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Macromolecular Prodrugs of Aspirin with HPMC: A Nano Particulate Drug Design, Characterization, and Pharmacokinetic Studies

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Abstract: This article presents the synthesis of novel hydroxypropylmethylcellulose (HPMC)-aspirin (ASP) conjugates, *i.e*. macromolecular prodrugs, through the reaction of HPMC with ASP after its *in situ* activation by 4-methylbenzenesulfonyl chloride. The highly pure ASP prodrugs obtained by this homogeneous and elegant esterification method were characterized using different spectroscopic and chromatographic techniques. Nanoparticulate drug design was successfully achieved by the conversion of free hydroxyls of the polymer into acetates. Transmission electron microscopy and scanning electron microscopy showed nanoparticle formation with the major population size distribution of around 450 nm. Nevertheless, the pharmacokinetics of the HPMC conjugates were studied using high performance liquid chromatography. The pharmacokinetic data indicated that a single dose of 132.6 mg of HPMC-ASP was well tolerated in animal studies without any adverse effects. The maximum plasma concentration (C_{max}) of HPMC-ASP was found to be 14.6 $\mu g \cdot L^{-1}$ with a t_{max} of 1 h. The plasma half-life and clearance and the volume of HPMC-ASP distribution were 4.6 h, 3.23 $L \cdot h^{-1}$, and 21.8 $L \cdot kg^{-1}$, respectively. The elimination of HPMC-ASP followed first-order kinetics with r^2 of 0.9643. The results presented in this paper show the great potential of HPMC-ASP as a more effective, safe, and stable prodrug.

Keywords: aspirin, biopolymers, hydroxypropylmethylcellulose, nanoparticles, polysaccharides, prodrugs.

Introduction

By the development of prodrugs, reduction in administration frequency, targeted drug delivery, better use of the active pharmaceutical ingredients, and patient compliance can be achieved.[1,2] Fabrication of macromolecular prodrugs of non-steroidal anti-inflammatory drugs (NSAIDs) by the covalent attachment of the drugs with biopolymers as an ester moiety is the forefront area of drug design. Esterified prodrugs can easily be hydrolyzed in the basic medium of the colon in particular. Biopolymers, especially, glycopolymers-drug conjugates can't be affected much by acidic hydrolysis in the stomach.[3] Therefore, by this ester linkage between NSAIDs, and glycopolymers, the stomach can easily be kept secure from the harmful effects of the NSAIDs. Indirectly, in this way, colon specific drug delivery can be achieved.[4] Glycopolymers have been used to deliver a wide variety of drugs including giant drug molecules, *e.g*. peptides, proteins, nucleic acids, NSAIDs, anti-cancer drugs, and acid sensitive drugs for colon targeting.[4-7]

The fabrication of macromolecular prodrugs of NSAIDs with polysaccharides is a well established area of research in drug design, and development.[8-10] However, there is no significant literature found for the use of cellulose ethers as drug careers. The widely used cellulose ether in biomedical, and pharmaceutical sciences is hydroxypropylmethylcellulose (HPMC). All sustained release studies, and targeted drug delivery from the HPMC, its gel, and matrix was studied so far as non-covalent interaction of HPMC with several drug molecules.[11-16] Nevertheless, we have recently reported the nano-rod formation of macromolecular prodrugs of salicylic acid onto HPMC[4] as the only significant work in the areas of covalent interaction of HPMC-NSAIDs conjugate as an ester moiety. Present study uses homogeneous reaction methodologies for the esterification of available free hydroxyl groups at hydroxypropyl moieties onto HPMC polymer backbone.[17-19]

The aim of this study was to fabricate acid resistant macromolecular prodrugs of aspirin (ASP) with HPMC to protect it from acidic digestion in the stomach as well as to

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protect the stomach from adverse effects of the drug. In addition to the synthesis of nonionic, acid resistant, neutral, and biocompatible novel conjugates, HPMC polymer offers two main advantages. Firstly, novel HPMC-esters of ASP can serve as macromolecular prodrugs for colon targeted drug delivery. Secondly, such macromolecular prodrugs can show controlled/sustained release of drugs after basic hydrolysis in the intestine. The aim was also to achieve a nanoparticulate macromolecular prodrug design. Present work was also focused to study self-assembly of HPMC-ASP conjugates in solution after slight structure modification (conversion of the free -OH on to HPMC polymer with acetic anhydride). Present study also aimed to develop an HPLC method for the determination of pharmacokinetic parameters of active drug ASP from HPMC-ASP conjugates after oral administration.

