Na-Montmorillonite-Dispersed Sustainable Polymer Nanocomposite Hydrogel Films for Anticancer Drug Delivery

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Supporting Information

ABSTRACT: Nanocomposite hydrogels have found a wide scope in regenerative medicine, tissue engineering, and smart drug delivery applications. The present study reports the formulations of biocompatible nanocomposite hydrogel films using carboxymethyl cellulose-hydroxyethyl cellulose-acrylonitrile-linseed oil polyol (CHAP) plain hydrogel and Na-montmorillonite (NaMMT) dispersed CHAP nanocomposite hydrogel films (NaCHAP) using solution blending technique. The structural, morphological, and mechanical properties of resultant nanocomposite hydrogel films were further investigated to analyze the effects of polyol and NaMMT on the characteristic properties. The synergistic effect of polyol and nanofillers on the mechanical strength and sustained drug-release behavior of the resultant hydrogel films was studied, which revealed that the increased cross-link density of hydrogels enhanced the elastic modulus (up to 99%) and improved the drug retention time (up to 72 h at both pHs 7.4 and 4.0). The release rate of cisplatin in nanocomposite hydrogel films was found to be higher in CHAP-1 (83 and 69%) and CHAP-3 (79 and 64%) than NaCHAP-3 (77 and 57%) and NaCHAP-4 (73 and 54%) at both pHs 4.0 and 7.4, respectively. These data confirmed that the release rate of cisplatin in nanocomposite hydrogel films was pH-responsive and increased with decrease of pH. All nanocomposite hydrogel films have exhibited excellent pH sensitivity under buffer solution of various pHs (1.0, 4.0, 7.4, and 9.0). The in vitro biocompatibility and cytotoxicity tests of these films were also conducted using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide assay of human embryonic kidney (HEK-293) and human breast cancer (MCF-7) cell lines up to 48 h, which shows their biocompatible nature. However, cisplatin-loaded nanocomposite hydrogel films effectively inhibited the growth of human breast MCF-7 cancer cells. These studies suggested that the proposed nanocomposite hydrogel films have shown promising application in therapeutics, especially for anticancer-targeted drug delivery.

INTRODUCTION

Cancer is a life-alarming disease responsible for an alarming rate of death worldwide. The regular treatment of cancer includes surgical procedure, radiotherapy, and chemotherapy.1 Chemotherapy is the utmost effective remedy for introducing the anticancer drugs into the patient at targeted point to eliminate the malignant cells. Among the abundant anticancer drugs, cisplatin (cis-diammine dichloroplatinum(II)) is the first-generation platinum-based chemotherapeutic drug, which is beneficial for the cure of gastrointestinal, head, neck, ovarian, breast, genitourinary, and lung cancers.2 However, the use of cisplatin in cancer therapy is still limited because of its nonspecific biodistribution and severe side effects, including neurotoxicity, ototoxicity, myelosuppression, nausea, and vomiting.3 In this regard, several attempts have been made to develop more efficient drug carriers such as polymeric micelles, dendrimers, liposomes, lipoprotein-based biodegradable nanoparticles, and hydrogels.4 Among these, hydrogels have emerged as the smart vectors and a promising alternative. These smart systems release the drug at the site of application in a sustained manner, due to their stimuli (temperature, pH, electric field)-responsive behavior.

Cellulose is one of the most important biodegradable polymers easily available in the market, and it could be considered as a possible candidate for fabricating hydrogels. Cellulose has been widely used in the textile, paper, and agriculture and food industries besides being used in specific-purpose biomaterials, such as hemodialysis devices, wound dressing, tissue engineering, and drug delivery due to its biocompatible, biodegradable, and nontoxic nature.5–7 How-
ever, due to its insolubility in water for fabrication of hydrogel, there is a large interest toward its derivatives. Various cellulose byproducts are derived from cellulose via chemical reactions, such as cellulose acetate, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose, carboxymethyl cellulose (CMC), methylcellulose, and cyanoethyl cellulose. Among the various derivatives, HEC and CMC had been produced extensively and are water-soluble biomaterials. However, CMC cannot be used as it tends to form intramolecular rather than intermolecular cross-links. For this reason, CMC, in its sodium salt form (NaCMC), has been used along with HEC, whose introduction avoids the formation of intramolecular cross-links because of the presence of polar functional (−OH) groups that enhances their in vivo degradation. HEC exhibits stability, suspension, dispersing ability, etc. The hydrogel films fabricated from CMC and HEC have many inherent advantages such as good film-forming capacity, biodegradability, biocompatibility, and natural abundance. Therefore, these hydrogels can be used to deliver a number of therapeutics (proteins, vaccines, enzymes, drugs). However, CMC- and HEC-based hydrogels are associated with poor mechanical properties, extensive swelling, rapid rupture, and fast in vitro biodegradability, which minimize their efficiency during the course of application. Hence, several methodologies have been established to improve and strengthen their inter/intramolecular interactions via increasing their cross-link density within the hydrogel structure through physical blending, interpenetrating networks, and the incorporation of different nanofillers. The utilization of nanofillers led to the development of highly stable nanocomposite hydrogels. Among various nanofillers, Na-montmorillonite (NaMMT), a smective-type clay, is composed of an expandable 2:1 type of alumino silicate clay mineral, which has a layered structure and also a relatively high cation exchange capacity, large specific surface area, good swelling capacity, and high platelet aspect ratio. The literature reveals that the use of low-cost nanoclay in the hydrogel matrix induces higher mechanical, thermal, hydrophilic, and organophilic properties in resultant nanocomposites. In addition, NaMMT is a versatile material for the preparation nanocomposite hydrogels for new drug-delivery systems, to promote the effective loading of drugs and their release rate reveals that the use of low-cost nanoclay in the hydrogel matrix induces higher mechanical, thermal, hydrophilic, and organophilic properties in resultant nanocomposites. For instance, Wang et al. reported quaternized chitosan/montmorillonite-based composites with enhanced drug encapsulation efficiency and decreased the drug release rate. Iliescu et al. prepared nanocomposite beads based on montmorillonite and sodium alginate as drug carriers. The literature further reveals that the delivery of cisplatin through various hydrogels exhibits short retention time. Thus, there is a need to develop hydrogels that can induce higher retention time for the delivery of cisplatin. In view of this, we have developed Na-montmorillonite-dispersed nanocomposite hydrogels to investigate the delivery of cisplatin and its retention time.

