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Formulation, Characterization and Optimization of Ketoprofen Loaded Hydrogel Films

Magharla Dasaratha Dhanaraju*, Vankayala Devendiran Sundar, Anilkumar Vadaga, Veeranjaneyulu N P.
Department of Pharmaceutical Technology, GIET School of Pharmacy, Chaitanya Knowledge City, Rajahmundry, AP – 533296.
***Corresponding author email:** mddhanaraju@yahoo.com

ABSTRACT
Hydrogel dressings comprised of hydrophilic, inflated, and soluble components are frequently available in the form of gel and film. They are only appropriate for the exterior of wounds because they merely comprise 70–90% water, which allows them to absorb excessive exudates. They have numerous advantages in drug delivery, bioengineering, sanitation products, farming, waste water treatment, textiles, and packaged food. Hydrogels generated from biodegradable polymers and their analogues have been extensively exploited in medication delivery and bioengineering purposes in recent years. Employing citric acid as a crosslinker, hydroxyethyl cellulose (HEC) hydrogel films were made in the current study for the controlled release of a model hydrophobic medication (Ketoprofen). Crosslinking enhanced the mechanical characteristics and fluid absorption capacity of dressings. Swelling, mechanical and mucoadhesive properties of blank and drug-loaded films were evaluated and compared. Drug loaded films showed better swelling and mucoadhesive profile. It can therefore be inferred that citric acid could be employed to create HEC hydrogel films. Altogether, the findings suggest that HEC hydrogel films are ideal for improved drug-loading and controlled release of weakly soluble medicines. The outcomes strongly suggest that hydrogel films can be utilized as possible wound healing materials.
Keywords: *Wound healing, wound dressings, hydrogels, hydroxyethyl cellulose, citric acid, Ketoprofen, swelling property, tensile strength, adhesion*

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INTRODUCTION

Wound healing is a complex and dynamic biological process that involves haemostasis/inflammation, cell proliferation, and tissue remodelling phases [1]. Wound dressings of various shapes, sizes, colours, and origins have been developed to create and maintain a moist environment, as well as to provide optimal conditions for wound healing [2]. The use of biopolymer-based hydrogels as wound dressings is popular owing to their biocompatibility and biodegradability. Despite recognized benefits, the use of hydrogels is still associated with some challenges because of a few limitations. These include mechanical weakness, water sensitivity, and instability under physiological conditions, with unpredictable behaviour in long-term applications [3].

Hydrogel dressings are most often accessible in gel and film forms and are totally fabricated of hydrophilic, expandable, and biodegradable constituents. Since they contain only 70-90% moisture, they should only be applied to top of wounds to soak up superfluous exude. Hydrogels generated from biodegradable polymers and their analogues have been extensively exploited in medication delivery and bioengineering purposes in recent years [4,5]. Addition of crosslinkers to hydrogels is one approach to improve the physical properties and stability of hydrogels via impeding the dissolution and disintegration of the polymer matrix. The selection of crosslinkers is important to avoid toxicity and undesirable reactions with the hydrogel polymer matrix [6]. However, they are useful, especially for the encapsulation of labile bioactive substances and living cells in hydrogels. Employing citric acid as a crosslinker, hydroxyethyl cellulose (HEC) hydrogel films were made in the current study for the controlled release of a ketoprofen [7].

MATERIAL AND METHODS

Ketoprofen drug was purchased from BEC Chemicals, Mumbai, India. 2-Hydroxyethyl cellulose, citric acid, β- Cyclodextrin was purchased from Sigma-Aldrich.

Methods

Preparation of Hydrogel films

Preparation of βCD grafted HEC Hydrogel films

Two beakers were taken and citric acid was added in one and beta cyclodextrin in other, followed by addition of hydroxyethyl cellulose (2% w/v) in each beaker. The beaker was then placed on a magnetic stirrer with 800 rpm at ambient temperature for 3 hours. It was then allowed to rest for 1 hour in order to allow air bubbles to escape before proceeding to the next step. The same procedure was repeated for 3% w/v HEC [8, 9].

For casting hydrogel films, the above mixtures (20mg of 2% w/v HEC, 30gm of 2% w/v HEC, 20gm of 3% w/v HEC and 30 gm of 3% w/v HEC) were poured into a petri dish of 9cm diameter and films were casted as described below.

- Hydrogel mixtures were poured into a petri dish and placed in an oven for 24 hours at 50°.
- This was followed by curing of dried films for 5 minutes at 145 ℃. They were washed using distilled water and pH was brought to neutral. They were washed again with ethyl alcohol for an hour to remove unreacted elements.
- The films were further dried for a day at ambient temperature and stored in a desiccators for future use. Four different HEC films were obtained:
  HEC-1 = 20g of 2% w/v HEC
  HEC-2 = 30g of 2% w/v HEC
  HEC-3 = 20g of 3% w/v HEC
  HEC-4 = 30g of 3% w/v HEC

Preparation of drug loaded films:

The hydrogel films are loaded with Ketoprofen before being tested for tensile strength, adhesion, water vapour transport rate, swelling capacity, and evaporated water loss. Ketoprofen-loaded films were obtained by immersing 3 squares of each film for 1 hour in a mixture of ethanol: distilled water in 8:2 ratios containing 0.05 g of Ketoprofen and covering the beaker with parafilm to prevent evaporation of the mixture. After an hour, squared films are placed in their correspondingly labelled petri dishes, where tests are carried out at 24, 48, 72, and 96 hours [10]. The drug-loaded films were weighed using a weighing balance and weights obtained are as follows:

HEC-1 = 0.346g
HEC-2 = 0.575g
HEC-3 = 0.572g
HEC-4 = 0.869g

These films were then squared by compressing in a square maker; a paper was placed under each film to prevent it from sticking to the square maker and breaking. The resultant squared films had the diameter of 20mm x 20mm. These films were then stored in a desiccators for future use. These squared films were again weighed and weights obtained are as follows:

HEC-1 = 0.028g
HEC-2 = 0.048g
HEC-3 = 0.040g
HEC-4 = 0.066g

Methods Used for Testing the Blank and Drug Loaded Films

Swelling study

For swelling study, 12 petri dishes are used each petri dish is labelled as follows in the table 1 below

Table 1: Swelling study

HEC-1			HEC-2			HEC-3			HEC-4		
A	B	C	A	B	C	A	B	C	A	B	C

Each squared film was placed in a petri dish and weighed as W_d. It was then immersed in SWF (pH 7.5, at 40°C), covered with a lid and left undisturbed for 24 hours. The films were then taken out and extra SWF was blotted using a tissue paper. The weight of each swollen film was noted as W_s. The films were immersed in SWF again for another 24 hours and the procedure was repeated for time intervals of 48 hr, 72 hr and 96 hr [11,12].The swelling capacity was calculated using the following formula.

$$R_s(\text{g/g}) = \frac{(W_s - W_d)}{W_d}$$

Where, R_s is swelling ratio
W_s is weight of swollen film
W_d is weight of dry film

Measurement is done triplicate to minimise the errors.

