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Multiple cross-linked hydroxypropylcellulose-succinate-salicylate: Prodrug design, characterization, stimuli responsive swelling-deswelling and sustained drug release

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ABSTRACT

Hydroxypropylcellulose-succinic anhydride (HPC-SAn) conjugate was synthesized homogeneously at 80 °C for 6 h under N_2 in *N,N*-dimethylacetamide (DMA). HPC-SAn conjugate was further covalently linked with salicylic acid (SA) drug using a versatile reagent $ZrOCl_2.8H_2O$ at 80 °C for 6 h. Multiple crosslinking of benign HPC-SAn-SA conjugate was achieved using oxalyl chloride. The resultant cross-linked prodrug (CL-HPC-SAn-SA conjugate) was characterized using different spectroscopic techniques. UV/Vis analysis of HPC-SAn-SA conjugate has indicated that it contains 26 mg of SA per 100 mg. CL-HPC-SAn-SA showed reasonably good swelling properties in water and at different physiological pH values (6.8 and 7.4). However, negligible swelling was observed at acidic pH (1.2). Kinetic studies revealed that CL-HPC-SAn-SA followed second order swelling kinetics. Additionally, CL-HPC-SAn-SA conjugate showed stimuli responsive (pH 7.4/1.2) swelling-deswelling properties. The effect of different pH (1-10) on swelling of CL-HPC-SAn-SA was also studied. Thermal analysis revealed that the cross-linked prodrug CL-HPC-SAn-SA was thermally more stable as compared to pure SA. This method of multiple crosslinking of drugs with polysaccharides and the resultant prodrugs are highly potential for pharmaceutical and medicinal applications.

1 Introduction

Different efforts have been made to synthesize useful polymeric prodrugs of salicylic acid (SA) over the years. Among synthetic polymers, poly (anhydride-ester) composed of alkyl chain and polyamidoamine dendrimer conjugates were used to esterify SA to form its prodrugs.[1,2] The later study showed sustained and colon targeted release of SA. Due to biocompatibility issues,

synthetic polymers are nowadays being replaced with naturally occurring hydrophilic, film or gel forming polysaccharides.[3,4] In this regard, cellulose ethers are attracting vigil eye of medicinal and material chemists working in the field of drug design and applications.[5] Recent reports witnessed HPC (a cellulose ether derivative) as a superb choice for prodrug formation because it is non-ionic, hydrophilic, biocompatible and has inbuilt oligo-hydroxypropyl linkers.[6]

Cross-linking (CL) of polymeric prodrugs could also be a useful reaction to develop hydrogels for sustained and targeted prodrug design. [7,8] Adverse aspects of toxic CL agents, like glutaradehyde,[9] can be avoided by using appropriate CL agents.[10] A useful CL agent is oxalyl chloride that cross-links OH groups by the evolution of gaseous by-products thereby generating ester linkage among polysaccharide chains with fairly high purity.[11]

Herein, we report fabrication and characterization of HPC-SAn-SA conjugate. Aims were to utilize oxalyl chloride as a CL agent in order to develop swelling behaviour in prodrugs. Therefore, HPC-SAn-SA conjugate was cross-linked to afford CL-HPC-SAn-SA conjugates. The novel synthesis protocol for HPC-SAn-SA conjugates and concept of multiple crosslinking in prodrug design of SA are being reported for the first time. Such prodrugs of SA may have potential applications against inflammatory bowel disease due to high swelling index, ease of metabolism in basic environment and reduced hydrolysis in gastric fluid.

2 Materials and methods

2.1 Materials

HPC (MS 3.46) used was purchased from Nanjing Yeshun Industry and International Trading Co. Ltd, Jiangsu, China. HPC was dried under vacuum at 110 °C for 5 h before use. Organic solvents used during study were of LabScan whereas zirconium oxychloride octahydrate ($ZrOCl_2.8H_2O$), SA, SAn, and oxalyl chloride were received from Fluka and used without further purification.

2.2 Measurements

FTIR (KBr) spectra were taken on an IR Prestige-21 (Shimadzu, Japan) instrument. ^{1}H and APT (attached proton test) ^{13}C NMR (δ ppm, 400 MHz) spectra were acquired on a Bruker NMR in DMSO-d_6 using TMS as an internal standard. The number of scans for ^{1}H and ^{13}C NMR were 16 and 2000, respectively. Thermogravimetric analysis was carried out to observe thermal stability and kinetics of the samples on SDT Q 600 (TA Instruments, USA) thermal analyzer. Thermal degradation was recorded at a heating rate of 15 °C/min from ambient-1000 °C. UV/Vis spectrophotometer used was PharmaSpec 1700 (Shimadzu, Japan).

2.3 Synthesis of HPC-SAn conjugates

HPC (2.0 g) was dissolved in DMA (30 mL) under nitrogen at 80 °C with constant stirring for 30 min. Succinic anhydride (1.95 g) was added into the polymer solution and stirred at 80 °C for 6 h. The product, i.e., HPC-SAn conjugate was isolated by precipitation of reaction mixture in diethyl ether (250 mL). Precipitates were washed thrice with acetone (100 mL) to remove unreacted SAn and succinic acid by-product. These precipitates were filtered, dried and then ground to powder (colourless).

Yield: 3.05 g (85%); Degree of substitution (DS) = 2.84/HPC-repeating unit (HPC-RU) as calculated by ^{1}H NMR spectroscopy; FTIR (KBr): 3462 ν(O-H), 2926 ν(C-H), 1738 ν($C=O_{Ester}$),

1369-1450 ν(CH_2), 1062 ν(C-O-C) cm^{-1}; ^{1}H NMR (DMSO-d_6, δ ppm): 1.05 (H 9), 3.07-4.67 (HPC H 1-8), 2.37 (H 11), 2.74 (H 12); APT ^{13}C NMR (DMSO-d_6, δ ppm): 27.48-28.61 (C 11,12), 16.79 (C 9), 65.04-67.34 (C 6-8), 75.35 (C 2), 77.31 (C 7), 81.13-83.09 (C 3-5), 102.23 (C 1), 169.63 (C 10), 174.21 (C 13).

2.4 Synthesis of HPC-SAn-SA conjugates

HPC-SAn conjugate (1.0 g, 1.97 mmol) isolated from previous reaction was dissolved in DMA (30 mL). To the solution of HPC-SAn conjugate, SA (1.28 g, 9.25 mmol) was added followed by the addition of $ZrOCl_2.8H_2O$ (0.634 g, 1.97 mmol, 21.29 mol% to SA) as a catalyst for esterification. Reaction was preceded under stirring at 80 °C for 6 h. Resultant product (HPC-SAn-SA conjugate) was obtained by precipitation of the reaction mixture in diethylether (250 mL). The product was purified by washing it with acetone (100 mL) thrice and dried under vacuum at 50 °C overnight.

Yield: 1.12 g (83%); Drug content (DC) = 26 mg/100 mg = DS 1.88/HPC-RU as calculated by UV-Vis spectrophotometry; FTIR (KBr): 3462 & 3541 ν(weak signal, O-H), 2927 ν(C-H), 1728 ν(C=O Ester), 1371-1448 ν(CH_2), 1053 ν(C-O-C) cm^{-1}; ^{1}H NMR (DMSO-d_6, δ ppm): 1.05 (H 9), 3.08-4.73 (HPC H 1-8), 2.48 (H 11), 2.53 (H 12), 6.73 (H 15), 7.26 (H 16), 7.74 (H 17); APT ^{13}C NMR (DMSO-d_6, δ ppm): 27.51-28.64 (C 11,12), 16.78 (C 9), 65.02 (C 6), 67.31 (C 8), 75.38 (C 2), 77.28 (C 7), 81.09-83.12 (C 3-5), 102.19 (C 1), 116.06 (C 18), 116.98 (C 15), 130.09 (C 17), 132.54 (C 16), 162.13 (C 14), 169.58 (C 10), 172.10 (C 13), 173.38 (C 19).