Considering the synergistic contribution of natural polymers and NaMMT in the development of biomaterials, our hypothesis in the present study was to disperse the NaMMT into the HEC. The CMC matrix shows controlled water uptake capacity and drug release rate, through illuminating the drug release time of the nanocomposite hydrogel films. To validate this hypothesis, we report a facile method to fabricate a novel nanocomposite hydrogel film using HEC, CMC,

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acrylonitrile (AN), sustainable polyols (cross-linking agents), and NaMMT (nanofillers) via solution blending technique for controlled release of cisplatin. The effect of concentration of 3 and 6 mg of NaMMT on the assembly, morphology, mechanical and swelling properties, biocompatibility, cytotoxicity, and drug release profile of these films was investigated by Fourier transform infrared (FT-IR), X-ray diffraction (XRD), scanning electron microscopy (SEM), tensile strength techniques, 3-(4,5-dimethylthiazole-2-yl-2,5-diphenyl tetrazolium bromide) (MTT) assays of normal human embryonic kidney (HEK-293) and human breast cancer (MCF-7) cell lines, and UV–vis spectrometry. Cisplatin can be taken as a model drug to prove that these nanocomposite hydrogel films are applicable for targeting chemotherapy. The prolonged and sustained release rate of cisplatin from the nanocomposite hydrogel films in the present study is found to be higher than that of others hydrogels for cisplatin delivery,3,19–22 which can be attributed to the selection of sustainable (biodegradable, biocompatible) polymers and Na-montmorillonite dispersion that showed efficiently drug delivery.

■ RESULTS

Synthesis and Formulation of CMC-HEC-AN-LP (CHAP) and NaCHAP Nanocomposite Hydrogel Films. The facile strategy is via solution blending for the preparation and formulation of plain and nanocomposite hydrogel films, using biodegradable and biocompatible CMC-HEC-AN, linseed oil polyl (LP), and NaMMT as matrix, cross-linker, and nanofillers, respectively, as per reaction scheme (Scheme 1). The interactions between different polar functionalities of CMC, HEC-AN, and polyl led to the formation of a stable CHAP hydrogel network. The processed hydrogel was further stabilized through the dispersion of NaMMT nanofillers in CHAP matrix solution (Scheme 1), resulting in the formation of NaCHAP nanocomposites. The solutions of these hydrogels were cast in Petri plates and placed in an vacuum oven at 27 °C for 4–5 days to obtain their respective films of around ∼75 μm thickness. The tensile strength test on these films was conducted on universal testing of machine, which shows that the nanocomposite hydrogel films have superior mechanical strength (Figure 3). These films (plain and nanocomposite hydrogels) were used for further studies.

FT-IR Spectroscopy of CHAP and NaCHAP Nanocomposite Hydrogel Films. The FT-IR spectra (Figure 1a) of CHAP-1, CHAP-3, and NaCHAP-3 confirm the successful formation of plain and nanocomposite hydrogels. The absorption peaks at 1544 and 1404 cm⁻¹ are assigned to the symmetric and asymmetric stretching of COO⁻ groups, while the peak at 1376 cm⁻¹ is attributed to –OH bending vibration.23 The peak at 1745 cm⁻¹ in CHAP-3 and NaCHAP-3 is attributed to the stretching of C=O bond, which confirms the formation of ester linkage and signifies the cross-linking between polymer matrix and polyl.24 The absence of this peak (1745 cm⁻¹) in CHAP-1 reveals the lack of cross-linking within the hydrogel structure.24 The absorption band with multiple peaks in the range of 1121–1017 cm⁻¹ is attributed to the formation of ether bonds in CMC backbone.25 The band at 2928 cm⁻¹ is due to C–H stretching vibration. The shifting of –OH bands (CHAP-1) from 3268 to 3260 cm⁻¹ (CHAP-3) and 3254 cm⁻¹ (NaCHAP-3) is due to the presence of electrostatic interactions between the –OH groups of NaMMT and those of CMC, HEC, and polyl.26 In addition, the two vibrational peaks at 3314 and 3268 cm⁻¹ in the case of CHAP-1 merged after the incorporation polyl and NaMMT into the CHAP matrix and appeared at 3254 cm⁻¹ of –OH groups, which indicate the occurrence of chemical interaction between –OH groups of polyl and NaMMT. These results suggest the formation of hydrogen bonding between CHAP hydrogel and NaMMT, resulting in the formation of nanocomposite (NaCHAP) hydrogels. The additional absorption peaks at 2111 and 2100 cm⁻¹ (C≡N group) were pragmatic in NaCHAP-3, CHAP-3, and CHAP-1, indicating superficial AN grafting on CHAP and NaCHAP nanocomposite hydrogel films.27

XRD Analysis of CHAP and NaCHAP Nanocomposite Hydrogel Films. Figure 1b represents the XRD patterns of CMC, CHAP-3, and NaCHAP-3. XRD patterns were recorded in the range of 5–60° (2θ). CMC exhibits a broad peak at about 20.2°, which indicates that the CMC possesses an amorphous structure.28 However, the diffraction patterns of CHAP-3 and NaCHAP-3 show the presence of a prominent peak at 25.2°, which exhibits a positive blue shift of 5.2°, i.e., from 20.2 to 25.2°, can be corroborated to the incorporation of HEC and polyl within the amorphous structure of CMC, resulting in the formation of amorphous structure of CHAP-3 and NaCHAP-3. In addition, the presence of additional sharp peaks at 17, 28, and 33° in CHAP-3 and NaCHAP-3 indicates grafting of acrylonitrile into CHAP-based polymer matrix.27 However, the NaCHAP-3...
exhibits a decrease in the intensity of the sharpness of the peak at 25.2° after the dispersion of NaMMT within the polymer matrix, which indicates the presence of strong interaction between the −OH groups of NaMMT and those of CHAP hydrogels. The X-ray diffractogram of NaCHAP-3 (nanocomposite) corresponds to a full width at half-maximum of 0.145, having an average particle size of 63 nm, calculated by Scherrer equation. The particle size of around 60–70 nm calculated with XRD is in good agreement with transmission electron microscopy (TEM) analysis.

