RAPID COMMUNICATION

HPMC-Salicylate Conjugates as Macromolecular Prodrugs: Design, Characterization, and Nano-Rods Formation

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INTRODUCTION

By the development of prodrugs, reduction in amount and frequency of dose can easily be achieved by more targeted drug delivery benefits of prodrugs, which indirectly increases the patient compliance.[1,2] In these days, fabrication of macromolecular prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs) by the covalent attachment of the drugs with biopolymers as an ester moiety is getting greater attention. In general, such ester conjugates of NSAIDs can easily be hydrolyzed at basic medium of colon in particular. Such linkages cannot be affected much by acidic hydrolysis in stomach. Hence, by this conversion of NSAIDs as an ester moiety onto glycopolymers, stomach can easily be kept secure by the harmful effects of the NSAIDs. Indirectly, by this way, colon specific drug delivery can easily be achieved.

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Polysaccharides (glycopolymers) are entering into colon targeted drug delivery with high potential.[3,4] Glycopolymers "the most promising macromolecular carriers" made possible to deliver a wide variety of drugs, e.g., peptides, proteins, nucleic acid, NSAIDs, anticancer drugs, and sensitive drugs to acidic environment directly to colon.[5–7] Use of glycopolymers are advantageous because these polymers target the site of action, increase the intensity and/or prolong pharmacologic action and/or reduce toxicity of small molecule drugs, proteins, or enzymes, and so forth.

A significant number of publications have been reported about the macromolecular prodrugs fabrication of NSAIDs with polysaccharides and other polymers in which useful results were obtained.[8–11] However, there is no literature found for the use of cellulose ethers as drug career by making its ester derivatives. Cellulose ethers are of great research interest in the areas of biomedical and pharmaceutical sciences, for example, as carriers for drug targeting, vaccine bullets, sustained release of drug and material for disintegration of matrix tablets. On the other hand widely used cellulose ether, HPMC is semisynthetic, nonionic,

Table 1. Reaction Conditions and Results of Esterification of HPMC Dissolved in DMAc Using *In Situ* Activated Salicylic Acid with Tos-Cl at 80 °C for 24 h

Sample	Molar Ratio[a]	DS[b] (mg/100 mg)	Yield (g)	Solubility
1	1:1:1:3	1.8	1.29	DMSO, DMAc, water
2	1:3:3:6	2.9	1.65	DMSO, DMAc, water
3	1:6:6:12	6.1	1.95	DMSO, DMAc, water

[a] HPMC:salicylic acid:Tos-Cl:TEA.
[b] DS was calculated by UV spectroscopy.

nontoxic, cheep, neutral, acid resistant and biocompatible, which makes it a valuable tool for the fabrication of macromolecular prodrugs. All the sustained release studies and targeted drug delivery from the HPMC, its gels and matrix were studied as noncovalent interaction of HPMC with several drug molecules.[12–17]

We are focused to design macromolecular prodrug of salicylic acid with HPMC by using a versatile and powerful reagent Tos-Cl[18–25] under homogeneous reaction conditions. Aim was also focused to fabricate novel amphiphilic HPMC-salicylic acid conjugates to get their double advantage. First, synthesis of novel macromolecular prodrugs of salicylic acid with HPMC for colon targeted drug delivery. Second, such macromolecular prodrugs itself is a very good system for sustained release of salicylic acid after hydrolysis in basic medium of colon. Third, these conjugates will be amphiphilic in nature hence self-assembled nanostructures can be observed which will add into design of sustained release formulations at nanolevel. Sustained release studies and formulation development of newly fabricated HPMC-NSAIDs conjugates are the future plans.

EXPERIMENTAL

Materials

Hydroxypropylmethylcellulose (HPMC-E5, United States Pharmacopoeia (USP) 26; with hydroxypropyl moiety 7.5% and O-methyl groups 28%) obtained from Zhejiang Zhongbao Imp and Exp Corp, was dried under vacuum at 110 °C for 8 h. Analytical grade organic solvents, reagents and other chemicals obtained from Fluka were used as received. Salicylic acid used was according to the USP standard.

