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Original Article

Hydrogel Films of Citric Acid Cross-linked Hydroxypropyl Methylcellulose/Methylcellulose for Hydrophilic Drug Delivery

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ABSTRACT

Aim: To develop hydrogel films for hydrophilic drug delivery with citric acid-cross-linked Hydroxypropyl Methylcellulose (HPMC) and Methylcellulose (MC). **Materials and Methods:** The existing study is concerned with the development of HPMC/MC hydrogel films employing citric acid as a cross-linking agent in order to accomplish drug loading while simultaneously releasing hydrophilic drugs (ciprofloxacin hydrochloride). Thermal analysis and ATR-FTIR spectroscopy were used to explore the hydrogel films. Drug loading, drug content, swelling ratio, *in vitro* drug release, carboxyl content, and hemolytic assay were all measured on the films. Cross-linking through ester development was revealed by ATR-FTIR spectra, whereas drug loading and crosslinking by means of ester formation were validated by thermal analysis as well as SEM. **Results:** A good equilibrium swelling ratio of 14.71 was found in phosphate buffer pH 7.4. With low carboxyl content, the citric acid-cross-linked hydrogel films swelled the most and showed better drug release. The addition of methylcellulose aided in the creation of firm, homogeneous hydrogel films for loading and dispensing ciprofloxacin hydrochloride. **Conclusion:** The hydrogel films' biocompatibility was demonstrated using a hemolytic experiment. It was observed that HPMC/MC hydrogel films that had been cross-linked with citric acid showed a tendency to sustain the release of hydrophilic drugs for up to 4 hr.

Keywords: Hydroxypropyl methylcellulose, Methylcellulose, Citric acid, Hydrophilic drug, Cross-linked hydrogel films, Ciprofloxacin hydrochloride, Drug delivery.

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INTRODUCTION

Hydrogels are cross-linked, hydrophilic polymers that exhibit a significant increase in size upon water absorption.[1] The degree of swelling can range from 10 to 1000 times the original weight due to the presence of chemical or physical cross-links within the network structure, rendering them insoluble in water.[2] While hydrophilic hydrogels are preferred for biological applications such as drug delivery, hydrophobic polymeric frameworks, such as Poly (Lactic Acid) (PLA) and Poly (Lactide-co-Glycolide) (PLGA), exhibit a low water uptake capacity of only 5-10 percent.[3] Hydrogels possess a porous nature, which enables drug loading within the polymeric matrix and facilitates drug release through the gel networks. The porosity of hydrogels can be regulated by controlling the density of cross-links within the polymeric matrix.[4] Hydrogels can be synthesized using both natural and synthetic polymers. Synthetic polymers are bio-inert and lack cell adhesion and tissue formation properties.[5] In contrast, hydrogels made from natural polymers offer physiologically recognizable moieties, biocompatibility, and biodegradability, promoting cellular activities.[3-6]

Hydrogels synthesized from natural polymers such as cellulose and its derivatives offer various advantages, including their abundance in nature, low cost, transparency, and easy accessibility. Hydroxypropyl Methylcellulose (HPMC) is a commonly employed thickening and gelling agent, owing to its good swelling index, and is frequently utilized in controlled-release formulations of water-soluble drugs.[7-9] Some researchers have utilized citric acid as a non-toxic and cost-effective cross-linking agent to prepare cellulose-based hydrogels, suitable for pharmaceutical applications.[1,7,10] Polymers, including Hydroxypropyl Methylcellulose (HPMC), Sodium Carboxymethyl Cellulose

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Table 1: Design summary for 3^2 factorial designs.

Factor	Level		
	-1	0	1
X_1 HPMC:MC	1	2	3
X_2 Citric acid (%)	15	20	25
Response			
Y_1-Carboxyl content (mEq/g)			
Y_2-Drug content (%)			
Y_3-Equilibrium Swelling Ratio (HCl)			
Y_4-Equlibrium Swelling Ratio (PBS)			
Y_5-Drug release after 2hr (HCl) (%)			
Y_6-Drug release after 2hr (PBS) (%)			

(Na-CMC), Methylcellulose (MC), and a mixture of Na-CMC and Hydroxyethyl Cellulose (HEC), have been cross-linked with citric acid to synthesize hydrogels. However, there is no known hydrogel that utilizes a combination of HPMC and MC. When plain hydrogels of HPMC are synthesized, they exhibit reduced mechanical strength and transparency. The literature suggests that adding methylcellulose to the hydrogel formulation can enhance its mechanical strength.[11] Therefore, in this study, methylcellulose is used in conjunction with HPMC to prepare hydrogels with enhanced mechanical strength. Both MC and HPMC have been utilized as thermoresponsive and bioadhesive materials, which exhibit gelation upon heating and have superior bioadhesive properties compared to other polymers.[12,13]

The objective of the present investigation was to synthesize citric acid-cross-linked Hydroxypropyl Methyl Cellulose/ Methyl Cellulose (HPMC/MC) hydrogel films. Ciprofloxacin hydrochloride, a broad-spectrum fluoroquinolone antibiotic that is administered intravenously and orally, belongs to the BCS class III and is rapidly eliminated from the body in its conventional dosage form. It exhibits stability under gastric pH conditions. However, owing to its therapeutic application, limited biological half-life (4 hr), repeated daily dosage (2-3 times), and 60% bioavailability, it necessitates formulation as a controlled release pharmaceutical product.[14] Therefore, the current study aimed to synthesize a hydrogel of ciprofloxacin hydrochloride utilizing cross-linked polymers such as HPMC and/or MC, through the utilization of a full factorial design approach.

