DESIGN, CHARACTERISATION, AND EVALUATION OF TERIFLUNOMIDE CONTAINING HYDROGEL DOSAGE FORM

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Abstract:

Purpose: The formulation of this hydrogel aims to treat osteoarthritis which is one of the most common inflammatory autoimmune disorders of joints. The use of conventional drugs like NSAIDs has various side effects. The main advantage of this dosage form is it will release the drug as per requirement which will avoid first-pass metabolism, give a synergistic effect and also lessen the side effects because of dose reduction.

Method: Using the Statistical model, one formulation is optimized where the percent drug release is considered. Hyaluronic acid and DMSO of different concentrations were used to produce hydrogel formulations of Teriflunomide. This hydrogel is prepared by the physical cross linking method. Various evaluation parameters such as Physical Properties, pH, viscosity, spreadability, Homogeneity swelling, thermal properties, assay, *in vitro* drug release, *and stability testing* were carried out.

Result: No considerable changes occurred in either physicochemical properties or Drug content and release of Drug after exposure to accelerated conditions of temperature (40 ± 2°C) and humidity conditions (75 ± 5%RH). The gel formulation consisting of 1.9% w/v hyaluronic acid was found to be suitable for topical application based on *in vitro* evaluation studies.

Conclusion: These results suggest the feasibility of the hydrogel formulation of Teriflunomide. The formulation of these injectable hydrogel containing teriflunomide will definitely. Dosage form development of hydrogel containing teriflunomide was done successfully using DoE.

Keywords- Teriflunomide, Injectable Hydrogel, Hyaluronic acid, Osteoarthritis.

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1. Introduction:

Osteoarthritis is an autoimmune disorder where structural or functional damage is produced due to the correlation of immunologically competent cells or antibodies against the standard component of the body [1]. Women are more prone to osteoarthritis than that of man the ratio is around three times more than men [2, 3]. Osteoarthritis causes pain, stiffness, and swelling in major joints present in the body [4]. There are many disadvantages to the conventional drugs which are being used in the treatment of osteoarthritis.

Drugs that are used in the treatment of osteoarthritis are non-steroidal anti-inflammatory drugs (NSAIDs), Corticosteroids, and others [5, 6]. These drugs are effective against OA but they have serious complications. NSAIDs are a widely used medication regimen for OA but they only show symptomatic relief they do not alter disease conditions. NSAIDs also have adverse effects like gastrointestinal ulceration along with high dose 3891equirement [7]. Corticosteroids like Glucocorticosteroids are also used but long-term use of these medications shows severe side effects [8]. DMARDs are one of the slow-acting drugs which instead of showing symptomatic relief they involve in the incipient stage of the disease [9]. Teriflunomide is an active

metabolite that inhibits dihydroorotate dehydrogenase (DHODH). This drug acts in the pyrimidine production pathway and also reduces the proliferation of stimulated lymphocytes. During the disease stage, there is an activation of macrophages in an inflammatory reaction in synovial fluid. [11, 12, 13]. Drug delivery of this drug by parenteral route shows the highest drug delivery with advantages like immediate effect; avoiding first-pass metabolism and gastrointestinal complications. Due to these advantages dosage frequency as well as the dose is also reduced [14].

There for these parental dosage form includes liposomes [6, 15], Dendrimers [16,17], micelle [18], nanocapsule [19], nanogel [20,21]. These methods of drug delivery are convenient and effective and also reduces side effect [8]. By taking into consideration all the factors present study focused on the development of a novel injectable hydrogel of teriflunomide to improve the therapeutic efficacy, faster effect, and also to minimize drug-related side effects.