Measurements

^{1}H NMR spectra (400 MHz, 16 scans) of the HPMC-salicylic acid conjugates were acquired in D_2O. ^{1}H, ^{1}H COSEY NMR spectrum was recorded at 40 °C in D_2O. FTIR spectra were measured on IR 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 °C up to 1150 °C. Elemental analysis was performed by CHNS 932 Analyze (Leco Cor. USA). Ultraviolet visible (UV) Pharmspec 1700 (SCHIMADZU, Japan) was used to quantify the degree of substitution of pendant groups on HPMC- nonsteroidal anti-inflammatory agents (NSAIDs) conjugates. Solutions were prepared in distilled water as solvent along with 1*N* NaOH solution and UV spectra were recorded from 190 to 400 nm. For GPC analysis, 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 eluant (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) using a Philips 420 instrument with an acceleration voltage of 120 kV.

Fabrication of HPMC-Salicylic Acid Conjugates, a Typical Example

For typical preparation, 2 g of dry HPMC was added to 30 mL DMAc. Mixture was kept under stirring for 2 h to obtain optically clear solution of HPMC. To the solution of 2 g HPMC in DMAc, 4.992 g Tos-Cl (3 mol/mol of HPMC), 7.386 mL triethylamine (6 mol/mol HPMC) and 3.90 g salicylic acid (3 mol/mol HPMC) were added. Reaction mixture was heated and stirred under nitrogen at 80 °C for 24 h. Isolation of the product was carried out by precipitation of reaction mixture into 200 mL diethyl ether. Precipitates were washed with diethyl ether thrice by stirring to remove any of the unreacted drug contents and impurities. Precipitates were then dried under vacuum at 50 °C for 24 h to yield product **2**.

Yield: 1.65 g. $DS_{SALICYLIC\ ACID}$ = 2.9 mg/100 mg of HMPC-salicylate (determined by UV spectroscopy). FTIR (KBr): 3446, 3433 υ (OH), 2931, 2839 υ (CH), 1625 υ ($C{=}O_{Ester}$) cm^{-1}. ^{1}H NMR (D_2O): δ (ppm) = 3.13

