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SYNTHESIS AND CHARACTERIZATION OF HYDROXY PROPYL METHYL CELLULOSE-BASED CITRIC ACID CROSSLINKED NANOGELS OBTAINED VIA GREEN PROTOCOL

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ABSTRACT
Nanogels are versatile tools in the hands of a biochemist, increasingly being used for medicinal applications and as bio-sensing tools. The utilization of biopolymer-based nano gels is a field that still has much exploration to be done. In this article, we will study the Hydroxypropyl Methyl Cellulose (HPMC)-based Citric Acid (CA) cross-linked nanogels synthesized via a green protocol. The nanogels were synthesized following a novel technique without using a surfactant at 55°C by employing citric acid as a crosslinker and Acryl Amide (AAm) as a co-polymer. The nanogels synthesized were categorized with swelling studies, FTIR, Particle size Analyzers and FESEM that provided proof for co-polymer synthesis and nanogel formation. The particle size analysis of HPMC-*co*-poly(AAm)-*cl*-CA nanogels indicated a particle size below 10nm with spherical nature that was supported by FESEM data that presented spherical particles with a size below 50nm. The swelling studies carried out at acidic (4.0) and basic pH (7.0) exhibited their pH sensitivity as the nanogels showed 40% higher swelling at 7.0 pH than 4.0 indicating these could be utilized for site-specific drug delivery.
Keywords: Biopolymer, Nanogels, Swelling, Polymers, Hydrogels.

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INTRODUCTION

Nanogels are nano-sized hydrogels with a size range of 10-200nm, having properties of both hydrogels and nanoparticles. Initially, the term 'nanogel' was used for a bifunctional system of polyionic and cross-linked polyethyleneimine (PEI) or non-ionic polymer and Poly Ethylene Glycol (PEG-*cl*-PEI) for antisense oligonucleotides delivery.[1,2] They have three-dimensional network nanostructures with a higher specific surface area than hydrogels. Due to their small size, nanogels respond rapidly to changes in the environment like temperature or pH changes. They are formed by physical or chemical cross-linking taking place between synthetic polymers or biopolymers through different functional groups like sulphonic ($-HSO_3$), amino ($-NH_2$), hydroxyl (-OH), and carboxyl (-COOH). In nanogels, the cross-linked polymers consist of amphiphilic or hydrophilic macromolecular chains which swell by holding the higher volume of water and maintain the structure intact without dissolving into the aqueous medium.[3] The capacity to absorb the water to a greater extent is due to the macromolecular chains and hydrophilic functional group present in the structure of the polymer.[4] The physiochemical interactions between the functional group of polymeric compounds and drug substances are responsible for the drug loading of nanogels which can host drug molecules in their polymeric network.[5] Owing to the nanogel properties, it carries several drugs to numerous organs. Nanogels are crucial in a variety of sectors, including organ targeting, gene delivery, diagnostics, and chemotherapy.[6,7] Biopolymer-based nanogels play a crucial role in drug delivery and tissue engineering applications.[8,9] These contain all the properties of synthetic counterparts and the added intrinsic properties such as biodegradability, and abundance in nature, are non-

toxic, renewable and are also cheap. Biopolymer based nanogels have functional groups like hydroxyl, amino, and carboxylic acid, which are used for cross-linking and for bio-conjugation with target cells.[10] Biopolymers modified with nanoparticles (NPs) have many uses in electronics, optics, catalysis, drug delivery and antimicrobial activity. The nanogel based drug delivery system is more productive in terms of solubility enhancement, improving *in-vivo* stability, drug pharmacokinetics and optimized biodistribution of the attached nucleic acids, proteins and drugs.[11,12] Biopolymers such as chitosan, starch, alginate and guar gum-based biomaterials in the form of hydrogels, xerogels, and microgels have been used for drug delivery.[13-16] Cellulose is the most abundant naturally occurring biopolymer found in plants and animals. It is biocompatible and biodegradable, which is extensively used in pharmaceutical applications.[17] In recent years, cellulose derivatives containing hydrophilic polymers which form gels in aqueous media have been used for developing controlled release technology for pharmaceutical products. HPMC (Hydroxy propyl methyl cellulose) can be used for controlled drug release owing to its non-toxic nature, good swelling properties and ability to accommodate higher drug levels.[18] HPMC is a stabilizer of emulsions and foams in the food industry and a controlled-release polymer for various dosage forms.[19,20] It is chemically inert and non-ionic and does not react with metal salts or ionic organics. Its property of enzyme resistance in an aqueous solution provides good viscosity stability during long-term storage. With higher viscosity in polymers, HPMC chains swell faster and inhibit further liquid uptake. This results in forming of a turbid gel that resists erosion and dilution, resulting in the slow diffusion of drugs.[21,22] In the present study, we have reported HPMC-based nanogels prepared by cross-linking with acryl amide using citric acid as a crosslinker by a surfactant-free method. The synthesized nanogels were characterized by "Fourier Transform Infrared Spectroscopy" (FTIR), "Field Emission Scanning Electron Microscope" (FESEM), and particle size analyzer to get evidence for cross-linking and particle size. These nanogels were prepared for potential application in a therapeutic application for targeted drug delivery.

