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Formulation *In Vitro* and *In Vivo* Evaluation of Ketorolac Topical Hydrogel

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) like Ketorolac Tromethamine are frequently prescribed to alleviate pain associated with osteoarthritis, ankylosing spondylitis, acute sciatica, rheumatoid arthritis, and low back pain. Hydrogels are polymeric three-dimensional networks that are able to consume significant volumes of water and remain insoluble in water due to their physical and chemical crosslinking. They respond to temperature, pH and ionic strength. They can be prepared by using natural polymers such as dextran, pectin, alginate, or synthetic polymers such as polyvinyl alcohol, polyethylene oxide, and poly–hydroxy ethyl methacrylate. Hydrogels are used to deliver several drugs. Today, hydrogels have found a wide range of applications due to their non-toxic nature and low cost. The purpose of the current research was to formulate and assess a topical gel based on hydrogel that contained Ketorolac Tromethamine to treat inflammation and pain while reducing the gastrointestinal side effects associated with oral treatment. According to the FTIR analysis, Ketorolac Tromethamine doesn't interact with other excipients in a significant way. The physical characteristics, pH level, extrudability, spreadability, swelling property, in-vitro drug release study of hydrogel formulations were assessed. Using a dialysis membrane and a phosphate buffer solution with a pH of 7.4, in vitro and ex vivo release studies were conducted on the Franz diffusion cell. Among all formulations, HF4 showed high spreadability of 25.2 ±0.3 gm./cm/sec, extrudability, swelling index, in vitro drug release, and ex vivo drug diffusion. HF4 hydrogel showed no signs of skin irritation. The final formulation HF4 hydrogel shows an equal analgesic and anti-inflammatory effect as standard Ketorolac gel. It was found from the drug release kinetics that the Topical Ketorolac hydrogel HF4 drug release mechanism follows the Higuchi model and zero order.

Keywords: Ketorolac Tromethamine, FTIR studies, Swelling, Drug Release Kinetics.

1. INTRODUCTION

Hydrogels are three-dimensionally structured polymeric networks that can absorb large volumes of biological fluids or water. Hydrophilic groups like -OH, -CONH, -$CONH_2$ and –SO_3H are found in polymers that form hydrogel structures, and these groups are considered responsible for the polymers affinity to absorb water [1, 2, 3]. The network's contribution from these groups and domains causes the polymer to be hydrated to varying degrees (sometimes more than 90% weight), depending on the aqueous environment's characteristics and the polymer's constitution [4]. Owing to the inclusion of chemical and/or physical crosslinks, such as entanglements, they are insoluble [5]. One of the most often prescribed drug categories are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). These medications can be applied topically or ingested orally to treat a variety of Rheumatic Disorders, such as Rheumatoid Arthritis (RA), Osteoarthritis (OA), Inflammation, lower back pain, and several joint ailments [6]. The Cyclooxygenase enzyme (COX) is temporarily inhibited by NSAIDS, which also results in a reduction in prostaglandin synthesis. However, due to the suppression of prostaglandins (PGs), which are involved in protecting the gastric mucosa when these medications are administered, they have negative effects that are concentrated primarily on the stomach [7]. Ketorolac tromethamine, a non-steroidal anti-inflammatory medication, has the chemical formula C15H12NO3.C4H12NO3. It is one of the potent NSAIDs. The inhibition of cyclooxygenase serves as the unifying mechanism for both its analgesic and anti-inflammatory effects. It has been studied in a wide range of pain states including postpartum and postoperative arthritic pain, pain from trauma, severe dental pain, renal, biliary colic, cancer, abdominal and gynecological pain. Pain ranging from moderate to severe is treated with it. Local application of medications to the skin or into the bloodstream after they have passed through the skin provides a number of advantages over oral and injectable drug administration. These supposedly positive effects include reduced

hepatic first-pass metabolism, improved patient compliance, and simplicity of transdermal delivery. Topical formulations are used for localized effects at the application site since the medication might penetrate into the deeper layers of the mucous or skin membranes [8]. Due to their significant physical and chemical characteristics, such as the controllable and prolonged drug - release in organisms, polymeric gels and hydrogels are currently used extensively in drug delivery systems; as a result, high local concentrations of medical preparations are maintained in the affected tissues for a long period of time [9]. The ability to distribute drugs more precisely to a specific site is the greatest advantage of topical delivery systems (local action).

ANATOMY OF HUMAN SKIN

Skin serves as a natural barrier for topical drug administration. The human skin is formed by three tissue layers. The outermost and non-viable layer of the skin, avascular, stratified, cellular epidermis, serves as a protective barrier for the body and is incredibly challenging to cross [10]. Intercellular lipids, which include ceramides, cholesterols, cholesterol esters, and free fatty acids, constitutes the stratum corneum. The arrangement and distinctive chemical makeup of these lipids provide skin a high degree of water impermeability. The stratum corneum's barrier function is given by patterned lipid lamel that is restricted to the extracellular gaps between corneocytes, making it difficult for water and other permeates to pass through membrane. Dermis is the next layer and is composed of blood vessels and connective tissue. The subcutaneous fat layer, which is located below the dermis, is lowest layer. The stratum corneum's barrier abilities must be overcome in order for therapeutic doses of the medicine to pass through skin [11]. A limited number of medications can be transported to the skin for local action at therapeutic doses due to the selective nature of skin barrier. A lipophilic substance can traverse the stratum corneum, but its rate of diffusion declines once it reaches the deeper, more aqueous layers of epidermis. Diffusion slows and the concentration gradient widens as a highly hydrophobic substance diffuses into the deeper layers of skin.

2. MATERIALS AND METHODS

2.1 MATERIALS

Ketorolac Tromethamine obtained from Lee Pharma Limited, Carbopol 971P obtained from Lubrizol Advanced Materials Europe BVBA Chaussee de Wavre 1945, Xanthan gum, Eudragit ES100, HPMC 15cps obtained from Research Lab Fine Chem Industries, Tween 80 from Finar limited Ahmedabad, Glycerin, Methyl Paraben from Oxford laboratory Mumbai and Distilled Water.