Experimental

Materials. Hydroxypropylmethylcellulose (HPMC-E5, USP 26; with a hydroxypropyl moiety 7.5%, and O-methyl groups 28%) obtained from Zhejiang Zhongbao Imp & Exp Corp, Ltd. was dried under vacuum at 110 °C for 8 h prior to use. Methanol, acetic acid, aspirin (ASP), and salicylic acid were used of E. Merck grade. Analytical grade organic solvents, reagents, and other chemicals obtained from Fluka were used as received.

Measurements. ^{1}H NMR (400 MHz) spectra of the HPMC-ASP conjugates were acquired in D_2O. After peracylation of the unmodified hydroxyl groups, the spectra of the samples were acquired in $CDCl_3$. FTIR spectra were measured on a Prestige-21 (Shimadzu, Japan) using the KBr pellet technique. Thermal decomposition temperatures (T_d) of the esters were determined by thermogravimetric analysis (TGA) on a SDT Q 600 (USA) thermo balance. The T_d was reported as the onset of significant weight loss from the heated sample. Samples (10 mg) were measured under air with a temperature increase of 10 °C/min from 35 up to 600 °C. Elemental analysis was performed using a CHNS 932, Leco (USA). UV-vis spectrophotometer Pharmspec 1700 (Schimadzu, Japan) was used to quantify the degree of substitution (DS) of the pendant groups on HPMC-NSAIDs conjugates. Solutions were prepared in distilled water as solvent along with 0.1 N NaOH solution, and UV spectra were measured at different wavelengths. For the GPC analysis, an Agilent Technologies 1200 (Germany) series equipment was used including degasser (DG-980-50), pump (PU-980), RI detector (RI-930), and UV-detector (UV-975) at 254 nm. Distilled water was used as an eluent (30 °C, 1 mL/min). The separation was carried out using columns from polymer standards service with 1000, 10,000, and 100,000 A°. Polystyrene standards were used for calibration. The products were characterized for aggregation in solution using transmission electron microscopy (TEM) on a Philips 420 instrument with an acceleration voltage of 120 kV. The HPLC system consisted of LC-10AT VP pump, UV-vis detector SPD-10A VP, SCL-10A VP system controller all from Shimadzu, Japan. The column used was a Shim-Pak ODS 5 μm (4.6×250-mm). Scanning electron microscopy (SEM) was carried out on a FEI Phenon (FEI, Eindhoven, The Netherlands) operating at 0.5 kV.

Fabrication of HPMC-ASP Conjugates, a Typical Example (Sample 3). For a typical preparation, dry HPMC (2 g) was added to dimethylacetamide (30 mL) solvent. The mixture was kept under stirring for 2 h to obtain an optically clear solution of HPMC polymer. To the solution of 2 g of HPMC in dimethylacetamide (DMAc), 4-methyl-benzenesulfonyl chloride (Tos-Cl; 4.99 g, 26.26 mmol), triethylamine (7.39 mL, 52.56 mmol), and ASP (4.73 g, 26.26 mmol) were added. The reaction mixture was heated, and stirred under nitrogen at 80 °C for 24 h. Isolation of the product was carried out by precipitation of the reaction mixture into acetone (150 mL). The precipitates were washed three times with acetone by stirring in order to remove any of the un-reacted drug contents. Precipitates were then dried under vacuum at 50 °C overnight. Yield 1.97 g; DS_{ASP}=8.4 mg/100 mg as determined by UV spectroscopy; FTIR (KBr) 3397 ν (OH), 2967 ν (CH), 1740 ν (C=O_{Ester}) cm^{-1}, 1454 ν (CH_2); ^{1}H NMR (D_2O) δ 2.04 (H-16), 2.95-4.75 (other AGU-H-1-5), 3.78 (H-7), 1.05 (H-9), 7.64 (H-11), 6.90 (H-12, 14), 7.34 (H-13), 3.15 (H-17).