EXPERIMENTAL

Materials

Hydroxy propyl methyl cellulose (HPMC) (S D Fine-Chem Limited, Mumbai, India), citric acid (CA) (S D Fine-Chem Limited, Mumbai, India), ammonium per sulphate (A) (S D Fine-Chem Limited, Mumbai, India) acryl amide (AAm) (S D Fine-Chem Limited, Mumbai, India), ethanol (S D Fine-Chem Limited, Mumbai, India), distilled water were used as obtained.

Synthesis of Nanogels

HPMC-*co*-poly(AAm)-*cl*-CA nanogels were synthesized using a novel technique employing no harsh chemicals. The nanogels were cross-linked using citric acid (CA). The HPMC-*co*-poly (AAm)-*cl*-CA hydrogel was first synthesized using A as an initiator (1% by weight of the reaction mixture) and 10 mg of HPMC, 5g of AAm and 2% by weight of CA as crosslinker under the reaction conditions of 70°C in the water bath. The hydrogel was then converted to nanogels by sonication at 55°C in ethanol, followed by homogenization at 1000 rpm for 24 h.

Characterization of Nanogels

Synthesized nanogels can be grouped as per the FESEM, FTIR spectroscopy, and particle size analyzer for inducing the evidence of particle size and network formation. In transmittance mode in KBr, the FTIR spectra are recorded on Perkin Elmer. The nanogels FESEM analysis was administered on the SEM after coating it with Au/Pd on 15kV with the help of identical magnification for inducing a result comparison. The synthesized nanogels were also subjected to swelling studies at 4 pH and 7 pH as a function of time at 37°C. For desirable pH, a solution was made from the pH tablets available commercially. The Cyber-scan pH confirmed the ultimate pH. Swelling measurement was studied as the function of temperature and pH. A known weight of dried nanogel is applied for all swelling experiments. In buffer solutions of 4 and 7 pH, the effect of pH on swelling was observed at 37ºC. At the pH mentioned above, for nanogels, the temperature sensitivity was estimated by swelling it at different temperature levels for 8 hours. The % swelling (Ps) was calculated using the following equation:[23]

$$Ps = (\text{Weight of swollen nanogel} - \text{Weight of dry nanogel} / \text{weight of dry nanogel}) \times 100 \quad (1)$$

The particle sizes were analyzed by "Dynamic Light Scattering" (DLS). It shows the size distributions of the nanogels by intensity. The particle size analysis was administrated by dispersion of the nanogels in ethanol.

RESULTS AND DISCUSSION

The surfactant-free method prepared the HPMC-*co*-poly(AAm)-*cl*-CA nanogels. The HPMC-*co*-poly(AAm)-*cl*-CA hydrogels were synthesized with CA as crosslinker at 70ºC which were then converted to nanogels by sonication at 55ºC, in ethanol followed by homogenization at 1000 rpm for 24 h. They were characterized by FTIR, particle size analysis, and FESEM. The FTIR characterization provided evidence for successful co-polymerization and cross-linking. Within the pure HPMC spectrum, its characteristic peaks are 3500-3400 cm^{-1} (-OH vibrational stretching), and peaks within the range 2850-3000 cm^{-1} represent the stretching vibration of –C-H bonds. Bands on 1065 cm^{-1} indicate the stretching vibration of –C-O bonds. The bending vibration of the –OH group on the HPMC appeared within the range 1330-1430 cm^{-1}. The spectrum of HPMC is confirmed through a literature study performed by S. Bashir *et al.* They observed characteristic peaks at 3519, 2867, 1386, and 1045 cm^{-1}.[24]