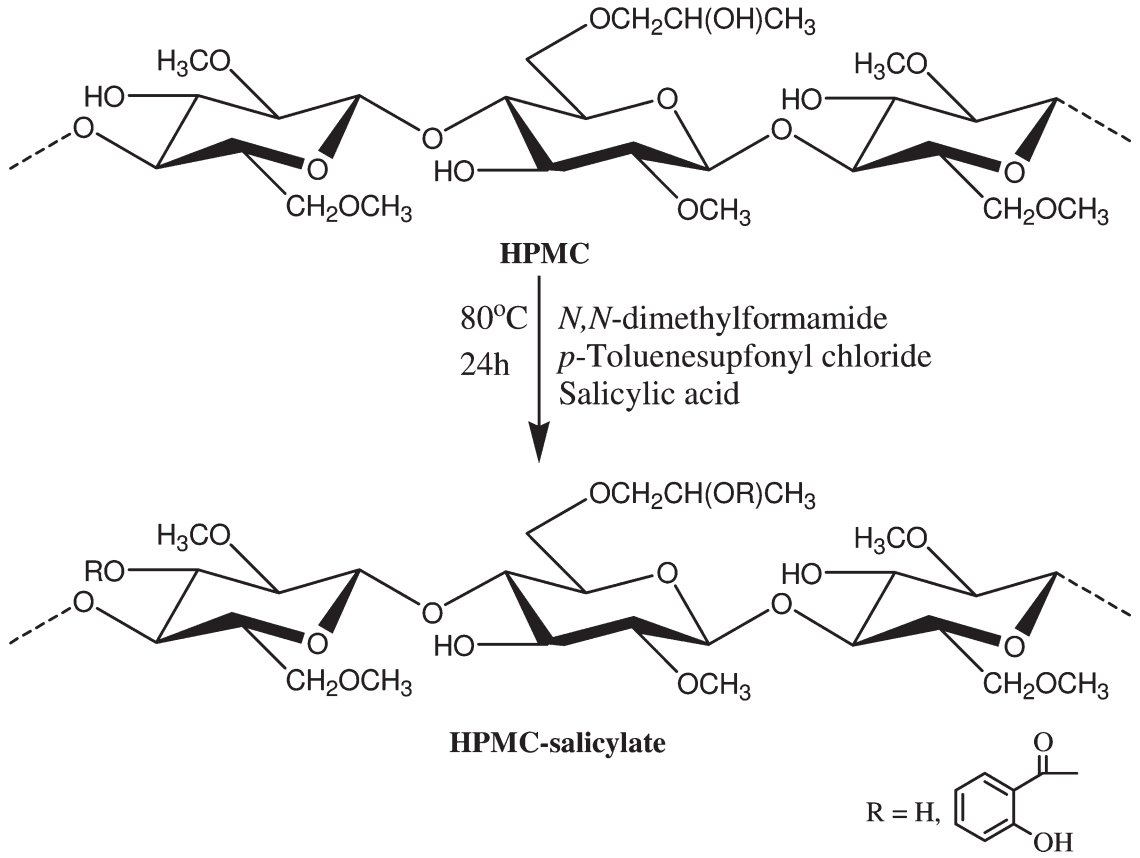

Figure 1. Synthesis of HPMC-salicylate applying *in situ* activation with Tos-Cl.

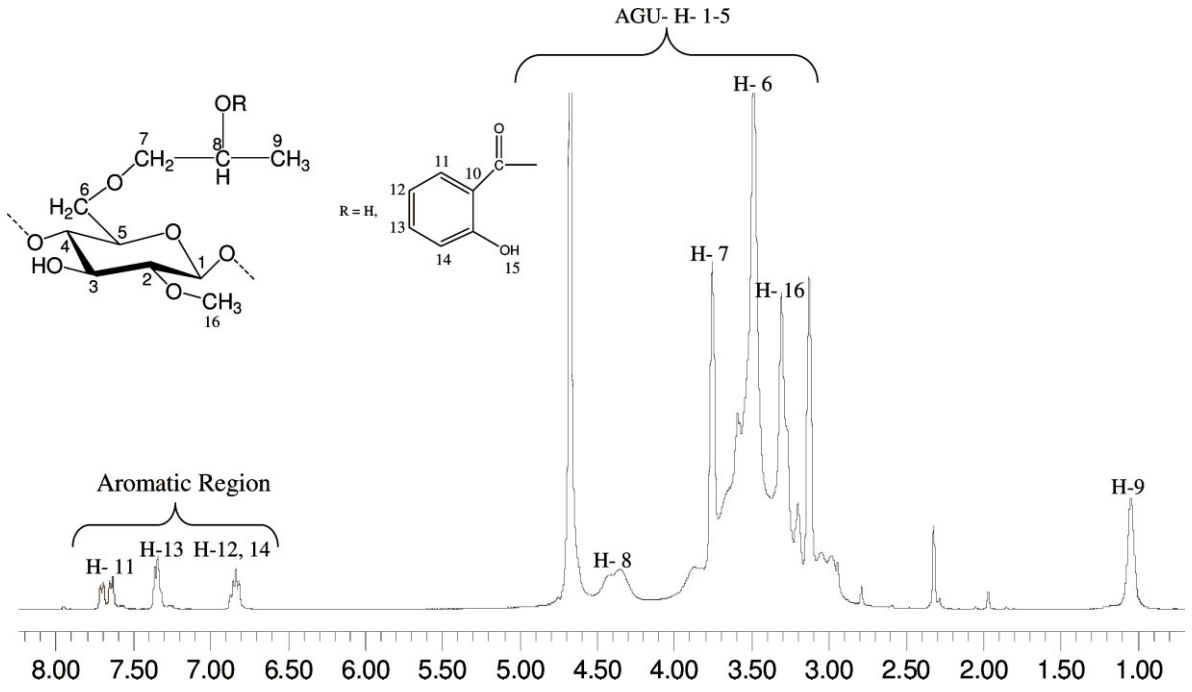

Figure 2. ^{1}H NMR (D_2O) spectrum of HPMC-salicylate **1**.