MATERIALS AND METHODS

Medispray Pvt. Ltd., Satara, provided a gift sample of ciprofloxacin hydrochloride, whereas S. D. Lab Chem., Mumbai, supplied HPMC-K100M (MW 700 kDa, DS 0.9 viscosity, 3400 cm/s, food grade), MC, and sodium hypophosphite. Citric acid (anhydrous) was obtained from Loba Chemie, also located in Mumbai. All other compounds were purchased from Sigma Aldrich, Mumbai and were of analytical quality.

Experimental design

Pharmaceutical scientists often face the challenge of finding the optimal combination of various processing parameters to achieve the best product.[14,16] Response Surface Methodology (RSM) is one of several experimental design types used to optimize drug delivery systems by developing polynomial relationships and mapping the response over the experimental domain.[17-19] The factorial design is another frequently used experimental design for generating and optimizing various pharmaceutical formulations and procedures, providing extensive information on the impacts of experimental variables.[20] While it requires a certain amount of time and trial runs, it is more adaptable and efficient.

In this study, a hydrogel of ciprofloxacin HCl was produced using HPMC and MC as polymers with citric acid as a cross-linking agent. An optimized batch was produced using a 3^2 factorial design. The objective of the study was to successfully prepare a better hydrogel using this polymer mixture for delivery of ciprofloxacin hydrochloride.

A factorial design was employed to analyze two factors, each of which had three levels, resulting in nine combinations in total. The factors studied were the ratio of HPMC to MC (X_1) and the concentration of citric acid (X_2), and the responses analyzed were carboxyl content (Y_1), drug content (Y_2), equilibrium swelling ratio in 0.1N HCl (Y_3), equilibrium swelling ratio in Phosphate Buffer Saline (PBS), pH 7.4 (Y_4), drug release in 0.1N HCl at 2 hr (Q2h) (Y_5), and in phosphate buffer after 2 hr (Q2h) (Y_6). Table 1 presents the processing parameters and levels along with the experimental values and outcomes.

Stat-Ease Inc.'s Design-Expert software was used for the creation and evaluation of the experimental design. The resulting polynomial mathematical model generated by the factorial design was represented by equation (1),

$$Y = b_0 + b_1X_1 + b_2 X_2 + b_3 X_1X_2 + b_4 X_1^2 + b_5 X_2^2 \quad (1)$$

Table 2: Observed responses for 3^2 factorial design.

Code	Independent Variable		Observed responses					
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
H1	1	15	393.7	3.2476	7.94	14.71	71.74	76.06
H2	2	15	420	8.5705	7.61	10.73	93.57	96.32
H3	3	15	454.54	6.8742	6.3	7.71	90.74	89.74
H4	1	20	490.79	5.2184	7.06	14.11	79.63	80.39
H5	2	20	620	6.4211	5.01	6.72	92.58	90.36
H6	3	20	1025.64	5.1994	4.5	5.27	88.92	84.95
H7	1	25	708.79	9.9973	6.24	12.29	89.12	86.98
H8	2	25	1075.5	5.3832	4.8	6.45	90.22	89.28
H9	3	25	2000	2.0156	1.75	3.56	83.01	68.61

where Y is the final result, b0 represents the starting point, and b_1, b_2, b_3, b_4, and b_5 are the regression coefficients. The model included three types of effects: linear (X_{12}, X_{22}), quadratic (X_1^2, X_2^2), and interactive (X_1,X_2). The model's significance value was determined using one-way ANOVA with a significance level of $p < 0.05$.

Preparation of HPMC/MC Hydrogel film

The HPMC:MC hydrogel films were produced with complete conversions. In the process, citric acid was added to an aqueous solution of HPMC:MC (2%) along with Sodium Hypophosphite (SHP) (20%) as a catalyst, at room temperature.[7] The solutions were allowed to settle overnight, and air bubbles were eliminated. The clear solutions were then poured into petri dishes and molded into circular discs with a diameter of 9 cm. Subsequently, the solvent was allowed to evaporate, and the mixture was dried at 50°C in a hot air oven for 24hr, resulting in the formation of films. The dry cast films were then crosslinked by heating at 160°C for 20 min. The curing time was increased to promote esterification between the hydroxyl groups of HPMC and citric acid, and to facilitate the release of water molecules, both of which were essential for cross-linking.[21-23]

The optimal curing temperature was employed to initiate the esterification reaction and to maintain the film during polymer decomposition. The dried hydrogel films were washed with distilled water and isopropyl alcohol for one hour to remove unreacted substances such as citric acid and HPMC molecules. After that, the hydrogel films were dried and treated at 50°C for 24 hr in a hot air oven before being placed in desiccators. To investigate the effect of polymer ratio and citric acid concentration on the properties of the hydrogel, their values were altered as shown in Table 2.