2. Formulation:

2.1. By Physical Cross-Linking [23,24, 25]

The gel can be defined as the semisolid preparations that contain a high ratio of solvent and gelling agent. They are transparent & translucent in nature. As per the USP gels are semisolid in nature, either they are suspension of small or large molecules penetrated in liquid (Gel system). During gel, the formation liquid is constrained between three-dimensional polymeric liquid. This gel system is visco-elastic in nature. Depending on the entrapped liquid gels are classified ad hydrogels or organogels. When the entrapped liquid is in the aqueous form it is hydrogels are formed and when it is in the combination of both aqueous and organic phases organogels are formed. Hydrogels are hydrophilic in nature. During the formation of hydrogel, there is a three-dimensional cross-linking polymer system in which a large amount of water or biological fluids can be entrapped in polymeric chains and form an aqueous semisolid or solid gel network [26]. Physical cross-linking involves various mechanisms like entangled chains, hydrogen bonding, hydrophobic interaction, and crystalline formation. Hydrogels formed after physical cross-linking is reversible in nature. (Fig.1)

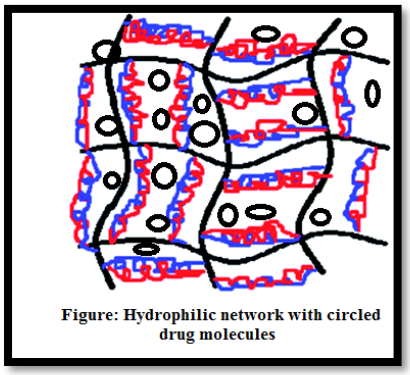

Fig.1: Physical Cross-Linking

The effectiveness of hydrogel relies critically upon quality attributes as listed in the table. The critical process parameter (CPP) and critical material attribute (CMA) are the two parameters that decide the quality of formulation. The CPP for the current formulation is temperature whereas CMA is the concentration of hyaluronic acid.

In the present investigation, the physical cross-linking method was used to formulate a hydrogel. The experimental design used was Central Composite Design. The independent variables used were percent composition of hyaluronic acid and heating temperature (25°C low level and 36°C high levels) with the dependent variable percent drug release. Alfa values (axial points) used were ±1.41.

In the present investigation, we have focused on Process and Formulation variables. While formulating Gel containing teriflunomide we have selected one process variable which is 'temperature'. The rationale for the selection of temperature as a variable was that temperature affects the features of the hydrogel. Pharmaceutical research scientists have reported that the gelation process performed at different temperatures (temperature has an influential role) can produce stable hydrogel (https://doi.org/10.1016/j.heliyon.2020.e03494). Few process variables like stirring time were also considered during the formulation of hydrogel containing both drugs. Factors and their levels were shown in following table 1.

Table 1. Factors and Their Levels

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	Hyaluronic Acid	%	Numeric	-1	1	-1	1
B	Heating Temperature	Degree Celcius	Numeric	-1	1	-1	1

The low level for HA was 1%w/w and the high level was 2%w/w the quantities lower than low coded values and higher than high coded values in formulation table 2 depicts the alfa axial points suggested by the software.

Table 2- Values obtained after application of design

Standard	Run	Factor1	Factor2
6	1	1.414214	0
2	2	1	-1
11	3	0	0
3	4	-1	1
12	5	0	0
7	6	0	-1.41421
4	7	1	1
1	8	-1	-1
8	9	0	1.414214
10	10	0	0
5	11	-1.41421	0
9	12	0	0
13	13	0	0

Table 3: Details of specifications used while applying experimental design

Design Summary									
Study type	Response surface		Runs	13					
Initial design	Central composite		Block	No Block					
Design Model	Quadratic								

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded	Mean	Std.Dev
A	Hyaluronic Acid	%	Numeric	-1	1	-1	1	0	0.784465
B	Heating Temperature	Degree Celcius	Numeric	-1	1	-1	1	0	0.784465
Response	Name	Units	Obs	Analysis	minimum	maximum	Mean	Std.dev	Ratio
Y1	% Release	%	0	Polynomial	polynomial	No Data	No Data	No Data	N/A

2.2. Preparation:

1.0 % w/w HA solution was prepared by dissolving HA in an equal portion of distilled water & DMSO. Solubilization of HA was catalyzed by warming the solution to 50-60°C. Subsequently, the solution is cooled. Two different solutions with the same concentration were cooled to 25° C and 36° C. After the formation of a clear solution, The drug was added to the viscous mass of gel prepared. Physical entanglement at lower temperatures was the principle of hydrogel formation. The same formulation was also prepared using 2.0 % w/w HA in solution.