2.2 METHODS

UV spectrum analysis

2.2.1 Determination of λmax

100mg of Ketorolac Tromethamine was accurately weighed and transferred into 100ml of distilled water volumetric flask. From the above solution 1µg/ml, 10µg/ml solution was obtained and scanned to get maximum absorbance. The λmax was recorded**.**

2.2.2 Construction of Calibration Curve

- **PREPARATION OF STANDARD STOCK SOLUTION**

100mg of Ketorolac was weighed accurately, transferred into 100ml volumetric flask and final volume was made up to 100ml using distilled water.

- **PREPARATION OF WORKING SOLUTION**

1ml of standard solution was taken and final volume was made upto10ml using distilled water.

- From the working solution, a range of concentrations of 1-5µg/ml were prepared were generated from this reserve solution, and the absorbance was scanned between 200-700nm in comparison to a blank using a UV spectrophotometer.

2.3 FTIR spectrophotometry

To ascertain any potential interactions between the chemical bonds of the drug and the excipients, IR spectroscopy test was carried out. Ketorolac Tromethamine's pure IR spectrum and also with other excipients were obtained. The samples were scanned between 400 and 4000 cm^{-1}.

2.4 Formulation of Ketorolac Tromethamine Hydrogel

Preparation of Ketorolac Tromethamine Hydrogels

According to the composition listed in Table 1, various gel formulations containing KT were developed. KT was incorporated at 2% w/w in each of the formulation to obtain homogeneous mass.

Preparation of Carbopol Hydrogel (HF1)

About 0.1g of Carbopol 971P was weighed accurately and added to the swirl of Ketorolac Tromethamine Solution of concentration 20mg/ml in 80ml beaker. A homogenizer was used to stir the contents of the beaker. Then required quantities of Glycerin, Tween 80 and Methyl Paraben was added and stirred thoroughly until a homogenous gel is formed.

Preparation of Xanthan gum Hydrogel (HF2)

About 0.1g of Xanthan gum was weighed accurately and added to the swirl of Ketorolac Tromethamine Solution of concentration 20mg/ml in a 80ml beaker. A homogenizer was used to stir the contents of the beaker. Then required quantities of

Glycerin, Tween 80 and Methyl Paraben was added and stirred thoroughly until a homogenous gel is formed.

Preparation of Carbopol and Xanthan gum Hydrogels (HF3, HF4, HF5)

A homogenizer was used to gently stir the weighed quantity of Carbopol 971P and Xanthan gum powder into the swirl of a 20 ml solution of Ketorolac Tromethamine solution at a concentration of 20 mg/ml in an 80 ml beaker. The gel bases were produced at various concentrations, including 1:1% (HF3), 1:2% (HF4), and 1:3% (HF5) respectively.

Preparation of HPMC Hydrogel (HF6)

About 0.1g of HPMC was weighed accurately and added to the swirl of Ketorolac Tromethamine Solution of concentration 20mg/ml in an 80ml beaker. A homogenizer was used to stir the contents of beaker. Then required quantities of Glycerin, Tween 80 and Methyl Paraben was added and stirred thoroughly until a homogenous gel is formed.

Preparation of Eudragit Hydrogel (HF7)

About 0.1g of Eudragit was weighed accurately and added to the swirl of Ketorolac Tromethamine Solution of concentration 20mg/ml in an 80ml beaker. A homogenizer was used to stir the contents of the beaker. Then required quantities of Glycerin, Tween 80 and Methyl Paraben was added and stirred thoroughly until a homogenous gel is formed.

Preparation of HPMC and Eudragit Hydrogels (HF8, HF9, HF10, HF11, HF12)

A homogenizer was used to gently stir the weighed quantity of HPMC and Eudragit powder into the swirl of a 20 ml solution of Ketorolac Tromethamine solution at a concentration of 20 mg/ml in an 80 ml beaker. The gel bases were produced at various concentrations, including 1:1% (HF8), 1:2% (HF9), 1:3% (HF10), 1:4% (HF11) and 1:5% (HF12) respectively.

Table-1: Formulation of Ketorolac Tromethamine Hydrogel

Ingredients	HF1	HF2	HF3	HF4	HF5	HF6	HF7	HF8	HF9	HF10	HF11	HF12
Drug- KT	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Carbopol(g)	0.1	-	0.1	0.1	0.1	-	-	-	-	-	-	-
Xanthan gum(g)	-	0.1	0.1	0.2	0.3	-	-	-	-	-	-	-
HPMC(g)	-	-	-	-	-	0.1	-	0.1	0.1	0.1	0.1	0.1
Eudragit(g)	-	-	-	-	-	-	0.1	0.1	0.2	0.3	0.4	0.5
Glycerin(g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Tween 80(ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water(ml)	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20	Upto 20

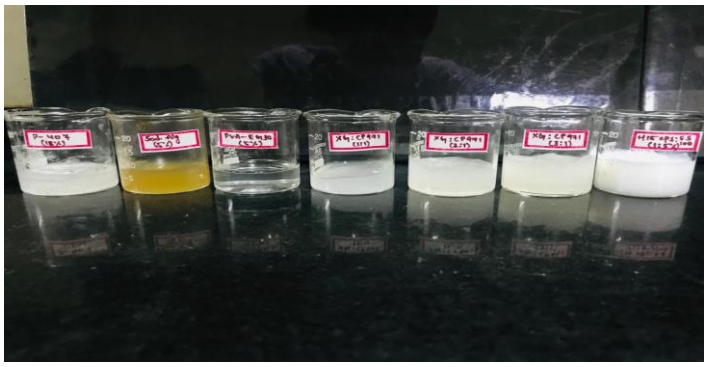

Fig-1: Ketorolac Hydrogel Formulations

3. Evaluation of Ketorolac Tromethamine Hydrogel

3.1 Physical Appearance

Physical characteristics including color and appearance were evaluated.