2.5 Synthesis of CL-HPC-SAn-SA conjugates

HPC-SAn-SA conjugates (2.0 g) were added to DMF (30 mL) and stirred (30 min) to obtain optically clear solution. The solution was then cooled up to -10 °C and oxalyl chloride (5 mL)

was added drop wise (in 5 min) using dropping funnel and stirred for 1 h. The reaction mixture was further stirred for 3 h at room temperature. In order to remove unreacted oxalyl chloride, the reaction mixture was therefore heated up to 70 °C for 2 h. Continuous supply of nitrogen was maintained during all steps of the reaction. The cross-linked product (CL-HPC-SAn-SA conjugate) was separated from reaction mixture by precipitation. Propanol (200 mL) produced fluffy precipitates of the product. Precipitates were washed with methanol (100 mL) thrice, filtered and dried under vacuum at 50 °C overnight to obtained white powder.

DC = 25 mg/100 mg = 1.78/HPC-RU as calculated by UV-Vis spectrophotometry; FTIR (KBr): 2935-2763 ν(C-H), 1734 ν(intense, C=O Ester), 1450 ν(CH_2), 1042 ν(C-O-C) cm^{-1}.

2.6 Calculation of DC (mg/100 mg) by UV/Vis spectrophotometry

DC was calculated using a reported UV/Vis spectrophotometric method.[6] Sample (20 mg) was hydrolysed using 0.1 N aq. NaOH solution (20 mL) under stirring at 80 °C for 5 h absorbance was noted after filtration. Solution of different known concentrations of standard SA were prepared and absorbance was noted to plot calibration curve. Amount of SA in samples was calculated using calibration curve of standard constructed at λ_{max} of 296 nm.

2.7 Calculation of DS value by ^{1}H NMR spectroscopy

The DS of succinyl moieties onto HPC polymer was calculated by the comparison of spectral intensities of different signals in ^{1}H NMR spectrum.[12]

2.8 Transmission electron microscopy (TEM)

The conjugate CL-HPC-SAn-SA (20 mg) was dissolved in DMSO (5 mL) and dialysed for 3 days against distilled water. The obtained suspension was diluted by the addition of milli-Q

water. Sample was drop casted onto carbon coated copper grids and dried under air before characterizing by transmission electron microscope (Philips EM420) operating at an acceleration voltage of 120 kV.

2.9 Swelling studies of cross-linked product CL-HPC-SAn-SA conjugate

To determine the swelling index (Q_t), known initial weight (W_i) of dry sample was added to distilled water. At different time intervals, final weight (W_f) of swollen products was noted. Following relation gave the value of Q_t (eq. 1);

$$Q_t = \frac{W_f - W_i}{W_i} \qquad \text{(eq. 1)}$$

Normalized equilibrium degree of swelling (Q_e) was calculated using eq. 2;

$$Q_e = \frac{W_\infty - W_i}{W_i} \qquad \text{(eq. 2)}$$

where, W_∞ is maximum swelling of the gel in maximum time.

Second order kinetics (eq. 3) provided the best fit on swelling data of the CL-HPC-SAn-SA conjugate;

$$\frac{t}{Q_t} = \frac{1}{kQ_e^2} + \frac{t}{Q_e} \qquad \text{(eq. 3)}$$

2.9.1 Preparation of buffer solutions of different pH

CL-HPC-SAn-SA conjugate was studied for its swelling properties at different pH values, i.e., 1.2, 6.8 and 7.4. Buffer solution of pH 1.2 was prepared by KCl and HCl, whereas potassium dihydrogen phosphate and NaOH were used to prepare buffer solution of pH 6.8 and pH 7.4.

2.9.2 Evaluation of pH responsive properties of CL-HPC-SAn-SA conjugate

Accurately weighed CL-HPC-SAn-SA (0.5 g each) was packed in 4 cellophane bags and hung in separate beakers (100 mL each) containing deionized water (50 mL) and buffer solutions (50 mL each) of pH 7.4, 6.8, and 1.2. Cellophane bags containing swollen CL-HPC-SAn-SA were taken out of medium at specific time intervals and hung for some time to remove extra water, weighed and placed again in respective media for further swelling. Swelling capacity (%) was calculated by using below given equation;

$$Swelling\ capacity\ (\%) = \frac{W_t - W_o - W_c}{W_o} \qquad \text{(eq. 4)}$$

where, W_c is the mass of wet cellophane bag, W_t is mass of cellophane bag containing swollen CL-HPC-SAn-SA conjugate and W_o is the mass of the pre-dried CL-HPC-SAn-SA conjugate.

2.9.3 pH responsive on-off swelling of CL-HPC-SAn-SA conjugate

In order to observe on-off swelling behaviour of CL-HPC-SAn-SA conjugate, it was packed in cellophane bags and tested against pH 1.2 and 7.4. Accurately weighed CL-HPC-SAn-SA conjugate (0.5 g) was packed in cellophane bag and hung in buffer solution (50 mL) of pH 7.4 taken in a beaker (100 mL) for 15 min. After removing the excess liquid, bag containing CL-HPC-SAn-SA conjugate was weighed and hung in pH 1.2 buffer (50 mL) for another 15 min. These on-off experiments were performed over four cycles.

2.10 Drug release and kinetics

In-vitro drug release studies were carried out at 37 °C using dialysis method in simulated gastric and intestinal environments (pH 1.2 and 7.4, respectively). Solutions of CL-HPC-SAn-SA conjugate (1% w/v) were made in phosphate buffered saline (PBS) of pH 1.2 and 7.4. Both solutions were sealed in separate dialysis tubing and dialyzed against 200 mL of PBS with prescribed pH values. While stirring the bags at 100 rpm, small

aliquots (2 mL) from each sample were withdrawn at specified time intervals with replacing an equal volume of PBS having respective pH. The withdrawn aliquots were diluted and their absorbance was recorded using a UV/Vis spectrophotometer. Cumulative drug released after hydrolysis of the prodrug was calculated from calibration curve of SA.

Since, drug release is dependent on hydrolysis of the ester bonds, therefore it followed a pseudo first order kinetic rate expression that is shown in eq. 5.[13]

$$ln(q_e - q_t) = ln\, q_e - kt \qquad \text{(eq. 5)}$$

where, q_e and q_t are equilibrium concentration and concentration at time t, while, k is the rate constant.