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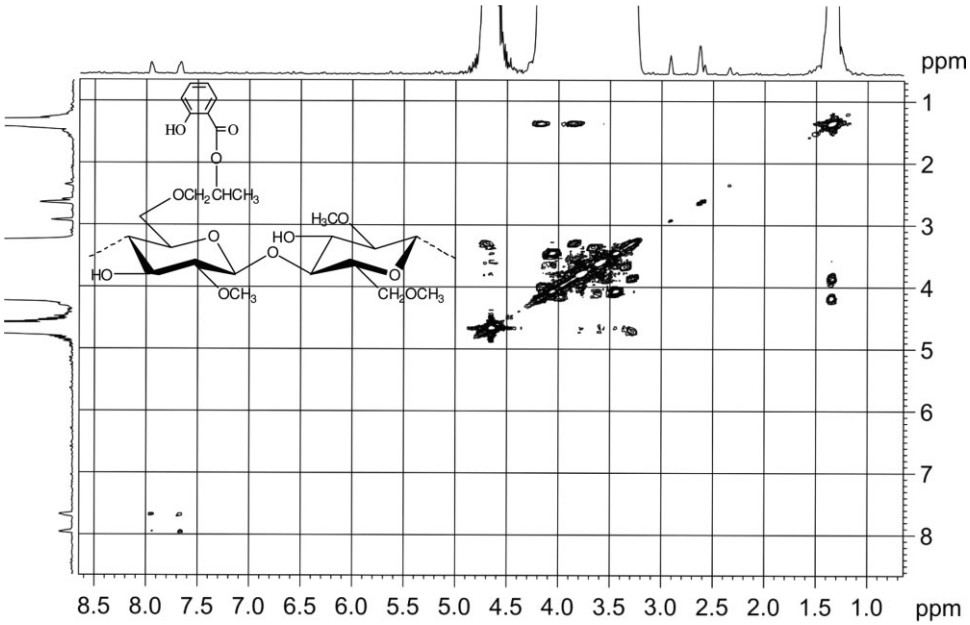

Figure 3. ^{1}H, ^{1}H COSY NMR (D_2O) Spectrum of HMPC-salicylate **2**.

(H-4), 2.85–4.80 (other anhydroglucose unit -H), 3.76 (H-7), 4.38 (H-8), 1.05 (H-9), 7.66 (H-11), 6.86 (H-12, 14), 7.36 (H-13)

UV Spectroscopic Analysis

For UV analysis 10 mg of sample was taken in round bottomed flask followed by the addition of 10 mL 1 N NaOH. 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}) 301 nm. The molar extinction coefficient was found to be 98.08.

Transmission Electron Microscopy

Nanoparticles were prepared using dialysis process. In a typical batch, 170 mg of HPMC-salicylate sample was dissolved in 5 mL of purified DMSO and was dialyzed against distilled water for 4 days. The suspension obtained was concentrated using rotary vacuum evaporator and investigated by transmission electron microscopy (TEM). A droplet (5 mL) of the freshly sonicated solution (self-assembled HPMC-salicylate aqueous solution) was placed on hydrophilized carbon films on copper wire grids and excess fluid was blotted off and air-dried on the grids.

RESULTS AND DISCUSSION

To prepare the macromolecular prodrugs of salicylic acid, HPMC dissolved in DMAc was allowed to react with salicylic acid and Tos-Cl. Triethylamie was used as a base. Reaction was carried out for 24 h at 80 °C. Tos-Cl first reacts with salicylic acid to yield different reactive intermediates, that is, acid chloride, acid anhydride and mixed anhydride of Tos-Cl and the salicylic acid. These intermediates are very reactive acylating agents produced *in situ* react with the -OH available on HPMC polymer backbone to yielded esters as macromolecular prodrugs, that is, HPMC-salicylic acid conjugates. The results are summarized in Table 1.

HPMC-salicylic acid conjugates (**1–3**) were prepared using different molar ratios (Fig. 1). For solubility of the product, 50 mg sample was dissolved in 10 mL solvent and stirred for 30 min at room temperature in closed vial. All of the samples were found soluble in DMSO, water and DMAc. All of the products were characterized by means of UV and FTIR spectroscopy, elemental analysis, TGA/DTA/DSC techniques, GPC, and

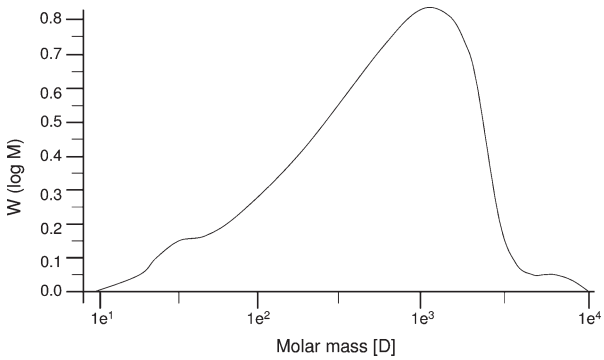

Figure 4. GPC spectrum of HPMC-salicylate **2**.