3.2 pH Determination

Using a pH meter, the pH values of 1% aqueous solutions of gels HF1-HF12 were measured.

3.3 Spreadability

It indicates how evenly the gel can spread when applied to skin or an affected area. The spreadability of a formulation influences its therapeutic effectiveness as well. Spreadability is measured in terms of the time in seconds it takes for two slides to separate from the gel that is placed in between them when a specific force is applied. Better spreadability is achieved with shorter gap times between two slides. It is computed utilizing the following formula:

S=M x L/T

Where,
M = wt. tied to upper slide
T = time taken to separate the slides
L = length of glass slides.

3.4 Extrudability

The amount of gel extruded from an aluminum collapsible tube when the weight in grams required to extrude at least a 0.5 cm ribbon of gel in 10 seconds was applied served as the basis for the method employed in the current study to assess the extrudability of gel formulations. The results of three separate evaluations of each formulation's extrudability are displayed.

3.5 Swelling Index

The swelling kinetics and equilibrium swelling ratio were determined using a phosphate buffer with a pH of 7.4. The dried hydrogel discs were first weighed before being soaked at room temperature in the buffer solution [13]. After a specific period of time, the discs were taken out of the buffer solution, wiped with butter paper to remove any extra surface water, and weighed once again. Swell patches were measured using an analytical balance. Studies on swelling were conducted until the patches' weight remained constant.

3.6 In Vitro Drug Release Study

We used Franz diffusion cells with a 25ml receptor compartment and an effective diffusion area of 1.8 cm2. The donor and receptor compartments were connected by a cellophane dialysis membrane with a molecular weight cut-off of 8000 Da that had been hydrated with the receptor medium for 12 hours. The releasing medium was a phosphate buffer with a pH of 7.4. A magnetic stirrer was used to continuously stir the substance of the receptor chamber during the experiment [14]. The experiment was carried out at 37.5±0.5 °C. The donor compartment received finite doses of the formulations, measured in approximately equal weight units. An aliquot of 5 ml was withdrawn at the different time intervals and replaced by an equal volume of the release medium maintained at the same temperature. A UV-Spectrophotometer was used to evaluate the samples' drug content.

3.7 Ex-Vivo Skin Permeation Study

Ex vivo diffusion was performed using porcine ear skin because it closely resembles human skin. The porcine ear was obtained from the local butcher shop and cleansed with saline solution before the skin covering and underlying cartilages were separated. The cartilages and fat layer were separated from the skin through dissection [15].

After the gel had been applied to the stratum corneum, drug diffusion was observed for eight hours. To assess the drug content in the UV spectrophotometer, aliquots of 1 ml were taken out at predetermined intervals and diluted with distilled water.

3.8 Assessment of the analgesic and anti-inflammatory effects

For studies on analgesics and inflammation, Albino Wistar rats (female) weighing 180-200 g were used. For research purposes, they were kept in the animal house. Under normal husbandry conditions, all the animals underwent a 7-day acclimatization period. The animals were given free access to a regular feed and were kept in strict hygienic conditions with unlimited access to water. Prior to the experiments, the Institutional Animal Ethical Committee's consent was obtained (IAEC) (01/IAEC/VIPER /M.Pharm/2022-2023).

The rats in the experiment were separated into 3 groups, each with three individuals. The topical method was used to administer all of the medications.

- Group (I): KT-marketed gel in the standard group
- Group (II): HF4 2%KT gel
- Group (III): Control group (blank gel)

Hot Plate Analgesic Method

The hot plate method of Eddy and Leimbach was used to evaluate the analgesic activity of the test drug (1953) [16]. Rats were placed on the hot plate 30 minutes after receiving group-specific drug doses. The hot plate was kept at a temperature of 55±0.20 °C. A timer was used to time the interval between licking and jumping, both of which are signs of pain [17]. The period of time between placing the animal on the hot plate and either it jumping or licking was measured as reaction time (latency period).

Carrageenan induced paw-edema method

Rat paw edema caused by carrageenan was used as a model to assess the HF4 gel's anti-inflammatory efficacy [18]. Female Wistar rats weighing 180–200 g were used. A 26 gauge needle was used to inject a solution of carrageenan in saline (1%w/v) to each rat's right hind paw was sub-plantar tissue [19]. Prior to and during the administration of the carrageenan solution, the paw thickness was measured. To prevent licking of the gel, 3M medical tape and gauge were used to cover the right hind paw after applying blank gel, HF4 gel, and commercial KT (n=3). Following an hour's interval, the paw volume was assessed using a plethysmometer and a mercury displacement technique.

Skin Irritancy Test

Various drugs may cause primary skin irritation when applied topically. A study on skin irritancy was conducted on rats that had unrestricted access to normal food and tap water. Two grams of gel for edema inhibition were applied onto the shaved dorsal skin of four rats and occluded with gauze and

bandage [20]. After 72 hours, the formula was removed, and the following erythematic symptom score was visually assessed:

1. Mild
2. Moderate
3. Severe erythema are different degrees of erythema.

4. RESULTS AND DISCUSSION

4.1 UV spectrum analysis

The drug sample for Ketorolac Tromethamine was subjected to UV spectroscopic analysis in distilled water, and the maximum absorption was found to be at 323nm.