Peracylation of HPMC-ASP Conjugates (Sample 3), a Typical Procedure. The sample (100 mg) was taken in reaction vessel containing pyridine (10 mL) followed by catalytic amount (4 mg) of 4-dimehtylaminopyridine. Acetic anhydride (10 mL) was added as an acylating agent. The reaction mixture was kept under stirring for 24 h at 60 °C. Peracetylated sample was precipitated, and washed with water for isolation, and purification. The product was dried overnight under vacuum at 50 °C to yield peracetylated sample **3**. The product is soluble in $CHCl_3$. FTIR (KBr) no free OH peak in spectrum; ^{1}H NMR ($CDCl_3$) δ 2.07 (Acetyl-H), 2.90-5.33 (other AGU-H), 1.05 (H-9), 7.0-7.4 (aromatic-H).

UV/Vis Spectroscopic Analysis. For the UV analysis sample (10 mg) was taken in a flask followed by the addition of 0.1 N NaOH (10 mL). The reaction mixture was stirred for 3 h at 80 °C for complete hydrolysis of ester moieties covalently attached on to the HPMC polymer backbone. After filtration, the volume was made up to 10 mL, and absorbance was recorded at wavelength (λ_{max}) 232.5 nm.

Transmission Electron Microscopy (TEM). Nanoparticles were prepared using a dialysis process. In a typical batch, peracylated HPMC-ASP conjugates (170 mg, sample **2**) were dissolved in purified dimethylsulfoxide (DMSO, 5 mL), and dialysed against DMSO for 3 days. The suspension obtained was concentrated using a rotary vacuum evaporator, and investigated by TEM. A droplet (5 mL) of the

freshly sonicated solution (self-assembled HPMC-ASP conjugate in DMSO) was placed on hydrophilized carbon films on copper wire grids, and excess fluid was blotted off, and air-dried on the grids.

Scanning Electron Microscopy (SEM). The samples were drop casted on silicon wafers mounted on carbon stubs (carbon adhesive Leit-Tabs No. G 3347) Plano (Wetzlar, Germany).

Pharmacokinetic Studies.

Preparation of Mobile Phase: Methanol-acetic acid system was prepared by mixing methanol, and acetic acid in a ratio of 20:1. The mobile phase was filtered, and degassed before use.

Preparation of Solutions of Standard ASP and Sample HPMC-ASP: A standard solution was prepared by dissolving, accurately weighed, ASP (100 mg) in mobile phase (100 mL). This solution (10 mL) was diluted with mobile phase (100 mL). The solution was filtered through a 0.45 μm filter paper, and degassed before use. A solution of sample HPMC-ASP was also prepared by this method as given above for standard ASP.

Chromatographic Conditions: The system optimization was carried out using various compositions of the mobile phase. Separation was achieved under isocratic conditions using a mobile phase of methanol: acetic acid (20:1) at a flow rate of 0.5 mL·min^{-1} with UV detection at 272 nm at ambient temperature. The volume of injection was 20 μL.

Method Validation: Method validation was carried out in the mobile phase as reported in a reference.[20]

Accuracy and Precision: The accuracy, and precision were determined by using a quality control sample, prepared by adding a known amount (lying in the middle range of entire standard curve) of standard, from three concentrations representing the entire range of a standard curve; one within 3×times of the lower limit of quantification (low quality control sample), one near the center (middle quality control), and one near the upper boundary of standard curve (high quality control). Measurements were made as ten replicates at each concentration, and the mean, and the coefficient of variance (CV) were calculated.

Linearity: Ten different concentrations of HPMC-ASP were prepared in the mobile phase. Twenty μL of each concentration were injected.

Limit of Detection (LOD): The solution of HPMC-ASP in the mobile phase was diluted to known concentrations to a final response equal to twice the signal-to-noise ratio.

Lower Limit of Quantification (LOQ): The LOQ was determined by using five samples independent of standards, and the CV was calculated at the 95% confidence level.

Specificity: The specificity of the method was established by using six different samples.