2.11 Thermal degradation kinetics

Drug (SA) and CL-HPC-SAn-SA conjugates were subjected to thermogravimetric analyses. Thermograms of the samples were recorded from ambient temperature to 1000 °C using ramp method at a heating rate of 15 °C/min under nitrogen atmosphere. Different thermal kinetic methods, i.e., Friedman,[14] Chang[15] and Broido[16] models were applied to thermal data for the calculation of kinetic parameters such as order of the reaction (n), pre-exponential factor (Z) and activation energy (Ea) of the samples. The n values were calculated from Chang model. Friedman, Chang and Broido methods use eq. 6-8, respectively.

$$ln\left(\frac{d\alpha}{dt}\right) = lnZ + n\, ln(1-\alpha) - \frac{Ea}{RT} \qquad \text{(eq. 6)}$$

$$ln\left[\frac{\frac{d\alpha}{dt}}{(1-\alpha)^n}\right] = lnZ - \frac{Ea}{RT} \qquad \text{(eq. 7)}$$

$$ln\left(ln\frac{1}{y}\right) = -\frac{Ea}{RT} + constant \qquad \text{(eq. 8)}$$

where, in eq. 6-8, R is gas constant; $d\alpha/dt$ is rate of weight loss; T is absolute temperature; 1-α is weight of sample left at a certain temperature; w_0 is initial weight; w_∞ is final weight; *y is* $(w_t - w_\infty)/(w_0 - w_\infty)$ and w_t is weight at a given time t.

The thermodynamic parameters, i.e., enthalpy (ΔH), entropy (ΔS) and Gibb's free energy (ΔG) were also calculated using Eyring method.[17] Moreover, index of thermal stability (ITS) and integral thermal decompositional temperatures (IPDT) of SA and CL-HPC-SAn-SA conjugate were also calculated using a reported method.[18]

3 Results and discussion

3.1 Synthesis, cross-linking and characterization of HPC-SAn-SA conjugate

HPC-SAn conjugate were synthesized employing homogeneous reaction conditions using DMA as a medium of reaction. SAn reacts with free hydroxyls of HPC to form ester bonds between HPC and SAn. The resultant HPC-SAn conjugate was isolated and purified by precipitation and washing. HPC-SAn conjugate was further reacted with SA (a non-steroidal anti-inflammatory drug) in the presence of an efficient catalyst, i.e., $ZrOCl_2.8H_2O$ to form HPC-SAn-SA conjugate. This reaction strategy for making multiple CL prodrug of SA is novel and being shown in Fig. 1.

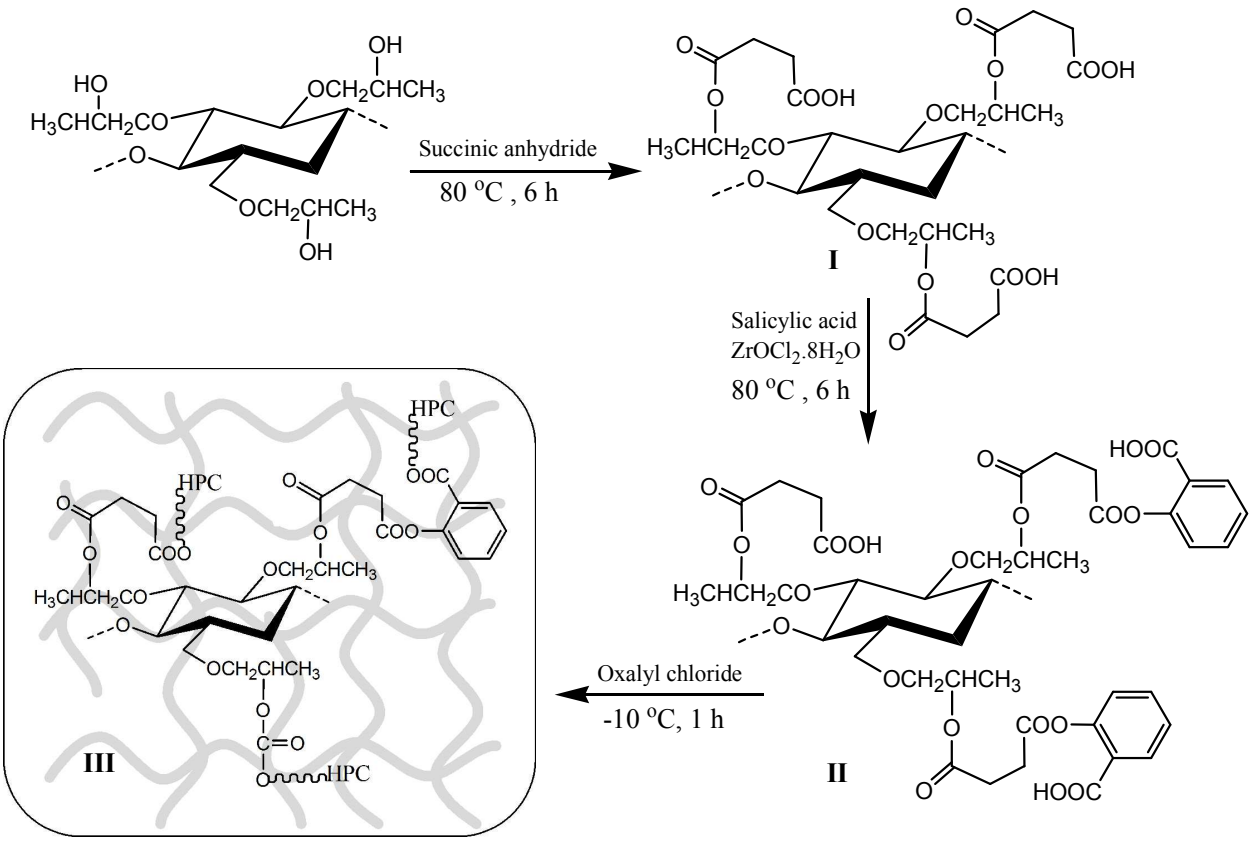
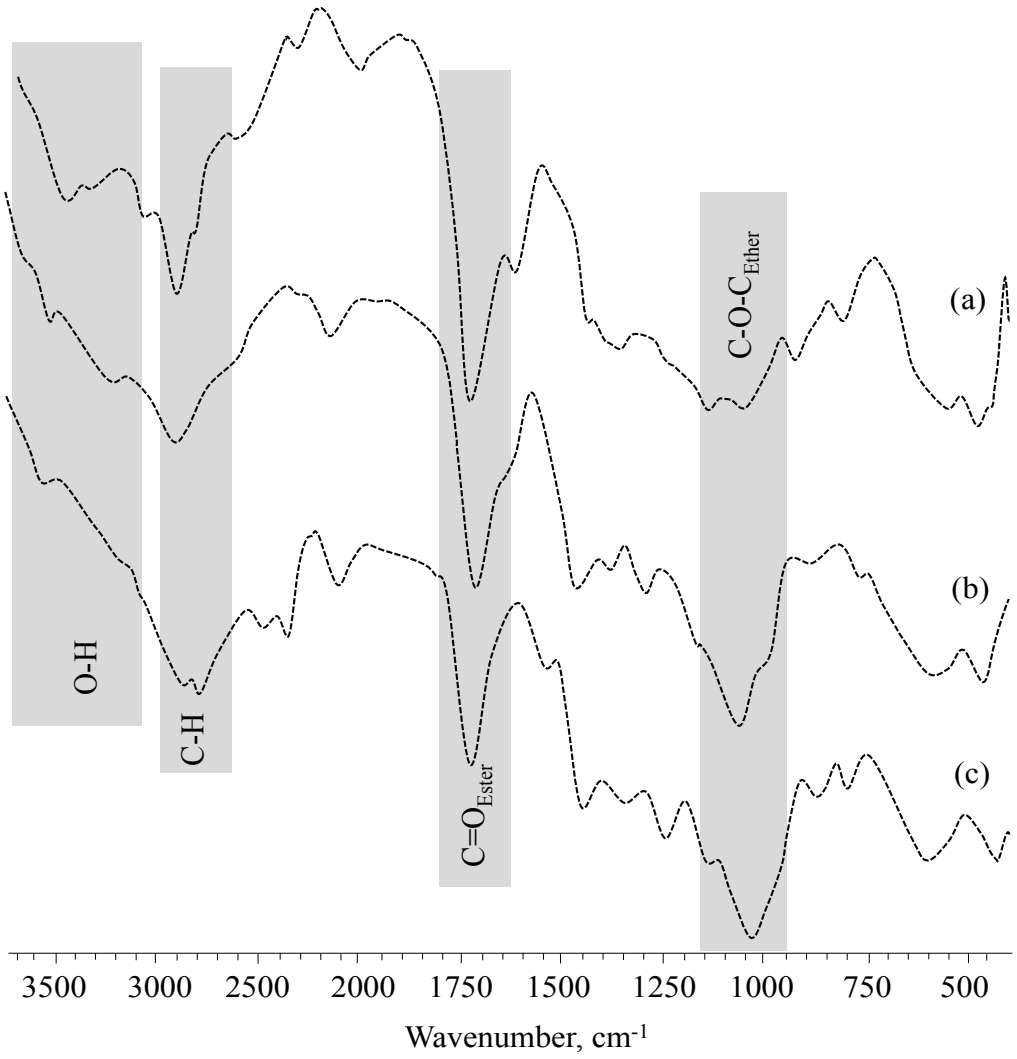