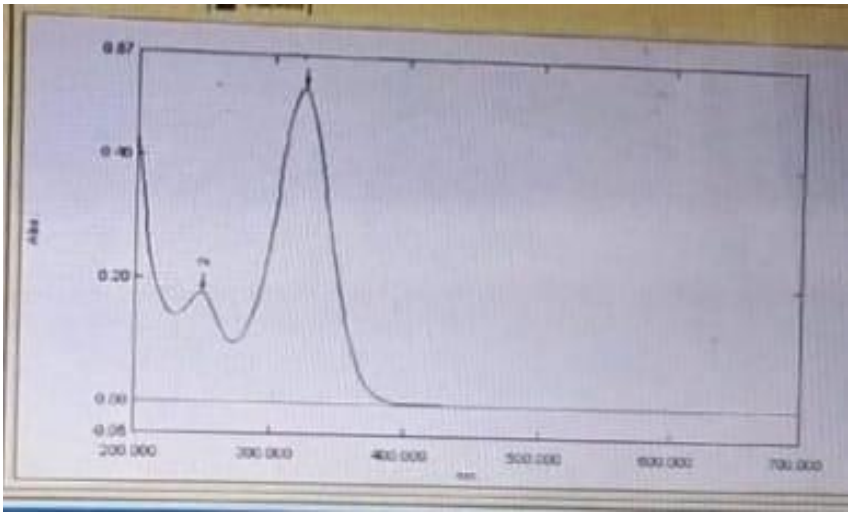

Fig-2: Ketorolac Tromethamine Maximum absorption

UV Calibration of Ketorolac Tromethamine

In the concentration range of 0-12 mg/ml, the drug calibration curve followed Beer Lambert's law (R^2 = 0.991), with the results depicted below.

Table-2: Linearity table of Ketorolac Tromethamine Drug

Conc in PPM	Absorbance at WL.323.0
1PPM	0.078
2PPM	0.121
3PPM	0.194
4PPM	0.254
5PPM	0.334

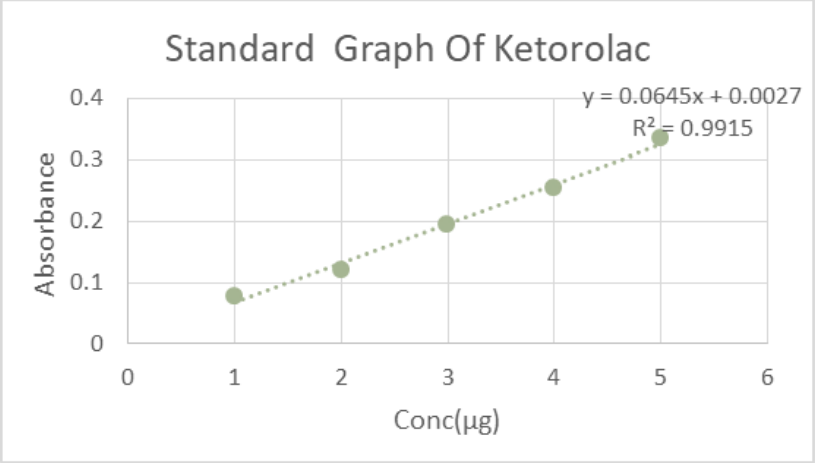

Fig 3: Standard Graph of Ketorolac Tromethamine

4.2 FTIR Analysis

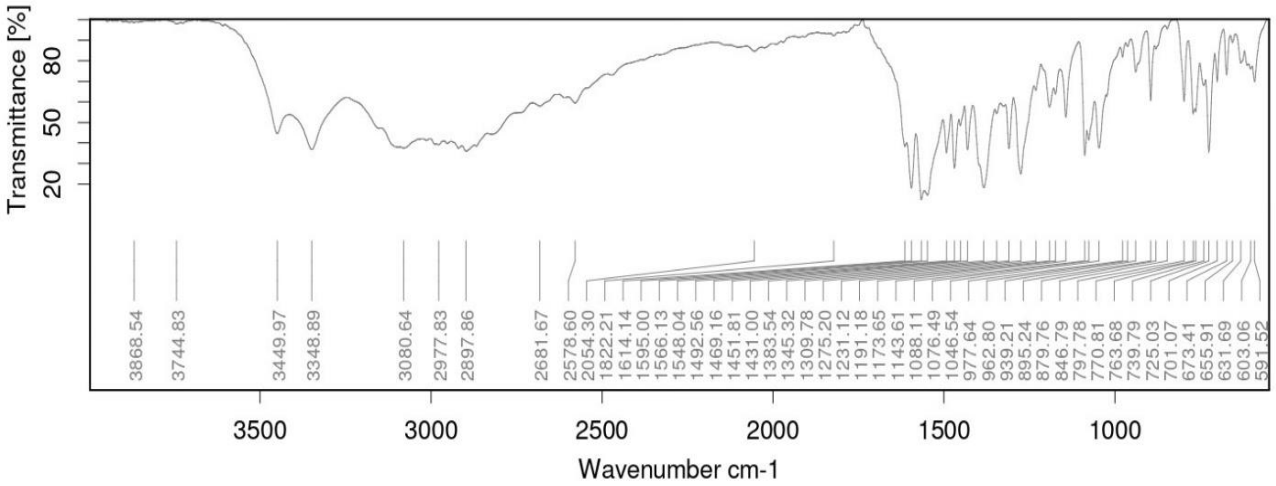

Fig-4: FTIR of Ketorolac Tromethamine Pure Drug

Table-3: FTIR Comparative Studies of Ketorolac Tromethamine Drug and Formulation

Wavelength cm^{-1}	Group	Compound Class
3900-3300	O-H stretching	Alcohol
3300-2700	N-H stretching	Amine salt
2700-2500	C-H stretching O-H stretching	Alkane Carboxylic acid
2500-2000	C=C=C stretching	Allene
2000-1500	C=O bending C=C bending C=N bending	Aromatic Compound Alkene Nitriles Carbene