Determination of carboxyl content

The carboxyl content of the hydrogel film was determined through an acid-base titration method.[24,25] The hydrogel film was completely dissolved in an adequate quantity of 0.1N sodium hydroxide after stirring for two hours using a magnetic stirrer. The ester linkages are broken by the sodium hydroxide, and the free carboxyl groups react with it to form sodium carboxylate (citrate). 0.1N HCl was used to titrate the excess 0.1N NaOH using phenolphthalein as an indicator. The carboxyl content of the hydrogel sheets was calculated as milliequivalents per 100g using the following formula:

$$\text{Carboxyl content} = \frac{(V_b - V_a) \times N \times 100}{W} \quad (2)$$

Where V_b and V_a are the volumes of 0.1N HCl in the presence and absence of the sample, respectively, N is the normality of the HCl solution, and W is the weight of the sample (g).

Characterization of Hydrogel film

IR Spectrum

The IR spectra of HPMC, MC, citric acid, ciprofloxacin hydrochloride, plain hydrogel film and drug-loaded hydrogel film were recorded using an ATR-FTIR spectrophotometer (Shimadzu, IR Affinity, Japan) with a resolution of 4 cm^{-1} and an average of 25 scans per spectrum in the range of 600-4000 cm^{-1}.

Thermal analysis

Thermal analysis of HPMC, MC, citric acid, ciprofloxacin hydrochloride, plain hydrogel film and drug-loaded hydrogel film was conducted using a Mettler-Toledo TGA/DSC thermogravimetric analyzer (Mettler-Toledo, Switzerland) in a nitrogen atmosphere at a flow rate of 10 mL/min and temperatures ranging from 30 to 300°C.

Determination of swelling ratio

The swelling ratio was determined by immersing predetermined weights of hydrogel films (0.2 g) in 0.1N HCl, Phosphate-Buffered Saline (PBS) pH 7.4, at 25°C for 5, 10, and 15 min.[26-29] Swollen hydrogel films were gently soaked with filter paper to remove surface liquid and weighed. The weight of the swollen hydrogel

(W_s) was measured and swelling ratio was calculated using the formula:

$$\text{Swelling ratio (g/g)} = \frac{W_s - W_d}{W_d} \quad (3)$$

where W_d is the weight of the dry hydrogel film. The absorbency was calculated for hydrogel sheets submerged in 0.1N HCl, PBS (pH 7.4), for 1hr and 24hr, and measurements were performed in triplicate.

Drug loading and *in vitro* release

Pre-weighed hydrogel films were immersed in 10 mL of distilled water containing 5 mg of ciprofloxacin hydrochloride until equilibrium swelling was achieved. Subsequently, the drug-loaded hydrogel films were dried for 24hr in a hot air oven at 40°C. A small piece of the drug-loaded hydrogel was weighed, broken into small pieces, and dissolved in 10 mL of distilled water to determine the amount of ciprofloxacin hydrochloride loaded. The liberated amount of ciprofloxacin hydrochloride in the solution after agitation on a magnetic stirrer for 24hr was quantified spectrophotometrically at λ_{max} of 271 nm. Dry hydrogel films loaded with ciprofloxacin hydrochloride (50 mg) were placed in 10 mL of 0.1N HCl, PBS (pH 7.4) at 37°C and samples were taken at definite time intervals. The withdrawn samples were immediately replaced with fresh media to maintain constant volume. The amount of ciprofloxacin hydrochloride released from the hydrogel films in 0.1N HCl and PBS (pH 7.4) was determined spectrophotometrically at λ_{max} of 277nm and 271 nm, respectively. The experiments were conducted in triplicate.[30-32]

Hemolysis assay

The hemolysis test was performed with some modifications in the previously reported method. The plain hydrogel sheets with an area of 2 cm^2 were prepared by equilibrating them in PBS (pH 7.4) for 60 min at 37°C, and then 0.5 mL of human CPD (Citrate-Phosphate-Dextrose) blood was added to each sample. After 20 min, hemolysis was stopped by adding 4.0 mL of 0.9% Sodium Chloride (NaCl) saline to each sample, which was then incubated for an additional 60 min at 37°C. To prepare positive and negative controls, 0.5 mL of human CPD blood was mixed with 4.0 mL of distilled water and 0.9 mL of normal saline, respectively. The samples were centrifuged at 3,500 rpm for 10 min to collect the supernatant and the solids. The absorbance of the supernatant was measured using a UV-visible spectrophotometer at 545 nm (Shimadzu, Japan).[33-35] The percentage of hemolysis was calculated using the following formula:

$$\text{Hemolysis (\%)} = \frac{A_{\text{Test sample}} - A_{\text{Control}}}{A_{\text{+ve Control}} - A_{\text{−ve Control}}} \times 100 \quad (4)$$

where 'A' represents the absorbance. The absorbance of positive and negative controls were recorded as 1.4969 and 0.002, respectively.

RESULTS AND DISCUSSION

Experimental design

A factorial approach was employed to investigate the impact of the independent variables. All nine possible combinations were tested, and the results for the independent variable are presented in Table 2.

The best-fitting model was used to analyze the impact of the independent factors. The polynomial equations for the responses included coefficients for the intercept, main effects, and interaction effects. The sign and size of the major effects indicate how much each element influenced the response in relation to another. 3D response surfaces were used to visualize how the ratio of polymer to citric acid concentration affected each response (see Figure 1). The significance of the model for all tested responses is presented in Tables 3 and 4, which include the ANOVA results and statistical parameters. As the signal-to-noise ratio was found to be too low (> 4) for acceptable accuracy, the models were also used to explore the design space.