Fig. 1 Synthesis of HPC-SAn conjugate (I), HPC-SAn-SA conjugate (II) and CL-HPC-SAn-SA conjugate (III).

HPC-SAn-SA conjugates were subjected to cross-linking in DMF solvent. Oxalyl chloride is used for cross-linking between OH groups as well as OH and COOH groups (see Fig. 1). Benefit associated with the use of oxalyl chloride is that by-products produced during reaction are gaseous (CO and HCl) or soluble (oxalic acid) hence easily removed by washing. The reaction produced water swellable and cross-linked HPC-SAn-SA conjugate, i.e., CL-HPC-SAn-SA conjugate. This strategy resulted in multiple cross-linking due to esterification of SA with OH groups of other chains of HPC or HPC-succinate as well as hydroxyls available on different polymer chains, etc.

HPC-SAn-SA conjugate and its cross-linked derivative CL-HPC-SAn-SA are novel products which were characterized by different spectroscopic techniques. FTIR spectra of HPC-SAn, HPC-SAn-SA and CL-HPC-SAn-SA conjugates are cumulatively shown in Fig. 2. FTIR spectrum of HPC-SAn conjugate witnessed the success of reaction by showing distinct ester carbonyl absorptions at 1738 cm^{-1}. Likewise, HPC-SAn-SA conjugate showed broad ester signal at 1728 cm^{-1}. Whereas, ester signals of CL-HPC-SAn-SA conjugate were shifted towards relatively higher absorption at 1734 cm^{-1} (intense peak) as expected due to cross-linking by ester formation as well. Reduction in hydroxyl absorptions of HPC in its esterified products is also observed indicating that OH groups were esterified. Indirect assessment of cross-linkages in CL-HPC-SAn-SA conjugate might be taken from the fact that said product swells in water as expected for cross-linked polysaccharides. All other vital signals of the drug, succinyl moieties and HPC were also present in spectra.



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228 **Fig. 2** FTIR spectra of (a) HPC-SAn, (b) HPC-SAn-SA and (c) CL-HPC-SAn-SA conjugates.

229

230 DC of SA in HPC-SAn-SA conjugate was determined in terms of mg of drug per 100 mg of
231 conjugate by using calibration curve of standard SA and found to be 26 mg/100 mg. The
232 overlayed UV spectral pattern of HPC-SAn-SA conjugate and CL-HPC-SAn-SA conjugate are
233 shown in Fig. 3.

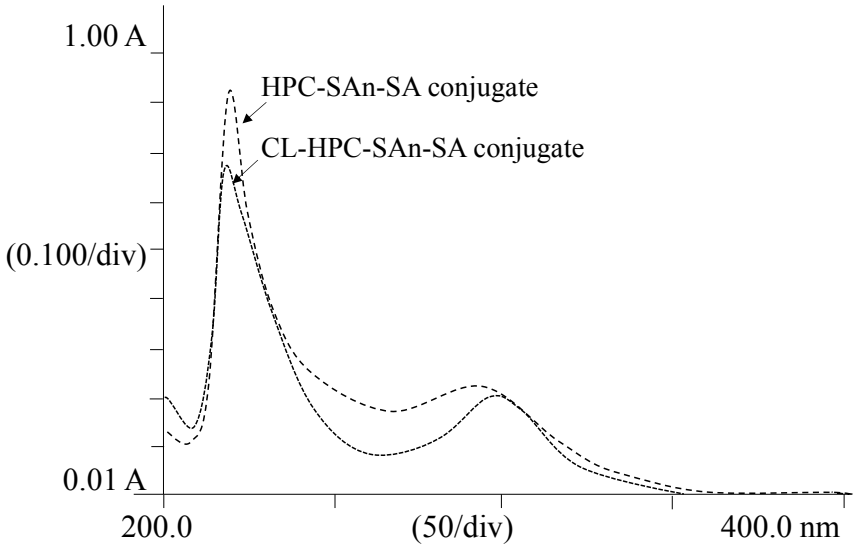


Fig. 3 UV/Vis spectral pattern of HPC-SAn-SA and CL- HPC-SAn-SA conjugates.

^{1}H NMR (400 MHz, 16 scans) spectrum of HPC-SAn conjugate was recorded in DMSO-d_6 (Fig. 4). Appearance of CH_2 signals (H 11,12) of SAn at δ 2.37-2.74, respectively indicated the successful esterification of SAn with HPC unlike pure succinic acid where both CH_2 give single peak due to identical environment. HPC repeating unit protons were detectable at δ 3.07-4.67 (H 1-8) ppm. The CH_3 protons of hydroxypropyl (HP) moieties of HPC were detectable at δ 1.05 ppm while signals of CH and CH_2 of HP moieties are overlapped with AGU signals.

APT ^{13}C NMR spectra (400 MHz, 2000 scans) of HPC-SAn-SA conjugate was recorded in DMSO-d_6. Spectrum of HPC-SAn-SA (see Fig. 4) confirmed the success of esterification by up field shift of ester carbonyl (C 13) signal from δ174.21 to 172.10 ppm. Other signal of ester carbonyl (C 10) appeared at δ 169.58 ppm. Signal of carboxyl carbon of SA can be observed at δ 173.38 ppm. Signals of aromatic carbons appeared at δ116.06-162.13 ppm. HPC repeating unit signals C 1, C 2 and C 6 appeared at δ 102.19, 75.38 and 65.02 ppm, respectively. Signals for C

8 and C 7 appeared at δ 67.31and 77.28 ppm, respectively. Methyl (C 9) of HPC was found at δ 16.78 ppm. Signals of C 11 and C 12 of succinic acid appeared at δ 27.51-28.64 ppm and overlapped signal indicated that both ends of succinyl moieties are occupied with ester bonds.