**SEM of CHAP and NaCHAP Nanocomposite Hydrogel Films.** The internal morphologies of CHAP-1, CHAP-3, and NaCHAP-3 films were recorded on SEM at two different magnifications, as shown in Figure 2. The surface morphology of CHAP-1 (Figure 2a,a") exhibits a relatively smooth and continuous structure, whereas the surface of CHAP-3 shows pores and a highly cross-linked structure (Figure 2b,b"). This can be attributed to the presence of a considerable amount of polyol (10 mL) on the surface of CMC/HEC, which resulted in the formation of a cross-link structure of polymer matrix with increased mechanical strength. On the other hand, the surface morphology of NaCHAP-3 (Figure 2c,c") shows a homogeneous distribution of NaMMT, implying good interaction between NaMMT and CMC/HEC/AN/PO, which is ascribed to the good compatibility achieved through the physical, chemical, and hydrogen bondings between CMC, HEC, AN-PO, and NaMMT.

**TEM of CHAP and NaCHAP Nanocomposite Hydrogel Films.** The size and distribution pattern of NaMMT nanoparticles within the polymer matrix were studied at different magnifications using TEM. The transmission electron micrographs of NaCHAP-3 (Figure S1) reveal that the nanoparticles of NaMMT are well dispersed within the polymer (CHAP) matrix and show spherical globular morphology of relatively uniform size in accordance with the particle size as estimated in the previous XRD section by the Scherrer equation (60–70 nm). The brighter region represents polymer matrix, while the dark spots are of NaMMT nanoparticles. The dispersion of dark-phase nanoparticles embedded within the bright polymer (CMC/HEC/AN/PO) reveals the grafting of polymer matrix on the surface of exfoliating NaMMT nanoparticles through hydrogen bonding besides the other strong electrostatic interactions between the functionalities of filler and matrix of nanocomposite hydrogels. This suggests that the exfoliated NaMMT nanoparticles

**Figure 2.** SEM images of freeze-dried liquid N₂ of CHAP-1 at 1500× and 500× (a and a’); CHAP-3 at 500× and 1000× (b and b’); and NaCHAP-3 at 20 000× and 5000× magnification (c and c’).
act as a physical cross-linker,\textsuperscript{17} which enhances the stability and tensile strength of nanocomposite hydrogels.

**Tensile Strength of CHAP and NaCHAP Nanocomposite Hydrogel Films.** The mechanical properties of CHAP NaCHAP hydrogel films were studied in terms of tensile strength and elastic modulus. These tests were performed on CHAP and NaCHAP films at room temperature (25°C). The stress–strain curves (Figure 3a) of these films confirmed that the nanocomposite hydrogel films exhibit significantly superior mechanical properties compared to the CHAP hydrogel film. These curves (Figure 3a) show that the robustness of nanocomposite hydrogel films increases with increased loading of NaMMT (3 and 6 mg). The tensile strength tests show an increasing trend, viz., 22.27, 22.96, 24.86, and 26.42 MPa, respectively, for CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 films, while the % tensile stresses of the CHAP-3, NaCHAP-3, and NaCHAP-4 films were found to be 8.88, 23.59, and 48.43%, respectively, higher than CHAP-1. The increase in tensile strength and % tensile-stress-bearing ability of nanocomposite hydrogel films can be attributed to the higher intermolecular interactions of nanofillers and polymer matrix as well as chain entanglement of the polyol with those of CMC/HEC molecules. On the other hand, the incorporation of NaMMT further improved the intermolecular interactions, consequently resulting in the increase of % tensile strength of nanocomposite films, i.e., NaCHAP-3 (23.59%) and NaCHAP-4 (48.43%) as compared to CHAP-3 (8.88%).\textsuperscript{31} The stress–strain curves for nanocomposite (NaCHAP-3 and NaCHAP-4) hydrogel films are linear in nature, while those of plain hydrogel (CHAP-1 and CHAP-3) films are parabolic in nature.

In addition, the elastic moduli of these films were determined using the slopes of the linear region of the stress–strain curves (Figure 3b), recorded in increasing order (3.63, 4.07, 5.01, and 6.34 GPa, for CHAP-1, CHAP-3, NaCHAP-3 and NaCHAP-4 respectively) of these films, and

![Figure 3. Stress–strain curves of CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 (a). Tensile stress and elastic modulus of CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 (b). The error bars in the graph represent standard deviations (n = 5).](image)

![Figure 4. Swelling ratios of CHAP and NaCHAP at pHs 1.0, 4.0, 7.4, and 9.0 (a). Swelling ratios of CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 at pHs 4.0 and 7.4 (b, c). The error bars in the graph represent standard deviations (n = 3).](image)
similar linear increase in % elastic modulus of these films was observed, i.e., 17, 45, and 99%, compared to CHAP-1. These results are in good agreement with those of % tensile strength. These investigations confirmed that the nanocomposite hydrogel films present superior mechanical properties to plain hydrogel films, which can be ascribed to the strong electrostatic/physical interactions of fillers (NaMMT) with polymer matrix (CHAP) through their polar functionalities.

**Swelling Ratios of CHAP and NaCHAP Nanocomposite Hydrogel Films.** Swelling capacity of hydrogels is one of the important characteristics that reveals their ability to retain and absorb solvents containing fertilizers, reagents, and drugs along with an ability for their controlled release, which make them potential candidates for application in the field of drug delivery. Thus, the swelling behaviors of CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 hydrogel films were investigated using phosphate buffer solutions of pHs 1.0, 4.0, 7.4, and 9.0 at room temperature (25 °C) (Figure 4a–c). These films show higher swelling ratios at pHs 7.4 and 9.0 without affecting stability, while in acidic medium (pHs 1.0 and 4.0), they exhibit a lower swelling ratio. The swelling ratios of hydrogel films increase on moving from acidic to slightly alkaline pH (Figure 4a). This change in pH sensitivity of the CHAP and NaCHAP films can be attributed to the hydrophilicity of the −OH groups in the hydrogels network. At low pH (pHs 1.0 and 4.0), most of the carboxylate anions were protonated, eliminating the anion–anion repulsion, leading to a remarkable decrease in swelling ratio (Figure 4a), while at higher pH (pHs 7.4 and 9.0), some of the carboxylate groups were ionized, causing electrostatic anion–anion repulsion, which leads to the improvement in the swelling ratios of these hydrogel films.

