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Examination of oxidized cellulose as a macromolecular prodrug carrier: preparation and characterization of an oxidized cellulose-phenylpropanolamine conjugate

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Abstract

The purpose of this study was to investigate the use of 6-carboxycellulose (OC), a biocompatible and bioresorbable polymer, as a prodrug carrier for amine drugs. Phenylpropanolamine hydrochloride (PPA . HCl) was used as a model drug. OC and PPA were reacted in dimethylformamide (DMF) in the presence of 1,3-dicyclohexylcarbodiimide (DCC) for 2.5 days at room temperature. Filtration, followed by washing with methanol, and subsequent drying under vacuum, produced the conjugate in 65–78% yield. The amount of PPA in the product, determined from the difference in the carboxylic content before and after the reaction, was 24.2% (w/w), corresponding to a degree of substitution (DS) value of 0.7. The Fourier transform-infra red (FT-IR) spectrum of the conjugate, compared with that of OC and PPA . HCl, showed a new band at about 1533 cm^{-1} attributable to a >C=O (amide II) stretching and N–H (amide I and amide II) bending vibrations, a decrease in intensity of the characteristic free carboxylic acid carbonyl stretching band at about 1748 cm^{-1}, and a strong band at 1663 cm^{-1} due to >C=O (amide I) stretching vibration, suggesting that the OC is linked to PPA via an amide bond. The solid-state carbon-13 cross polarization/magic angle spinning nuclear magnetic resonance (^{13}CCP/MAS NMR) spectrum of the conjugate was also consistent with this structure. The release studies performed in pH 4.5, 5.5, and 7.4 buffer solutions and in rat liver homogenate (pH 7.4), showed the conjugate to be more susceptible to hydrolysis at a lower pH and in the presence of rat liver homogenate. In conclusion, the results presented show that OC can be covalently linked to amine drugs via an amide bond in DMF using DCC as a coupling agent, and provide a macromolecular prodrug delivery system.

Keywords: Oxidized cellulose; 6-Carboxycellulose; Oxidized cellulose conjugate; Macromolecular prodrug; Polymer conjugate; Phenylpropanolamine; Carbodiimide

1. Introduction

Macromolecular prodrugs have been extensively investigated to improve the therapeutic effects and/or to reduce the toxic effects of drugs.

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They achieve this by altering drug pharmacokinetics and/or improving drug specificity to the active site(s). The principles and potential applications of macromolecular prodrugs in drug targeting, in general, and cancer chemotherapy, in particular, have been recently reviewed (Sezaki and Hashida, 1984; Duncan, 1992; Putnam and Kopecek, 1995; Kumar and Banker, 1996; Hoffman, 1998).

To be used as a macromolecular prodrug carrier, the polymer must be biodegradable and biocompatible and must contain appropriate functional sites for chemical conjugation. Further, the polymer and its degradation product(s) must be non-immunogenic and non-toxic and be effectively metabolized/excreted from the body. A variety of natural and synthetic biodegradable polymers that meet these criteria have been extensively investigated as macromolecular prodrug carriers, including albumin, chitins, dextrans, poly(ethylene glycol), poly(galactouronic acid), poly(*N*-(2-hydroxypropylmethacrylamide)) (HPMA) copolymers, poly(*N*-(2-hydroxypropylmethacrylamide-vinylpyrrolidone)) copolymers, poly(L-aspartic acid), poly(hydroxyethyl-L-glutamine), poly-L-lysine, poly(a-L-glutamic acid) (PGA), polyethylene glycols, poly(divinyl ether-co-maleic anhydride) copolymers (DIVEMA), and poly(styrene-co-maleic anhydride) copolymers (SMA, Sezaki and Hashida, 1984; Duncan, 1992; Putnam and Kopecek, 1995; Kumar and Banker, 1996). Several of these systems have reached clinical trials and show considerable promise. However, efforts are continuing to search for new macromolecular prodrug carrier systems.

Oxidized celluloses (OC) containing carboxylic groups (Fig. 1) represent an important class of biocompatible and bioabsorbable polymers (Ashton, 1968; Stillwell et al., 1997). Currently, OC containing 16–24% carboxylic content is commercially available in a sterilized knitted fabric or powder form for use in humans to stop bleeding during surgery and to prevent the formation of adhesions following surgery (Stillwell et al., 1997). These materials have been widely used in medicine for several decades. Studies show that OC with as low as 3% carboxylic content is bioabsorbable (Ashton, 1968). Recently, OC has also been found to possess antibacterial (Abaev et al., 1986), antitumor (Tokunaga and Naruse, 1998), immunostimulant (Otterlei et al., 1992), and wound healing (Finn et al., 1992; Pollack and Bouwsma, 1992) properties. Owing to the presence of carboxylic groups, OC has also been extensively investigated as an immobilizing matrix for a variety of amine drugs (Yasnitskii and Dol'berg, 1973; Dol'berg et al., 1973, 1974; Balakleevskii et al., 1986; Firsov et al., 1987; Sosnin et al., 1993; Kosterova et al., 1993; Kaputskii et al., 1995; Zimatkina et al., 1996); for enzymes (Alinovskaya et al., 1988, 1989); and for bovine serum albumin (Kumar and Deshpande, 2001).

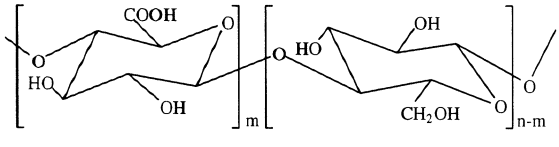


Fig. 1. Oxidized cellulose (OC).