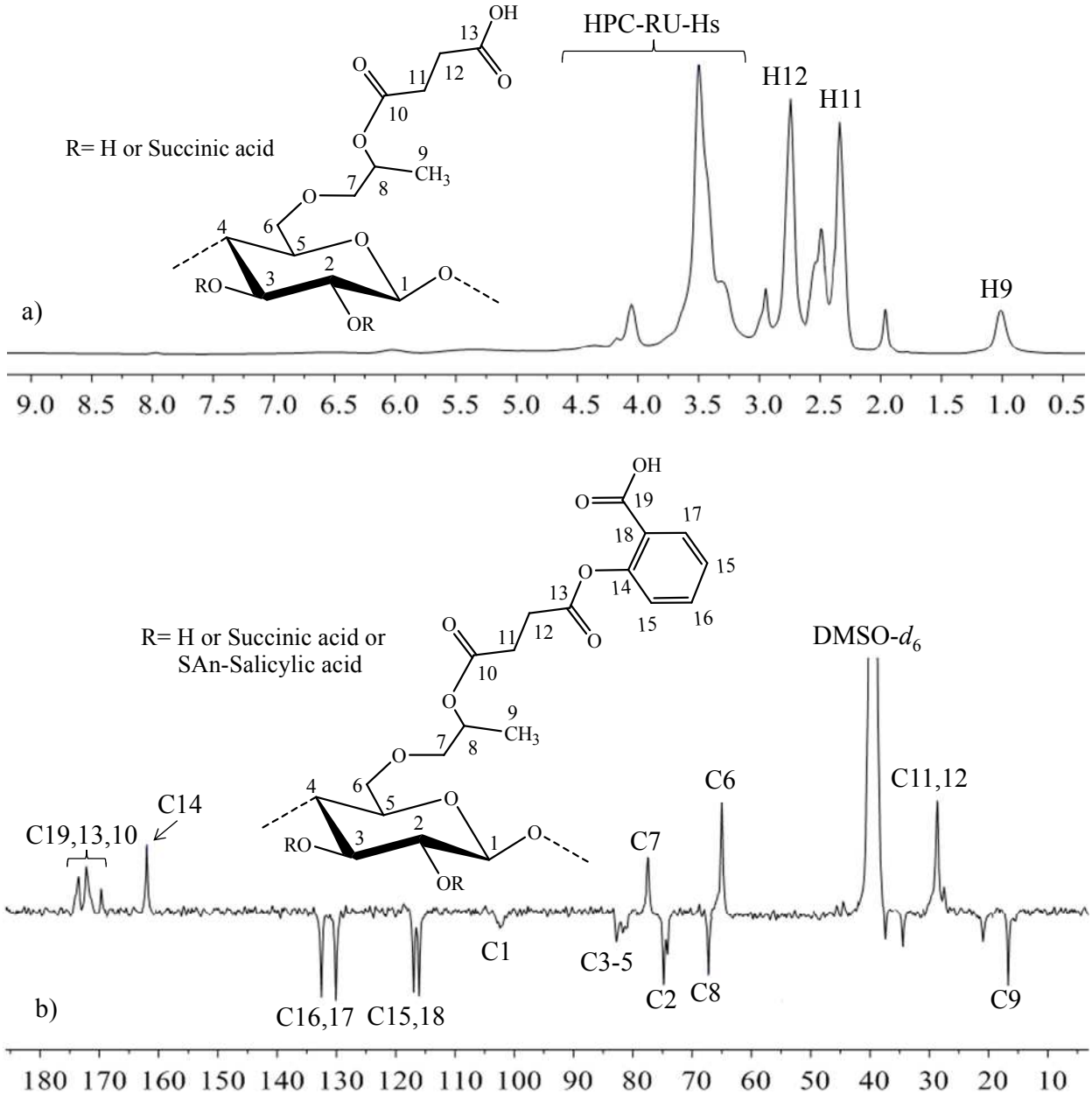
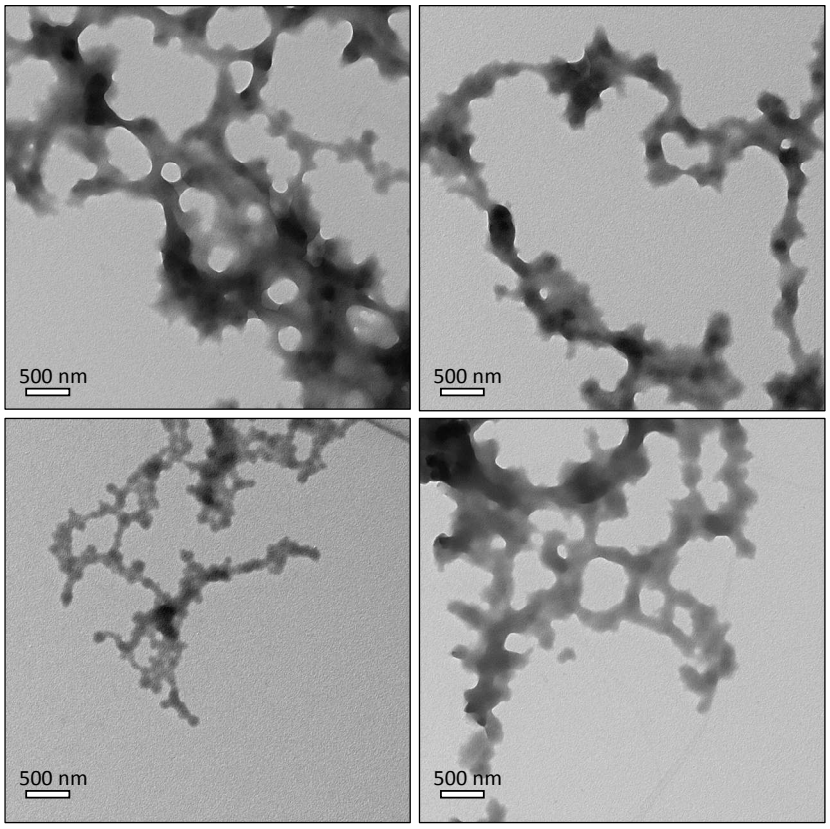


Fig. 4 ^{1}H and APT ^{13}C NMR (400 MHz, DMSO-d_6, ppm) spectra of HPC-SAn (a) and HPC-SAn-SA conjugate (b), respectively.

255

256 ### 3.2 Transmission electron microscopic analysis

257 Fig. 5 shows TEM images of the cross-linked prodrug of SA, i.e., CL-HPC-SAn-SA conjugate.
258 Upon exposure of CL-HPC-SAn-SA conjugate to solvent diffusion (dissolved in DMSO and
259 dialyzed vs. water), it self-assembled into cross-linked nanowires of 102-214 nm diameter.



260

261 **Fig. 5** TEM image of CL-HPC-SAn-SA conjugate showing nanowire formation at DMSO/water
262 interface.