Water vapor transmission rate (WVTR)

Twelve scintillated glass vials of 25ml volume and 8mm size were used. Three films of each type were used in this method and labelled as: HEC-1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B and 4C. 20mL of SWF was added to each vial and their mouth was covered with a squared film using a glue [13]. Each vial was weighed as $W_b$and then placed in an oven at 40°C for 24 hours. The vials were weighed again individually as W_{af} and placed in an oven again for another 24 hrs. The procedure was repeated for time intervals of 48 hr, 72 hr and 96 hrs (Thomas, 2007). WVTR was calculated using the following formula:

$$WVTR = \frac{W_b - W_{af}}{A} \times 10^6 \frac{g}{m^2} day^{-1}$$

Where, W_b is the weight of vial before placing in oven

W_{af} is the weight of vial after taking out of oven

A is the area of mouth of vial

Evaporative Water Loss (EWL):

Twelve square films, three of each type were taken in a petri dish and labelled as HEC-1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B and 4C. 1.5 mL of SWF was added to each petri dish and kept at ambient temperature for 24 hrs. The films were then removed and dried another 24 hr and weight of the film was noted as W_i. The procedure was repeated for specific time intervals of 48 hr, 72 hr and 96 hr and weights were noted each time [14]. EWL was calculated using the following formula:

$$EWL\ (\%) = \frac{W_t}{W_i} X\ 100$$

where, W_t is the weight of film after a specific time 't'

W_i is the weight of film after 24 hrs (initial incubation)

Adhesion Test

Preparation of gelatine for adhesion test of the blank and drug loaded films

300mL of de-ionised water was taken in a beaker and placed on a hot plate. When the desired temperature was reached, 20.01g of gelatin was slowly added to the beaker and kept aside until it dissolved completely [15]. 20g of this gel was then poured into petri dish and allowed to cool for 10 min, followed by refrigerating until it transforms into gel. The obtained gelatin was used for assessment of adhesive test.

Method of adhesive test

For evaluating the adhesive qualities and stickiness of films, texture analyser (stable microsystem Ltd) with texture exponent 32 software was employed. Before beginning the test, 1.5 ml of SWF was coated on a petri dish made of gelatin to simulate a chronic wound's surface, and it was then inserted on the texture analyser on the stationary platform. Using double-sided adhesive tape, sample films were affixed to the 35mm diameter cylindrical steel probe [16]. The following settings were used to obtain measurements: pre-test speed 0.50mm/sec, test speed 1.00 mm/sec, post- test speed 1.00 mm/sec, applied force 1.000 N, return distance 10.000 mm, contact time 60.00 sec, trigger type -Auto, trigger force 0.049 N. The test was conducted for drug-loaded and blank films. Three HEC films of each typewere used in this experiment. The adhesive properties of the films are calculated using the mean and standard deviation.

Tensile Strength

The TA. HD analyzer (Stable Microsystem Ltd, Surrey, U.K.), having a load capacity of 5 kg for data plotting and the texture Exponent 32 software program for visualization, was utilized for determining the tensile strength of the films. To fit inside the texture analyzer grips, the films were cut using a 3D rectangular-shaped bar to a size of 60 mm x10mm. The grips were marked at 10mm length from both ends. To evaluate the films' ability to withstand stretching, a 40mm gap of the film is kept in the middle. The film's average thickness is noted.Texture analyser settings are done as follows for blank films: test mode is tension, pre-test speed 60.0 mm/min, test speed 60.0 mm/min, post- test speed 120.0 mm/min, distance 99.000 mm, trigger type Auto force (Force), trigger force 0.010 N [17].

The stretching strength of Ketoprofen-loaded hydrogel films was analysed and drug loading capacity was determined.

Thickness of the films

Thickness of all the prepared films (20mg of 2% w/v HEC, 30gm of 2% w/v HEC, 20gm of 3% w/v HEC and 30 gm of 3% w/v HEC) were measured using a screw guage and three different readings obtained for each film are tabulated below.

Table 2: Thickness of the films

	HEC-1	HEC-2	HEC-3	HEC-4
A	0.7 mm	0.11mm	0.13 mm	0.125 mm
B	0.75 mm	0.9 mm	0.10 mm	0.17 mm
C	0.8 mm	0.10 mm	0.09 mm	0.15 m

RESULTS AND DISCUSSION

Hydrogel films were prepared by the using hydroxyethyl cellulose (HEC), β-CB and citric acid as cross-linking agent and then loaded with Ketoprofen. Both blank and drug-loaded films were evaluated for swelling capacity, mechanical properties, evaporative water loss (EWL), Equilibrium water content (EWC) water absorption (AW) and mucoadhesive properties

Swelling test:

Swelling ratio was calculated from the equation

$$\textbf{(IS) \%} = \frac{Wst - Wi}{wd} \times 100 \text{ -------- eq.1}$$

W_i is the initial weight of the film before hydration.

W_{st} is the swollen weight of films at different swollen time intervals at 24,48,72 and 96 hours respectively.

W_d is the dry weight of the film samples before hydration.

With the eq.1 swelling index of blank and drug loaded films are calculated and graphs are plotted

By taking the triplicates of A, B and C to minimize the errors for each percentage and weight of the film mean ± standard deviations are calculated for the graphical analysis of the blank and Ketoprofen-loaded films.

The swelling behavior of the films was evaluated in SWF (pH 7.5) and the effect of drug was also investigated. For blank films, 2% 30 g HEC films demonstrated minimum swelling capacity of 233.4667 ± 26.85086 at 24 hr interval and 2% 20 g HEC films showed maximum swelling capacity of 459.9333 ± 102.6371 at 24 hr interval. Among drug loaded films, 2% 20 g HEC showed minimum swelling capacity of 126.8633 ± 38.3022 at 24hr interval and a maximum swelling capacity of 696.7767± 369.4763 was reported in 2% 20 g HEC at 48 hr interval. Drug loaded films showed better swelling capacity compared to the blank films. The optimal swelling ratio of the film is determined by the percentage of polymer and amount of citric acid used [18]. As demonstrated by previous research, the occurrence of hydrophilic COOH groups in the polymer increases the swelling capacity of the composition due to the hydrophilic nature of these functional groups. The inclusion of citric acid could be accountable for the enhanced swelling capacity of the formulation. In addition, the chemical makeup of β-CD may enhance the three-dimensional network configuration during cross-linking interactions, hence boosting the swelling capacity of the formulation. Moreover, the swelling ratio of the hydrogel improves with the increase in its porosity. A possible explanation is that the hydrogel's high porosity produces a large volume to hold a significant amount of water in the hydrogel matrix.

For 2% 20 g HEC at 0, 24, 48, 72, and 96 hours

Table 3: Swelling index of 2% 20 g HEC films at different time intervals

A	B	C	**MEAN**	**ST**
0	0	0	**0**	**0**
320	563.3	496.5	**459.9333**	**102.6371**
277.1	370	355.1	**334.0667**	**40.73822**
291.4	319	403.44	**337.9467**	**47.66181**
294.2	496.1	413.7	**401.3333**	**82.88789**

For 2% 30 g HEC at 0, 24, 48, 72, and 96 hours

Table 4: Swelling index of 2% 30 g HEC films at different time intervals

IS A	B	C	**M**	**ST**
0	0	0	**0**	**0**
216	213	271.4	**233.4667**	**26.85086**
242.6	225	350	**272.5333**	**55.24644**
227.8	254	310.7	**264.1667**	**264.1667**
262.2	240.9	371.4	**291.5**	**57.1631**

For 3% 20 g HEC at 0, 24, 48, 72, and 96 hours

Table 5: Swelling index of 3% 20 g HEC films at different time intervals

IS A	IS B	IS C	**Mean**	**STD**
0	0	0	**0**	**0**
315	263	304	**294**	**22.37558**
362.5	247.6	286.6	**298.9**	**47.70723**
287.5	266	340	**297.8333**	**31.08143**
330	380	344	**351.3333**	**21.06076**