In this paper, an investigation of the use of OC as a macromolecular prodrug carrier for amine drugs is presented. Phenylpropanolamine hydrochloride (PPA . HCl) was used as a model drug. Specifically, the reaction of OC with PPA . HCl in dimethylformamide (DMF) in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and in water containing 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC . HCl) was studied. The goals of the study were to establish the method of preparing the conjugate, to identify the linkage between OC and PPA, and to evaluate the PPA release characteristics of the conjugate in vitro. Carbodiimides have been extensively used in organic synthesis and biotechnology (Williams and Ibrahim, 1981; Wong, 1991), including peptide synthesis (Wang et al., 1987) and modifications of polysaccharides (Danishefsky and Siskovic, 1971) and proteins (Hoare and Koshland, 1967; Wilchek et al., 1967; Yamda et al., 1981; Mauk and Mauk, 1989). However, except for a recent study (Taylor, 1991) that describes the reaction of polyuronic acids with water-soluble carbodiimides, leading to the formation of lactones or intramolecular esters, no report was found in the literature on the use of

carbodiimides in the preparation of OC conjugates with amine drugs.

2. Experimental

2.1. Materials

OC containing 3.5 mmole g^{-1} (or 15.7%, w/w) of carboxylic content was obtained from Eastman Chemical Co. (Kingsport, TN). The molecular weight of the OC as determined by the viscosity method was 6385. PPA . HCl (Lot No. 06011BS), DCC (Lot No. 12113CR), sodium 1-heptanesulfonate (high performance liquid chromatography (HPLC) grade, Lot No. 984631), and triethylamine (HPLC grade, Lot No. 963375) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC . HCl; Lot No. 55H0800) was received from Sigma Chemical Co. (St. Louis, MO). Dimethylformamide and sodium phosphate monobasic (Lot No. HB253) were obtained from Fisher Scientific (Fair Lawn, NJ) and Spectrum Chemical Mfg. Corp. (Gardena, CA), respectively.

2.2. Methods

2.2.1. Analysis of PPA

The analysis of PPA was performed by HPLC. A fully automated Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan), consisting of a LC-6A pump, a SCL-6A system controller, a SIL-6A autoinjector, a SPD-6AV ultraviolet–visible detector, and a C-R5A data processor/recorder, was used. The separation was carried out on a Zorbax Rx-C8 reversed-phase column (5 μm; 4.6×250 mm^2, Mac-Mod Analytical, Inc., Chadds Ford, PA) with an Alltech Hypersil BDS C8 guard column (5 μm, Lot No. 5/120/4682, Alltech Associate, Inc., Deerfield, IL). The mobile phase was composed of a 6.5:10 (v/v) ratio of methanol and an aqueous solution that contained sodium 1-heptanesulfonate (4.04 g/2000 ml), sodium phosphate monobasic (5.56 g/2000 ml) and triethyleneamine phosphate (40 ml of 0.25 M) in deionized water. It was filtered through a 0.45 μm nylon filter membrane (Chrom. Tech. Inc., Apple Valley, MN) and degassed for 10 min before use in a Bransonic Ultrasonic Cleaner (Model 5200, Bransonic Ultrasonics Corporation, Danbury, CT). The flow rate was maintained at 1 ml min^{-1}, and the effluent was detected at 254 nm. Three injections of 20 μl each were made for each sample. The amount of PPA present in the test solution was calculated using the standard curve constructed before each assay.

2.2.2. Preparation of oxidized cellulose–phenylpropanolamine (OC–PPA) conjugate

Appropriate amounts of OC (equivalent to 0.08–0.09 mole l^{-1} of COOH), DCC (0.34–0.37 mole l^{-1}), and PPA (0.27–0.72 mole l^{-1}) were placed in DMF and stirred at room temperature. The concentration of free PPA in the reaction mixture was periodically monitored by HPLC. The reaction supernatant (250 μl) were withdrawn, appropriately diluted with the HPLC mobile phase, and then analyzed. The reaction was stopped when the amount of PPA in the reaction medium no longer declined. The reaction mixture was then filtered, and the white to off-white residue obtained was thoroughly washed with methanol and dried at 50°C in a vacuum desiccator.

For reactions in water, the same procedure as described above was used except that DCC was replaced with an equivalent amount of EDAC . HCl.

2.2.3. Determination of PPA content in the conjugate

The determination of the PPA content in the conjugate was determined from the difference in the free carboxylic groups present in the conjugate and in the OC sample that had been treated with the carbodiimide alone under identical conditions used for the reaction with PPA . HCl. All samples were dried overnight in a vacuum desiccator at 50°C before use. The quantitation of free carboxyl content in the sample was determined by the USP method (USP, 1995). Briefly, about 0.5 g of the sample was accurately weighed and then sus-

pended in 50 ml of 2% (w/w) calcium acetate solution. The mixture was allowed to stand at room temperature for 30 min. After adding two drops of phenolphthalein indicator, the mixture was titrated with a standardized 0.1 N NaOH solution until a pink color was obtained. A blank titration using 50 ml of 2% w/w calcium acetate solution was conducted in the same manner. The amount of PPA in the conjugate was calculated using the equation:

$$\text{PPA content (\%, w/w)} = \frac{(C-S)151.2/1000}{\text{Sample weight (g)}}\,100$$

where C was millimoles of free carboxylic groups per gram of the carbodiimide-treated OC sample, S the millimoles of free carboxyl group per gram of OC–PPA conjugate, and 151.2 is the molecular weight of phenylpropanolamine free base.