263 ### 3.3 Swelling studies of CL-HPC-SAn-SA conjugate

264 #### 3.3.1 Swelling behaviour and kinetics of CL-HPC-SAn-SA conjugate in water

Stimuli (temperature, pH, ionic strength) responsive hydrogels are smart materials for controlled release of drugs.[19-21] As we are focused to develop multiple crosslinking in aforesaid conjugate therefore there is a possibility that the cross-linked product may show stimuli responsive properties. In order to assess the swelling properties of CL-HPC-SAn-SA conjugate, swelling ratio of cross-linked product was determined thrice in water and buffers of pH 1.2, 6.8 and 7.4 and mean values are being shown in Fig. 6. The results revealed that CL-HPC-SAn-SA conjugate got significant water-swelling ratio. It was noted that swelling rate was faster in first 200 min and then it slowed down. After 350 min, the equilibrium of swelling was established. Observed high rate of swelling can be attributed to the crosslinking of HPC-SAn-SA conjugate. Normalized degree of swelling and normalized equilibrium degree of swelling were calculated and graphs were plotted to calculate order of swelling of CL-HPC-SAn-SA conjugate. The kinetic results have indicated that CL-HPC-SAn-SA conjugate followed second order swelling kinetics in water and buffers of pH 1.2, 6.8 and 7.4 (Fig. 7).

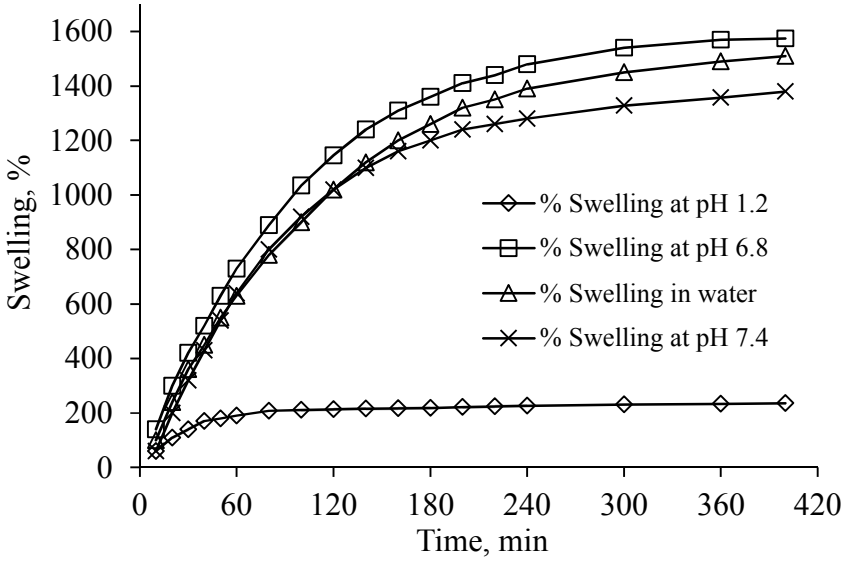


Fig. 6 Graphical representation of percentage swelling of CL-HPC-SAn-SA conjugate at pH 1.2, 6.8, 7.4 and in distilled water.

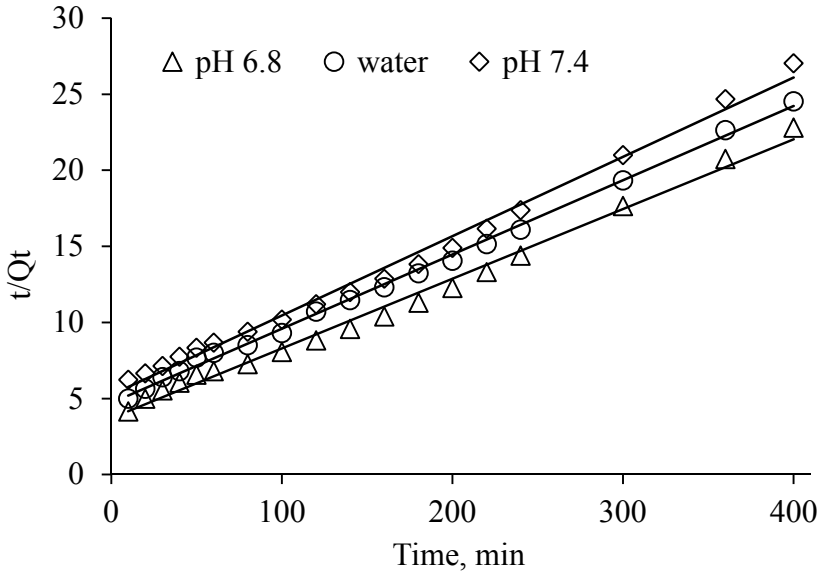


Fig. 7 Swelling kinetics of CL-HPC-SAn-SA conjugate at pH 6.8, 7.4 and in distilled water.

3.3.2 pH responsive swelling and kinetics of CL-HPC-SAn-SA conjugate

Hydrogels often show sensitivity to pH of the aqueous media which makes them materials of choice for controlled/sustained release of several classes of drugs.[22-24] Responsive swelling of the newly designed CL-HPC-SAn-SA conjugate was studied in water and at different physiological pH values (pH 1.2, 6.8 and 7.4, see Fig. 6). It was noted that CL-HPC-SAn-SA conjugate did not significantly swell at pH 1.2. Therefore, no significant release of SA at this pH is expected from CL-HPC-SAn-SA conjugate. This may lead to the assumption that CL-HPC-SAn-SA conjugate

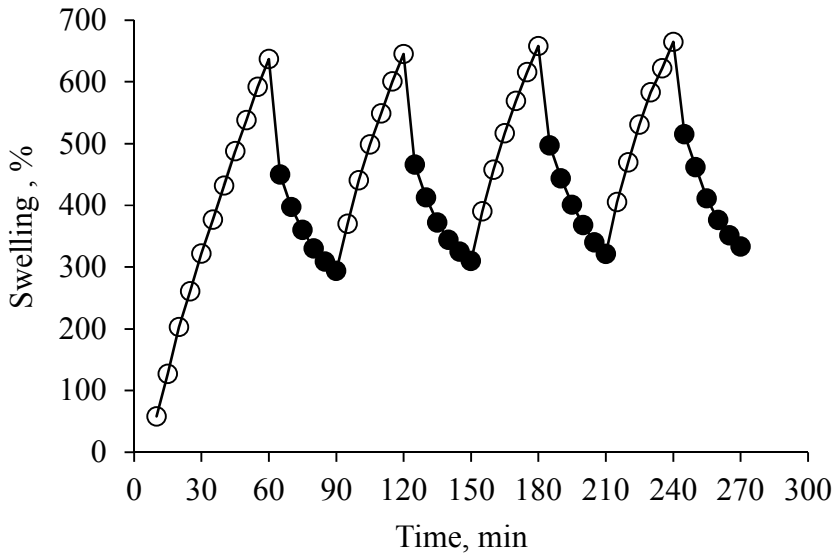
will be stomach safe and will not release significant amount of SA in stomach. In this way, side effects of SA on stomach can be reduced.

At pH 6.8 and 7.4, maximum swelling is observed for CL-HPC-SAn-SA conjugate. This is important to note that on reaching intestine, the conjugate starts swelling and releases SA at faster rate particularly in later part of intestine. In this way, intestine targeted drug delivery can be achieved.

Swelling data revealed that CL-HPC-SAn-SA conjugate show comparable swelling in water as well as at pH 7.4 and 6.8. However, swelling ratio was found to be negligible at pH 1.2 because unmodified COOH groups of SAn moieties form hydrogen bonding with the still free hydroxyls in HPC polymer. Kinetics analyses of swelling data of CL-HPC-SAn-SA conjugate showed that swelling followed second order kinetics at pH 6.8, 7.4 and in water (see Fig. 7).