The polynomial equations for responses are given below:

$$\text{Carboxyl content (mEq/g)} (Y_1) = 798.77 + 314.48\,X_1 + 419.34\,X_2 + 307.59 X_1 X_2 \quad (5)$$

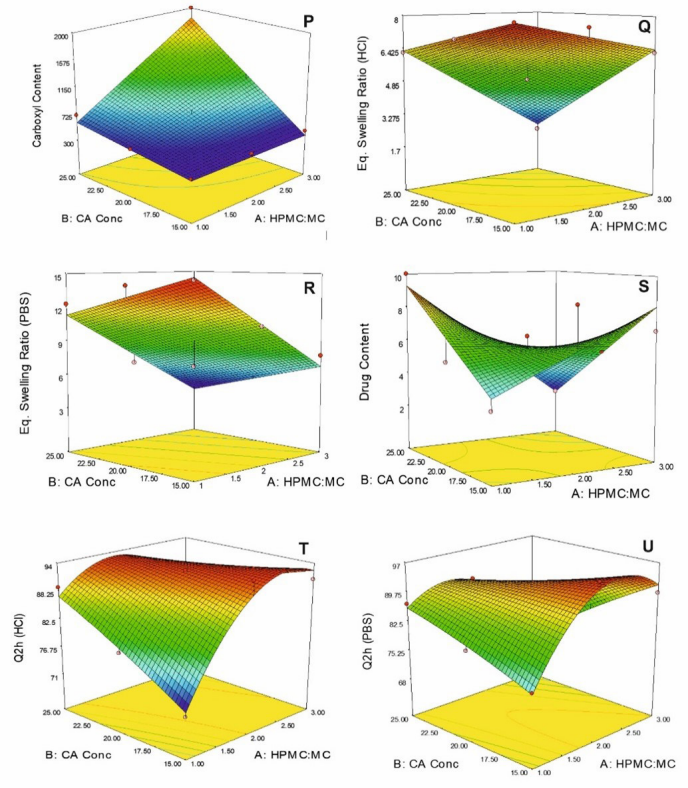

Figure 1: 3D surface response plot for carboxyl content (P), equilibrium swelling ratio in 0.1N HCl (Q), equilibrium swelling ratio in PBS, pH 7.4 (R), drug content (S), drug released after 2hr in HCl (T) and drug released after 2hr in PBS, pH 7.4 (U).

$$\text{Drug Content (\%) } (Y_2) = 5.88 - 0.73X_1 - 0.22X_2 - 2.90\ X_1X_2 \quad (6)$$

$$\text{Eq. Swelling Ratio (0.1N HCl) } Y3 = 5.69 - 1.45X_1 - 1.51X_2 - 0.71X_1X_2 \quad (7)$$

$$\text{Eq. Swelling Ratio (PBS) } Y4 = 9.06 - 4.09\ X_1 - 1.81X_2 \quad (8)$$

$$\text{Q2h (0.1N HCl) } Y5 = 92.55 + 3.70\ X_1 + 1.05\ X_2 - 6.28X_1X_2 - 8.26\ X_1^2 - 0.64\ X_2^2 \quad (9)$$

$$\text{Q2h (PBS) } Y6 = 92.48 - 0.022X_1 - 2.87\ X_2 - 8.01X_1X_2 - 10.86\ X_1^2 - 0.74\ X_2^2 \quad (10)$$

Citric acid crosslinked HPMC/MC hydrogel films

The esterification-cross-linking method was utilized to synthesize HPMC:MC hydrogel films, where both HPMC-MC cross-linking and interpolymer crosslinking could be involved in the hydrogel formation process. The potential crosslinking between HPMC, citric acid, and MC during the hydrogel synthesis is illustrated in Figure 2. The thickness of the films was found to be approximately 150 µm. The citric acid concentration, curing temperature, and curing time required for the production of hydrogels with good consistency were chosen based on the results of the preformulation experiments. In previous study, citric acid cross-linked hydrogels were prepared at the curing temperature in between 80 to 150°C, and the curing period ranged from 5 min to 24hr.[22,23,36,37] In this study, HPMC-MC hydrogel films with good elastic consistency were prepared at a curing temperature of 160°C and a curing time of 20 min.

Effect of polymer ratio and citric acid concentration on carboxyl content

The impact of the HPMC/MC ratio and citric acid on carboxyl content was investigated and it was found that an increase in the polymer ratio, as well as citric acid concentration, increased the carboxyl content and hence, the extent of crosslinking (see Figure 1P), which may be due to high number of reactive hydroxyl groups in HPMC compared to MC. The extent of crosslinking also increased with the concentration of citric acid, resulting in more carboxyl groups in the hydrogel's polymeric network. Equation (5) demonstrates that the citric acid had greater influence on the carboxyl content of the hydrogel films than the polymer ratio.[1,7]

ATR-FTIR analysis

Figure 3 shows the overlay ATR-FTIR spectra of plain hydrogel film, HPMC, MC, citric acid, ciprofloxacin hydrochloride and drug-loaded hydrogel film. The IR spectrum of plain hydrogel demonstrates the presence of specific peaks at 3462.22 cm^{-1}, 2922.16 cm^{-1}, 1728.22 cm^{-1}, 1327.03 cm^{-1}, 1195.87 cm^{-1} and 929.69 cm^{-1}, representing O-H vibrational stretching corresponding to OH and COOH groups, C-H stretching due to alkyl groups (methyl or hydroxypropyl groups), ester carbonyl stretching which ensures formation of ester crosslinks, C-O stretching of ester group, C-O stretching of primary alcohol, and C-C stretching in benzene, respectively.