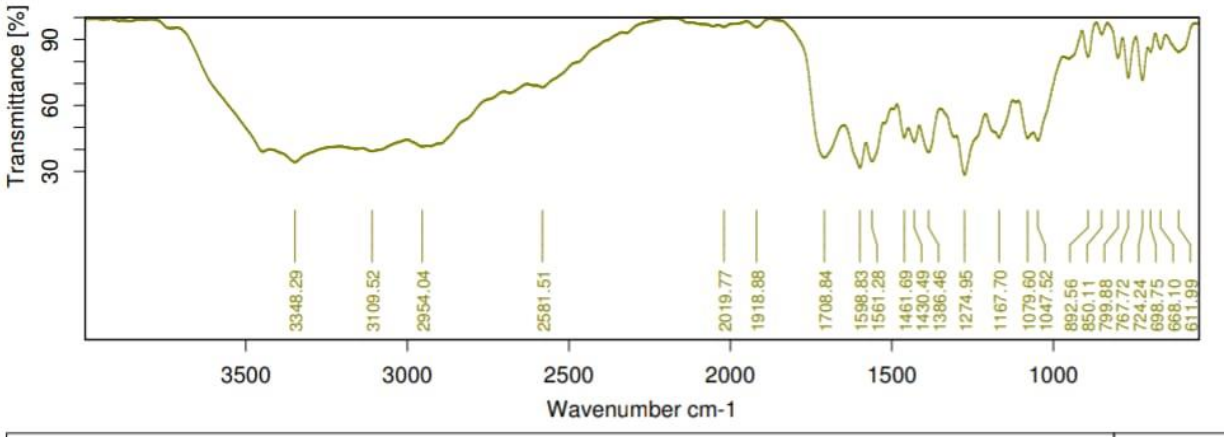

Fig-5: Topical Ketorolac Tromethamine Hydrogel formulation (HF4) spectrum

4.3 Physical Examination

The prepared ketorolac gel formulations had a homogenous, smooth appearance and were translucent white viscous. All of the formulations had a fair amount of spreadability, but HF4 had the greatest spreadability and excrudability. All of the prepared formulations had pH levels between 6.5 and 7.0, which were reevaluated as acceptable to reduce the risk of irritation when applied to the skin.

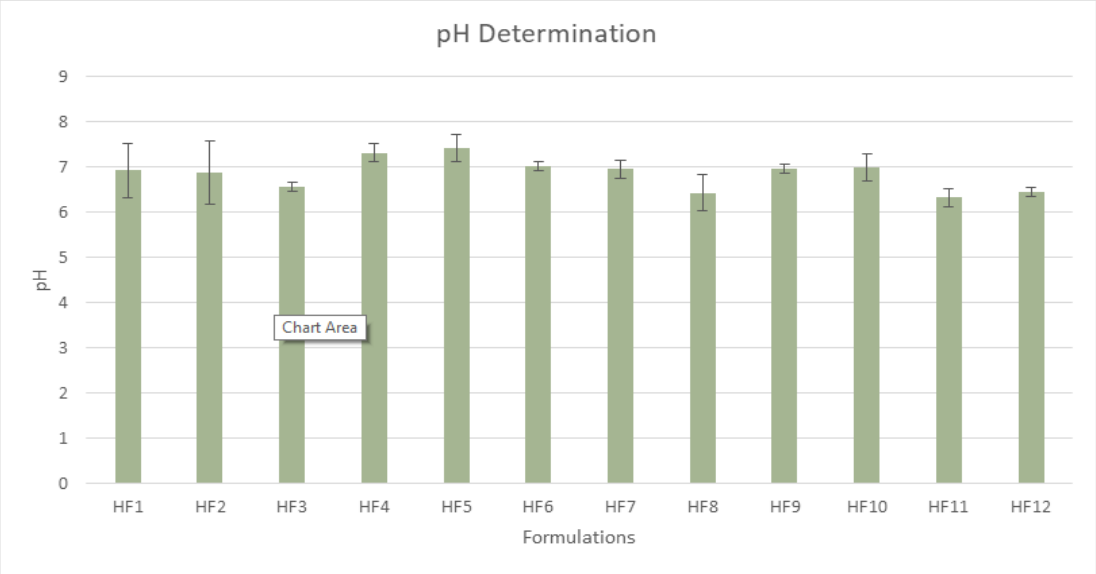

Fig-6: pH of prepared formulations

Table-4: Evaluation of Ketorolac Hydrogel Formulations

Formulation	pH	Spreadability (g.cm/sec)	Extrudability	Swelling Index	Mean % drug release ± SD
HF1	6.91±0.6	18.6±0.1	+	83.9±0.56	96.2±0.12 (1hr)
HF2	6.85±0.7	23.01±0.51	+ +	98.8±0.77	98.1±0.05 (1hr)
HF3	6.54±0.1	18.08±0.1	+ + +	115.4±0.42	98.1±0.05 (2hr)
HF4	7.3±0.2	25.2±0.3	+ + +	168.9±0.58	99.2±0.1 (10hr)
HF5	7.4±0.3	28.23±0.4	+ + +	172.7±0.41	97.9±0.1 (4hr)
HF6	7.01±0.1	26.9±0.6	+ +	78.1±0.39	97.8±0.1 (4hr)
HF7	6.94±0.2	19.5±0.8	+ +	68.9±0.29	98.7±0.15 (2hr)
HF8	6.41±0.4	23.9±0.8	+ +	98.2±0.46	98.6±0.2 (1hr)
HF9	6.95±0.1	19.3±0.4	+ + +	94.8±0.54	98.5±0.28 (1hr)
HF10	6.98±0.3	23.8±0.2	+ +	96.4±0.8	95.4±0.2 (2hr)
HF11	6.31±0.2	15.3±0.02	+	109.6±0.47	99.4±0.25 (6hr)
HF12	6.43±01	17.01±0.7	+	112.1±0.37	98.5±0.40 (6hr)

4.4 Swelling Index

The developed hydrogel formulation was characterized by measuring the swelling index and its release to determine the effect at pH 6.8. Optimized hydrogel formulation (HF4 and HF5) showed substantial swelling and drug release at pH 6.8. The results are demonstrated in table 3.