3.3.3 pH responsive on-off-switching

Reversible on-off-switching with respect to pH change is a hot topic of research nowadays.[25-27] Therefore, CL-HPC-SAn-SA conjugate was subjected to pH responsive on-off swelling studies. CL-HPC-SAn-SA conjugate showed quick pulsatile and on-off switching pH sensitive behaviour. Fig. 8 shows swelling and de-swelling behavior of hydrogel at pH 7.4 and 1.2, respectively. The sharp and reversible swelling of CL-HPC-SAn-SA conjugate makes it suitable candidate for controlled drug (SA) delivery systems. After four swelling-deswelling (on-off) cycles at pH 7.4 and 1.2, the CL-HPC-SAn-SA conjugate still showed pH-sensitivity which is highly reversible.



312

313 **Fig. 8** On-off swelling behaviour of CL-HPC-SAn-SA conjugate at pH 7.4 (on) and 1.2 (off)
314 over four cycles.

315

316 **3.3.4 Effect of pH on equilibrium swelling**

317 Equilibrium swelling of CL-HPC-SAn-SA conjugate was measured in triplicate at different pH
318 of solutions ranged from 1.0-9.5 (Fig. 9). It has been now academically well understood that by
319 the addition of cations in polysaccharidal hydrogels, shrinkage is observed due to decrease in
320 hydrogen bonding and by increasing pH, more swelling is observed due to repulsion among like
321 charges. Moreover, maximum swelling of 15.2 and 13.1 g/g was obtained at pH 6 and 7,
322 respectively which is abruptly dropped afterwards due to start of dissolution. However, from Fig.
323 6, it is also obvious that maximum swelling for CL-HPC-SAn-SA conjugate was noted at pH 6.8.
324 The CL-HPC-SAn-SA conjugate completely dissolved at pH 9.5. Similar way of pH-dependent
325 swelling have been reported in the case of other related hydrogel systems. [25,27,28]

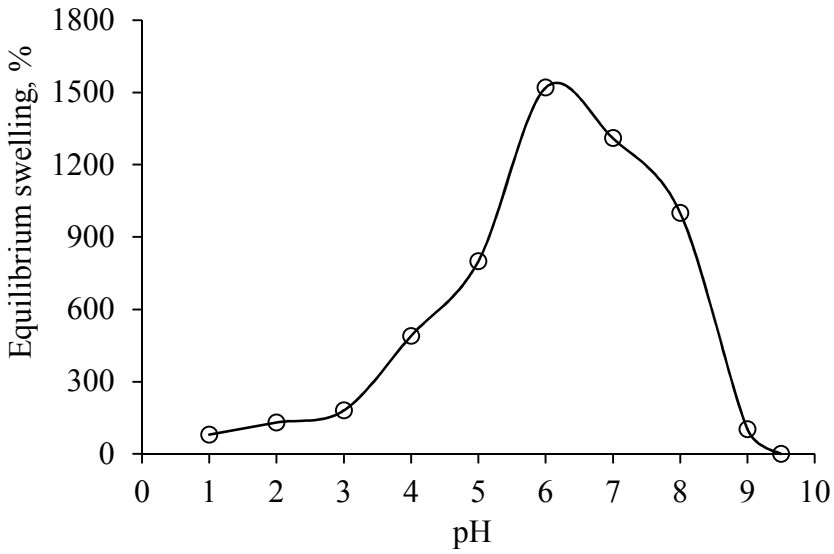


Fig. 9 Effect of pH of solutions on equilibrium swelling of CL-HPC-SAn-SA-conjugate.

3.4 Drug release studies

The hydrolytic release of SA from newly fabricated CL-HPC-SAn-SA conjugate was investigated at simulated gastric and intestinal pH (Fig. 10a). All the measurements were carried out in triplicate and the mean values have been shown. SA release is significantly faster at pH 7.4 than 1.2. The amounts of SA released in first 30 min were 1.01 and 2.12% at pH 1.2 and 7.4, respectively. CL-HPC-SAn-SA showed relatively much faster hydrolysis in simulated intestinal pH 7.4 (68%) as compared to pH 1.2 (10%) within 12 h. Therefore, it can be proposed that CL-HPC-SAn-SA conjugate can act as a prodrug of SA for sustained release. The *ln* (q_e-q_t) *vs*. time plot (Fig. 10b) confirmed that drug release kinetics followed pseudo first order kinetics. The drug release from conjugate is time dependent and increases with passage of time.

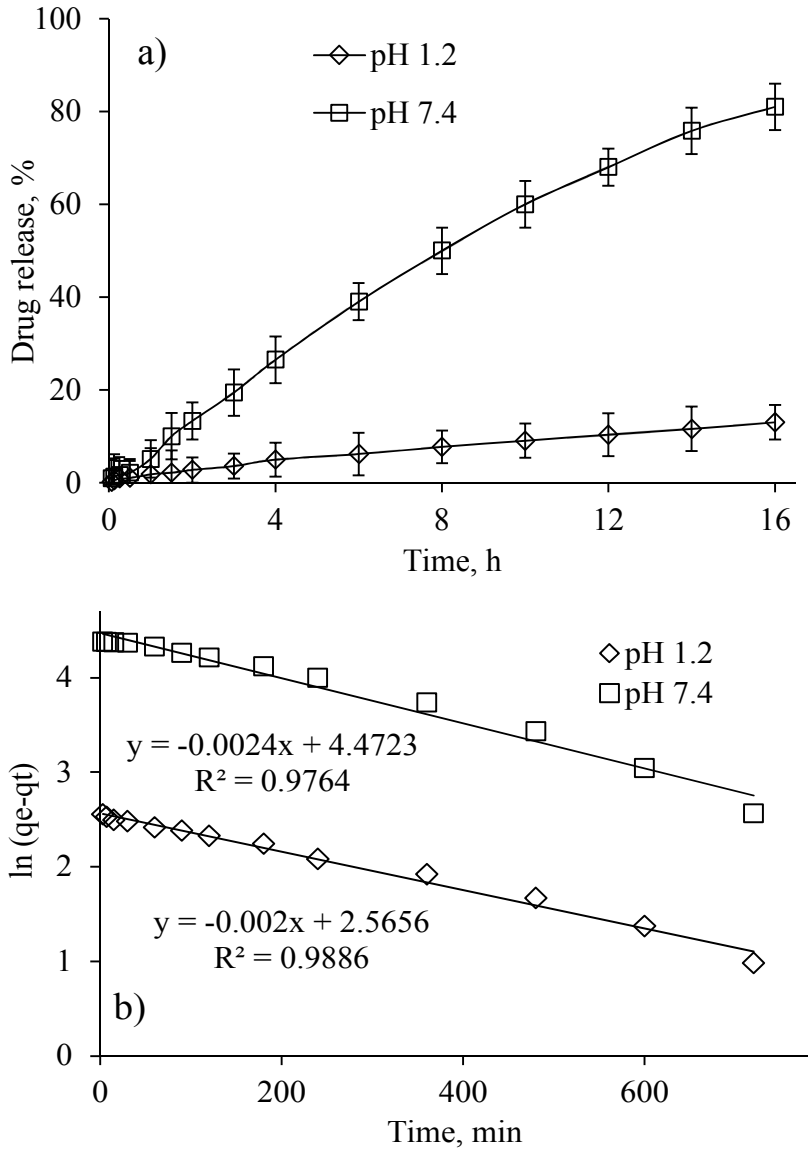


Fig. 10 a) Drug release and b) pseudo first order release kinetics of SA from CL-HPC-SAn-SA conjugate.