Among all of these hydrogel films, the CHAP-1 film exhibits the highest swelling ratio at pHs 4.0 and 7.4, i.e., 58.99 and 33.99% (CHAP-1) > 46.66 and 27.00% (CHAP-3) > 42.59 and 24.55% (NaCHAP-3) > 38.07 and 21.34% (NaCHAP-4) respectively (Figure 4b,c). The augmentation in the swelling behavior of CHAP-1 can be attributed to the presence of free −OH groups of CMC and HEC, which interact with water molecules, making the hydrogel more hydrophilic, which helps in imbining the large amount of water. However, the reduction in the swelling ability of CHAP-3 can be due to the generation of more cross-linking points through the introduction of polyol segments within the hydrogel networks. This led to the formation of a rigid and highly cross-linked structure, reducing the available free space within the hydrogel. However, in the case of nanocomposite hydrogels (NaCHAP-3 and NaCHAP-4), the presence of NaMMT further increases the cross-linked structure and reduces the spaces of microchannels and pores of hydrogels, resulting in the decrease of swelling ratio compared to plain hydrogels. However, the mechanical stability of nanocomposite hydrogels increases and attained the optimum swelling ratio.

**Equilibrium Water Swelling Studies of CHAP and NaCHAP Nanocomposite Hydrogel Films.** The equilibrium swelling behaviors (%EWS) of CHAP and NaCHAP hydrogel films was studied at pHs 4.0, 7.4, and 9.0 and room temperature (25 °C). It was noted that CHAP-1 has higher values of %EWS than CHAP-3, NaCHAP-3, and NaCHAP-4 hydrogels (Table S2). In the case of CHAP-3, the reduction in %EWS may be due to the loss of hydrophilicity of the hydrogel induced by the addition of polyol, which acts as a cross-linker. On the other hand, the NaCHAP-3 and NaCHAP-4 nanocomposite hydrogels exhibit a decrease in %EWS. The presence of NaMMT in nanocomposite hydrogels led to the formation of more cross-linked structure that reduces the free space of pores and microchannels, which led to decrease in %EWS and further increase of the loading of NaMMT in nanocomposite hydrogels.

**DEGRADABILITY STUDY**

**Hydrolytic Degradation of CHAP and NaCHAP Nanocomposite Hydrogel Films.** The biodegradability of CHAP and NaCHAP hydrogels films strongly influences the release of drug into their target side without inducing any toxicity. Thus, the hydrolytic degradation of these hydrogel (CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4) films was investigated at pH 7.4 (Figure S2a), which involves the hydrolysis of the functional groups enhancing the possibility for the formation of labile bonds. The hydrolytic degradation studies revealed that initially the hydrogel films showed a rapid increase in weight through swelling, followed by a weight loss due to the deswelling and degradation of hydrogel films. It was observed that there was successive weight loss after attaining %EWS. The degradation test confirmed that initially the mass loss started in the CHAP-1 films, followed by CHAP-3, NaCHAP-3, and NaCHAP-4. The decrease in weight of these films was recorded at a regular interval of time for a period of 28 days, which confirms the degradation process of these films. The presence of hydrophilic groups in CHAP-1 led to the improvement in the water uptake ability of the film, which enhances the autocalytic degradation. However, the higher hydrolytic degradability stability of CHAP-3 than CHAP-1 is due to the presence of polyol, which improves the cross-linked structure of hydrogels. The NaCHAP-3 and NaCHAP-4 films presented higher strength in the solution of pH 7.4, which start to decompose only after 72 h. The improvement in the degradation stability of nanocomposite hydrogels can be confirmed by the formation of strong electrostatic interactions between polar functional groups like −OH, −COO, etc., of CMC/HEC and polyol with those of Na-montmorillonite.

**Soil Burial Degradation of CHAP and NaCHAP Nanocomposite Hydrogel Films.** A soil burial study was conducted on CHAP and NaCHAP nanocomposite hydrogel films for 42 days in beakers containing 30% moist soil. The humidity of the soil can be maintained by adding water on every second day to overcome the loss of water occurring through evaporation. The CHAP and NaCHAP films of 1 cm × 1 cm dimensions were charged in various soil-containing beakers. The mass loss in films after immersing in soil was studied by weighing the samples on a Sartorius analytical balance (with accuracy of ±0.0002) before and after degradation at a given time interval (i.e., 1, 2, 3, 7, 14, ..., 42 days) (Figure S2b). The weight loss occurred primarily in the case of CHAP-1 and CHAP-3 films after 72 h, while the NaCHAP-3 and NaCHAP-4 films showed degradation only after 144 h, much slower than the degradation of plain hydrogel films. This can be due to the presence of Na-montmorillonite nanofillers, which induces the cross-linked network of NaCHAP-3 and NaCHAP-4 nanocomposite films that restricts the movement of polymer chains and leads to reduced degradation rate compared to CHAP-1 and CHAP-3 films. The initial weight loss and insignificant mechanical strength in CHAP-1 and CHAP-3 are also evident from storage and loss modulus compared to those of NaCHAP-3 and NaCHAP-4 films (Figure 3). The increased water absorption...
capability of CHAP-1 helps to induce a more flexible structure of plain hydrogel films containing CMC/HEC, which might enhance the phenomenon of autocatalytic degradation.\(^{25}\)