Table 4: formulation of hydrogel containing TRF and HA

Sr.No	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	Teriflunomide (mg)	100	100	100	100	100	100	100	100	100	100
2	Hyaluronic acid HA* (Wt%)	2.1	2	1	0.9	0	1.9	2	0.9	0	0
3	DMSO (ml) + WFI (ml) Q.S	10	10	10	10	10	10	10	10	10	10
* Values of HA were represented as low coded, high coded, and mean suggested by central composite design. Moreover, the Low level for HA was 1%w/w and the high level was 2%w/w, quantities lower than low coded values and higher than high coded values in the formulation table depict the alfa axial points suggested by the software.											

3. Evaluation:

3.1. Formulation optimization

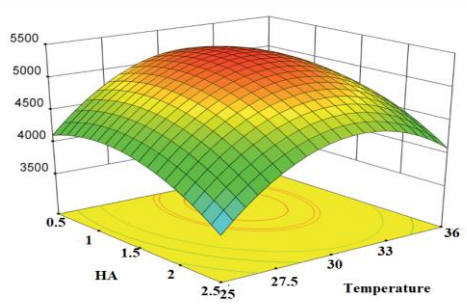

Fig.2: 3D Counter Plot

The ANOVA model fitted well with the response data. The model statistics are given in Table 5. The model passed the diagnostic tests for any outliers including a normal probability plot. The 3D response plot between temperature and concentration of HA was depicted in Figure. The figure shows the close proximity of the model prediction with the experimental values signifying the validity of the model selected. The data of predicted and actual were also shown in the subsequent figure. The optimized process conditions for the response variable were HA

concentration of 1.9% w/w and a temperature of 36 ºC.

The % release is more sensitive to the concentration of HA than that of the process variable (temperature). Anova further revealed the most influencing variable was the concentration of HA. A validating experiment was also carried out for the confirmation of the model suggested by the design expert also showed that the response variable was consistent with that of the variables suggested by the software and hence validated the model. The regression equation for quadratic terms was suggested as follows,

$$Y1 = 0.62 + 0.043X1 + 0.15X2 - 0.043X1X2 - 0.082X1^2$$

The regression equation suggests the influence of individual factors as well as the interaction between them. It was observed from the regression equation that the X1 i.e. concentration of HA is the most influencing factor than the other factor X2 (Temp.). moreover, there was a list of interactions between the factors. Hence combined effect of concentration of HA and temperature was least noted by the model.

From the response surface graph, ANOVA, and validating experiment it may be concluded that **the F6 formulation** can be considered the optimized formulation. Moreover, the predicted values, do not show any significant nonlinear pattern indicating non-normality in the error term.

Table 5: Model statistics

Source	Y1			
	Estimated Effect	*F* Value	*p* Value	R^2
Model	1223.3	50.6	0.0020 *	0.989
X1	950.7	386.3	<0.0001 *	
X2	294.5	37.0	0.0017 *	
X1X2	294.2	18.5	0.0077 *	
$X1^2$	221.7	9.7	0.0264 *	
$X2^2$	85.7	1.4	0.282	
		1.5	0.274	
	Source		Lack of Fit	
	Y1		0.0801	

Note: * indicates significant effect of this factor on the dependent response.
Abbreviations: $X1^2$, $X2^2$ are the quadratic terms for the factors, X1X2, interaction terms between the factors.

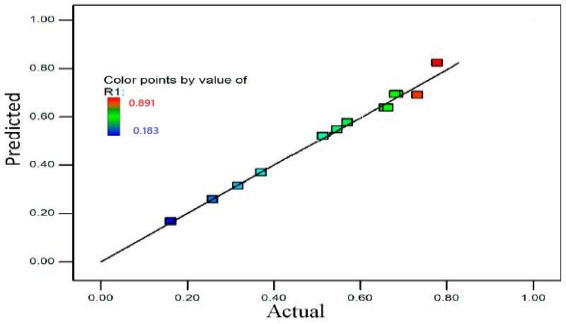

Fig 3: Plot of predicted vs. actual data of the model

3.2. Physical evaluation and Consistency

The physical evaluation of semisolid preparation was performed. The color, homogeneity & consistency was examined. The pH was checked every time before use so as to confirm its stability of it within the biological fluids.