For 3% 30 g HEC at 0, 24, 48, 72, and 96 hours

Table 6: Swelling index of 3% 30 g HEC films at different time intervals

IS A	IS B	IS C	**MEAN**	**STD**
0	0	0	**0**	**0**
285.7	245.7	194	**241.8**	**37.5378**
307.1	286.4	206.9	**266.8**	**43.19051**
311.9	286.4	212.5	**270.2667**	**42.15292**
361.9	276.2	227	**288.3667**	**55.74061**

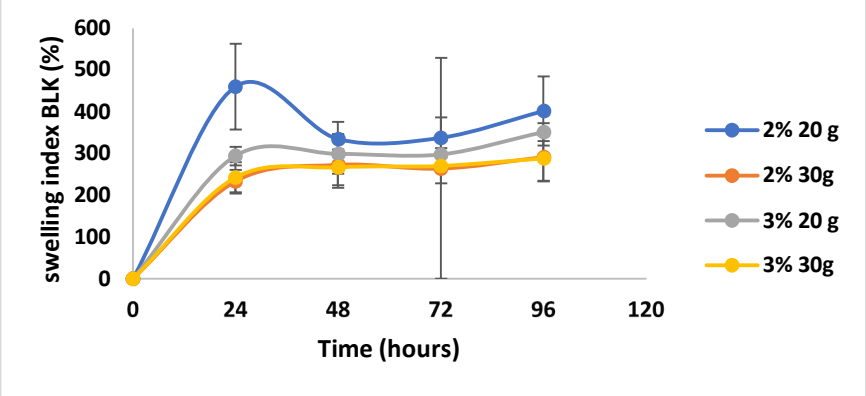

Figure 1: **Swelling index for blank films for 2% 20 g HEC, 2% 30 g HEC, 3% 20 g HEC and 3% 30 g HEC** n=3 ± SD

For preparing drug loaded films, 0.05 grams of KETOPROFEN drug is added to the films of same percentages and weights and difference between the swelling index percentage of blank and drug loaded films are observed [19].

For KETOPROFEN-loaded 2% 20 g HEC filmsat 0, 24, 48, 72, and 96 hours

Table 7: Swelling index of Ketoprofen-loaded 2% 20 g HEC films at different time intervals

IS A	IS B	IS C	**MEAN**	**st**
0	0	0	**0**	**0**
173.3	375	587	**378.4333**	**168.9098**
323.33	567	1200	**696.7767**	**369.4763**
86.66	575	800	**487.22**	**297.7611**
103.33	532	350	**328.4433**	**175.6664**

For KETOPROFEN-loaded 2% 30 g HEC filmsat 0, 24, 48, 72, and 96 hours

Table 8: Swelling index of Ketoprofen-loaded 2% 30 g HEC films at different time intervals

IS A	B	C	**MEAN**	**std**
0	0	0	**0**	**0**
104.6	180.76	95.23	**126.8633**	**38.3022**
206.97	307.69	419.04	**311.2333**	**86.61346**
176.74	288.46	180.95	**215.3833**	**51.70158**
116.27	211.53		**163.9**	**47.63**

For Ketoprofen-loaded 3% 20 g HEC filmsat 0, 24, 48, 72, and 96 hours

Table 9: Swelling index of Ketoprofen-loaded 3% 20 g HEC films at different time intervals

IS A	is B	is C	**M**	**std**
0	0	0	**0**	**0**
362.1	443.2	416	**407.1**	**33.70173**
356	618.9	557.1	**510.6667**	**112.2383**
245.9	543.2	333	**374.0333**	**124.7922**
237.8	432	383	**350.9333**	**82.46055**

For Ketoprofen-loaded 3% 30 g HEC filmsat 0, 24, 48, 72, and 96 hours

Table 10: Swelling index of Ketoprofen-loaded 3% 30 g HEC films at different time intervals

IS A	B	C	**MEAN**	**STD**
0	0	0	**0**	**0**
174	190.5	214.6	**193.0333**	**16.6714**
249	256.6	214.6	**240.0667**	**18.27299**
145	152.8	180.4	**159.4**	**15.18684**
160.7	200	156	**172.2333**	**19.72753**

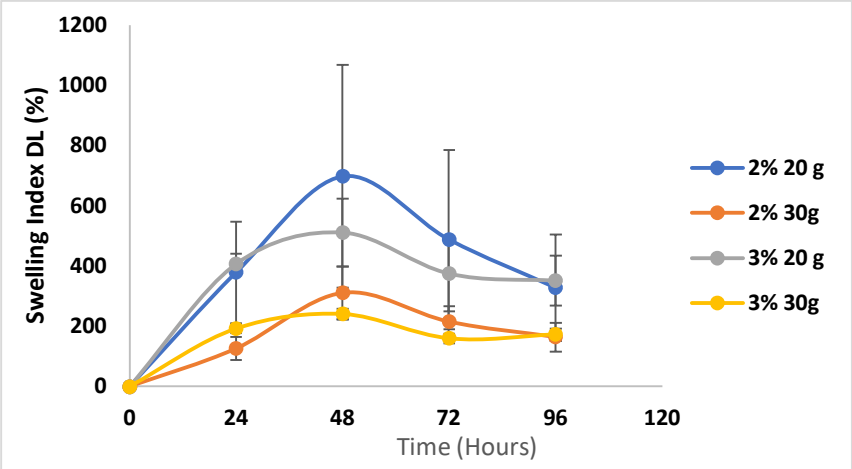

Figure 2: Swelling index drug loaded 2% 20 g HEC, 2% 30 g HEC, 3% 20 g HEC and 3% 30 g HEC

n = 3 ± SD

Time was taken on x- axis and mean values on the y- axis and after that standard deviation is added to know the deviation in the same sample.Mean of a, b, c was taken to avoid errors in the experiment.

Water vapour transmission rate.

$$\mathbf{WVTR} = \frac{Wi - Wt}{A} \times 10^6\ \mathbf{g/m^2\ day^{-1}} \text{ ---------- } \mathbf{eq.2}$$

W_i = initial weight of the film samples before placing into the oven.

W_t = weight of the samples after placing into the oven. At 24-, 48-, 72- and 96-hours of time intervals respectively.

A = area of the mouth of the scintillated glass vials = 201.06 mm.

By taking the mean and standard deviation of the A, B and C triplicates of 2% 20 g HEC, 2% 30 g HEC, 3% 20 g HEC and 3% 30 g HEC n=3 ± SD. WVTR is calculated as per eq.2 graph plotted is as follows

WVTR of blank films:

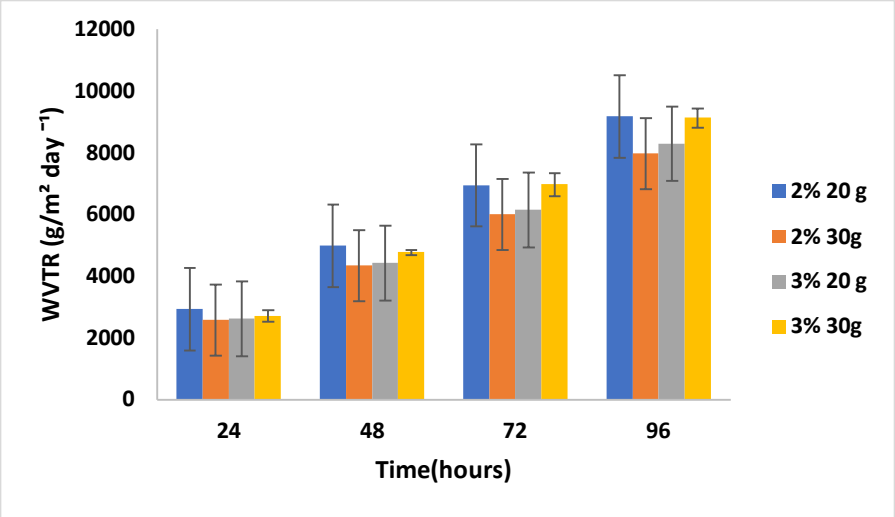

Figure 3: WVTR blank 2% 20 g HEC, 2% 30 g HEC, 3% 20 g HEC and 3% 30 g HEC n=3 ± SD for 24, 48, 72 and 96 hours.