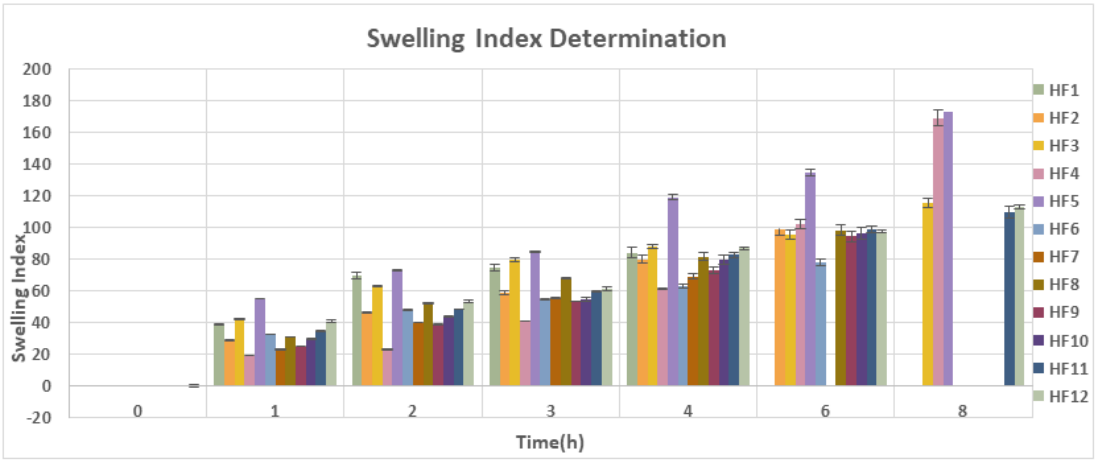

Fig-7: Swelling index of prepared formulations (HF1-HF12)

4.5 In vitro drug release study

The franz diffusion cell was used to conduct drug diffusion studies on all HF1–HF12 formulations. The cumulative percent release of KT-loaded gel from HF4 has strong penetration. According to the findings of these investigations HF4 formulation made of Carbopol and Xanthun gum shows controlled release of over the course of 10 hours when compared to others.

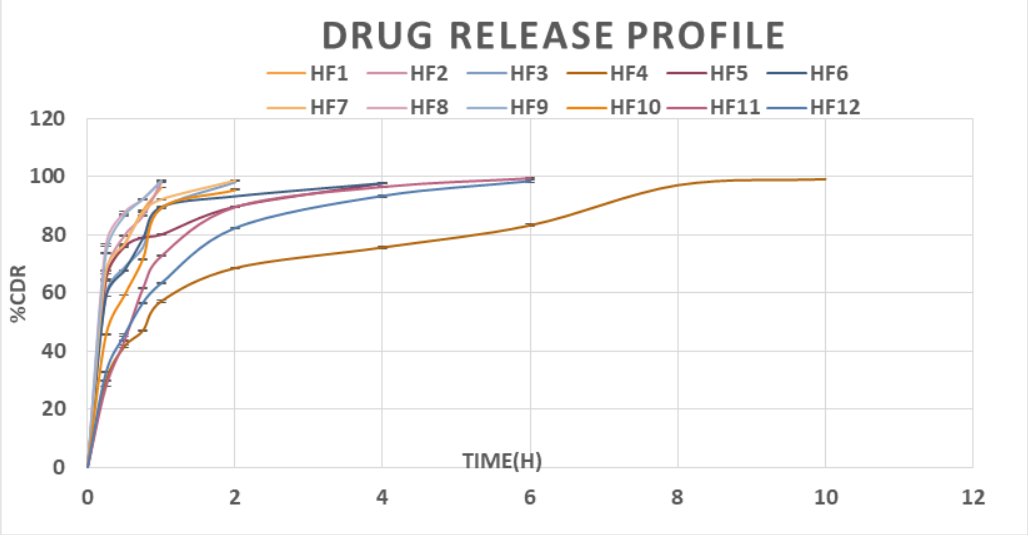

Fig-8: Drug release of Ketorolac Tromethamine Hydrogel Formulations

Table-5: In vitro Drug release profile of Ketorolac Tromethamine Hydrogel Formulations

%CDR												
Time	**HF1**	**HF2**	**HF3**	**HF4**	**HF5**	**HF6**	**HF7**	**HF8**	**HF9**	**HF10**	**HF11**	**HF12**
0	0	0	0	0	0	0		0	0	0	0	0
0.25	64.4±0.26	66.6±0.1	59.6±0.1	29.7±0.05	64.9±0.05	58.8±0.1	67.7±0.2	76.5±0.5	73.6±0.25	45.6±0.17	27.8±0.05	32.6±0.25
0.5	76.7±0.17	79.7±0.1	68.8±0.1	41.6±0.2	75.9±0.05	67.7±0.2	76.7±0.1	87.7±0.2	86.5±0.15	59.2±0.1	43.5±0.2	45.3±0.34
0.75	88.4±0.26	86.53±0.15	75.7±0..5	47.08±0.01	79.1±0.11	78.6±0.15	87.7±0.1	92.3±0.2	92.3±0.11	71.6±0.15	61.5±0.25	56.6±0.20
1	96.2±0.12	98.1±0.05	89.2±0.1	57.2±0.1	80.1±0.05	89.6±0.05	92.2±0.15	98.6±0.2	98.5±0.28	89.3±0.25	72.7±0.2	63.4±0.32
2	_	_	98.1±0.05	68.6±0.1	89.7±0.1	93.4±0.1	98.7±0.15	_	_	95.4±0.2	89.5±0.2	82.4±0.26
4	_	_	_	75.7±0.1	97.9±0.1	97.8±0.1	_	_	_	_	96.5±0.2	93.4±0.41
6	_	_	_	83.3±0.2	_	_	_	_	_	_	99.4±0.25	98.5±0.40
8	_	_	_	97.1±0.06	_	_	_	_	_	_	_	_
10	_	_	_	99.2±0.1	_	_	_	_	_	_	_	_

4.6. Drug Release Kinetics

It was found from the drug release kinetics that the Topical Ketorolac hydrogel HF4 drug release mechanism follows the Higuchi model and zero order. The drug release mechanism is by Non-Fickian Transport.

