pathways are frequently very complex and usually gave low product yield. In this study, we have tried to synthesis *p*-aminobenzoylhydroxypropyl cellulose by the reaction of HPC with *p*-nitrobenzoyl chloride using pyridine as a catalyst, the nitro group was then subjected to reduce to an amino group by using sodium dithionite as a non-toxic reducing agent[22].The original HPC was soluble in water but after the incorporation of aromatic nitro groups to the polymer chain, it could not preserve its water solubility property. *p*-Nitrobenzoylhydroxypropyl cellulose has solubility only in organic solvents like dimethylsulfoxide (DMSO), dimethylformamide (DMF), dimetylacetamide (DMA), tetrahydrofuran (THF) and etc., but on the other hand, sodium dithionite has not solubility in these organic solvents. Many efforts were performed to achieve complete conversion but could not find the satisfactory results, which might be caused by reduction in heterogeneous phase the reducing agent may not be in an active form.

For acquiring the primary amino functionality on HPC, we approached in another way that was esterification reaction between PABA and HPC. In this method, the primary amino group of PABA was first protected by 98% formic acid according to the method in the literature[23]. The protection of primary amino group was helpful to avoid from self-coupling of PABA during the reaction due to its bi-functional groups nature. 4-*N*-Formylaminobenzoic acid (4-f-PABA) was successfully esterified with HPC using *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC.HCl) as a condensating agent and 4-(dimethylamino)pyridine (DMAP) as a base[2]. Finally, the protecting group was tried to remove by stirring the product in 0.5 mol/lHCl according to the prescribed method[23]. However, HPC-PABA with free amino group was not successfully achieved by hydrolysis of HPC-4-f-PABA. The result demonstrated that the ester bond between HPC and protected PABA was broken, that may be due to the strong acid strength and high temperature assisted the hydrolysis of ester bond.

Finally, a novel and the simplest synthesis route of HPC-PABA ester conjugate was adopted for achieving the macromolecular carrier with primary aromatic amino functionality. HPC was first modified with chloroacetyl chloride to provide chloroacetate groups (Fig.1) using similar process to the previously report with some modification [24].

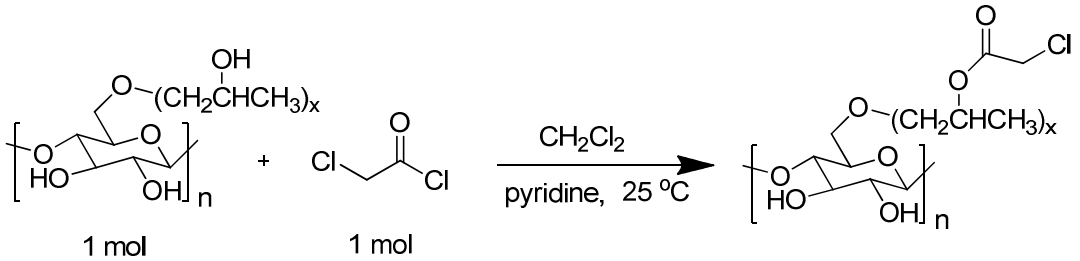

Figure 1 Reaction scheme for the synthesis of chloroacetylated HPC ester conjugate

For achieving the proper distribution of chloroacetate functionality in HPC chain,the esterification was performed in dried dichloromethane. The product, chloroacetylated HPC was easily obtained in high purity by dialysis against distilled water, since the by-product of the reaction, pyridinium chloride was soluble in water.

The coupling of bioactive carboxylic acid of PABA to HPC functionalized with chloroacetate groups was carried out by using the sodium-*p*-amino benzoate in DMSO (Fig.2). The sodium salt of PABA was easily prepared by reacting equivalent amounts of the acid with sodium hydroxide. The solution was left standing overnight to evaporate slowly. It was a novel methodology for the synthesis of macromolecular carrier and interestingly there was no need of any coupling agent and base for completing this type of esterification reaction. Highly pure HPC-PABA ester conjugate was fabricated by this elegant method because the impurity (NaCl) was soluble in distilled water, hence removed simply by dialysis. In this strategy there were no hard challenges like complete reduction of nitro group or complete deprotection of protected amino group that were faced in the beginning of this research work. Regarding solubility, HPC-PABA ester conjugate was soluble in many organic solvents like DMSO, DMA, DMF, THF, MeOH and EtOH, but it was not soluble in water.