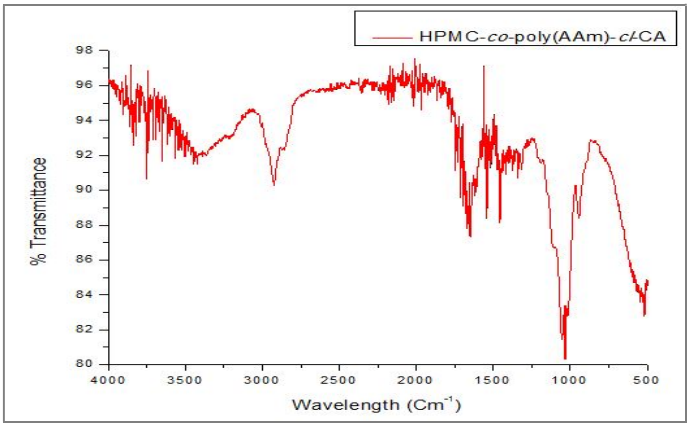

Fig.-1: FTIR Spectra of HPMC-*co*-poly(AAm)-*cl*-CA Nanogels

However, in HPMC-*co*-poly(AAm)-*cl*-CA nanogels spectrum, it is evident that all absorption peaks characteristic of HPMC are present, in addition to many additional peaks. Two peaks on 1540 cm^{-1} and 1648 cm^{-1} for amide groups of polyacrylamide chains. The bands at 2923 cm^{-1} and 1028 cm^{-1} indicate the –O-H stretching (R-C(O)-OH) and –C-OH stretching. There is a decrease in the value of $–CH_2$ rocking vibration, i.e., 521cm^{-1}. Thus, these bands proved the formation of HPMC-based nanogels cross-linked with citric acid.

Particle Size Analysis

The particle size of HPMC-co-poly(AAm)-cl-CA nanogels was assessed by the dispersion of nanogels in ethanol. On particle size analysis, the HPMC-*co*-poly(AAm)-*cl*-CA nanogels indicated a particle size below 10nm with probable spherical nature (Fig.-2). FESEM data further strengthened these results.

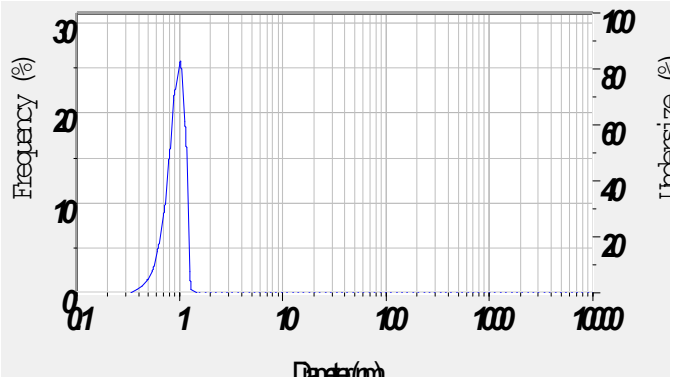

Fig.-2: Particle Size Analysis of HPMC-*co*-poly(AAm)-*cl*-CA Nanogels

FE-SEM Analysis

HPMC-*co*-poly(AAm)-*cl*-CA nanogels were also characterized by FESEM. Figure-3 shows the field-emission scanning electron micrographs of HPMC-*co*-poly(AAm)-*cl*-CA nanogels. Surface morphology depicts spherical particles with particle sizes below 50 nm. The variation in particle size observed on comparison of the Particle size Analysis and FESEM data is due to the nanogels undergoing agglomeration. Thus it was observed that the size and distribution of HPMC-*co*-poly(AAm)-*cl*-CA nanogels were smaller than those observed in FESEM.