Quality Control: The quality control samples were used to accept or reject the run. The replicate measurements were made at three concentrations, one at the lower limit of quantification, one in the mid range, and one approaching higher end of the range.

Stability Study: Stability study of the sample in the mobile phase was carried out to evaluate the time period that encompasses the duration of typical sample preparation, sample handling, and analytical run time which was about 30 min. The stability study was extended to 72 h in this work.

Participants and Study Design. Five healthy rabbits were selected. The rabbits were kept on fast for at least 10 h (overnight). A single dose of 132.6 mg of the test product, HPMC-ASP was administered to 5 rabbits along with 340 mL of water. The rabbits were kept fasting for 5 h after the administration of the drug. They were allowed to take water 1 h after administration of the drug during this fast.

Specimen Collection. Venous blood samples (3-5 mL) were collected from each rabbit by using disposable syringes, branulas, and butterflies under aseptic conditions. Blood was collected from the antecubital vein. Heparin (Leo, Denmark) was used as an anticoagulant. The blood samples were collected in centrifuge test tubes, which were already heparinized, and arranged in order on test tube racks, and labeled accordingly with great accuracy. Post dose blood samples were collected after 0.083, 0.16, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, and 12.0 h. The blood samples were centrifuged at 3000×g for 3 min. Plasma was separated by using micropipette with sucker.

Storage of Sample: The plasma was stored in capped test tubes separately, sealed with aluminum foil, and labeled accordingly. The plasma samples were frozen at -5 °C in a freezer (PEL FR-320) until analyzed.

Pharmacokinetic Parameters: Concentration-time curves were plotted, and the parameters $AUC_{0\text{-}t}$, the area under the curve from time zero to time t, and $AUC_{0\text{-}\infty}$, the area under the curve from time zero to time infinity, were determined using the following formula;

$$AUC_{0\text{-}\infty} = AUC_{last} + C_t/ke$$

Other parameters studied were half-life of the drug ($t_{½}$)= 0.693/ke, the peak drug concentration (C_{max}), the time to peak drug concentration (t_{max}), clearance (Cl)=dose/$AUC_{0\text{-}\infty}$, and volume of distribution (V_d)=Cl/ke. The area under the concentration-time curve was calculated by the linear trapezoidal method. The terminal rate constant, ke was determined by regression analysis of at least three data points in the terminal phase. The statistical analysis was performed by use of Statgraphics® 5.1.

Results and Discussion

Synthesis and Characterization. Pre-dried hydroxypropylmethylcellulose (HPMC) was dissolved in dimethylacetamide (DMAc), and allowed to react with Aspirin (ASP), and 4-methyl-benzenesulfonyl chloride (Tos-Cl) in the presence of triethylamine as a base. Reaction was carried out for 24 h at 80 °C. Homogeneous esterification was carried out

Table I. Reaction Conditions and Results of Esterification of HPMC Dissolved in DMAc Using *In situ* Activated ASP with Tos-Cl at 80 °C for 24 h

Sample	Molar Ratio[a]	Yield (g)	Solubility	DS[b]
1	1:1:1:3	1.29	DMSO, DMAc, Water	2.8
2	1:2:2:4	1.65	DMSO, DMAc, Water	4.4
3	1:3:3:6	1.97	DMSO, DMAc, Water	8.4
4	1:6:6:12	2.15	DMSO	9.2

[a]HPMC:ASP:Tos-Cl:Triethylamine. [b]DS of ASP (mg drug/100 mg sample) was calculated by UV-vis spectroscopy.

as one pot synthesis. Tos-Cl first reacts with ASP to yield different reactive intermediates, *i.e.* acid chloride, acid anhydride, and mixed anhydride of Tos-Cl, and ASP. These intermediates are highly reactive acylating agents produced *in situ* and they react with the -OH available on HPMC polymer backbone to yield esters as macromolecular prodrugs, *i.e.* HPMC-ASP conjugates. The reaction conditions, and results of all reactions are summarized in Table I.