**In Vitro Cell Assay of CHAP and NaCHAP Nanocomposite Hydrogel Films.** The MTT assay studies of (%) cell viability have indicated that the CHAP and NaCHAP nanocomposite hydrogel films do not cause any numerical deterioration in cell viability up to 48 h treatment (Figure 5a). Different concentrations of the resultant hydrogel films were used to investigate the toxicity on human embryonic kidney (HEK-293) cells, and it was observed that these hydrogel films have biocompatible properties. In the case of CHAP-1 and CHAP-3, there is only 15 and 9% cytotoxicity (i.e., \(\geq 85\) and 92% cell viability) compared to that of control (100% cell viability) at 100 \(\mu\)g/mL after 48 h incubation. However, the (%) cell viability decreases with increased concentration, i.e., at 200 \(\mu\)g/mL, and shows marginal toxicity (28–22%) with 72–77% cell viability. The NaMMT-modified nanocomposite hydrogel films (NaCHAP-3 and NaCHAP-4) have \(\geq 81.44\) and 73.33% cell viabilities at 200 \(\mu\)g/mL, which demonstrated that the (%) cell viability of the nanocomposite hydrogel films increased after the dispersion of NaMMT in CMC-HEC-AN-polyol polymer matrix.\(^{36}\) From the graph (Figure 5a), it has been confirmed that these hydrogels did not show any significant toxicity even at higher doses (200 \(\mu\)g/mL). In addition, the morphology of the cell with CHAP and NaCHAP (CMC-HEC-AN-polyol biopolymer-modified NaMMT) disks treatment was studied using an inverted microscope system, which scanned the entire probes. The study on cellular morphology shows no alteration in the original morphology of cells used in the case of hydrogels in comparison to that of control cells even at the highest dose of treatment (i.e., 200 \(\mu\)g/mL) after 48 h, suggesting that the interaction of both CHAP and NaCHAP nanocomposite hydrogel films with HEK-293 cells did not have negative effect on cell life and morphology (Figure 5b). The MTT assays indicate that these hydrogels are found to be biocompatible at these tested concentrations (i.e., 25–200 \(\mu\)g/mL). The inspiring biocompatibilities of CHAP and NaCHAP suggested that the resultant nanocomposite hydrogel films can be successfully applied for drug-delivery systems.

**Drug Loading of CHAP and NaCHAP Nanocomposite Hydrogel Films.** Cisplatin was selected as a model drug to assess both drug loading and controlled release behavior of nanocomposite hydrogel films. The method used for drug loading onto the hydrogel films has been described in the previous characterization section. In all of the cases (CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4), the hydrogel films were dispersed in drug solution of known concentration for 72 h. The transparent hydrogel films in the swollen states in pH 4.0 and 7.4 drug appeared yellow with a more tightly compact form upon complexation with cisplatin. The loading of cisplatin drug in CHAP-1 hydrogel showed a higher % (17 and 30.03%) loading compared to CHAP-3 (14 and 27.27%), NaCHAP-3 (10.71 and 23.42%), and NaCHAP-4 (7.14 and 21.44%) after 72 h at both pHs 4.0 and 7.4, which can be attributed to the higher swelling behavior and interactions of drug molecules with the polar functional groups of constituent moieties of CHAP-1 and CHAP-3, whereas the loading of cisplatin was found to be comparatively slightly lower in the

![Figure 5](image-url)
case of NaCHAP-3 and NaCHAP-4 at both pHs (4.0 and 7.4). The slightly lower loading of cisplatin drug in nanocomposite hydrogels is due to the random distribution of NaMMT nanofillers into the CHAP polymeric matrix, which resulted in the formation of highly cross-linked structures, thereby reducing the free space within the polymer matrix, which led to the reduction of the penetration of drug molecules. Furthermore, these hydrogel films showed higher drug loading at pH 7.4 than at pH 4.0, due to higher degree of swelling at pH 7.4.

Drug Release of CHAP and NaCHAP Nanocomposite Hydrogel Films. The in vitro cisplatin release profile was investigated by dispersing the cisplatin drug-loaded CHAP-3, NaCHAP-3, and NaCHAP-4 hydrogel films in the phosphate buffer solutions of pHs 4.0 and 7.4 for a defined period of time (Figure 6a,b). From the graph, it has been observed that in vitro the release showed a biphasic pattern; the initial release was attributed to the burst release, which disappears within a limited hours; the continuing elongated pattern showed a linear sustained release. The drug release data demonstrated that these nanocomposite hydrogels act as an optimal device for a sustained release of anticancer drug. The sustained release phase could have been due to the cross-linked porous and polar structure of hydrogel, achieved via dispersion of NaMMT nanofillers and polycrylic acid into the polymer matrix, which reduces the diffusion process for the drug entrapped within the inner part of hydrogels and act as a drug reservoir for cancer treatment. It is observed from Figure 6a,b that the amount of drug release was found to be much higher (CHAP-1 (83%) > CHAP-3 (79%) > NaCHAP-3 (77%) > NaCHAP-4 (73%)) at pH 7.4 than at pH 4.0 (CHAP-1 (69%) > CHAP-3 (64%) > NaCHAP-3 (57%) > NaCHAP-4 (54%)), which lasted less than 72 h, exhibiting a pH-dependent behavior.

Initially, during the first 2 h, the burst release of drug from CHAP-1 (38 and 24%) and CHAP-3 (20 and 22%) film at pHs 7.4 and 4.0, respectively, was approximately observed. The subsequent increase in the drug releasing rate at pH 7.4 can be attributed to the poor electrostatic interaction between the drug molecules and hydrogels at high pH. The decrease in the release behavior of cisplatin at pH 4.0 was consistent with the results from the swelling studies at low-pH solutions. In addition, the higher drug release rates of CHAP-1 and CHAP-3 films compared to those of NaCHAP-3 and NaCHAP-4 at both pHs 4.0 and 7.4 can be assigned to the higher swelling ability due to more porous and flexible structure, which led for the faster and higher release rate of the drug. Due to the porous structure of this film, cisplatin is more weakly complexed to the carboxylate group of CMC/HEC, whereas in the case of NaCHAP-3 and NaCHAP-4, after the burst phase in the first few hours, the release of cisplatin exhibited a tendency to decrease with the increase of the NaMMT content in the hydrogel composition. The sustained release rate can be attributed to the intermolecular interaction formed by the dispersion of Na-montmorillonite nanoparticles within the polymer matrix. In the present study, it was found that the release rate of cisplatin loaded in (CHAP) hydrogel and Na-montmorillonite-based nanocomposite hydrogel films (NaCHAP) was sustained at pHs 4.0 and 7.4. Although other workers have reported the delivery of cisplatin through hydrogel, the retention time for the same is not sufficiently high (only 15, 18, and 24 h, Table S3) compared to the present nanocomposite hydrogels, which found a reasonably high retention time (72 h). The prolonged sustained and higher efficient release rates of cisplatin from nanocomposite hydrogels in the present study were found to be higher than those of the previously reported system (Table S3), which can be attributed to the selection of sustainable (biodegradable, biocompatible) polymers and Na-montmorillonite dispersion that showed high efficiency in drug-delivery systems.