For 2% 20 g HEC WVTR BLANK at 0, 24, 48, 72, and 96 hours

Table 11: WVTR of 2% 20 g HEC films at different time intervals

A	B	C	**Mean**	**SD**
0	0	0	**0**	**0**
2859	2990	2924	**2924.333**	**53.48105**
4764	5102.95	5097	**4987.983**	**158.3988**
6800	7087	6948	**6945**	**117.1865**
9002.28	9345	9171	**9172.76**	**139.9204**

For 2% 30 g HEC WVTR BLANK at 0, 24, 48, 72, and 96 hours

Table 12: WVTR of 2% 30 g HEC films at different time intervals

A	B	C	**MEAN**	**STD**
0	0	0	**0**	**0**
2655.92	2641	2437.08	**2578**	**99.83148**
4550.88	4327.06	4177.85	**4351.93**	**153.3009**
6217.04	5958.42	5839.05	**6004.837**	**157.7656**
8236.34	7823.53	7838.45	**7966.107**	**191.1809**

FOR 3% 20 g HEC WVTR BLANK at 0, 24, 48, 72, and 96 hours

Table 13: WVTR of 3% 20 g HEC films at different time intervals

WVTR A	B	C	**MEAN**	**STD**
0	0	0	**0**	**0**
2581.31	2731.3	2546.5	**2619.703**	**80.1802**
4371.82	4575	4312.14	**4419.653**	**112.5163**
6082.76	6300	6072.81	**6151.857**	**104.8319**
8230	8614	8037.4	**8293.8**	**239.68**

FOR 3% 30 g HEC WVTR BLANK at 0, 24, 48, 72, and 96 hours

Table 14: WVTR of 3% 30 g HEC films at different time intervals

A	B	C	MEAN	ST
0	0	0	**0**	**0**
2461.95	2800.15	2874.76	**2712.287**	**179.6163**
4705.06	4719.98	4884.11	**4769.717**	**81.11731**
7420.67	6510.49	6982.99	**6971.383**	**371.6701**
9415.09	8698.89	9260.91	**9124.963**	**307.7843**

WVTR of Ketoprofen-loaded films:

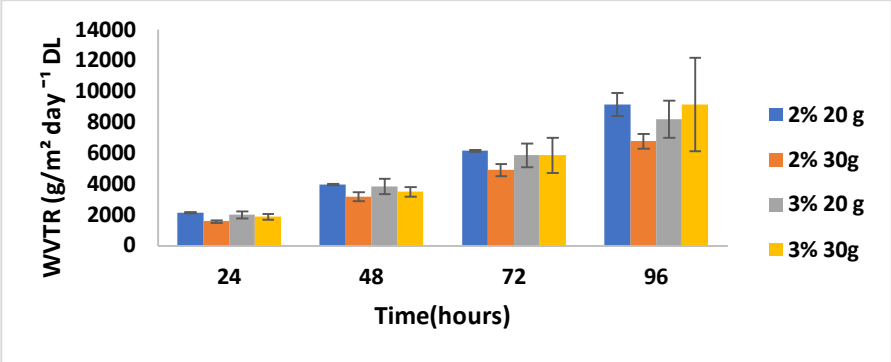

Figure 4: WVTR of drug loaded films with Ketoprofen 2% 20 g HEC, 2% 30 g HEC, 3% 20 g HEC and 3% 30 g HEC n=3 ± SD at 24-, 48-, 72- and 96-hours' time on x- axis wvtr mean values on y-axis.

Table 15: WVTR of Ketoprofen-loaded 2% 20 g HEC films at different time intervals

A	B	C	MEAN	STD
2088.92	2138.66	2183.42	**2137**	**38.59732**
3978.91	3929.17	3998.8	**3968.96**	**29.28408**
6217.04	6147.41	6067.84	**6144.097**	**60.95569**
10195.96	8753.6	8475.08	**9141.547**	**754.2033**

Table 16: WVTR of Ketoprofen-loaded 2% 30 g HEC films at different time intervals

A	B	C	MEAN	ST
1691.03	1492.09	1531.88	**1571.667**	**85.9516**
3581.02	2934.44	3018.99	**3178.15**	**286.9557**
5471	4575.74	4710.03	**4918.923**	**394.208**
7440.47	6425.94	6470.7	**6779.037**	**468.0608**

Table 17: WVTR of Ketoprofen-loaded 3% 20 g HEC films at different time intervals

A	B	C	MEAN	ST
2337.61	1894.95	1790.51	**2007.69**	**237.153**
4526.01	3585.99	3431.81	**3847.937**	**483.5842**
6913.53	5475.97	5172.59	**5854.03**	**759.3484**
9847.8	7614.64	7112.3	**8191.58**	**1188.945**

Table 18: WVTR of Ketoprofen-loaded 3% 30 g HEC films at different time intervals

A	B	C	MEAN	ST
2088.93	1641.3	1889.98	**1873.403**	**183.1197**
3680.49	3058.78	3730.23	**3489.833**	**305.4764**
5520.74	4675.22	7395.8	**5863.92**	**1136.872**
7659.41	6401.07	13379.09	**9146.523**	**3036.645**

WVTR values of blank films and Ketoprofen-loaded films were compared with the help of mean and standard deviation

Among blank films, 2% 30 g HEC films displayed minimum WVTR i.e., 2578 ± 99.83148at 24 hours interval and 2% 20g HEC films showed maximum WVTR of 9172.76 ± 139.9204at 96 hr interval. For drug-loaded films, 3% 30 g HEC films showed maximum WVTR of 9146.523 ± 3036.645at 96 hours whereas 2% 30 g HEC films showed minimum WVTR of 1571.667 ± 85.9516at 24 hours. KETOPROFEN loaded films exhibited higher WVTR values ranging from 1571.667 ± 85.9516 at 24 hr to9146.523 ± 3036.645g/m^2day^{-1} at 96 hr, than the BLK films(ranging between 2578 ± 99.83148 at 24 hr and9172.76 ± 139.9204g/m^2day^{-1}at 96 hr interval). This is believed to be because the addition of the medicine (Ketoprofen) increases inter-molecular chain mobility, resulting in a loosening of the matrix that lets more water to permeate through it [20].