HPMC-ASP conjugates (**1-4**) were prepared using different molar ratios of reactants, according to the scheme given in Figure 1, using homogeneous reaction methodologies. All of the samples synthesized were soluble in DMSO, water, and DMAc however; exception was the sample **4** which was insoluble in water. The insolubility of the sample **4** in water may be due to increased substitution of ASP onto the HPMC polymer which is achieved by using higher molar ratios of ASP to HPMC.

All of the products were thoroughly characterized by several chromatographic, spectroscopic, and thermal analysis techniques. The FTIR Spectra showed the success of the esterification reaction from the carbonyl, aromatic, and ester absorptions. The HPMC-ASP conjugates have shown a characteristic peak in the FTIR spectra typical for the ester

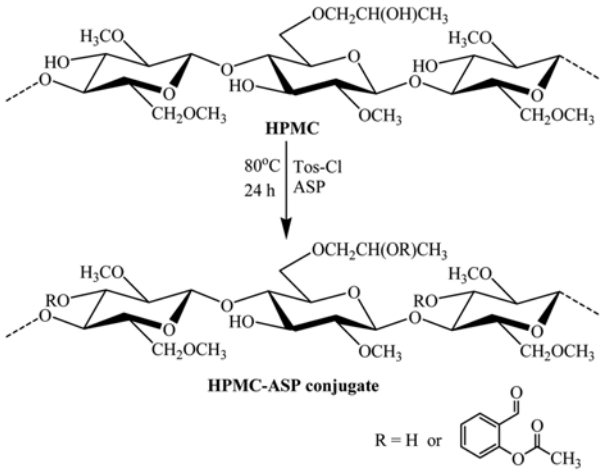

Figure 1. Synthesis of HPMC-ASP conjugates applying *in situ* activation of ASP with Tos-Cl.

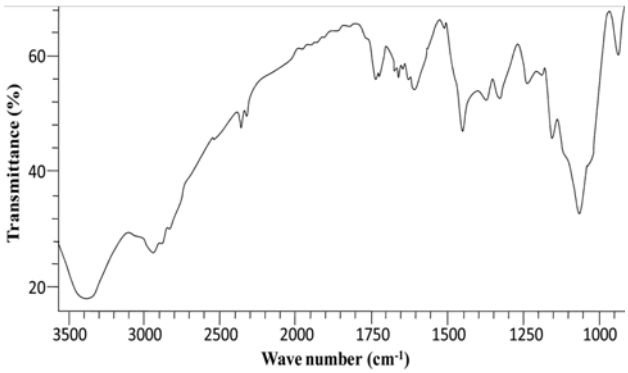

Figure 2. FTIR (KBr) spectrum of HPMC-acetylsalicylate **2**.

moiety in the range of 1739-1745 cm^{-1} ($C=O_{Ester}$). The spectra of **1-4** displayed hydroxyl group absorptions at about 3387-3397 cm^{-1}, aromatic C-H absorption at 2967-2969 cm^{-1}, and a CH_2 absorption at 1452-1454 cm^{-1}. As a typical example FTIR spectrum of sample **2** is shown in Figure 2.

As acylation reagent Tos-Cl was used therefore, elemental analysis was carried out to check the presence of sulphur in products. Elemental analysis revealed the absence of sulphur in all samples showing that there is no introduction of tosylate groups either covalently bounded or as an impurity.

^{1}H NMR spectra of HPMC-ASP conjugates **1-3** were recorded in D_2O. A typical ^{1}H NMR spectrum of sample **2** is shown in Figure 3. Spectrum revealed the presence of an aromatic ring attached to the HPMC polymer backbone as aromatic protons were detectable at δ 7.64 (H-10), 6.90 (H-11, 13), and 7.34 (H-12) ppm. It shows that the unsaturated system is not destroyed during the reaction. Protons of the HPMC polymer backbone anhydroglucose unit (AGU) were detectable at δ 3.5 (H-6), and 2.95-4.75 (other AGU-H) ppm. The protons of the hydroxypropyl moiety of HPMC polymer were detectable at δ 3.78 (H-7), 1.05 (H-9) ppm, and the H-8 signal overlapped with the AGU signals. The FTIR spectrum has already indicated aromatic ring absorptions.