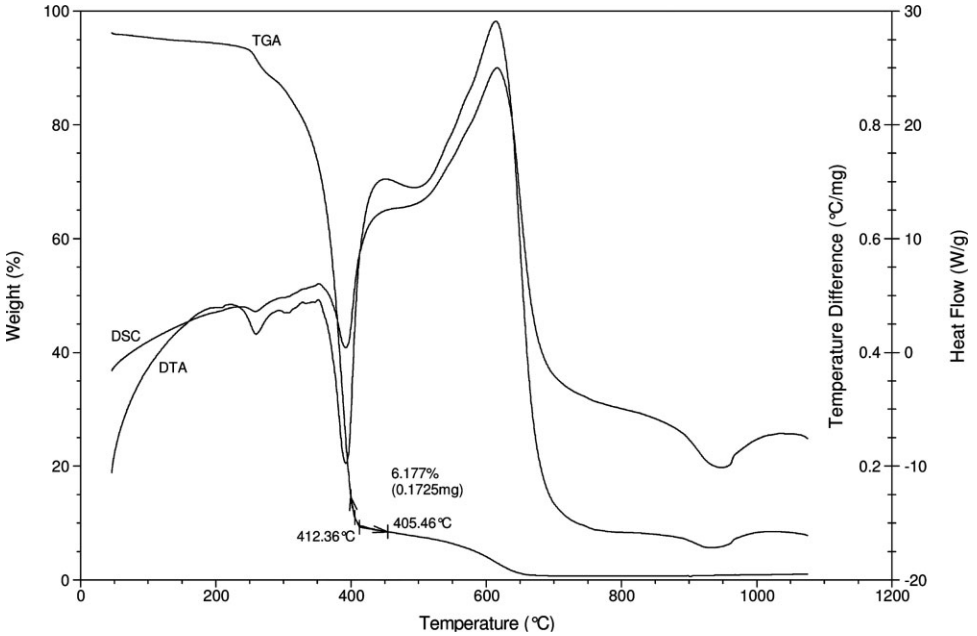

Figure 5. DTA, TGA and DSC spectra of HPMC-salicylate **2**.

NMR spectroscopy as well as ^{1}H NMR spectroscopy after peracylation.

HPMC-salicylic acid conjugates have shown a characteristic peak in FT-IR (KBr) spectra typical for the ester moiety at about 1740 cm^{-1} (C=O$_{Ester}$). The spectrum displayed hydroxyl group absorption at 3340 cm^{-1}, aromatic C—H absorption at 2962 cm^{-1} and CH_2 absorption at 1446 cm^{-1}. FTIR Spectra showed the success of reaction due to carbonyl, aromatic, and ester absorptions. Elemental analysis has revealed the absence of sulfur in all of the samples showing that there is no introduction of tosylate groups either covalently bounded or as an impurity.

All of the samples were soluble in water hence, ^{1}H NMR spectra of HPMC-salicylates (**1–3**) were recorded in D_2O. A typical ^{1}H NMR spectrum of sample **1** is shown in Figure 2. All the spectra (1–3) have revealed the presence of aromatic ring attached to HPMC polymer backbone as aromatic protons were detectable at δ 7.66 (H-11), 6.86 (H-12, 14), and 7.36 (H-13) ppm, showed that the unsaturated system is not destroyed during the conversion. The protons of HPMC polymer backbone anhydroglucose unit (AGU) were detected at δ 3.13 (H-4) and 2.85–4.80 (other AGU-H) ppm. The protons of hydroxypropyl moiety of HPMC polymer were detected at δ 3.76 (H-7), 4.38 (H-8), and 1.05 (H-9) ppm. FTIR spectrum has already indicated aromatic ring absorptions. A representative ^{1}H, ^{1}H COSEY NMR spectrum (40 °C) has been shown in Figure 3. The spectrum has indicated well-resolved sugar signals along with substitution of salicylic acid group onto polymer as aromatic signals can be viewed.