In Vitro Antiproliferative Efficacies of CHAP and NaCHAP Nanocomposite Hydrogels. MTT assay on breast cancer (MCF-7) cells after treatment indicated that CHAP and NaCHAP hydrogels did not induce significant antiproliferative effect in the dose range of 10–160 μg/mL (Figure 5c). Different concentrations (10, 20, 40, 60, 80, and 160 μg/mL) of CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 hydrogel films were used to investigate the antiproliferative effect on human breast cancer (MCF-7) cells, and no significant difference in cell viability after the addition of disks compared to control was observed (Figure 5c). Although CHAP-1 and CHAP-3 were found to be slightly cytotoxic (i.e., 30 and 37%) at higher concentration (160 μg/mL), which is maximum cytotoxicity compared to all of the studied hydrogels. In our study, we have found that the cell viability is approximately ≥70% at 160 μg/mL, which was relatively high, indicating that the resultant hydrogel films had no significant growth inhibition of MCF-7 cells without cisplatin.

In Vitro Antiproliferative Efficacies of Cisplatin-Loaded CHAP and NaCHAP Nanocomposite Hydrogels.
The anticancer activities of free cisplatin and cisplatin-loaded CHAP and NaCHAP nanocomposite hydrogel films against MCF-7 cells were examined through MTT assay. The MTT assay on MCF-7 cells after treatment indicated that cisplatin-loaded CHAP and NaCHAP hydrogel samples show significant antiproliferative effect in the dose range of 50–200 μg/mL (Figure 5d). The biocompatibilities of CHAP and NaCHAP were first examined before the incorporation of cisplatin. The CHAP and NaCHAP nanocomposite hydrogels were found to be nontoxic (200 μg/mL), which indicate high biocompatibility, and did not affect the results of the cell viability in the cisplatin carriers. To evaluate the antiproliferative effect of free cisplatin and cisplatin-loaded CHAP and NaCHAP nanocomposite hydrogel films, we fixed the concentration of cisplatin at 0.75 mg/mL. Different concentrations (50, 100, 150, and 200 μg/mL) of CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 hydrogel films were used to investigate the antiproliferative effect on breast cancer (MCF-7) cells up to 48 h (Figure 5d). The nanocomposite hydrogels (CHAP-1 (54%), CHAP-3 (57%), NaCHAP-3 (59%), and NaCHAP-4 (63%) showed a slow decline of cancer cells (half-maximal inhibitory concentration (IC50) 139.14, 169.52, 159, and 176 μg/mL, respectively), after being incubated for 48 h. In contrast, cisplatin (Figure S4) shows antiproliferative activity at lower doses (IC50 123.24 μg/mL) that killed 56% cancer cells (44% cell viability) and left only 18% of the cell viability at high concentration (IC50 176 μg/mL). Overall, the cytotoxic effects of the treated hydrogels (CHAP and NaCHAP) were lesser than those of the cisplatin-free drug. This can be due to the fact that the nanocomposite hydrogels exhibit lower antiproliferative activity compared to free cisplatin, which can be attributed to the sluggish internalization of the polysaccharide-based hydrogels into the cell and the slow release pattern of cisplatin, while the higher cytotoxicity of the cisplatin-free drug can be attributed to the fast release of cisplatin due to the unmodified drug carrier. The results after 48 h of incubation suggest that the CHAP and NaCHAP nanocomposite hydrogels provided an increased growth inhibition of human breast cancer cells, indicating a sustained release of cisplatin with time.

## CONCLUSIONS

The CHAP and Na-montmorillonite-dispersed sustainable polymer nanocomposite hydrogel (NaCHAP) films were synthesized using CMC-HEC-AN-LP via solution blending techniques. The study revealed that the unique characteristic properties are associated with the introduction of NaMMT in the CHAP matrix, which resulted in the enhanced stability of nanocomposite hydrogels inducing restricted hydrophilicity in swelling ratio measurement and tensile strength. The in vitro cytotoxicity test of CHAP plain hydrogel and NaCHAP nanocomposite hydrogels indicated that they are highly biocompatible and nontoxic, which is suitable to be used in drug-delivery systems. The release rate of cisplatin in nanocomposite hydrogels was found to be higher in CHAP-1 (83 and 69%) and CHAP-3 (79 and 64%) than in NaCHAP-3 (77 and 57%) and NaCHAP-4 (73 and 54%) at pHs 4.0 and 7.4, respectively. The in vitro cytotoxicity of cisplatin-loaded nanocomposite hydrogels test indicates that these films effectively inhibited the growth of human breast MCF-7 cancer cell line. These results suggested that in future, CHAP and NaCHAP nanocomposite hydrogel films find promising platforms to construct pH-responsive controlled anticancer (in vivo, in the form of capsules) drug-delivery systems.