Evaporated Water Loss (EWL)

Formula used to calculate as (water loss % = $\frac{Wt}{W0}$×100) ---------eq.3

W_t =weight of the film after time t, (after time interval 24 hours)

W_0 = weight of the film after 24 hours of immersion in SWF (simulated wound fluid)

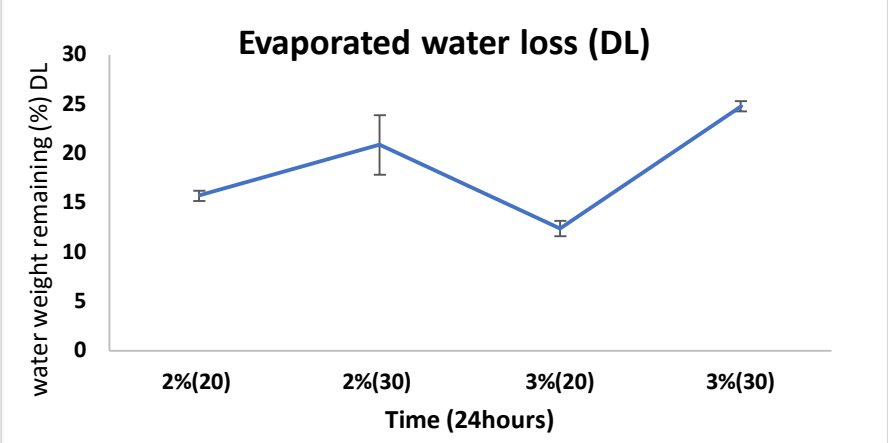

Figure 5: EWL of Ketoprofen loaded films

Table 19: EWL of Ketoprofen-loaded HEC films

	A	B	C	MEAN	STD
2% 20 HEC	15.789	16.32	15.02	**15.70967**	**0.533679**
2% 30 HEC	20.66	17.24	24.65	**20.85**	**3.028102**
3% 20 HEC	13.51	11.98	11.7	**12.39667**	**0.795501**
3% 30 HEC	24.44	24.44	25.52	**24.8**	**0.509117**

Mean values are taken on y- axis and film percentages of 2% 20 g HEC, 2% 30 g HEC, 3% 20 g HEC and 3% 30 g HEC on x- axis

The flow of water molecules across the film is theorized to happen via suitably large pores which randomly develop in the film. Among the Ketoprofen-loaded film formulations, minimum EWL was shown by 3% 20g HEC films (12.39667 ± 0.795501) and maximum EWL was shown by 3% 30g HEC films (24.8 ± 0.509117), as shown in Table 3.17.EWL of the films increased with increased concentration of HEC; it can be attributed to the hydrophilic nature of HEC and more the hydrophilicity, more water loss a film shows [21].

Equilibrium Water Content (EWC)

EWC% = $\frac{Ws-Wi}{Ws} \times 100$ ----------eq.4

W_s = weight of the swollen film after time t (24) hours

W_i = initial weight of the film (dry film)

From the blank swelling data n=3 ± SD taking a, b, and c as triplicates of each sample n=3 ±SD to minimize the error

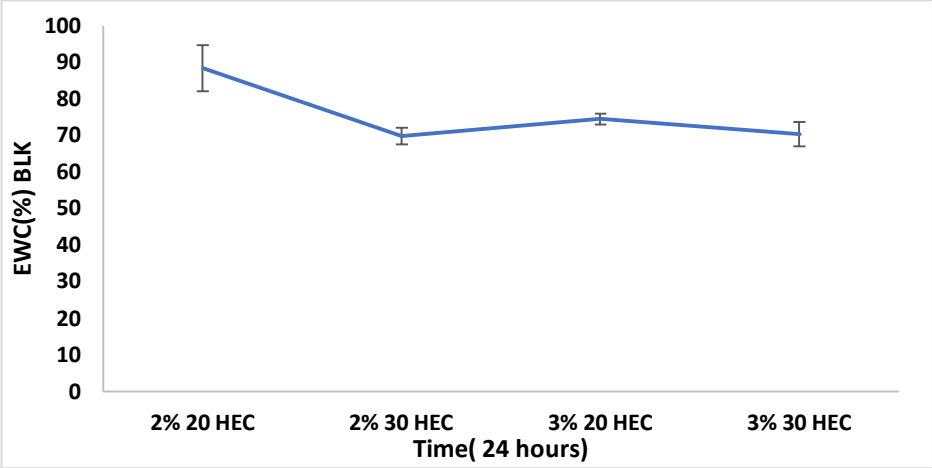

Figure 6: EWC (%) BLK For blank samples at 24 hours

Table 20: EWC of blank HEC films

24HRS	A	B	C	mean	STD
2% 20 HEC	97.27	84.97	83.23	**88.49**	**6.248904**
2% 30 HEC	68.39	68.11	73.07	**69.85667**	**2.275043**
3% 20 HEC	75.9	72.48	75.27	**74.55**	**1.486136**
3% 30 HEC	74.07	71.07	66.03	**70.39**	**3.317348**

Mean values on y – axis, samples on x- axis (2% 20 g HEC, 2% 30 g HEC, 3% 20 g HEC and 3% 30 g HEC) for time interval of 24 hours

Table 21: EWC of Ketoprofen-loaded HEC films

	A	B	C	MEAN	ST
2% 20 HEC	95.1	78.9	85.4	**86.46667**	**6.656492**
2% 30 HEC	51.1	64.3	48.7	**54.7**	**5.939697**
3% 20 HEC	76	81.5	80.6	**79.36667**	**2.40878**
3% 30 HEC	63.5	65.5	68.2	**65.73333**	**1.667833**

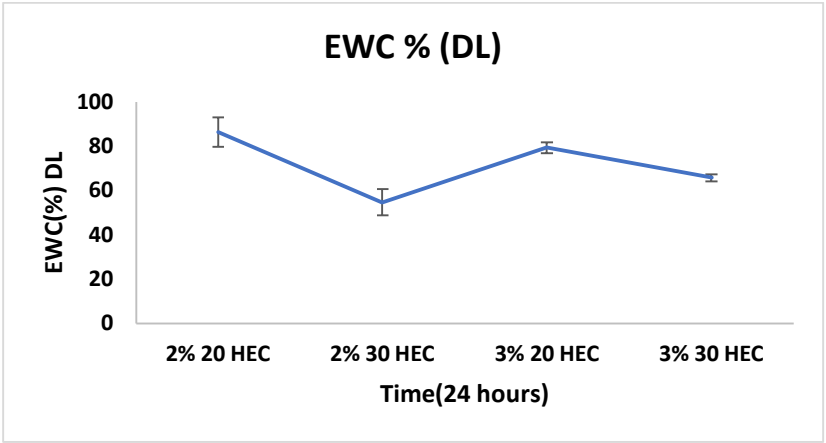

Figure 7: EWC % of Ketoprofen-loaded HEC films after 24 hours. n = 3± SD

Among blank films, 2% 20g HEC films showed maximum EWC% of 88.49 ± 6.248904at 24 hr interval and 2% 30g HEC films showed minimum EWC % of 69.85667 ± 2.275043, as shown in Table 3.18.Among the Ketoprofen-loaded films, maximum EWC % at 24 hr interval was shown by 2% 20g HEC filmsranging around86.46667 ± 6.656492. Minimum EWC % was shown by 2% 30g HEC films (54.7 ± 5.939697), as shown in Table 3.19.Compared to the Ketoprofen-loaded films, blank films had greater EWC%, which may be attributed to the amount of hydroxyalkyl groups present in the HEC polymer [22].