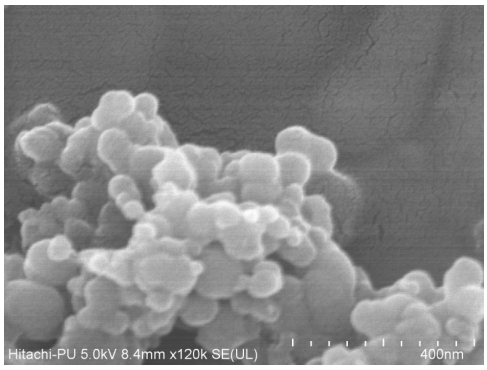

Fig.-3: FESEM Image of HPMC-*co*-poly(AAm)-*cl*-Citric Acid

Swelling Study of the Nanogels

The swelling characteristics of HPMC-*co*-poly(AAm)-*cl*-CA nanogels were studied at 37°C at different pH(4, 7 pH). Weighing the dry and wet nanogels allowed us to determine the swelling ratio. The swelling study was carried out over a span of 72 hours till equilibrium swelling attained. With time and pH shift, the nanogels showed substantial volume phase changes. The manifestation of the time-dependent stretching of the cross-linked chains of nanogels, permits more water inside the interior of the nanogel and is responsible for rise in water absorption with an increase in time. Ionization, which enables water to be absorbed from the hydrophilic functional group connected to the cross-linked polymer backbone, is what gives nanogels their ability to swell.It was observed that HPMC-*co*-poly(AAm)-*cl*-CA nanogel exhibited high Ps in alkaline pH than with acidic pH (Figure 4 representing Qt, vs Square Root of Time Swelling Data of the Hydroxyl Propyl Cellulose, Based Nanogels at Different pH).

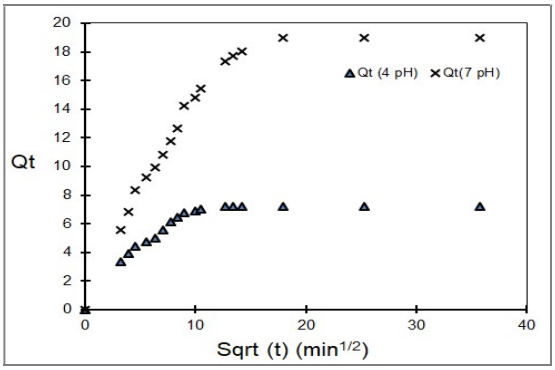

Fig.-4: Qt, vs Square Root of Time Swelling Data of the Hydroxyl Propyl Cellulose, Based Nanogels at Different pH

Since there is more physical cross-linking between the H^+ ions and the functional group of the nanogel network at lower pH values, the nanogel network tends to contract and the swelling ratio falls. At higher pH levels, the -OH ions are higher, and H^+ ions are less, due to which the formation of additional cross-linkages decreases. The poly(AAm) carboxylic group was not ionized in acidic environments, which caused the nanogel to shrivel. The carboxylic group was more likely to ionize, interact with water, and produce higher Ps values in the alkaline media. Using the most fundamental Fick's rule, the diffusion of polymeric structures and swelling kinetics are described in equation (2):

$$F = S_t/S_e = kt^n \quad (2)$$

In this equation, the swelling fraction is represented by "F", the equilibrium swelling nanogel content by "S_e", the solvent's diffusion exponential by "n", and the constant that varies depending on the network structure of the gel by "k". The slope of a line derived from the lnF-lnt graph data of the area where the swelling has not yet achieved equilibrium may be used to determine the magnitude of the diffusion exponential (n) (Figure 5 represents a graph between lnF and ln t at pH 4 mad pH 7, respectively). Here the value of n signifies the mechanism of swelling kinetics. The relaxation rate is substantially higher than the diffusion rate for n = 0.5. It reflects Fickian or Case 1 transport behavior when pure drug diffusion release is taken into account and the relaxation coefficient is minimal. For n=1, the diffusion is faster than the relaxation rate and is called zero-order release kinetics. For n laying between 0.5 and 1, there is anomalous diffusion and non-Fickian where diffusion is analogous to structural relaxation. The sorption curves are similar to Fickian curves for n values less than 0.5, but the final equilibrium is extremely sluggish; this process is called pseudo-Fickian behaviour.[25-27]