For the purpose of substitution of free hydroxyl groups

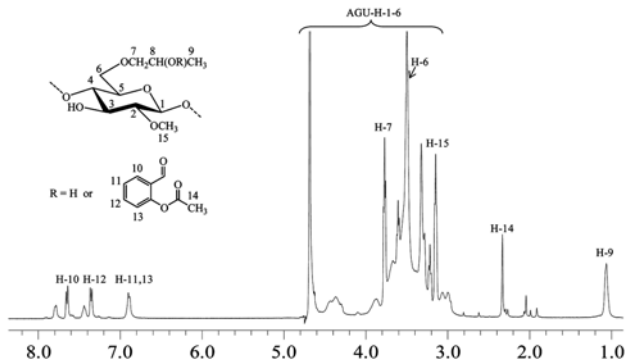

Figure 3. 400 MHz ^{1}H NMR (D_2O) spectrum of HPMC-ASP conjugates **2**.

available after reaction on the polymer, acetylation was carried out using acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP) as catalyst, and pyridine as solvent. The ^{1}H NMR spectra of the HPMC-ASP conjugates **1**-**4** after peracylation with acetic anhydride were recorded in $CDCl_3$. This conversion was performed just to vary the polarity of the HPMC-ASP conjugate to get nanostructures in solutions. The ^{1}H NMR spectra revealed the presence of an aromatic ring attached to the HPMC polymer backbone, and sugar regions of the HPMC polymer. The only change in the products was the introduction of the acetyl methyl groups from the acetic anhydride. The methyl-H of this acetyl group appeared at δ 2.01 ppm. Remaining signals of AGU-H, and the aromatic regions appeared similar to the spectrum before acetylation (see Figure 3).

The gel permeation chromatography (GPC) was carried out to investigate the hydrolytic degradation of the polymer chain during the reaction. The results of GPC have indicated that HPMC-ASP conjugates were synthesized without any significant degradation of the polymer backbone as bimodal absorptions are absent. For instance, the GPC spectrum of sample **2** is shown in Figure 4.

Thermal decomposition temperatures (T_d) were obtained from the thermal analysis for HPMC-ASP conjugates **1**-**4** (T_d 377, 379, 380, and 393 °C, respectively). Therefore, an increased stability trend in the HPMC-ASP conjugates was found with increasing degree of substitution (DS) of ASP onto HPMC polymer. Thermal data showed that HPMC-ASP conjugates are thermally quite stable as pure HPMC (T_d 408 °C). It is important to note here that ASP becomes more stable after attachment onto HPMC as higher T_d values were obtained for the conjugates in comparison with pure ASP (T_d 311 °C). Therefore, extra stability of the ASP was achieved by making its macromolecular prodrugs.

The DS of the HPMC-ASP conjugates **1**-**4** was investigated by UV spectroscopy. Calibration curve for the drug was made at the wavelength 232.5 nm. The samples (10 mg) were hydrolyzed in 0.1 N NaOH, and the free drug was analyzed by UV spectroscopy after making the suitable dilutions, wherever necessary. The absorbance was changed into concentration using the equation generated by regression analysis from the calibration graph, and the dilution factor was also adjusted. The DS expressed in mg of drug/100 mg of the sample was calculated from UV analyses (see Table I).

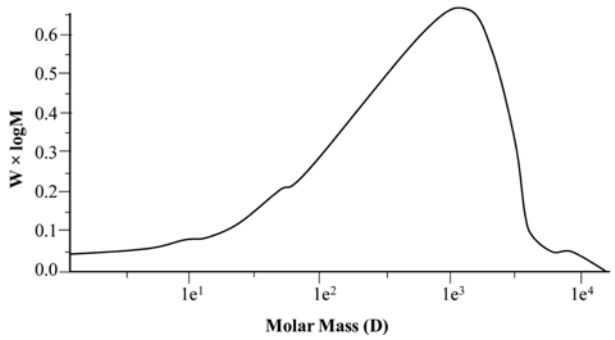

Figure 4. A GPC spectrum of HPMC-acetylsalicylate **2**.