Water Absorption (AW) %

$$\text{AW (\%)} = \frac{Ws - Wi}{Wi} \times 100 \text{ ----------eq.5}$$

W_s = swollen weight of the film samples.
W_i = initial weight of the film before immersion into SWF (simulated wound fluid)

AW% of Blank Films After 24 Hours

Table 22: AW% of blank HEC films

AW (%) BLK	A	B	C	MEAN	STD
2% 20 HEC	320	563.3	496.5	**459.9333**	**102.6371**
2% 30 HEC	216	213	271.4	**233.4667**	**26.85086**
3% 20 HEC	315	263	304	**294**	**22.37558**
3% 30 HEC	285.7	245.7	194	**241.8**	**37.5378**

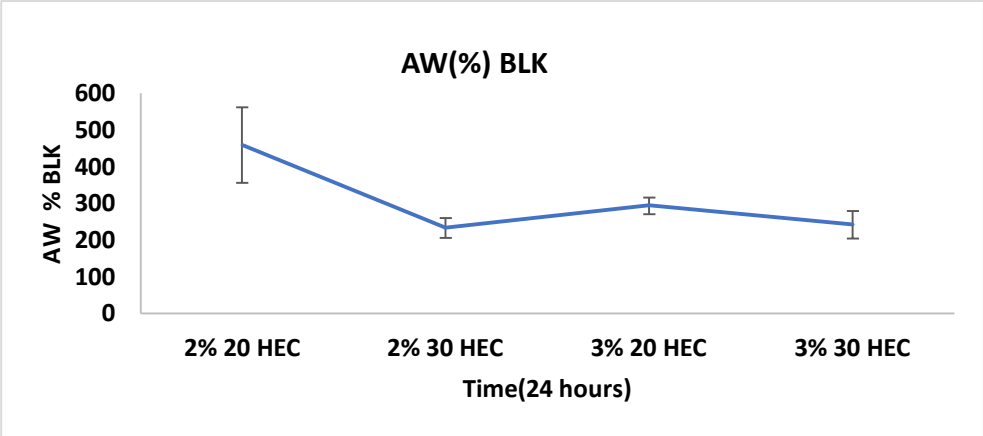

Figure 8: AW% OF blank films after 24 hrs.

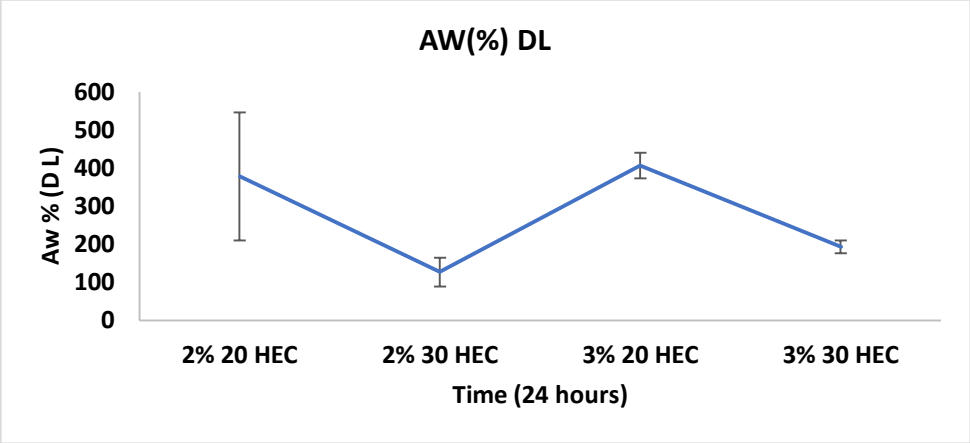

Figure 9: AW (%) of Drug loaded films.

AW% of Ketoprofen-loaded films

Table 23: AW% of Ketoprofen-loaded HEC films

	A	B	C	MEAN	STD
2% 20 HEC	173.3	375	587	**378.4333**	**168.9098**
2% 30 HEC	104.6	180.76	95.23	**126.8633**	**38.3022**
3% 20 HEC	362.1	443.2	416	**407.1**	**33.70173**
3% 30 HEC	174	190.5	214.6	**193.0333**	**16.6714**

Among the blank films, 2 % 30g HEC films showed minimum AW% of 233.4667 ± 26.85086 and maximum AW% was seen in 2% 20g HEC films, 459.9333 ± 102.6371, as shown in table 3.20. The evaporation from the dense makeup of the matrix reduces their absorption rate because of the diffusion of water from the hydrogel's external surface and into its core. Cross-linking HEC and citric acid are thought to increase

porosity of hydrogels, which would be accountable for the formulation's enhanced water absorption [23].After loading the films with Ketoprofen, maximum AW% was seen in 3 % 20g HEC films (407.1 ± 33.70173) and minimum in 2% 30 g HEC (126.8633 ± 38.3022), as shown in Table 3.21.

Adhesion Test

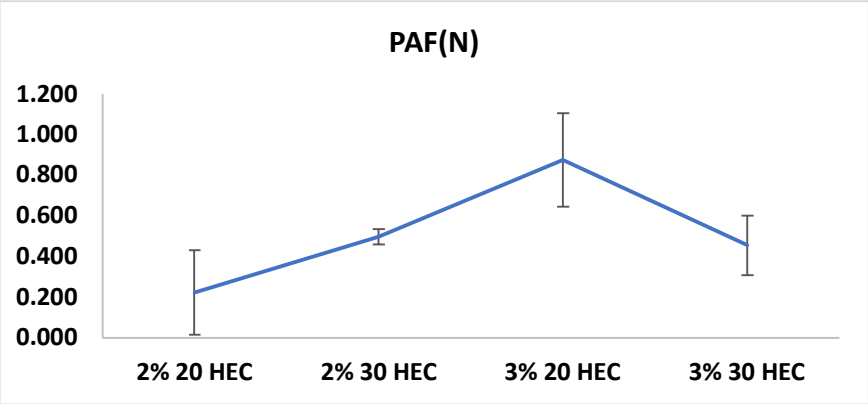

Figure 10: Peak adhesive force of blank films

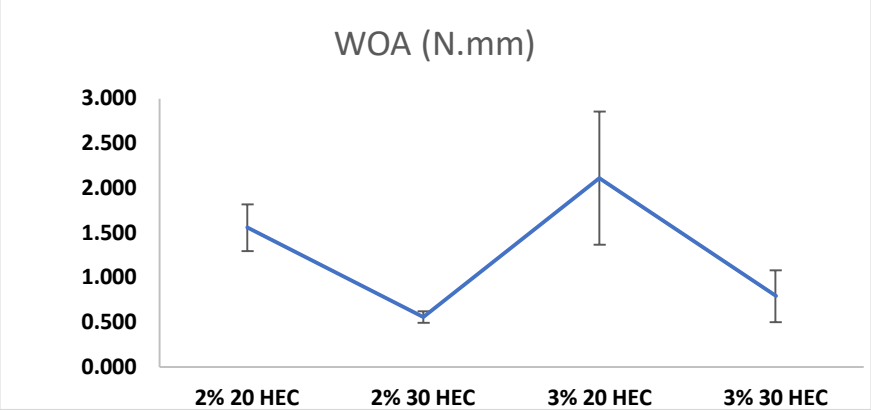

Figure 11: WOA for blank films

Peak adhesive force (PAF) for blank films was taken on x- axis and peak positive force on y- axis n = 3 ± SD
WOA of adhesion for blank films was taken on x- axis and positive area mean values of a, b, and c values on y – axis n = 3 ±SD