The spectrum of pure ciprofloxacin hydrochloride shows the presence of characteristic peaks at 3531.66 cm^{-1}, 3373.50 cm^{-1}, 3091.89 cm^{-1}, 3022.45 cm^{-1}, 2821.86 cm^{-1}, 2931.80 cm^{-1}, 2916.37 cm^{-1}, 2686.84 cm^{-1}, 2617.40 cm^{-1}, 1699.29 cm^{-1}, 1616.35

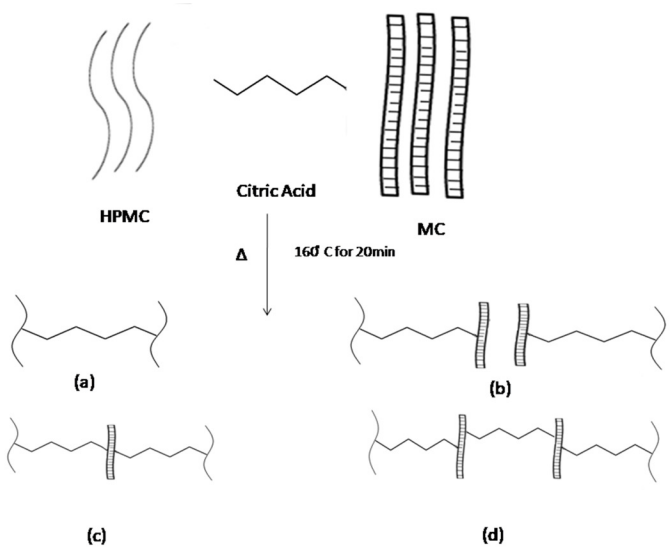

Figure 2: Possible crosslinking reaction between HPMC, citric acid and MC.

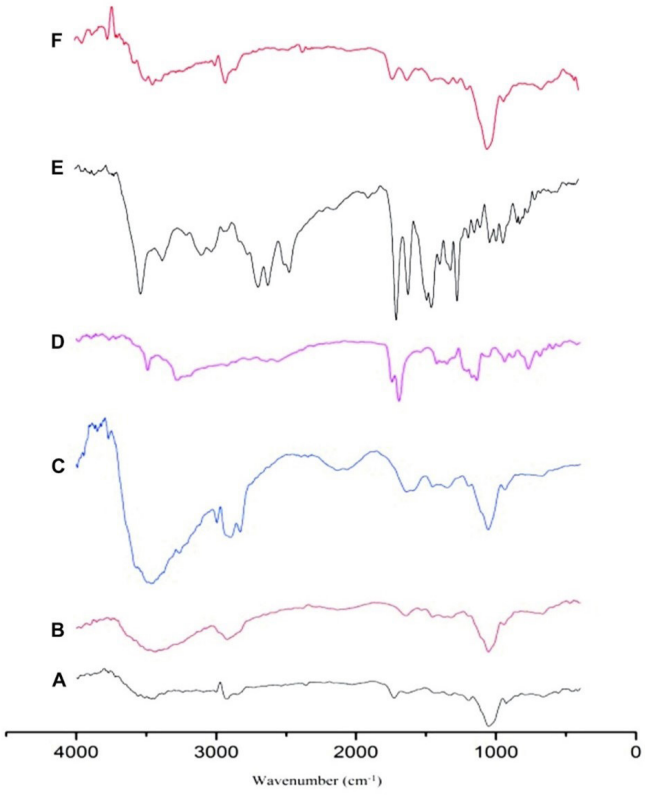

Figure 3: ATR-FTIR spectra of plain hydrogel (A), HPMC (B), MC (C), citric acid (D), ciprofloxacin hydrochloride (E) and drug-loaded hydrogel film (F).

cm^{-1}, 1483.26 cm^{-1}, 1450 cm^{-1}, 1265.30 cm^{-1}, and 1313.52 cm^{-1}, representing O-H vibrational stretching due to intermolecular hydrogen-bonded alcohols and acids, N-H stretching due to piperazinyl, N-H stretching due to secondary amine, C-N stretching, aliphatic C-H stretching, N-C stretching denoting primary amine, C=O stretching of carboxyl group, C=O stretching of quinolone, C-N stretching, C-F stretching, and C-O stretching in alcohols, respectively. The spectra of ciprofloxacin hydrochloride exhibit all peaks at the same wavelength and with the same intensity in both plain and drug-loaded hydrogel, suggesting that there was no undesired interaction between the components of hydrogel and pure drug utilized in the study.[38] Consequently, it is evident that ciprofloxacin hydrochloride is compatible with HPMC, MC, and citric acid.