2.2.4. Scanning Electron Microscopy (SEM)

Samples were loaded on a stub covered with liquid graphite and then coated with gold, followed by a layer of carbon, for 4 min in an Emitech K550 coater. SEM photographs were then taken on a Hitachi S-4000 SEM using Polaroid film.

2.2.5. Solid-state carbon-13 cross-polarization/magic angle spinning nuclear magnetic resonance (^{13}C-CP/MAS NMR) spectroscopy

Each sample was carefully packed into a NMR rotor. The spectrum was obtained on a Bruker MSL-300 spectrometer set up with a CP/MAS probe using the true 90° pulse calibration time of 6 μs, a proton transmitter dead time of 2 μs, and a contact time for polarization transfer with Hartmann-Hahn match of 3 μs. The spin rate, relaxation time and acquisition time used were 5000 rpm, 3 and 0.053 s, respectively. In order to identify spin side bands, each sample was run at a spin rate of 4500 rpm. The spectra were obtained using Bruker's Nuts data processing software.

2.2.6. Fourier-transform infrared (FT-IR) spectroscopy

The FT-IR spectra of samples were obtained as KBr pellets on a Nicolet 5DXB FT-IR spectrophotometer, equipped with Omnic data processing software.

2.2.7. In vitro PPA release studies in buffer solutions

The release media used in this study were isotonic pH 5.5 and 7.4 phosphate buffer solutions and an isotonic pH 4.0 acetate buffer solution. About 30 mg of OC–PPA conjugate powder (45–106 μm) was accurately weighed and transferred to a screw-top plastic centrifuge tube. To each tube, 10 ml of the release medium was added using a 5 ml Oxford pipetter. The tube was shaken in an incubator-shaker (Lab-line Instruments Inc., Melrose Park, IL), maintained at 37°C, at an agitation rate of 100 cpm. At predetermined time intervals, the sample tube was removed from the incubator shaker and centrifuged for 10 min at 7000 rpm using an ICE HN-S Centrifuge (Damon Corp., Needham Heights, MA). The supernatant (0.5 ml) was withdrawn and a fresh buffer solution was immediately added to the release medium to replace the removed sample. The withdrawn sample was diluted with 0.01 N HCl to 10 ml and then analyzed by HPLC. The amount of PPA in the sample was calculated using the standard curve constructed prior to each assay.

2.2.8. In vitro PPA release studies in rat liver homogenate

Fresh rat livers were obtained and immediately stored in 0.25 M sucrose solution. They were minced with scissors and then homogenized in pH 7.4 phosphate buffer using an Kika-Werk Ultra-Turrax Model SDT homogenizer (Tekmar Company, Cincinnati, OH). The liver homogenate, which contained 20% (w/w) liver, was then centrifuged at 1700 rpm for 10 min ($\sim$ 6500 g min at an average radial distance of 20 cm). After centrifugation, the homogenate (liquid portion) was removed and stored immediately at -70°C in a freezer. The residue that settled at the bottom of the centrifuge tube was discarded.

About 20 mg of OC–PPA conjugate powder (particle size 45–108 μm) was weighed accurately and transferred into a 10 ml glass test tube. To each tube, 1 ml of freshly thawed rat liver ho-

Table 1
The effect of the mole ratio of OC:PPA . HCl:DCC on the PPA content (%, w/w) of the OC–PPA conjugate

Mole ratio of OC:PPA . HCl:DCC	PPA content (%, w/w, S.D.)[a]
1:3:4	0
1:6:4	24.0 (1.1)
1:7:4	24.2 (1.2)
1:8:4	25.5 (1.0)

[a] $n = 3$.

mogenate was added and then incubated in a controlled environment incubator shaker at 37°C and agitated at 100 cpm. At each sampling time, the tubes were removed and centrifuged at 10 000 rpm for 15 min using an ICE HN-S centrifuge. A 0.5 ml of aliquot was withdrawn, mixed with an equal volume of 1 N HCl, and then centrifuged again. The supernatant was appropriately diluted with methanol and filtered by a syringe filter unit (Nylon 0.45 μm). The filtrate was collected and analyzed by HPLC. An equal volume of fresh liver homogenate was added to the remaining sample in the tube at each time point and the experiment was continued.

3. Results and discussion

3.1. Preparation and characterization of OC–PPA conjugate

The carbodiimide-mediated coupling reactions between carboxylic acids and amines or alcohols can be carried out in aqueous as well as organic media. Frequently, carbodiimides are used in excess to ensure completion of the reaction. The OC used in this study contained 20% w/w (i.e. 3.5 mmole g^{-1}) carboxylic content. It was practically insoluble in water but showed partial solubility in DMF. Thus, the preparation of the conjugate was investigated in DMF. Table 1 lists the different mole ratios of OC-COOH:PPA . HCl:DCC used to prepare the conjugate in DMF, at room temperature, for a period of 60 h and the amount of PPA determined in the conjugate by the USP titration method. These results show that a minimum of about a 1:6:4 mole ratio of OC-COOH:PPA . HCl:DCC is required to produce the conjugate. The PPA content determined in the product was 24% (w/w) (or 1.6 mmole g^{-1}), corresponding to a degree of substitution (DS) of 0.7.