4.7 Ex-Vivo Diffusion

The pig ear skin was used to assess the optimised formulation for ex vivo diffusion. The hydrogel's flow was discovered to be 77.43 g/hr/cm2. This could be interpreted as a high flux value reflecting the gel's good penetration properties.

4.8 Hot Plate Analgesic method

Table displays the mean change in the latency period for responses on the hot plate analgesiometer.

The reaction time for the test formulation increased statistically significantly, similar to the Standard marketed gel. The test response was compared with the standard Ketorolac gel from 0-8hrs using

ANOVA, Dunnets test. It was found P<0.05 shows that prepared formulation to be effective in showing Analgesic effect.

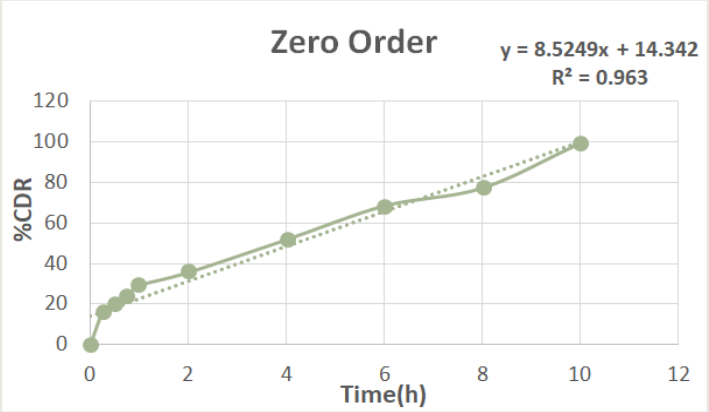

Fig-9: Zero order release kinetics of HF4

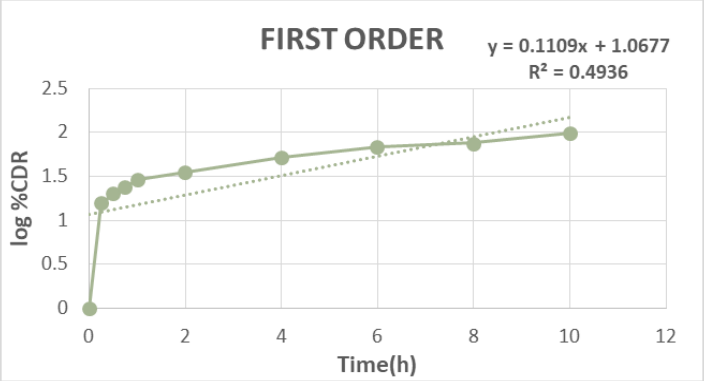

Fig-10: First order release kinetics of HF4

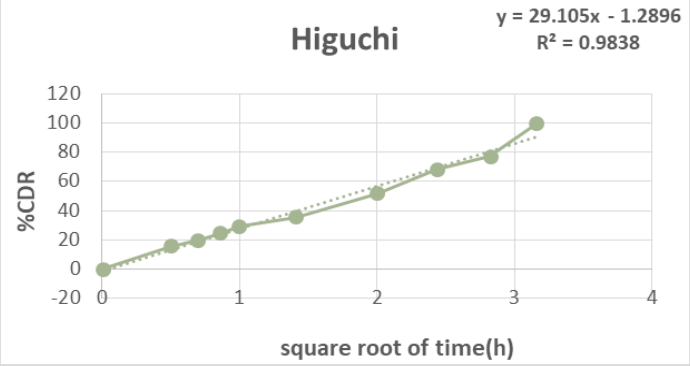

Fig-11: Higuchi release kinetics of HF4

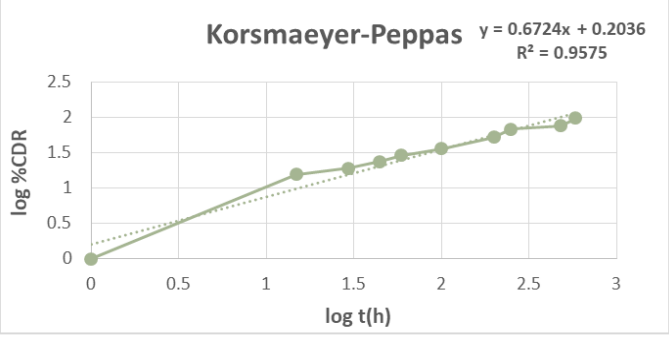

Fig-12: Korsemeyer- Peppas release kinetics of HF4

Table-6: Results of HF4 Formulation analgesic activity

	0 Mins	30 Mins	60 Mins	2 hours	3 hours	4 hours	6 hours	8hours
Normal	2.54±0.4	2.47±0.41	2.67±0.31	2.78±0.33	2.81±0.28	2.84±0.32	2.88±0.39	2.91±0.26
Standard	2.58±0.51	3.12±0.49	3.78±0.48$^{\#}$	4.47±0.48$^{\#}$	4.94±0.35$^{\#\#}$	5.42±0.44$^{\#\#}$	5.53±0.56$^{\#\#}$	3.41±0.5$^{\#}$
Test Formulation	2.48±0.2	3.21±0.26	3.54±0.28*	4.98±0.29*	5.05±0.34**	4.74±0.44*	4.64±0.57*	3.01±0.37

#- Significant; *- significant; ##- Highly significant; **- Highly significant; *-Relatively highly significant**