## EXPERIMENTAL SECTION

### Materials

Hydroxyethyl cellulose (HEC), G053006, CAS No. (9004-62-0), viscosity 270 mPa s, melting point 140 °C (284 °F; 413 K), Loba Chemie; carboxymethyl cellulose (CMC) (sodium salt) CAS No: 9004-32-4, viscosity 400–800 cP; and Na-montmorillonite nanoclay (30–60 mesh size), surface area 250 m²/g were supplied by Sigma-Aldrich. Acrylonitrile (AN) was obtained from CDH Laboratory (India). 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium (MTT), Dulbecco’s modified Eagle’s medium (DMEM), and 0.25% trypsin and 0.02% ethylenediaminetetra-acetic acid mixture were purchased from HiMedia (India). Phosphate buffer tablets of pHs 1.0, 4.0, 7.4, and 9.0 were purchased from SD Fine Chemicals Ltd., India. Cisplatin (cis-diamine platinum(II) dichloride), an anticancer drug (mol wt, 300.05; ≥99.9% trace metals basis; CAS Number 15663-27-1), and fetal bovine serum (FBS) were obtained from Gibco (South Africa). The linsed oil polyl (refractive index, 1.489; specific gravity, 1.063; and inherent viscosity, 0.9214) was processed as per the reported method. All of the reagents were used as such.

### Synthesis of CMC-HEC-AN-Polyol (CHAP) and CMC-HEC-AN-Polyol-NaMMT (NaCHAP) Nanocomposite Hydrogel Films

The CHAP and NaCHAP nanocomposite hydrogel films were primed via a simple solution blending technique. CMC (2%) and HEC aqueous solutions (2%) (20 mL each) were charged in a 200 mL beaker, followed by the addition of 5 mL of acrylonitrile (AN), dimethylamino pyridine, and N,N-dimethylformamide as the solvent. The mixture was stirred for 1 h at room temperature (25 °C) until the films were obtained. Plane CHAP hydrogel films were also prepared using a similar method. CHAP and NaCHAP nanocomposite hydrogel thin films (∼75 μm thickness) were cut into 1 cm × 1 cm dimensions to use them for further studies.

### FT-IR Analysis

The CHAP and NaCHAP were dried at room temperature (25 °C) for 48 h to obtain the constant weight. The chemical structures of these hydrogel films were investigated using FT-IR spectra recorded in the range of 400–4000 cm⁻¹ using an FT-IR spectrophotometer (PerkinElmer Cetus Instruments, Norwalk, CT, Central Instrumentation facility, Jamia Millia Islamia).

### SEM and TEM Analysis

The morphologies of dried and swelled CHAP and NaCHAP nanocomposite hydrogel films were investigated by SEM (environmental scanning electron microscope model FEI Quanta 200 F with Oxford EDS system JE 250 X Max 80). The nanocomposite hydrogel films were primarily swelled in media of different pHs (4.0 and 7.4) for 24 h. The interior structure of the swollen films was analyzed after freeze drying by tumbling in liquid nitrogen and lyophilized for 24 h to ensure the complete drying and to retain the porous structure without any split. Prior to their measurement, the freeze-dried samples were attached to brass holders coated with a thin film of gold for 120 s in a chemical vapor deposition machine. Transmission electron microscopy (TEM) was used to characterize the size and shape of NaMMT-dispersed...
nanocomposite hydrogels (NaCHAP). The images of NaCHAP polymer solutions were obtained by a transmission electron microscope (a model Philips Morgani, 268) operating at 80 kV (AIIMS, New Delhi, India). The crushed and powdered films were dispersed in distilled water. The dilute suspensions of these films were initially sonicated for 30–60 min under ice–water bath for sonication; therefore, numerous droplets of sonicated suspension were deposited onto a standard carbon-coated copper grid. The grid was then stained with uranyl acetate solution (2 wt %) for 30 s overnight for air drying in a vacuum oven at 25 °C. The grid was then placed in the TEM hole for image acquisition.

**Mechanical Strength and X-ray Diffraction (XRD) Studies.** The tensile strength analysis of CHAP and NaCHAP nanocomposite hydrogel films was investigated at room temperature (25 °C) using break stress and strain tests conducted on the films of 25 mm gauge at a crosshead speed of 5 mm/min, by Instron universal machine (model no. 8871). These tests were repeated three times on both CHAP and NaCHAP films, and the average values were recorded. X-ray diffraction of these samples was conducted on a Siemens X-ray diffractometer model D5000 equipped with Ni-filtered Cu Kα radiation (k = 1.5406) within the angle range 2θ = 5–60°. The diffractometer was functioned with 1° diverging, receiving slits at 50 kW and 40 mA, and a continuous scan was recorded.

**Swelling and Equilibrium Swelling Ratios Measurement.** The swelling performances of CHAP and NaCHAP nanocomposite hydrogel films were measured at 25° for 96 h. These films were taken in 30 mL test tubes containing solutions of pHs 1.0, 4.0, 7.4, and 9.0. After assigned time intervals (10, 20, 30, 60, 120,..., 5000 min), the inflamed films were cautiously taken out of these solutions, sponged with a filter paper (Whatman, 41 number) for removing the additional water present on the surface, and then weighed again. These films were again restocked with new solutions in a parallel way. The swelling ratios of these nanocomposite hydrogel films were calculated using the equation

\[
\text{swelling ratio (SR)} = \frac{W_s - W_d}{W_d}
\]  

where \(W_s\) and \(W_d\) are the weights of the swollen and dehydrated CHAP and NaCHAP films, respectively.

The equilibrium water contents (%EWS) of CHAP and NaCHAP nanocomposite hydrogel films were calculated after 72 h. The % EWS was recorded only after the swollen samples further swelled and attained equilibrium. The %EWS of the enflamed sample was calculated using the following equation

\[
\%\text{EWS} = \frac{W_{\text{sw}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100
\]  

where \(W_{\text{sw}}\) and \(W_{\text{dry}}\) are the weights of swollen and dehydrated CHAP and NaCHAP films, respectively.

**Biodegradability Studies.** Hydrolytic Degradation. The degradability experiments on CHAP and NaCHAP nanocomposite hydrogel films were conducted at pHs 4.0 and 7.4 and 25 °C. The weight loss of swollen nanocomposite hydrogel films was frequently studied at specified intervals of times (i.e., 1, 2, 3, 4, 5, 6, 7,..., 28 days) using gravimetric technique. The average values of three tests were recorded. The degradability ratio was obtained by the difference of mass loss of CHAP and NaCHAP films by using the following equation

\[
\text{wet mass change (\%)} = \frac{W_t - W_e}{W_e} \times 100
\]  

where \(W_t\) and \(W_e\) signify the mass loss of nanocomposite hydrogel films and their original equilibrium swollen states and at time \(t\) at different pH solutions, respectively.