The Fourier transform-infrared (FT-IR) spectra of OC, the OC–PPA conjugate, and PPA . HCl are compared in Fig. 2. The spectrum of OC shows the characteristic carbonyl peak due to free carboxylic group at about 1733 cm^{-1}. The peaks at about 1641 and 2905 cm^{-1} in the spectrum are due to water molecules adsorbed onto OC and C–H stretching vibration belonging to the anhydroglucose unit, respectively (Klemm et al., 1998). In the spectrum of the OC–PPA conjugate, the >C=O (free COOH) stretching vibration band appears at 1748 cm^{-1} and shows significantly reduced intensity compared with that for OC. The peak at about 1533 cm^{-1} in the spectrum is due to >C=O (amide II) stretching and N–H (amide I and amide II) bending vibrations (Williams and Fleming, 1966; Pavia et al., 1979), whereas that at 1663 cm^{-1} is attributed to >C=O (amide I) stretching vibration (Williams and Fleming, 1966). The peaks at 2860 and 2933 cm^{-1} are due to C–H (aliphatic) stretching vibrations belonging to PPA and OC, whereas two very small peaks appearing at about 3040 and 3094 cm^{-1} are assigned to C–H (aromatic) stretching vibration due to the phenyl group of PPA.

The solid-state ^{13}C-CP/MAS NMR spectra of OC and the OC–PPA conjugate, along with the solution ^{13}C-NMR spectrum of PPA . HCl, are shown in Fig. 3. The peaks in the spectrum of OC were assigned based on recent solution and solid-state ^{13}C-NMR studies reported in the literature (Andersson et al., 1990; Heinze, 1990; Heinze et al., 1993; Kumar and Yang, 1999), whereas in the case of PPA . HCl the peak assignments were made using estimates of substituent effects. A comparison of the spectra of OC, the OC–PPA conjugate, and PPA clearly shows that the peaks at about 153, 142, 128, and 50, and 17 ppm in the spectrum of the OC–PPA conjugate are due to carbons associated with PPA linked to OC via an amide linkage. Recently, Rahn et al. (1995) also observed the amide carbonyl carbon at 155 ppm

in the ^{13}C-NMR spectrum of carboxycellulose benzylamide prepared by reacting OC first with $SOCl_2$ and then with benzylamine. The FT-IR spectrum of this product exhibited C=O (amide I) stretching and N–H (amide II) bending vibrations at 1656 and 1528 cm^{-1}, respectively. As noted above, the corresponding peaks in the OC–PPA conjugate appeared at 1663 and 1533 cm^{-1}.

The preparation of the OC–PPA conjugate in water using EDAC . HCl, a water-soluble carbodiimide, and different ratios of OC:EDAC . HCl:PPA . HCl, was also investigated. The FT-IR spectrum and the ^{13}C-CP/MAS NMR spectra of the product prepared using a 1:4:6 molar ratio of OC, EDAC . HCl, and PPA . HCl at room temperature for 60 h are shown in Fig. 4. As can be seen, the band at 1538 cm^{-1} due to >C=O (amide II) stretching vibra-