3.5 Thermal analysis

Thermogravimetric analyses of SA and CL-HPC-SAn-SA conjugate were carried out in order to determine their thermal stability. Fig. 7 is showing comparative degradation pattern of SA and

CL-HPC-SAn-SA conjugate. TG curve of CL-HPC-SAn-SA conjugate revealed two step degradation in contrast to SA which exhibited single step degradation. SA showed complete degradation from 125-199 °C (Tdi and Tdf, respectively). A 34% mass of CL-HPC-SAn-SA conjugate was lost in first degradation step whereas the remaining mass of the sample degraded slowly and completed at 856 °C. It is inferred from these results that drug (SA) got significant thermal stability after conjugate formation and cross-linking. Results of thermal analyses of SA and CL-HPC-SAn-SA conjugate are summarized in Table 1. The increased stability of SA in prodrug (CL-HPC-SAn-SA conjugate) has greater benefits because stable drugs may show improved pharmaceutical performance parameters, e.g., shelf-life.[28]

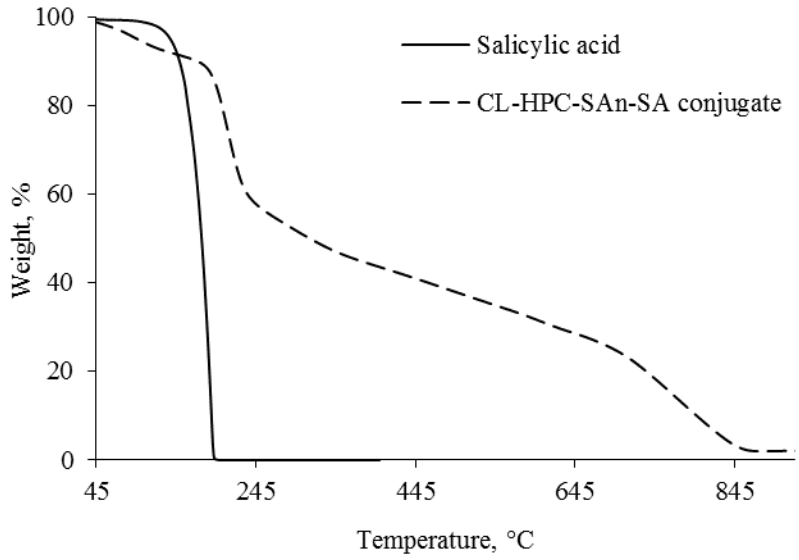


Fig. 11 Overlay TG curves of SA and CL-HPC-SAn-SA conjugate.

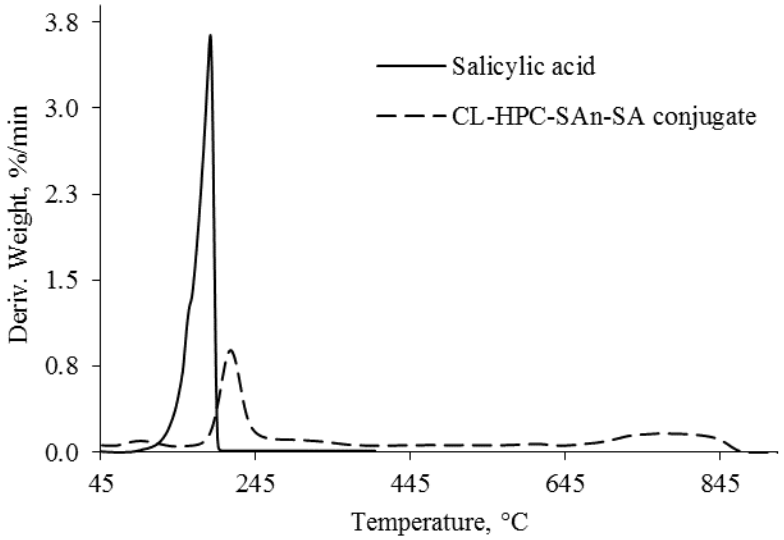


Fig. 12 Overlay DTG curves of SA and CL-HPC-SAn-SA conjugate.

Table 1 Thermal decomposition temperatures and char yield values of salicylic acid (SA) and CL-HPC-SAn-SA conjugate.

Sample	Steps	Tdi (°C)	Tdm (°C)	Tdf (°C)	Weight loss % at Tdf
SA	I	125	188	199	99.91
CL-HPC-SAn-SA conjugate	I	180	212	261	33.83
	II	697	778	856	97.22

Different kinetic and thermodynamic parameters were calculated in order to assess the stability, order of reaction *n* and *Ea*. The *Ea* values for first degradation step of CL-HPC-SAn-

SA conjugate were found in the range of 163.52-165.25 kJ/mol whereas for SA were found 96.77-109.54 kJ/mol as calculated by Friedman, Broido and Chang methods. Higher *Ea* values of CL-HPC-SAn-SA conjugate indicated that extra thermal stability was imparted to SA after conjugate formation and cross-linking. Degradation of SA and CL-HPC-SAn-SA conjugate followed first order kinetics. All thermal kinetic and thermodynamic parameters of SA and CL-HPC-SAn-SA conjugate are summarized in Table 2 for comparison. Higher values of thermodynamic parameters, ITS and IPDT of the CL-HPC-SAn-SA conjugate as compared to SA also indicated the thermal stability imparted to multiple cross-linked conjugate (prodrug) of SA.

Table 2 Thermal kinetic and thermodynamic parameters of salicylic acid (SA) and CL-HPC-SAn-SA conjugate.

Sample	Method	Step	R^2	*n*	*Ea* (kJ/mol)	ln*Z*	Δ*H*	Δ*S*	Δ*G*	ITS	IPDT (°C)
SA	Friedman	I	0.999	-	96.77	26.59	92.45	-38.75	110.32	0.30	168
	Chang		0.999	1	99.67	27.82	95.83	-28.24	108.85		
	Broido		0.999	-	109.54	28.85	105.73	-18.89	114.44		
SAn-SA conjugate	Friedman	I	0.997	-	165.25	41.36	161.12	87.27	118.87	0.33	421
	Chang		0.998	1	164.47	41.88	160.43	91.56	116.01		

Broido		0.999	-	163.52	40.43	159.48	79.45	120.94
Friedman		0.998	-	115.16	11.64	106.45	-182.12	297.88
Chang	II	0.997	1	112.13	11.34	103.39	-184.88	297.68
Broido		0.996	-	110.67	12.65	101.93	-174.05	284.89

4 Conclusions

The reagent $ZrOCl_2.8H_2O$ appeared highly valuable for macromolecular prodrug formation of SA and structurally related drugs with polysaccharidal hydroxyl groups via esterification. To impart the swelling characteristics in HPC-SAn-SA conjugate, cross-linking of the prodrug was achieved using oxalyl chloride. Thermally stable and cross-linked prodrug CL-HPC-SAn-SA is a novel material designed for the sustained drug release through its swelling at intestinal pH. The cross-linked prodrug of SA appeared advantageous as it showed remarkable swelling properties in water and at pH 6.8 and 7.4. However, negligible swelling was observed at pH 1.2 indicating that the cross-linked prodrug of SA is safe for stomach and will be colon targeted having additional benefit of sustained release. The cross-linked prodrug CL-HPC-SAn-SA also showed pH responsive swelling properties which further add to stomach safe and intestine targeted potential drug design. Present study can easily be modelled for carboxylic acid containing drugs with OH containing polymers and vice versa.

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