Transmission electron microscopy (TEM) was carried out for imaging the molecular assembly on carbon coated TEM grids which showed particulate aggregation forming the nanoparticle (sample **2** after peracetylation). We believe that the balance of hydrophobicity, and hydrophilicity in conjugates helped in the aggregation of the hydrophilic HPMC chain. Hydrophobic character of conjugates was increased by conversion of free hydroxyl groups onto the HPMC polymer backbone even after the HPMC- conjugates formation. Free hydroxyl groups were peracetylated with acetic anhydride in the presence of 4-dimethylaminopyridine as a catalyst, and pyridine as a base, and solvent. TEM images of the HPMC- conjugates **2** after acetylation are shown in Figure 5(a) and (b). Other samples **1**, **3**, and **4** did not show the formation of nanoparticles before or after peracetylation. Nevertheless, a nanoparticulate drug design was achieved for the HMPC- conjugates **2**.

The Figure 6(a) and (b) shows scanning electron microscopy (SEM) images of HMPC-ASP (sample **2** after peracetylation) nanoparticles. The overview SEM image in Figure 6(a) shows that the nanoaprticles are in the size regime from 250 nm to 550 nm but the majority of the nanoparticles have size of approximately 450 nm. These nanoaprticles are quite stable in water, as confirmed by the SEM image in Figure

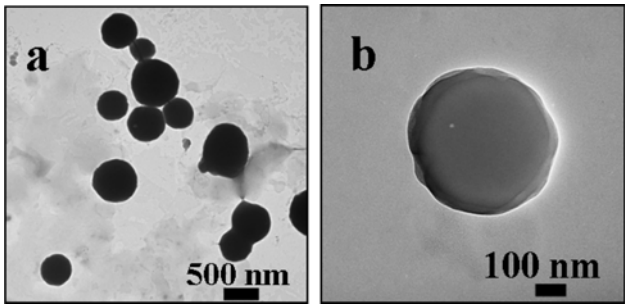

Figure 5. TEM images of the HMPC-ASP conjugates **2** after peracetylation (a) overview and (b) single nanoparticle.

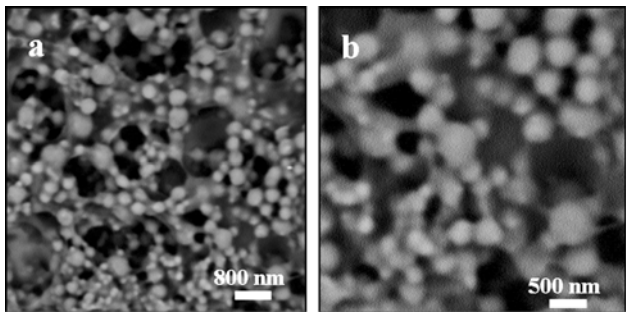

Figure 6. SEM images of HMPC-ASP conjugates **2** after peracetylation showing nanoparticles formation (a) overview after 2 days and (b) overview image after 3 weeks.

Table II. Validation Parameters of HPMC-ASP at Three Different Concentration Levels

Parameter	HPMC-ASP (Mean)
Precision (CV, within Day/ between Days)	1) 0.08/0.15 at 20 μg·mL^{-1} 2) 0.1/0.15 at 100 μg·mL^{-1} 3) 0.09/0.13 at 200 μg·mL^{-1}
Accuracy (% Recovery)	1) 96 at 20 μg·mL^{-1} 2) 95.8 at 100 μg·mL^{-1} 3) 96.1 at 200 μg·mL^{-1}
LOD	20 ng·mL^{-1}
LOQ	40 ng·mL^{-1}
Concentration Range	20-200 μg·mL^{-1}

Table III. Pharmacokinetic Data after a Single Oral Dose of 132.6 mg HPMC-ASP Conjugate Containing of 5.9 mg Active Drug ASP

Parameter	ASP from HPMC-ASP
t_{max}	1.0 h
C_{max}	14.6 μg·L^{-1}
$t_{1/2}$	4.6 h
$AUC_{0-\infty h}$	41.06 h·μg·L^{-1}
V_d[a]	21.8 L·kg^{-1}
Cl[b]	3.23 L·h^{-1}

[a]V_d=Volume of distribution. [b]Cl: Total body clearance for extra- vascular administration.