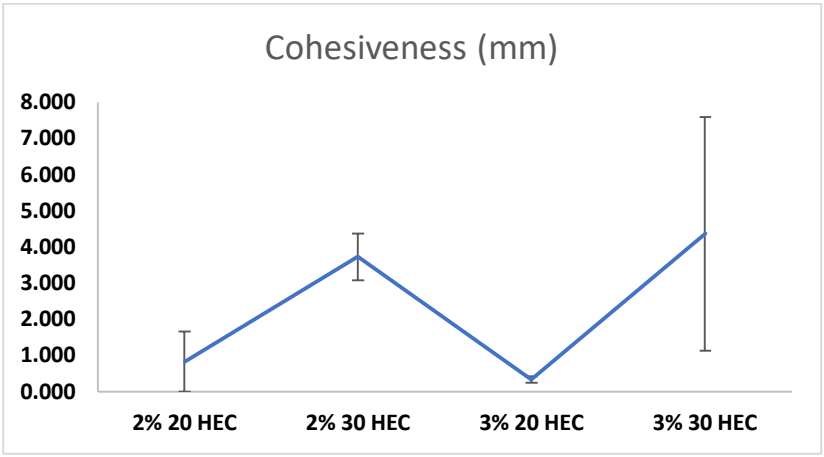

Figure 12: Cohesiveness of blank films

Cohesiveness of blank films was taken on x- axis and separation distance mean values on y – axis

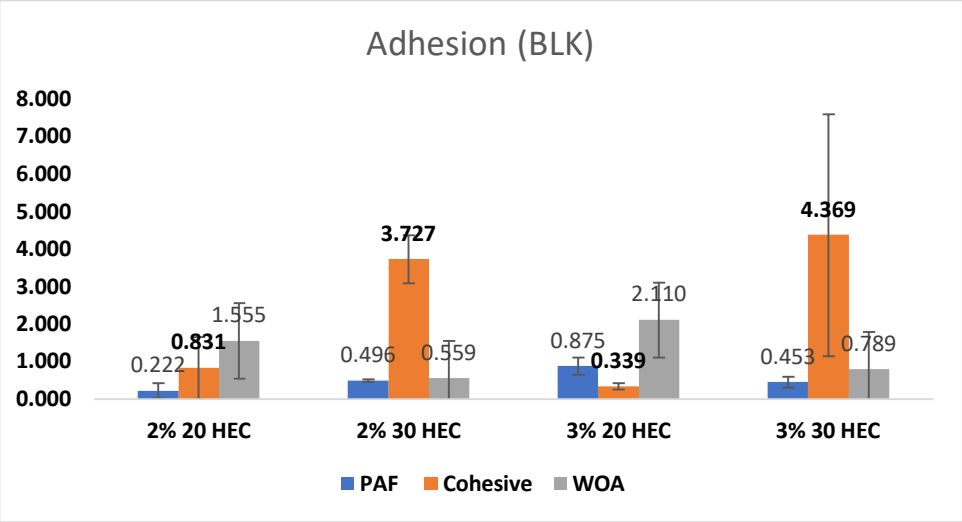

Figure 13: Bar graph showing adhesion of blank films

Table 24: PAF, WOA and Cohesiveness of blank HEC films

BLANK ADD	PAF mean	STD	WOA mean	STD	Cohesive mean	STD
2% 20 HEC	0.222	0.209	1.555	0.263	0.831	0.827
2% 30 HEC	0.496	0.038	0.559	0.066	3.727	0.646
3% 20 HEC	0.875	0.230	2.110	0.746	0.339	0.087
3% 30 HEC	0.453	0.147	0.789	0.290	4.369	3.226

For the blank films, 3% 20g HEC films showed maximum PAF values of 0.875 ± 0.230 and minimum by 2% 20g HEC films (0.222 ± 0.209). WOA values were maximum for 3% 20g HEC films (2.110 ± 0.746) and minimum for 2% 30g HEC films (0.559 ± 0.066). Cohesive values were maximum for 3% 30g HEC film (4.369 ± 3.226) and minimum for 3% 20g HEC film (0.339 ± 0.087). Table 3.22 shows PAF, WOA and cohesiveness of blank HEC films. The physicochemical features of the hydrogel films, including their distribution of pore sizes and resultant ability to absorb water have a direct effect on these three parameters. Specifically, PAF was utilized as a metric of in vitro wound adhesion efficacy [24].

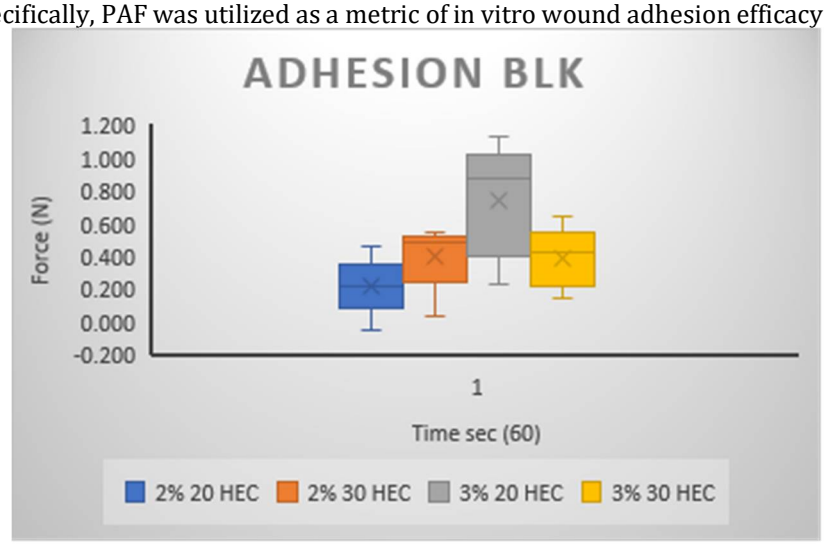

Figure 14: Box plots of peak positive force for blank samples

Table 25: Mean of triplicates for adhesion test of HEC films

	2% 20 HEC	2% 30 HEC	3% 20 HEC	3% 30 HEC
	0.465	0.547	1.136	0.645
	-0.045	0.485	0.575	0.289
	0.246	0.456	0.913	0.427
mean	0.222	0.496	0.875	0.453
std	0.209	0.038	0.230	0.147

Mean of triplicates n = 3

For adhesion test peak positive force box plot is plotted with the help of mean and standard deviation For Ketoprofen-loaded films, PAF values were maximum for 2% 20g HEC (0.582 ± 0.046) and minimum for 3% 30g HEC (0.215 ± 0.176). WOA values were maximum for 2% 20g HEC films (0.955 ± 0.644) and minimum for 3% 20g HEC films (0.132 ± 0.128). Maximum Cohesiveness was seen in 3% 20g HEC films (8.504 ± 0.710) and minimum was seen in 2% 20g HEC films (3.474 ± 2.870). Table 3.24 shows the PAF, WOA and cohesive values of 2% 20g HEC and 3% 20g HEC films. The strong mucoadhesion of the films suggests that they would probably stay intact with the wound surface for an extended period of time, hence possibly reducing the requirement for frequent dressing changes that induce patient non-adherence due to discomfort encountered while removing the wound dressing. The discrepancies in all three parameters might be related to the hydrophilic character of HEC, which increases the interactions between the film and the SWF, most likely as a result of the film's increased initial water uptake.