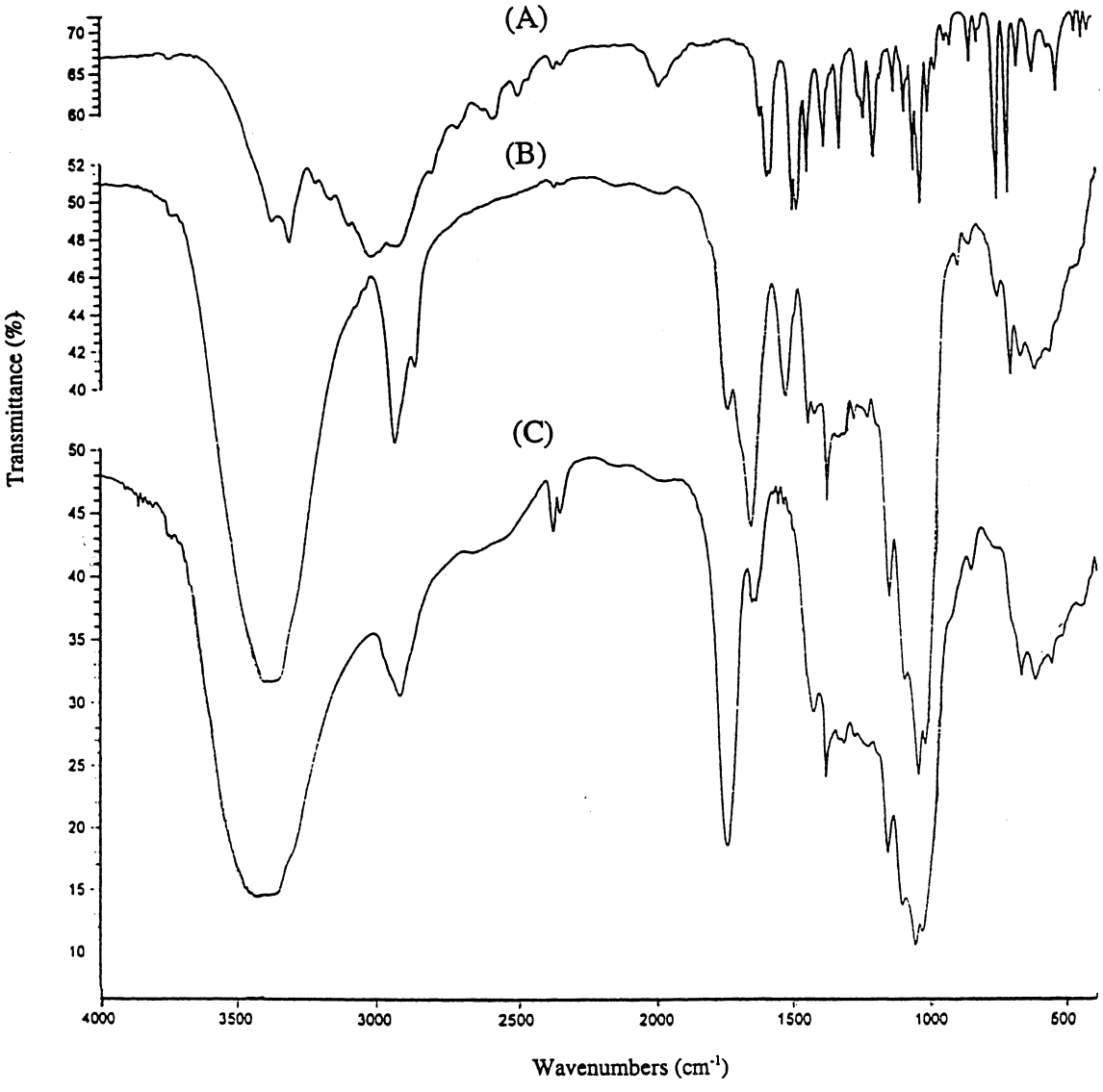


Fig. 2. FT-IR spectra of (A) PPA . HCl (B) the OC–PPA conjugate, and (C) OC.

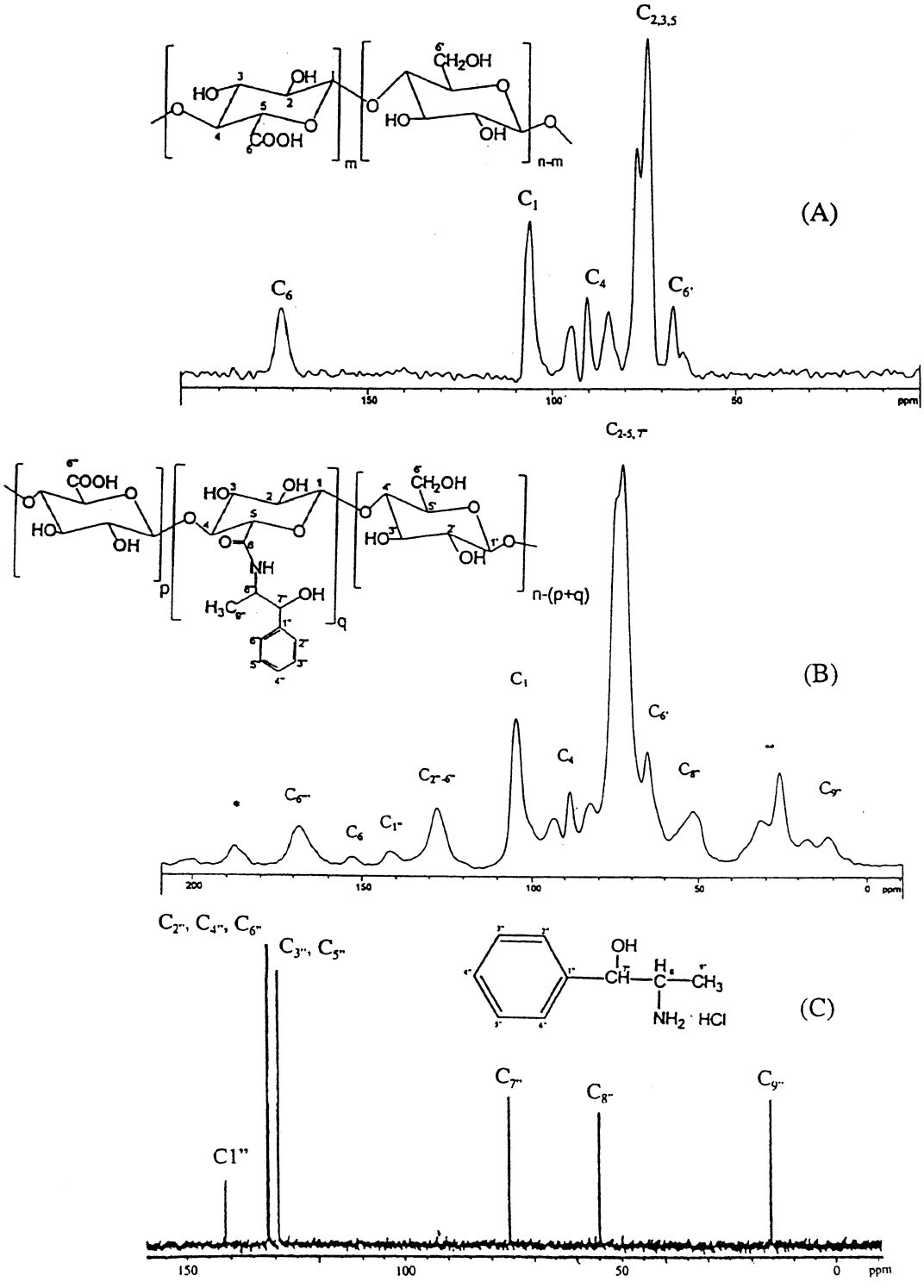


Fig. 3. ^{13}C-NMR spectra of (A) OC and (B) the OC–PPA conjugate, and (C) PPA . HCl, *, spin side band; **, OC–DCC.