DSC analysis

Figure 4A depicts DSC thermogram of citric acid, MC, HPMC and plain hydrogel film. The DSC thermogram of HPMC-K100 displayed a small endothermic peak at 348.42°C, indicating the initiation of thermal decomposition as well as a broad endothermic peak at 370.41°C, which complete decomposition at temperatures above 360°C. In the case of methyl cellulose, a small endothermic peak at 339.70°C corresponded to the initiation of thermal decomposition, while a broad endothermic peak at 370.41°C indicated completion of decomposition. Anhydrous citric acid demonstrated a small endothermic peak at 155.46°C, which corresponds to the melting point of citric acid. Additionally, a broad endothermic peak was observed at 161.70°C and 194.23°C, which may be attributed to the decomposition of citric acid. The plain hydrogel formulation exhibited no citric acid peak in its DSC thermogram, as citric acid underwent esterification and all residual traces of free citric acid were eliminated during washing with isopropyl alcohol and distilled water. The plain hydrogel exhibited a small endothermic peak at 91.40°C indicating the loss of bound water and slightly elevated endothermic peak at 370.41°C, which corresponds to the decomposition of both MC and HPMC.

The DSC analysis of ciprofloxacin hydrochloride revealed several endothermic peaks. A small endothermic peak observed at 121.70°C is attributed to the removal of moisture content present in the drug sample. Another endothermic peak at 140.75°C may be associated with the elimination of water, while a third peak at 188.45°C corresponds to the glass transition temperature of the drug. Additionally, an endothermic peak at 323.70°C signifies the melting point of ciprofloxacin hydrochloride.

Furthermore, the DSC thermogram of ciprofloxacin hydrochloride loaded hydrogel displayed a small endothermic peak at 92.16°C and 149.09°C, indicating the loss of bound water. The strong endothermic peaks at 348.04°C correspond to the decomposition of MC and HPMC. However, the melting peak of ciprofloxacin hydrochloride was not detected in the DSC analysis

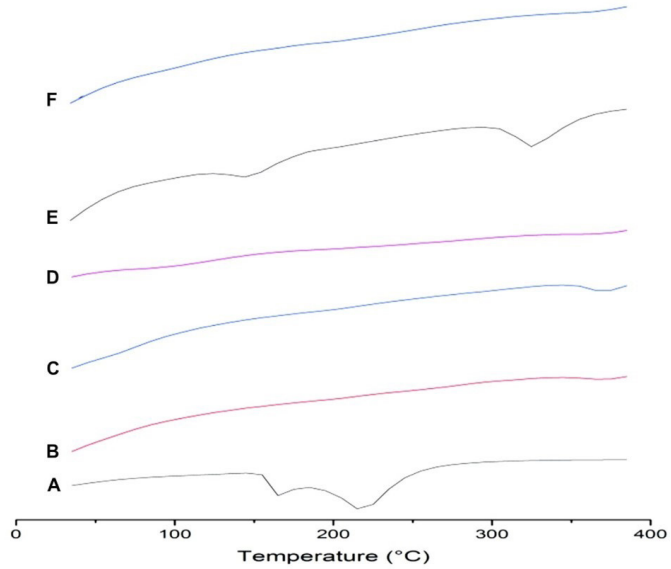

Figure 4: DSC thermogram of citric acid (A), MC (B), HPMC (C), plain hydrogel (D), ciprofloxacin hydrochloride (E) and drug-loaded hydrogel film (F).

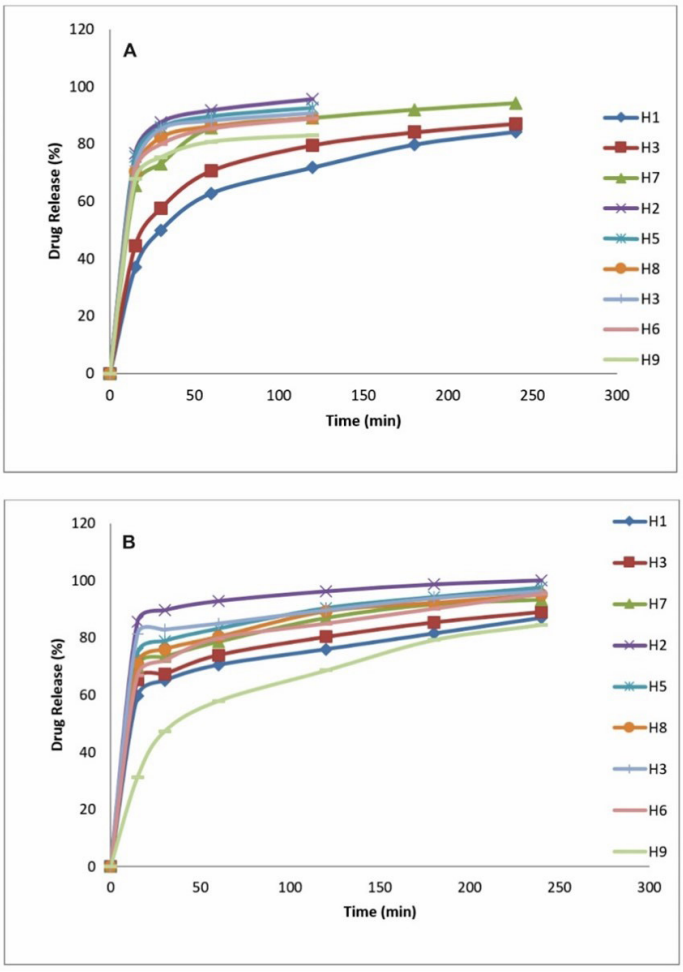

Figure 5: Drug release from HPMC/MC hydrogel films in 0.1 N HCl (A) and PBS, pH 7.4 (B).

of drug loaded hydrogel, indicating the possibility of molecular dispersion of ciprofloxacin hydrochloride within the polymeric matrix of cellulose-based hydrogel.[38]

Effect of variables on equilibrium swelling ratio

The impact of citric acid on the equilibrium swelling degree in 0.1 N HCl is highlighted by Equation (7) and Figure 1Q. The results demonstrate that changes in the polymer ratio can cause

Table 3: Summary of ANOVA for responses.