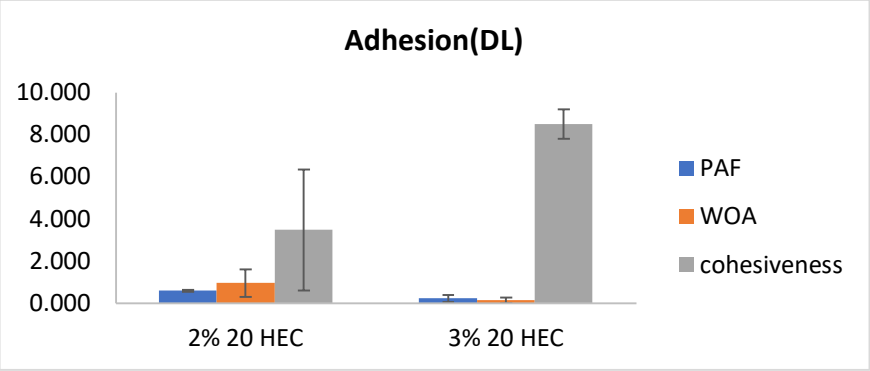

Figure 15: Bar graph representing adhesions for drug loaded films 2% 20g HEC and 3% 20g HEC

Table 26: PAF, WOA and Cohesiveness of Ketoprofen-loaded HEC films

	PAF	ST	WOA	ST	cohesiveness	st
2% 20 HEC	0.582	0.046	0.955	0.644	3.474	2.870
3% 20 HEC	0.215	0.176	0.132	0.128	8.504	0.710

Mean of PAF peak adhesive force, for WOA positive area mean and cohesiveness is done by mean of separation distance

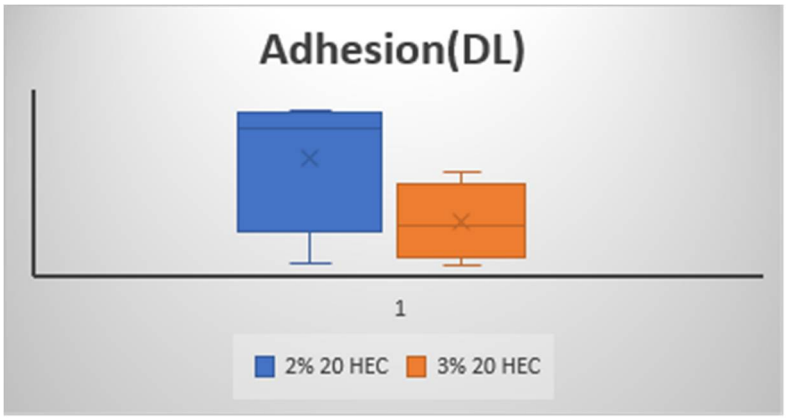

Figure 16: Box plot showing positive adhesive force for drug loaded samples 2% 20 HEC and 3% 30 HEC

Table 27: Adhesive force for drug loaded samples 2% 20 HEC and 3% 30 HEC

	2% 20 HEC	3% 20 HEC
A	0.536	0.390
B	0.629	0.039
C	0.582	0.215
MEAN	0.046	0.176

Adhesion tests for 2% 30 g HEC and 3% 30 HEC films could not be performed further because drug loaded films rolled and didn't support for the testing.

Tensile Strength

Among the blank films, 3% 20g HEC films showed maximum tensile strength i.e., 37.4025 ± 6.460865388 and 2% 30g HEC films showed minimum strength (12.523 ± 2.626994523), as shown in Table 3.26. Among Ketoprofen-loaded films, 2% 20g HEC film showed maximum tensile strength (23.444 ± 4.0099502) and 2% 30g HEC films showed minimum strength (11.806 ± 7.56445193), as shown in Table 3.27. Compared to Ketoprofen-loaded films, blank films showed better tensile strength. Apart from HEC polymer, citric acid and β-CD were also included as cross-linkers in the hydrogel matrix, which may enhance the mechanical profile of hydrogel matrix by serving as fillers [25]. Nevertheless, tensile strength dropped when Ketoprofen was incorporated to the films; this could be attributed to a marked decline in polymer crystallinity caused by the drug.

Table 28: Tensile strength for blank HEC films

PEAK +VE FORCE	**2% 20 HEC**	2% 30 HEC	3% 20 HEC	3% 30 HEC
A	37.945	12.956	32.982	34.891
B	36.393	12.09	41.823	27.728
C	33.249	18.045	48.77	5.578
MEAN	35.86233333	12.523	37.4025	22.73233
ST	1.953511255	2.626994523	6.460865388	12.47746

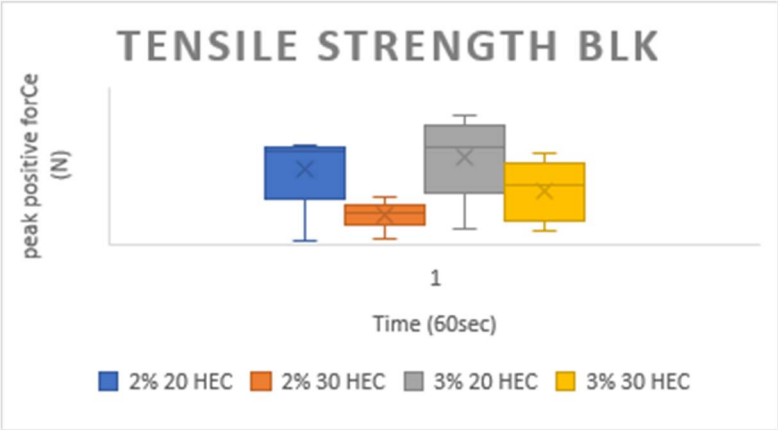

Figure 17: Box plot is plotted for blank films

By taking mean n= 3 for triplicates A, B, and C on y -axis and samples on x- axis in 60 sec time

For tensile strength of drug loaded films mean and standard deviation for triplicates of samples to minimize error

Table 29: Tensile strength for Ketoprofen-loaded HEC films

Tensile (DL)	2% 20 HEC	2% 30 HEC	3% 20 HEC	3% 30 HEC
A	19.322	4.241	19.945	18.565
B	22.133	19.370	25.775	
C	28.878		13.198	
mean	23.444	11.806	19.639	18.565
STD	4.0099502	7.56445193	5.13937549	0

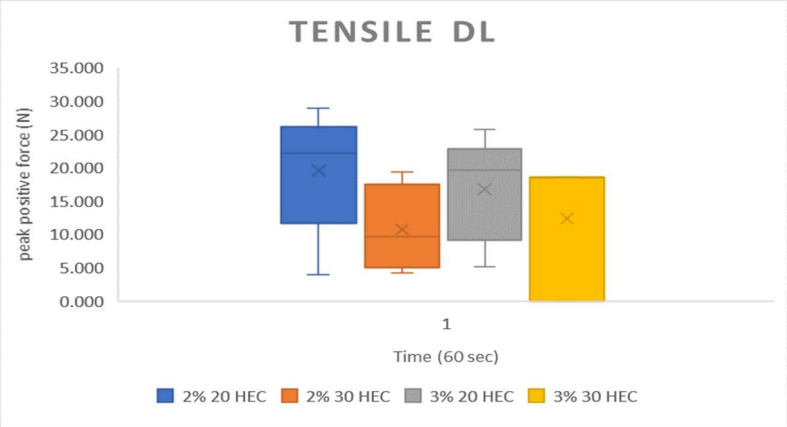

Figure 18: Peak positive force (N) on y- axis of mean of triplicates a, b, and c of different samples for this 3% 30 g HEC it has done with only one A because of shortage of films.

CONCLUSION

The results of the investigation indicate that citric acid has the potential to crosslink HEC and create hydrogel. HEC films found to be the most effective, with better mechanical properties and fluid absorption capacity. Ketoprofen-loaded HEC films exhibited superior swelling, water absorption, and diffusion when compared to blank films. Therefore, it may be inferred that drug-loaded films have substantial swelling and mucoadhesive qualities. Their mechanical properties, however, were inferior to those of blank films. Therefore, the produced HEC hydrogel films are suitable for the sustained release of hydrophobic medicines. The developments will significantly increase the ability of hydrogel-based delivery of drugs to efficiently deliver the hydrophobic medicines at the desirable rate and site.

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