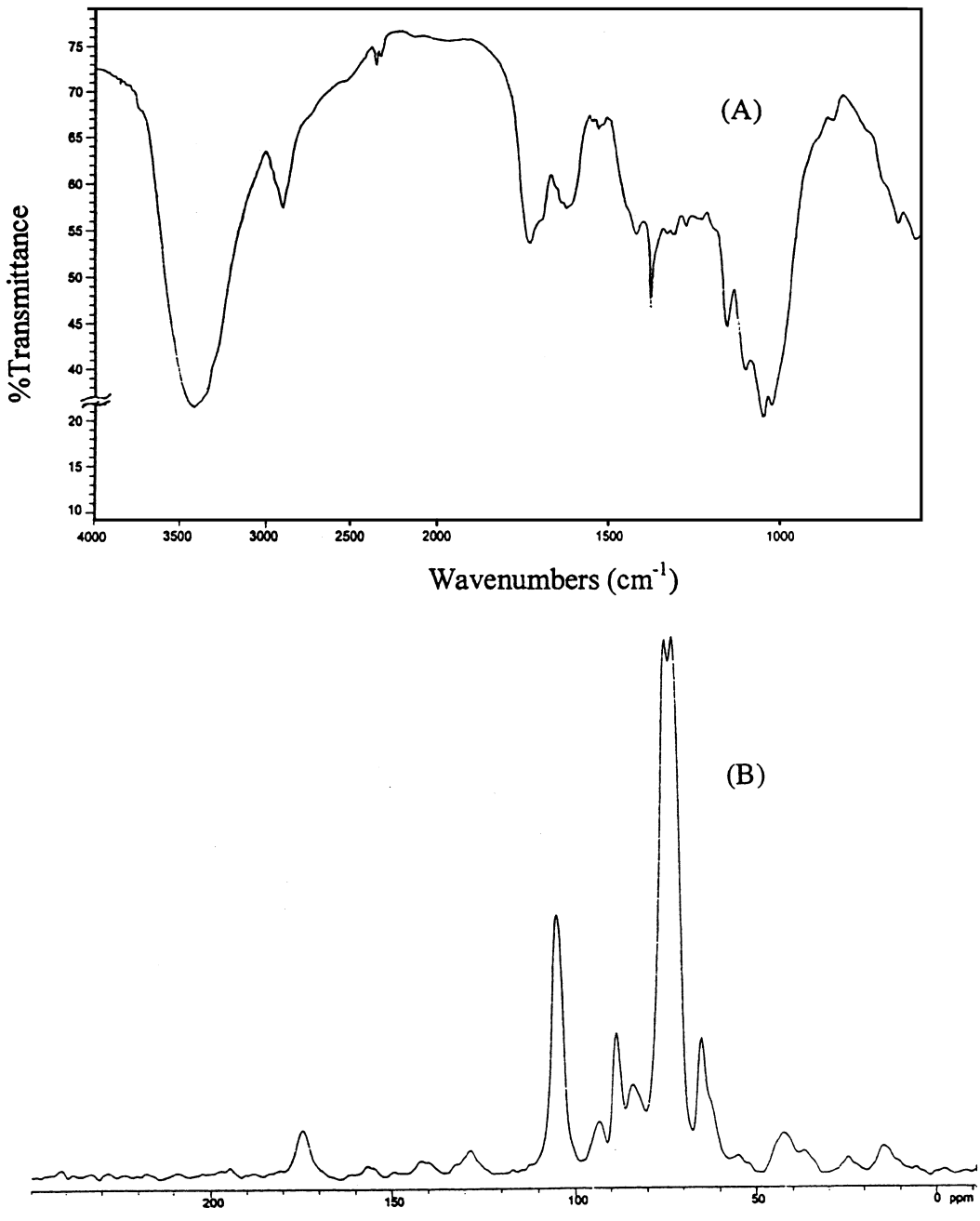


Fig. 4. (A) FT-IR and (B) solid-state ^{13}C-CP/MAS NMR spectra of the product prepared in the EDAC . HCl/water reaction system.

tion in the FT-IR spectrum, (Fig. 4A), is barely visible, while the signals due to amide (C-6) and aromatic (C1″–C6″) carbons in the ^{13}C-NMR spectrum, (Fig. 4B), are much weaker in intensity, compared with the corresponding peaks in the respective spectrum of the OC–PPA conjugate