6(b) which was recorded after three weeks. The nanoparticles retained their morphology.

Pharmacokinetic Studies. The determination of HPMC-ASP was carried out by the use of the validated HPLC method. The limit of detection (LOD), and limit of quantification (LOQ) values for HPMC-ASP were found 20 and 40 ng·mL^{-1}, respectively (Table II). The between days precision near the limit of detection (LOD), in terms of the coefficient of variation (CV), ranged from 0.08 to 0.15, and accuracy, in terms of percent recovery, was found to be greater than 96.0% for HPMC-ASP (see Table II). All performance parameters clearly established the validity of the HPLC method for this study. The pharmacokinetic data following the single oral administration of 132.6 mg HPMC-ASP (5.9 mg active drug ASP) is given in Table III. The plasma concentration-time curve is shown in Figure 7.

The areas under the curves ($AUC_{0-\infty h}$) of the ASP 41.06 h·μg·L^{-1} from the HPMC-ASP conjugate **2**, shows that significant amounts of ASP remain available in the plasma for a longer period of time. The literature shows that after administration of pure ASP, the level of ASP in the plasma rises rapidly to reach maximum with negligible amount remaining after 2 h.[21,22] The elimination of ASP from the HPMC-ASP conjugate **2** follows the first order kinetics with r^2 0.9643. It was noted that about 3.09% (*i.e.* $AUC_{0-\infty h}$×100/ dose) of ASP from HPMC-ASP conjugates of the oral dose

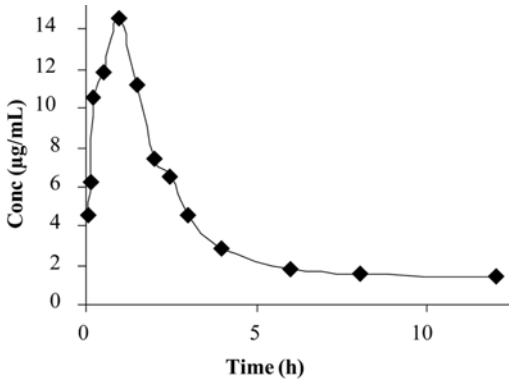

Figure 7. Plasma concentration curve of active drug ASP from HPMC-ASP conjugates **2**.

reaches systemic circulation at t_{max} (1 h). The C_{max} was found to be 14.6 μg·L^{-1} at a t_{max} 1 h, which is about 3% of the administered dose of the HPMC-ASP conjugates **2**. While, normally in case of pure ASP, it is about 4% of the administered dose between 14-15 min.[21-23] The $t_{1/2}$ was 4.6 h, which is closer to the ideal value for once a day dosing for sample **2**. The volume of distribution (V_d), and clearance (Cl) values for ASP released from the HPMC-ASP conjugates **2** were 21.8 L·kg^{-1} and 3.23 L·h^{-1}, respectively. The large V_d, and Cl value may be due to the complex behavior of swelling, dissolution and hydrolysis of HPMC-ASP conjugates in GI tract hence prolong the plasma levels.

Conclusions

Macromolecular prodrugs of aspirin were successfully synthesized and characterized. A nanoparticulate drug design was achieved for ASP. The fabrication of pure, organosoluble, and novel derivatives of neutral, and non-ionic hydroxypropylmethylcellulose (HPMC), *i.e.* HPMC-ASP conjugates (macromolecular prodrugs) was achieved by using homogeneous reaction methodologies. The products have acid resistant HPMC polymer backbone that makes it possible to keep the stomach safe from the harmful effects of such acidic drugs. Nanoparticles of HPMC-ASP conjugates were observed by TEM that may show a better pharmacokinetic profile, and sustained release of the active drug at basic pH of the colon. An HPLC-UV method was successfully developed to study the pharmacokinetic profile of HPMC-ASP conjugates. Present study has shown a better pharmacokinetic profile as compared with pure ASP. Especially, an increased $t_{1/2}$ was obtained by converting ASP to the HPMC-ASP (prodrug). Moreover, formulation designs of newly fabricated HPMC-ASP conjugates are the future plans.

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