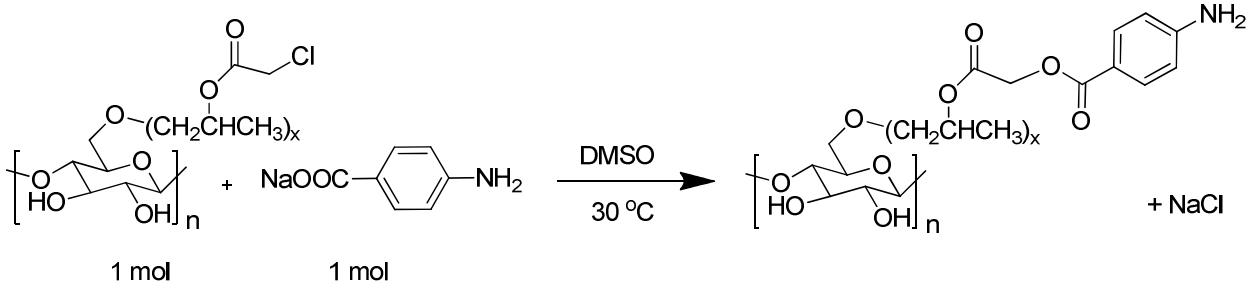

Figure 2 Synthesis scheme for the preparation of HPC-PABA ester conjugate

Figures 3a-c show the FT-IR spectra of unmodified HPC (Fig. 3a), partially modified HPC with chloroacetate groups (Fig. 3b), and HPC-PABA ester conjugate (Fig. 3c). Unmodified HPC (Fig. 3a) shows broad transmission bands at 3600-3100cm^{-1}of hydroxyl stretching vibration. This band is obviously reduced after modification in the spectrum of the obtained chloroacetylated HPC (Fig. 3b). Moreover, FT-IR of chloroacetylated HPC displays new characteristic bands of the attached ester groups appeared at 1754 cm^{-1}belongs to the stretching vibration of carbonyl of the ester, at 1754 cm^{-1}belongs to –C-O stretching vibration, and at 781 cm^{-1}of -CH_2Cl. In addition, the FT-IR spectrum of HPC-PABA ester conjugate (Fig. 3c) demonstrated the absorption bands of –C=O stretching vibration at 1757 cm^{-1} (HPC-O-**C=O**) and a new band at 1711cm^{-1} of -CH_2-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 3357 and 3244cm^{-1}, C–N stretching at 1281cm^{-1}, N–H bend at 1619.cm^{-1} and -C=C, and C-H in the benzene ring at1605 and 771 cm^{-1} were observed. The FT-IR results indicated that the desired product was successfully obtained by this procedure.

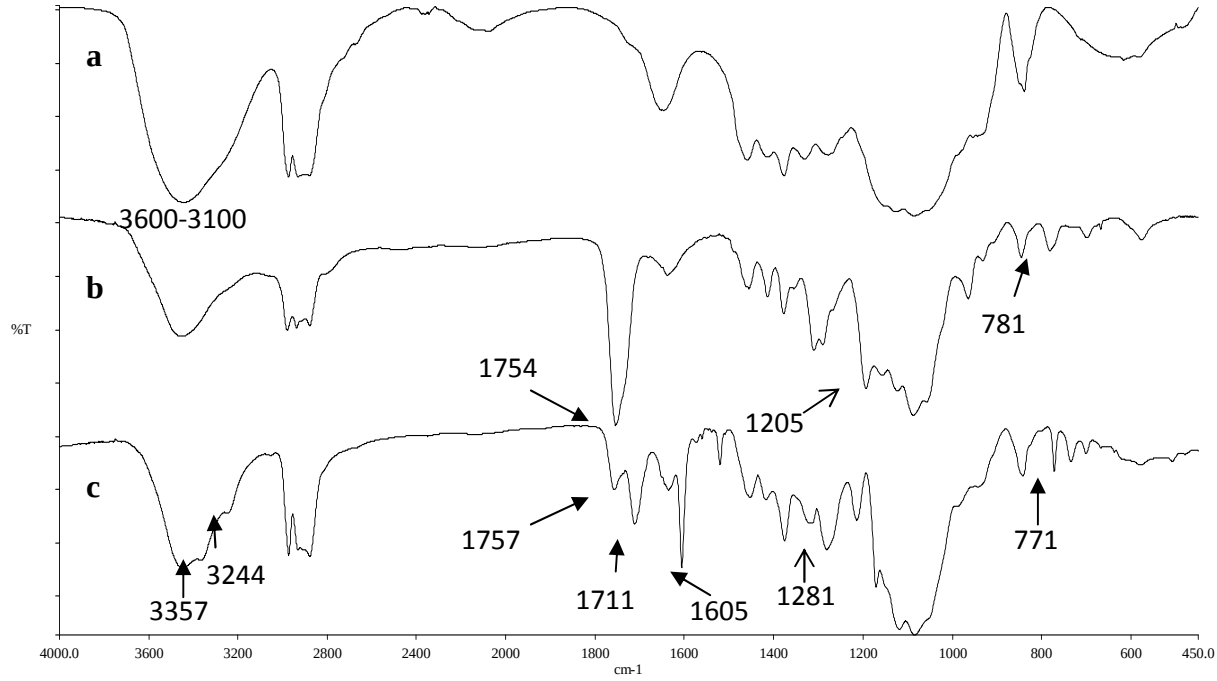

Figure 3FTIR spectra of: a) HPC, b) chloroacetylated HPC (DS 0.52), c) HPC-PABA ester conjugate (DS = 2.53)