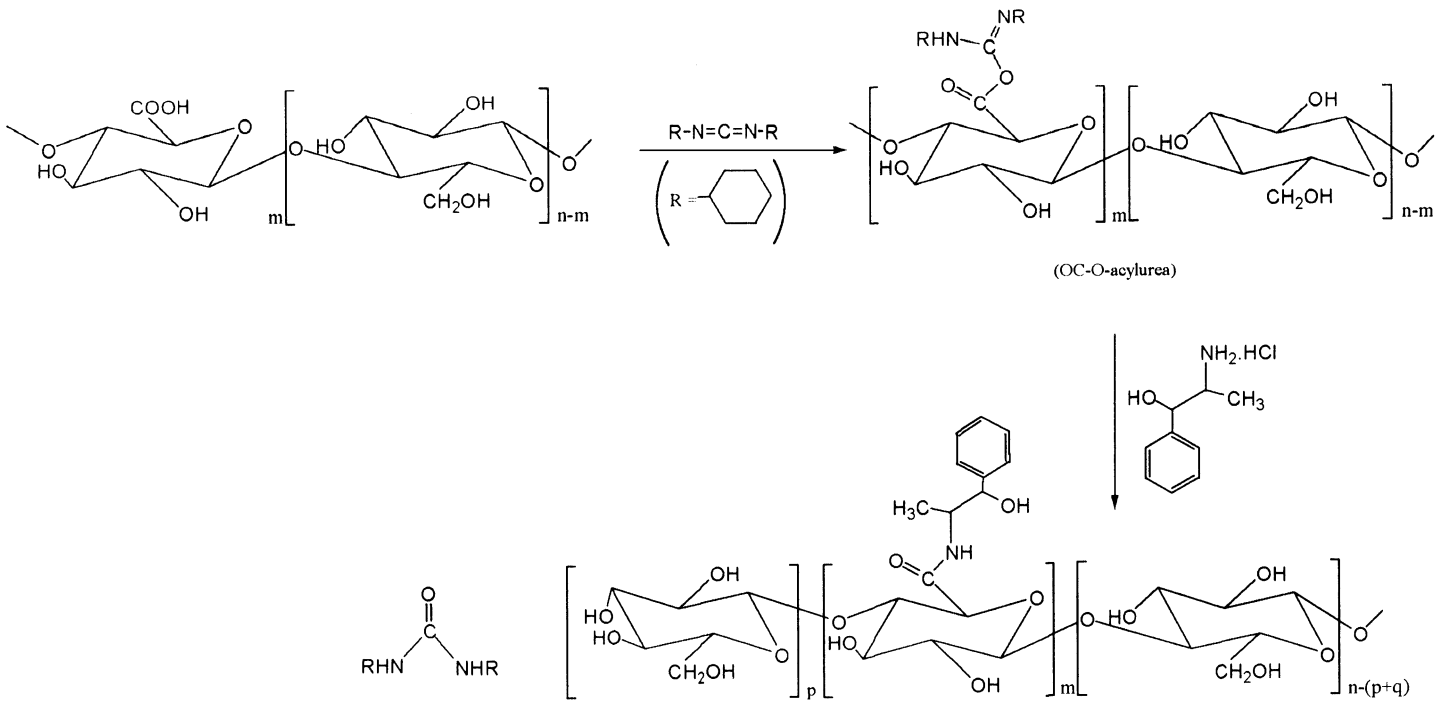


Fig. 5. The reaction mechanism of the formation of OC–PPA conjugate in DCC/DMF.

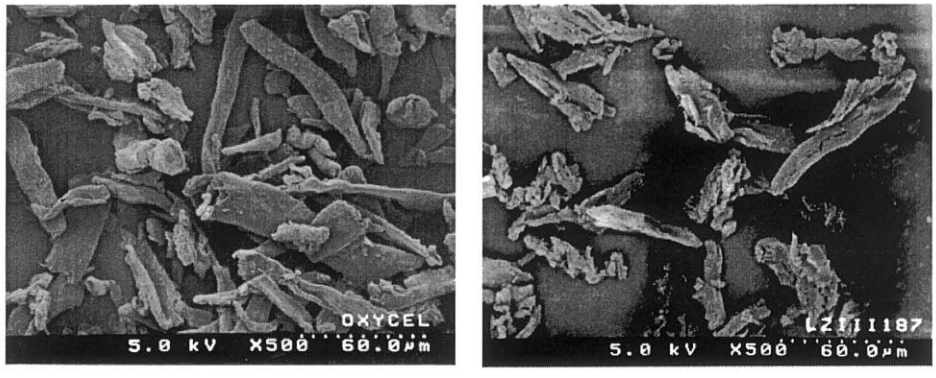
prepared in DMF (Figs. 1 and 2). These results suggest that the coupling reaction between OC and PPA in the EDAC . HCl/water system was not as effective as in the DCC/DMF system. Recently, Nakajima and Ikada (1995) reported that compounds with cyclizable carboxylic groups (e.g. maleic acid and poly(acrylic acid)) readily react with EDAC . HCl in aqueous media and produce carboxylic anhydrides, which in the presence of an amine, readily converts to an amide. With non-cyclizable carboxylic acids (e.g. fumaric acid and poly(ethylene glycol), however, only a trace of amide was formed. Further, they found that EDAC . HCl is stable at neutral and higher pH conditions, but rapidly loses its activity in aqueous media of low pH (3.5–4.5) and converts to a urea derivative. When used in excess, EDAC . HCl reacts with carboxylic groups, irrespective of whether the latter is cyclizable or non-cyclizable, producing a stable *N*-acylurea derivative. Thus, it appears that the small amounts of OC–PPA conjugate formed in the EDAC · HCl/water reaction system could be due to the inability of the carboxylic acids on OC to cyclize and produce a carboxylic anhydride in situ. In the case of the DCC/DMF-mediated reaction, the formation of the OC–PPA conjugate may have involved an initial reaction between OC and DCC, producing an OC-*o*-acylurea intermediate, followed by a nucleophilic attack by PPA on the carbonyl carbon of the carboxylic groups of OC (Fig. 5), as has been reported by Williams and Ibrahim (1981).

3.2. Morphology of the OC–PPA conjugate

The SEM photographs of the starting OC powder and the OC–PPA conjugate prepared by reacting a 1:4:6 mole ratio of OC-COOH:DCC: PPA . HCl in DMF at room temperature for 60 h are shown in Fig. 6. Both OC and the OC–PPA conjugate are shown to be fibrous materials. In general, the OC fibers appear somewhat longer and more swollen, with possibly a smoother surface with fewer fractures compared with that of the OC–PPA conjugate fibers.