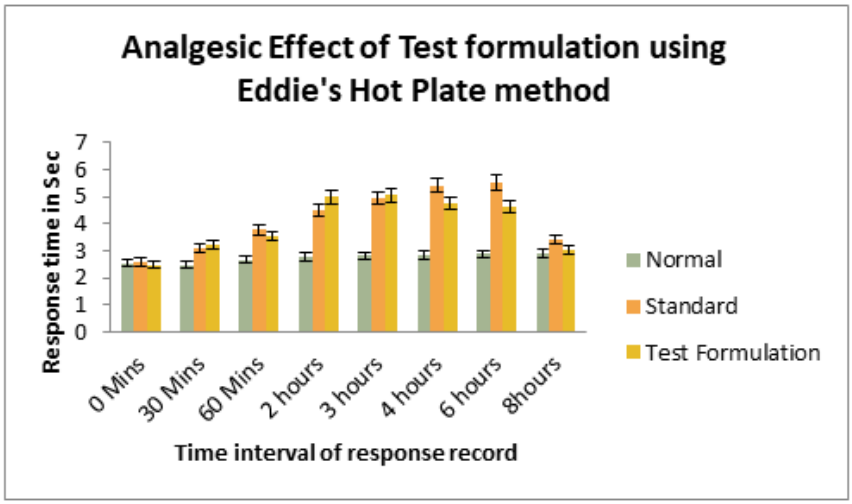

Fig-13: Graph of analgesic activity of HF4 Formulation

4.9 Carrageenan Induced Paw Method

Table displays basal mean paw size and the difference from basal size following the administration of formulations at various time intervals. For all three groups, i.e., Test formulation and Standard gel, there is a statistically significant reduction in paw size than control at all time periods. Additionally, it demonstrates that the test formulation results in statistically significant reduction in paw size after 3 hours. The test response was compared with the standard Ketorolac gel from 0-8hrs using ANOVA, Dunnets test. It was found P<0.05 shows that prepared formulation to be effective in showing Anti-inflammatory effect

Table-7: Results of HF4 Formulation anti-inflammatory activity

	0 Mins	30 Mins	60 Mins	2 hours	3 hours	4 hours	6 hours	8hours
Normal	3.44±0.21	3.45±0.24	3.47±0.31	3.4±0.19	3.49±0.22	3.47±0.26	3.54±0.31	3.64±0.28
Toxic Control	7.42±0.41	7.54±0.43	7.57±0.39	7.94±0.37	7.87±0.41	7.41±0.43	6.47±0.41	6.21±0.37
Standard	7.87±0.5	7.04±0.48	6.78±0.43	6.47±0.47	4.94±0.46	3.42±0.31	3.33±0.34	3.31±0.31
Test Formulation	7.88±0.47	7.21±0.43	6.44±0.0.33	5.98±0.26	5.05±0.28	4.74±0.38	3.41±0.27	3.21±0.28

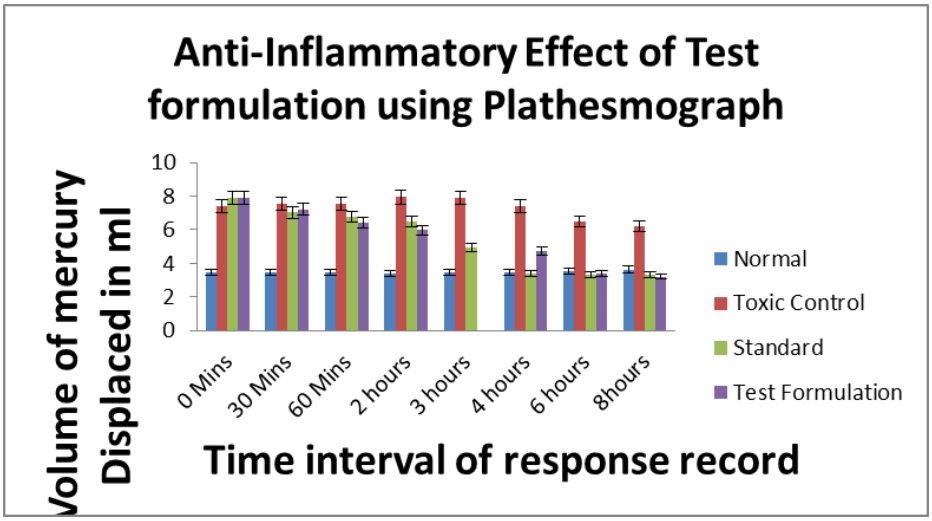

Fig-14: Graph of anti-inflammatory activity of HF4 Formulation

4.10 Skin Irritancy Test

The results illustrated that, there were no any signs of irritation after 72hours (showed zero of erythema score) which indicated that the HF4 hydrogel formulation is as safe as marketed gel.

5. CONCLUSION

Numerous topical hydrogel formulations have been successfully developed with ketorolac tromethamine. The physical characteristics, pH, excrudability, spreadability, swelling property, in-vitro drug release study of hydrogel formulations were assessed. The pH of the formulations is were in the range of 6.31- 7.4, reducing any skin irritation, and the HF4 formulation produces high spreadability of 25.2 0.3 gm-cm/sec than other formulations. According to in vitro experiments on drug flux via a cellophane membrane, KT topical formulations might be ranked in the following order: HF4>HF12>HF11>Hf6>HF5>HF7>HF3>HF10>HF2> HF1>HF8>HF9. The release of HF4 in vitro was greater than others (99.2±0.1). Utilizing a dialysis membrane and a phosphate buffer solution with a pH of 7.4, UV analysis, FTIR spectroscopy, and in vitro release study were carried out on a Franz diffusion cell. The FTIR research shows that there is no consistent interaction among Ketorolac Tromethamine and any of the excipients. After 72 hours of application to rats' shaved dorsal skin, HF4 hydrogel showed no signs of irritation. The outcomes of this study offer additional proof of the analgesic and anti-inflammatory effects of Ketorolac Tromethamine. The final formulation HF4 hydrogel shows equal analgesic and anti-inflammatory effect as Standard Ketorolac gel.

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