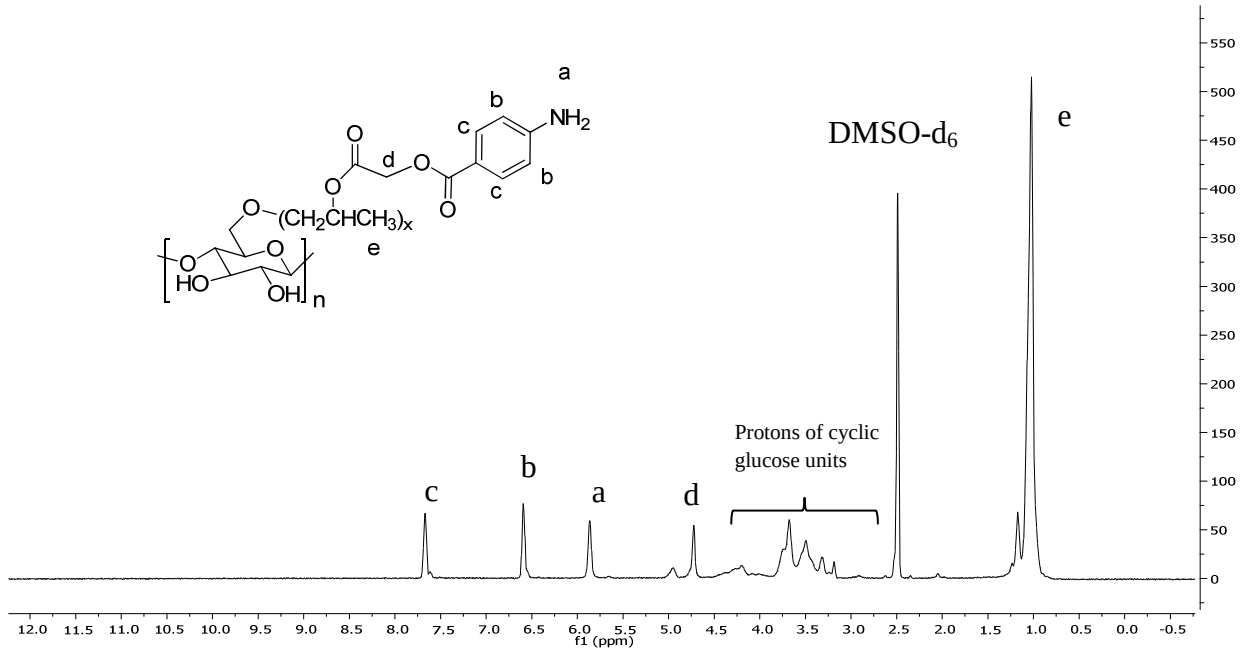

Figure 4[1]H NMR spectra of HPC-PABA ester conjugate (500 MHz, in DMSO-d_6)

The structure of modified HPC was confirmed by[1]H NMR spectra with a 500 MHz Unity Inova, Varian Nuclear Magnetic Resonance Spectrometer in DMSO-$d_6$and displayed in Fig.4. The peak at 2.49 ppm is due to DMSO-d_6. The spectrum of HPC-PABA ester conjugate shows broad peaks at 1.0 -1.25 ppm from the methyl groups in the hydroxypropyl moieties**.** Peaks in a range of 3.19 – 4.2 ppm belong to protons of cyclic glucose units. The characteristics resonance peaks at 7.67 and 6.59 ppm are ascribed to the protons of aromatic moiety of PABA. The peak at 5.86 ppm belongs to protons of the primary amino group of PABA and a characteristic peak of protons of –CO-**C**$\mathbf{H_2}$-O- appeared at 4.72 ppm. A tiny peak detected at 4.95 ppm belongs to the resonance of the methine protons of modified HPC. The DS of HPC-PABA ester conjugate can be easily calculated form the spectrum of[1]H-NMR by using the integration ratio of the peaks appearing due to protons of the cyclic glucose units of HPC and

peaks appearing due to protons of aromatic moiety of PABA and the result demonstrated that the product was obtained with 2.53 degree of substitution (DS).

Both FT-IR and ^{1}H-NMR characterization results confirmed that HPC-PABA ester conjugate was synthesized successfully and could be obtained in purified form.

CONCLUSION

Fabrication of a novel macromolecular carrier that is HPC-PABA ester conjugate was completed in a two easy steps that were performed homogeneously. Organosoluble macromolecular conjugate with primary aromatic amine functionality were synthesized and characterized effectively with excellent percentage yield and purity. HPC-PABA ester conjugate can be a useful carrier for different biomedical and pharmaceutical applications.

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