3.3. In vitro PPA release studies

The design of polymeric prodrugs requires that the parent drug be regenerated in vivo by either chemical or enzymatic hydrolysis. The conditions used for chemical hydrolysis usually include buffer solutions with pH values ranging between 4 and 7.4. The reason for using the mild acidic pH is that endosomes and lysosomes have a reported pH of 5.0–5.5 and 4.5–5.5, respectively (Kumar and Banker, 1996). The OC–PPA conjugate used in this study contained 24.2% (w/w) PPA. The release media used were pH 4.0, 5.5, and 7.4 buffer solutions. Fig. 7 shows the release profiles of PPA over 35 days. The plots show that the



(A) (B)

Fig. 6. SEM photographs of (A) OC and (B) the OC–PPA conjugate.

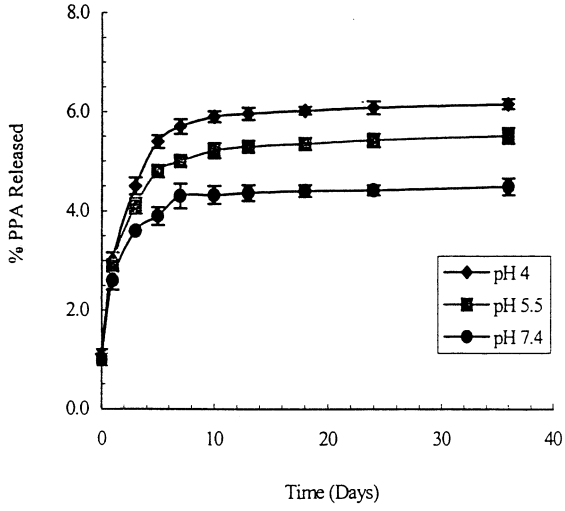


Fig. 7. Release of PPA from the OC–PPA conjugate in various pH buffer solutions.

conjugate had an initial burst release, during day one, based on the total amount released, followed by a slow release of PPA over a period of 1 week. The total amount of drug released during this period was about 6%. After 1 week, the release of PPA was very slow, causing only a small increase in the cumulative PPA release after this time point. The extent of PPA released as a function of pH showed the following order, pH 4.5 > pH 5.5 > pH 7.4, suggesting that the amide linkage in the conjugate is more susceptible to hydrolysis in lower acidic pH conditions. The release of PPA seen at the initial or zero time point is probably due to free or ionically entrapped PPA that was present on the surface and in fractures of the particles.

To test the susceptibility of a polymeric prodrug to enzymatic hydrolysis, the conjugate is usually incubated with a specific lysosomal enzyme (e.g. cathepsin or cathepsin D, (Dosio et al., 1997), a non-specific lysosomal enzyme (i.e. a mixture containing acid hydrolases, esterases, peptidases, glycosidases, and phosphatases, (Trouet et al., 1982), or a mouse (Dosio et al., 1997) or rat (Song et al., 1996) liver homogenate containing intracellular enzymes. In the present study, the OC–PPA conjugate was incubated with a 20% rat liver homogenate (pH 7.4). The percent PPA released over a period of 7 days is shown in Fig. 8. After incubation for 7 days, about 12% of the PPA released from the conjugate. This corresponded to more than double the amounts of PPA released in pH 7.4 buffer alone (Fig. 7), suggesting that the amide linkage in the OC–PPA conjugate is more susceptible to enzymatic hydrolysis than to chemical hydrolysis.

4. Conclusions

OC, DCC and PPA when reacted in DMF in a 1:4:6 mole ratio produces a covalently-linked OC conjugate containing about 24% (w/w) PPA, corresponding to a DS of about 0.7. A similar reaction in water using EDAC . HCl as a coupling agent, instead of DCC, by contrast, produced a conjugate with very little PPA. The FT-IR and solid state ^{13}C-CP/MAS NMR spectra of the conjugate revealed that the OC is linked to PPA via an amide bond. The release studies in pH 4.5, 5.5, and 7.4 buffer solutions and in rat liver homogenate showed the conjugate to be more susceptible to hydrolysis under acidic pH conditions, and in the presence of rat liver homogenate (pH 7.4). The results suggest that OC can be successfully used to prepare an amide-linked conjugate with amine drugs and used as a bioabsorbable macromolecular prodrug delivery system.

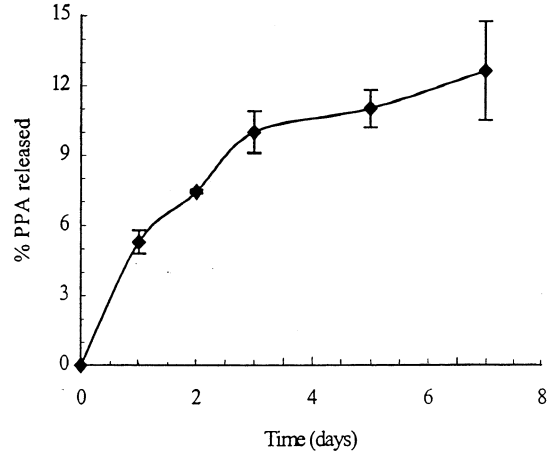


Fig. 8. Release of PPA from the OC–PPA conjugate in 20% rat liver homogenate.

Further studies to prepare OC-amine drug conjugates linked via a variety of different chain length spacer arms, to modulate the release characteristics of the conjugates, are in progress.

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