GPC was applied to investigate hydrolytic degradation of the polymer chain during the reaction. GPC results have indicated that HPMC-salicylates were synthesized with almost no degradation of polymer backbone. For instance, GPC spectrum of sample **2** is shown in Figure 4.

Thermal decomposition temperatures Td (405 °C) of HPMC-salicylates were obtained from thermo gravimetric analysis. A simultaneous thermal analysis (TGA, DTA and DSC) is being shown in Figure 5 for sample **2**. Spectra have shown that HPMC-salicylic acid conjugates are thermally as stable as native HPMC (Td 408 °C).

The Degree of substitution (DS) of HPMC-salicylic acid conjugates (**1–3**) was calculated by UV spectroscopy. Calibration curve for the drug was made according to British Pharmacopoeia (BP) standard at the

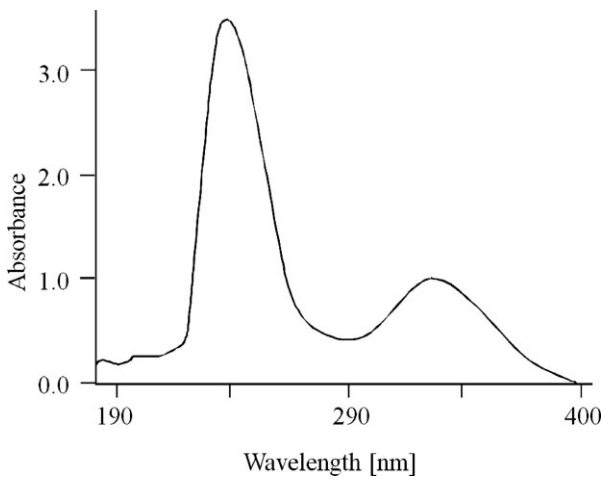

Figure 6. A typical UV absorption spectrum of HPMC-salicylate **2**.

Table 2. Results of UV Spectroscopy: Absorption, Concentration, and DS of HPMC-Salicylates Measured from Respective Calibration Graph

Sample	Absorbance	Concentration (ppm)	Adjusted Concentration (ppm)	DS (mg/ 100 mg)
1	0.4928	18.95	18.95	1.8
2	0.7552	29.05	29.05	2.9
3	0.7943	30.55	61.10	6.1

wavelength 301 nm for salicylic acid. The samples (10 mg) were hydrolyzed in 1 N NaOH (10 mL) at 80 °C for 1 h and the free drug was analyzed by UV spectroscopy after making suitable dilutions, wherever necessary. A typical spectrum of sample **2** is shown in Figure 6.

Solution of sample **3** was diluted two times while solutions of other samples were used as such. 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 is expressed as milligrams of drug per 100 mg of the sample and was possible to calculate from UV analyses. Results from UV spectroscopy are summarized in Table 2.

TEM imaging of the molecular assembly on carbon coated TEM grids showed particulate aggregation in nano-rod shape. We believe that formation of such nano-rods is because of hydrophobic aromatic portion of salicylate moieties, which help aggregation of hydrophilic HPMC chain. TEM images of HPMC-salicylate **2** are being shown in Figure 7. HMPC-salicylate nano-rods were readily soluble in aqueous and organic media.