**Soil Burial Test.** The soil burial study on CHAP and NaCHAP films was conducted in temperate soil for 42 days. The soil used was obtained from Indian Agricultural Institute (PUSA, New Delhi, India). Beakers were charged with 30% moist soil with slightly acidity (pH 6.7). Different specimens of size 1 cm × 1 cm were charged in separate beakers containing soil, and the weight loss was carefully measured using a Sartorius analytical single pan balance (with sensitivity of ±0.0001 g) at different (1, 2, 3, 4, 5, 6, 7,..., 28 days) intervals for 42 days.

**Cell Culture.** In Vitro Cytotoxicity Assay. The MTT assay is generally used to validate the biocompatibility/viability/antiproliferative properties of any biological and synthetic material in vitro. Thus, the cytotoxicities of CHAP and NaCHAP nanocomposite hydrogel films on two different (normal human embryonic kidney (HEK-293) and human breast cancers (MCF-7) cells) cell lines were evaluated under (in vitro condition. These cells were procured from National Centre for Cell Sciences (Pune, India). The cell line with epithelial morphology is commonly used for the determination of biocompatibility and cytotoxicity study. The cells were cultured in T-25 and T-75 culture flasks in Dulbecco’s modified Eagle’s medium (DMEM, HiMedia), which contains 10% fetal bovine serum and penicillin/streptomycin (HiMedia), at 37 °C and 5% CO₂ in humidified chamber (Nuaire CO₂ incubator) under MTT assay. The MTT is a metabolic substrate that reduces in amount by the mitochondrial succinate dehydrogenase enzyme that further forms formazan crystals after association with live cells in dimethyl sulfoxide (DMSO) solution. The OD of the live cells was measured at 570 nm by a UV spectrophotometer. Further, the biocompatibility and cytotoxicity of CHAP and NaCHAP were evaluated through MTT assay.

**MTT Assay of Nanocomposite Hydrogel Films.** To attain the MTT assay for the evaluation of biocompatibility and cytotoxicity, the CHAP and NaCHAP nanocomposite hydrogel films were crushed into powder by using a sterilized mortar and pestle. The crushed samples were dispersed in aqueous solutions to prepare the working solutions of 1 mg/mL concentration. Before its treatment with cells, the aqueous solution was sonicated at a very high speed for 1 h. The T-75 flask of cells was harvested using 0.25% trypsin, and the cells were counted on a Neubauer chamber. A total of 1 × 10⁶ cells/well were sowed in a flat-bottom 96-well plate (150 μL/well) for 24 h. Both cell (HEK-293 and human breast cancer (MCF-7)) lines after 24 h were treated with hydrogel doses ranging from 50 to 200 and 10 to 160 μg/mL, respectively, for 48 h. After 48 h of treatment, the medium was isolated and the cells were cultivated with 20 μL/well of MTT solution for 4 h at 37 °C, resulting in the formation of formazan crystals, through mitochondrial reduction of MTT. These crystals were soothed upon treatment with DMSO solutions of 150 μL/well, and absorbance was recorded at 570 nm after 15 min of incubation through the iMark Microplate Reader (BioRad). The % cytotoxicity and biocompatibility were measured as a fraction of control.
Cisplatin-Loaded Nanocomposite Hydrogels. The in vitro loading of cisplatin onto the hydrogels (CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4) was carried out at pHs 4.0 and 7.4. The dry nanocomposite hydrogel films (1 cm × 1 cm) were weighed and soaked in 10 mL phosphate-buffered saline (PBS) (pHs 4.0 and 7.4) containing cisplatin drug (6 mM) at a given pH for 72 h. The amount of cisplatin loaded onto the hydrogel films was measured by UV spectroscopy (a Lambda 950 UV–vis spectrophotometer; PerkinElmer). The complex hydrogel films were washed with respective PBS before drying. The maximum loading of cisplatin was determined after 72 h of treatment, i.e., on turning the transparent drying. The maximum loading of cisplatin was analyzed at wavelengths of 301 and 308 nm at pHs 4.0 and 7.4, respectively. The % drug loading was calculated using the following equation:

\[
\text{drug loading (%)} = \frac{\text{weight of drug in nanocomposite hydrogel}}{\text{weight of nanocomposite hydrogel}} \times 100
\]  

Cisplatin Release from Nanocomposite Hydrogels. The in vitro release experiments on the cisplatin-loaded plain and nanocomposite hydrogels (CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4) films were carried out by placing them in 10 mL of PBS (pHs 4.0 and 7.4) in a test tube for 72 h at room temperature. At certain time intervals (10 min, 30 min, 60 min, ... 72 h), 3 mL of the solution was withdrawn and the same amount of respective fresh PBS solution was added back to the beaker to maintain a constant volume. The amount of cisplatin released was analyzed by measuring the absorbance spectra for different concentrations of standard solutions of cisplatin drug. The drug release studies were performed in triplicate, and their average value was derived using the following equation:

\[
\text{drug release ( %)} = \frac{\text{concentration} \times \text{dissolution bath volume} \times \text{dilution factor}}{1000}
\]  

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01691.

Reaction scheme to prepare CHAP and NaCHAP nanocomposite hydrogel films (Scheme S1); TEM images of NaCHAP-3 (Figure S1); hydrolytic and soil burial degradation of CHAP-1, CHAP-3, NaCHAP-3, and NaCHAP-4 films (Figure S2); in vitro cytotoxicity of free cisplatin evaluated by MTT assay test on human breast (MCF-7) (Figure S3); feed composition of CHAP- and NaCHAP-based nanocomposite hydrogel films (Table S1); %EWS of plain and nanocomposite hydrogel films at various pH solutions (Table S2); comparative studies of present work with earlier reported system using cisplatin drug (Table S3) (PDF)
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