Source	Sum of squares	d.f.	Mean square	F value	*p*-value Prob>F
Y_1-Carboxyl content (mEq/g) (2FI Model)					
Model	2.027E+006	3	6.756E+005	32.17	0.0011
X_1	5.934E+005	1	5.934E+005	28.25	0.0032
X_2	1.055E+006	1	1.055E+006	50.23	0.0009
X_1X_2	3.785E+005	1	3.785E+005	18.02	0.0081
Y_2- Drug content (%) (2FI Model)					
Model	37.16	3	12.39	5.47	0.049
X_1	3.19	1	3.19	1.41	0.0001
X_1X_2	33.69	1	33.69	14.87	0.0119
Y_3- Equilibrium swelling ratio in 0.1N HCl (2FI)					
Model	28.30	3	9.43	39.39	0.0007
X_1	12.59	1	12.59	52.56	0.0008
X_2	13.68	1	13.68	57.13	0.0006
X_1X_2	2.03	1	2.03	8.48	0.0333
Y_4 - Equlibrium swelling ratio in PBS pH (Linear Model)					
Model	120.23	2	60.12	39.48	0.0004
X_1	100.61	1	100.61	66.07	0.0002
X_2	19.62	1	19.62	12.88	0.0115
Y_5- Q2h in 0.1N HCl (%) (Quadratic)					
Model	383.63	5	76.73	9.22	0.0485
X_1	81.99	1	81.99	9.85	0.0517
X_1X_2	157.63	1	157.63	18.93	0.0224
X_1^2	136.57	1	136.57	16.40	0.0271
Y_6- Q2h in PBS (%) (Quadratic Model)					
Model	543.57	5	108.71	11.98	0.0339
X_1X_2	256.08	1	256.80	28.30	0.0130
X_1^2	236.10	1	236.10	26.02	0.0146

Table 4: Statistical Parameters.

Parameters	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
Std Dev	144.93	1.51	0.49	1.23	2.89	3.01
Mean	798.77	5.88	5.69	9.06	86.61	84.74
CV %	18.14	25.59	8.60	13.62	3.33	3.55
PRESS	5.617E+005	47.00	2.96	16.58	304.49	283.96
R-Squared	0.9507	0.7664	0.9594	0.9294	0.9389	0.9523
AdeqPrecision	15.190	7.238	18.136	16.57	8.63	10.022

Y_1- carboxyl content (mEq/g); Y_2- drug content (%); Y_3- equilibrium swelling ratio in 0.1N HCl; Y_4 - equlibrium swelling ratio in PBS pH; Y_5- Q2h in 0.1N HCl (%);Y_6- Q2h in PBS (%).

significant fluctuations in the degree of equilibrium swelling in 0.1 N HCl. The equilibrium swelling ratio in 0.1N HCl (Y3), as shown in Equation (4), reveals a negative and prominent effect of citric acid on the swelling degree in 0.1 N HCl. As the concentration of citric acid increases, the extent of cross-linking also increases, which results in reduced water retention and, ultimately, a decreased swelling degree in 0.1 N HCl. Variations in the polymer ratio also led to significant changes in the equilibrium swelling degree in 0.1 N HCl. Specifically, in the 1:1 polymer ratio, the concentration of MC is greater than that of HPMC. This decreased concentration of -OH groups in the 1:1 polymer ratio leads to a reduction in crosslinking, resulting in an increased number of free polymeric chains. This leads to the formation of a loose polymeric network, which enhances water retention and ultimately results in increased swelling.[7]

Equation (8) and Figure 1R reveal that the equilibrium swelling ratio in PBS is primarily influenced by the polymer ratio rather than the concentration of citric acid. The swelling of hydrogels decreases as the concentration of citric acid increases due to increased cross-linking, which results in the formation of a dense polymeric network that has reduced water retention and ultimately leads to decreased swelling. Additionally, increasing the polymer ratio leads to an increase in the number of hydroxyl groups and an increased extent of cross-linking, resulting in decreased swelling in PBS, pH 7.4. The concentration of methylcellulose is higher in the 1:1 polymer ratio, which reduces the number of OH groups and leads to decreased cross-linking. This, in turn, results in increased water retention and a softer hydrogel compared to the hydrogels prepared using polymeric ratios of 2:1 and 3:1.

Effect variables on drug content and drug release

According to Equation (6), the drug content was notably influenced by the interaction effect of polymer ratio and citric acid. As depicted in Figure 1S, drug content was found to be maximum when amount of HPMC is high and concentration of citric acid is low, and vice-versa.