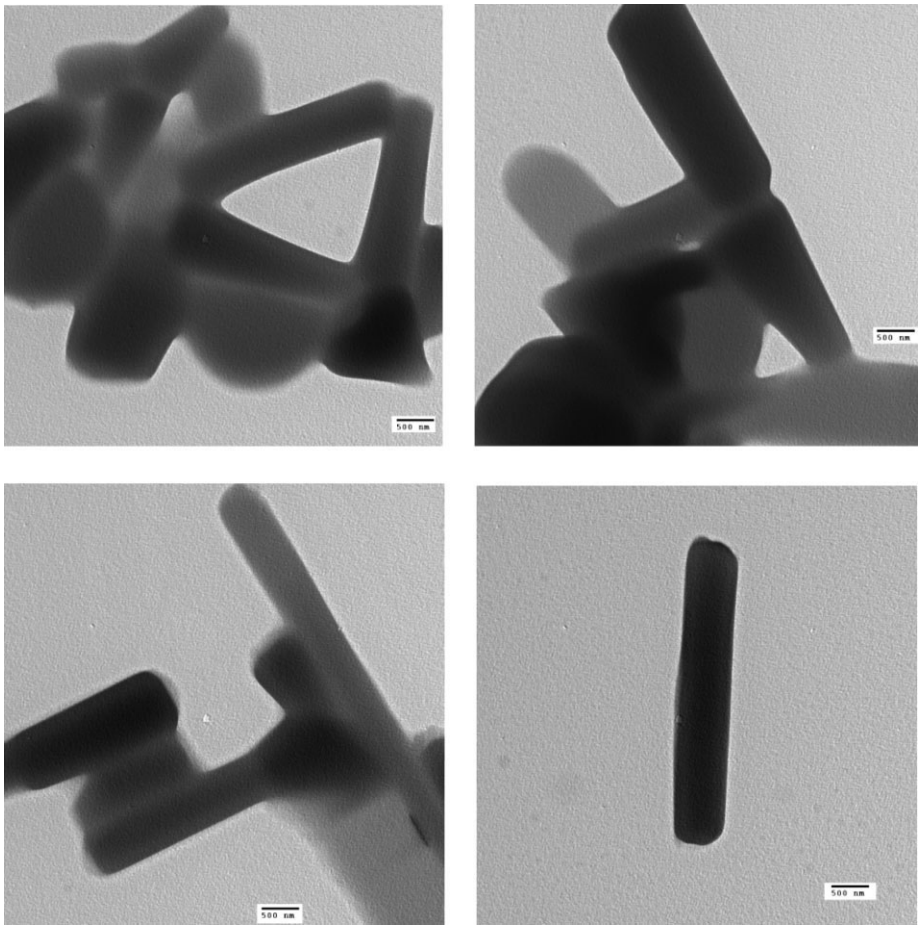

Figure 7. Transmition electron microscopy of HMPC-salicylate **2** showing nano-rod formation.

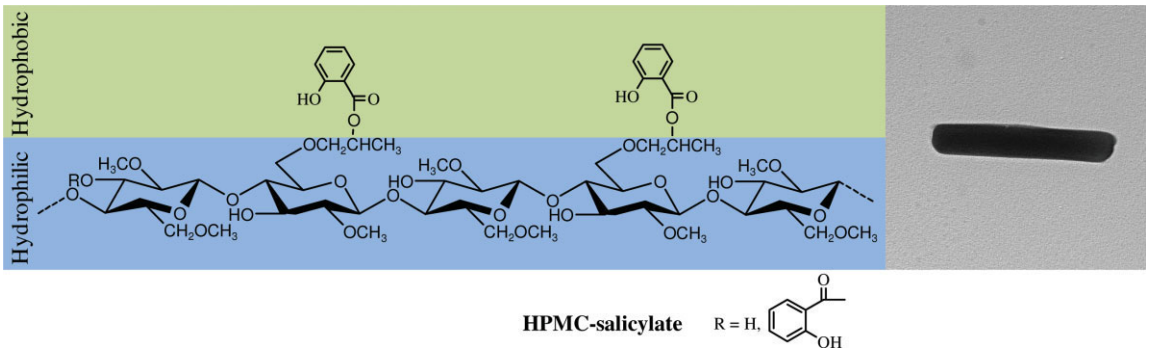

Figure 8. Illustration of nano-rods formation of HMPC-salicylates observed in TEM.

An illustration of nano-rods assembly of macromolecular prodrug, that is, HPMC-salicylate is given in Figure 8. After the attachment of hydrophobic salicylic acid moieties onto hydrophilic HPMC polymer backbone, amphiphilic character was established which tends to form nano-rods.

CONCLUSIONS

Fabrication of novel macromolecular prodrugs of salicylic acid with neutral and nonionic hydroxypropylmethylcellulose (HPMC) was achieved by using homogeneous reaction conditions. These amphiphilic macromolecular prodrugs of salicylic acid were thoroughly characterized by NMR, UV, TGA, DTA, DSC, and TEM. All the products obtained were highly pure and organosoluble. Nano-rods were observed by TEM in solution, hence, we believe that macromolecular prodrug will show sustained release at basic pH of colon. Nevertheless, colon targeted drug designing at nanolevel was achieved. Moreover, we are studying pharmaceutical properties, that is, formulation development and sustained release of drugs from nano-rods conjugates.

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