Applying Fick's second rule of diffusion, which is utilized for polymer composites and is provided in equation 2, one may explain how nanogels swell and absorb water[28]:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-\left(\frac{D(2n+1)^2 t}{h^2}\right)\pi^2\right] \quad (3)$$

Here, the moisture contents at time t and equilibrium are represented by M_t and M_∞. The sample thickness is represented by h, and the diffusion coefficient by D. Equation 2 becomes less complex at first absorption (an initial linear section of the curve), ($M_t/M_\infty \leq 0.5$).

$$\frac{M_t}{M_\infty} = \left(\frac{4}{h}\right)\left(\frac{D}{\pi}\right)^{1/2} t^{1/2} \quad (4)$$

Which can be further reduced to equation (5):

$$M_t = kt^{1/2} \quad (5)$$

where the slope of the M_t vs. $t^{1/2}$ curve is given by k. Therefore, with the known thickness of the sample, D may be determined from the M_t vs $t^{1/2}$ slope graph.[28]

Using Fick's first diffusion law, the diffusion coefficients (D) may also be determined using (6):

$$dQ/dt = ADK_d(C_o - C)/h \quad (6)$$

where mass transfer rate is given by dQ/dt, the thickness of the hydrogel by h, the film surface area by A, the partition coefficient by K_d, and C and C_o are the drug concentrations in the receptor and donor cells, respectively. The partition coefficient value can be calculated through hydrogel by using the following equation (7):

$$K_d = [V_s(C_0 - C_s)]/V_m C_s \quad (7)$$

Where V_m was measured by using the equation

$$V_m = \pi r^2 h \quad (8)$$

Where the solution volume is given by V_s, the polymer film volume by V_m, the concentrations of solute and initial solute by C_s and C_o at equilibrium, h is the equilibrium thickness of hydrogel, and r is the radius of the hydrogel.[29] The plot of M_t, here represented as Qt, vs square root of time, is represented in Fig.-4 for the synthesized nanogels at pH 4 and 7.0. The water swelling capacity for the non-biodegradable composites is explained by Fick's second diffusion law (Crank *et al*., 1975).[28] This time-dependent diffusion of the water molecules inside the nanogel help in explaining the swelling behavior of the nanogels. The following equation (9) helps determine the fractional swelling and swelling coefficient for the nanogels.

$$\ln F = n \ln t + \ln k \quad (9)$$

Here, fractional swelling is represented by F, swelling rate constant by k, the exponent of swelling and diffusion by n, and time by t. Figure-5 shows a graph between lnF and ln t that provides the n and k values. To understand diffusion inside the nanogels, the values for the swelling exponents 'n' and swelling rate constant k were determined from the intercepts and slopes. The results showed that the diffusion didn't follow Fick's rule and it is a quasi-Fickian diffusion with values of $n<0.5$.[30]

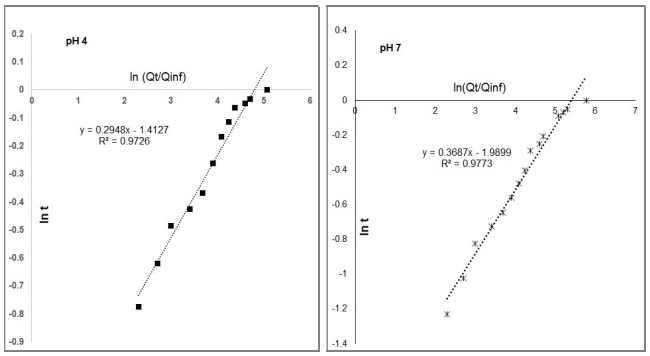

Fig.-5: A Graph Between lnF and ln t at pH 4 mad pH 7, Respectively

CONCLUSION

In the present study, a simple method successfully used hydroxyl propyl methyl cellulose as a backbone to synthesize HPMC-*co*-poly(AAm)-*cl*-CA nanogel. Various physicochemical characterizations, like FTIR, FESEM, and particle size analysis, were carried out. It has been observed that HPMC-based nanogels are of particle size below 10 nm with spherical nature that suffers accumulation over time. It has also been observed that these nanogels are pH sensitive and show potential for use in pH-specific drug delivery and release. These nanogels are biodegradable and biocompatible, which are easy to synthesize and have a prospective application in drug separation and delivery processes.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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