Equation (9) and Figure 1T demonstrates a significant impact of the interaction between the polymer ratio and concentration of citric acid on drug release in 0.1N HCl. In batch H2, the highest drug release of 93.57% was achieved in 0.1 N HCl due to the appropriate concentration of citric acid (15%) and polymer ratio (2:1), which resulted in increased swelling and pore size, leading to enhanced drug entrapment and release (see Figure 5). However, in batch H1, the lower concentration of citric acid (15%) led to inadequate crosslinking, causing the obstruction of pores by free polymeric chains and decreased entrapment of drug particles, resulting in reduced drug release. In batch H3, with a higher polymer ratio of 3:1, the drug release was improved compared to batch H1 due to increased swelling, but it was lower than batch H2 because of a lower drug content (8.575%). A similar trend was observed in batches H4 to H6 and H8, except for batches H7 and H9, where the high and low drug content, respectively, affected the drug release. As the drug was freely soluble in 0.1 N HCl, the maximum drug release was observed within 2 hr.

In phosphate buffer at pH 7.4, drug release was sustained for a duration of up to 4 hr. The ionization of –COOH groups leads to the formation of free –COO- ions that repel each other, resulting in increased spacing between adjacent polymeric chains. This phenomenon causes swelling to increase, leading to the sustained release of the drug for a period of 4 hr (Figure 5B). Equation (7) and Figure 1U reveals that the drug release in phosphate buffer was mainly affected by interaction between polymer ratio and citric acid concentration, followed by concentration of citric acid. Both the variables had negative impact on the release of drug from the hydrogel matrix. Considering high drug content, swelling ratio and better ability to control the release, batch H2 was found to be optimized batch among the prepared batches.

Hemocompatibility study

A hemolysis assay was performed on the optimized batch to assess the compatibility of the prepared hydrogel with red blood cells. The ideal hydrogel should maintain its regular physiology, rheology, and integrity when in contact with blood. The hemolytic potential of the hydrogel film measures the degree of hemolysis that occurs when the hydrogel comes into contact with blood. The hydrogels demonstrated a negligible hemolytic potential of 1.49% which was within the permissible range of 5%.[33,34] This finding demonstrates that the prepared HPMC:MC hydrogel offers highly favorable blood compatibility.

CONCLUSION

The combination of HPMC/MC can be crosslinked effectively using citric acid. The cross-linking process was characterized using DSC or FTIR analysis. FTIR spectra were collected at various reaction times and an excess citric acid concentration was used to investigate the different cross-linking reactions. The batch with a polymer ratio of 1:1 and 15% citric acid showed the highest equilibrium swelling ratio in phosphate buffer, pH 7.4, and 0.1N HCl. The hydrogel produced using the method described in this study has significant advantages in terms of lowering primary and production costs and not using any harmful intermediates. HPMC/MC hydrogel films prepared with a polymer ratio of 1:1 and 25% citric acid exhibited a high loading of ciprofloxacin hydrochloride. The films produced using a blend of the two polymers showed superior physico-pharmaceutical characteristics compared to those made with either polymer alone. The films made with HPMC/MC at a 2:1 ratio with 15% citric acid (batch H2) demonstrated the best functionality among the polymer blends tested. Based on the overall results of this study, citric acid crosslinked HPMC/MC hydrogel films could be a promising biomaterial for wound treatment. However, further

investigation is necessary to confirm their effectiveness as wound dressings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATR-FTIR: Attenuated total reflection-Fourier-transform infrared; **BCS:** Biopharmaceutics Classification System; **CA:** Citric acid; **CM:** Carboxymethyl cellulose; **CPD:** Citrate-phosphate-dextrose; **DSC:** Differential scanning calorimetry; **HEC:** Hydroxyethyl cellulose; **HPMC:** Hydroxypropyl methyl cellulose; **MC:** Methyl cellulose; **PLA:** Poly (lactic acid); **PLGA:** Poly (lactide-co-glycolide); **PBS:** Phosphate buffered saline; **RSM:** Response surface methodology; **SEM:** Scanning electron microscopy; **SHP:** Sodium hypophosphite; **TGA:** Thermo gravimetric analyzer.

SUMMARY

The current study involved the development of Hydroxypropyl Methylcellulose/Methylcellulose (HPMC/MC) hydrogel films that were cross-linked with citric acid for the delivery of a water-soluble drug, ciprofloxacin. A 3^2 full factorial design was employed for batch optimization. The films were characterized using thermal analysis and ATR-FTIR spectroscopy to confirm the formation of crosslinks. The hydrogel films were evaluated for various properties, including carboxyl content, swellability, drug loading, drug release, and hemocompatibility. The results showed that the addition of methylcellulose aided in creating firm and homogeneous hydrogel films. The hydrogel batches with low carboxyl content exhibited the good swelling and sustained the drug release for longer duration. The formation of ester cross-links was confirmed using ATR-FTIR spectra and DSC thermograms. The hydrogel films were also found to be biocompatible through hemolytic assay. The combination of HPMC/MC with citric acid showed the highest equilibrium swelling ratio in phosphate buffer at pH 7.4 and 0.1N HCl. Notably, the method used in this study has advantages in terms of lowering primary and production costs and not using any harmful intermediates. Among the polymer blends tested, the films made with HPMC/MC at a 2:1 ratio with 15% citric acid demonstrated the best functionality. In conclusion, the citric acid crosslinked HPMC/MC hydrogel films possess superior physico-pharmaceutical characteristics and biocompatibility, making them a promising biomaterial for wound treatment.

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