3.2.1. Viscosity and Homogeneity:

Brookfield viscometer was used for the evaluation of the viscosity of hydrogels. To check the viscosity of the formulation 1, 3 & 5 grams the formulation was dissolved in 25 ml purified water for 24 hours.

3.3. Drug Content:

The assay of the drug was performed by extraction of drug from 1gm of gel formulation with 20ml of phosphate buffer pH is 7.4 for 30min. the membrane filter of pore size 0.45 µm is used for the filtration of the resultant mixture. The solution was diluted with phosphate buffer pH 7.4 and its absorbance was checked spectrophotometrically at its lambda max with the help of a calibration curve, the concentration of the drug was also estimated.

3.4. Swelling studies:

At a definite period of time and at room temperature, a 0.2gms sample of dried hydrogel is immersed in 200ml of distilled water. The sample was stirred slightly with a magnetic stirrer. After specified time dispersion of water-swollen gel particles was formed and filtered through the Buchner funnel. A filtered sample was weighed. After the specified time sample was removed from the water so as to check the absorption rate. The absorption rate was measured repeatedly for three times and the swelling ratio was calculated with the help of the following equation

$$\text{Wt} = \frac{Mw - Md}{Md}$$

Where,
Wt- Swelling ratio at time t
Mw- Weight of swollen hydrogel at time t
Md- Weight of the dried gel

3.5. Thermal properties characterization.

To determine the thermal properties of the gel thermo gravimetric analysis is done. Thermo analyzer system apparatus is used to collect the thermo gravimetric data of dried gel. The sample ranging from 5-10 mg were weighed and heated from 25°C to 600°C with a scanning rate of 10°C min^{-1}. Differential Scanning colorimetry is also used to check the thermal properties of the gels. With the help of a standard medium, the temperature and heat flow was calibrated. The sufficiently dried gel is taken as a sample and weighed separately with weights ranging from 3-5mg. the samples were equilibrated at 25°C and ramped up at 10°C/min to 360°C and after that total heat flow was noted.

3.6. In vitro drug release study:

Franz diffusion cell is used for in vitro diffusion study. The phosphate buffer pH is 7.4 at 37±1ºC was used as diffusion media. 25 ml of 7.5 pH phosphate buffer was added to the acceptor compartment whereas the donor compartment is filled with hydrogel. The hydrogel is permitted into the acceptor compartment from the donor compartment through a semipermeable membrane (S50). At predetermined intervals, 1 ml sample was withdrawn and replaced with an equal amount of fresh buffer (pH 7.4).

3.7. Accelerated Stability Studies:

All the stability studies were performed on the optimized formulation as per ICH Guidelines. Hence as per ICH guidelines formulation is packed in an aluminum tube and subjected to accelerated conditions for 3months. Temperature Conditions as per ICH guidelines (40 ± 20°C) and relative humidity 75 ± 5%. After every month for up to 3 months sample was collected and analyzed for changes in pH, Drug content, and in vitro drug release. All the tests were carried out in triplicate and the mean value is taken into consideration if any change is observed it was taken into consideration.

4. Results of Evaluation:

4.1. Physical evaluation and Consistency

After physical evaluation, it was found that the formulation was clear transparent semisolid in nature with entrapment of air bubbles in negligible proportion.

Fig 4: Physical appearance

Table 6: visual appearance

Formulation code	Visual appearance
F1	Thick, Translucent
F2	Thick, Translucent
F3	Thick, Translucent
F4	Thick, Translucent
F5	Thick, Translucent
F6	Thick, Translucent
F7	Thick, Translucent
F8	Thick, Translucent
F9	Thick, Translucent
F10	Thick, Translucent

4.2. Viscosity and Homogeneity

Results of the viscosity measurement indicate that hydrogels cooled to 27ºC were found to be more viscous than the hydrogels which were called to 37ºC. The result stated that viscosity is dependent on the concentration as the concentration increases viscosity is also increased. Hence the viscosity of HA (2 % w/w) was found to have more than the viscosity of HA having a concentration of HA at 1% w/w. compiled in the following tabulated format.

Table 7: Viscosity of Formulations

Formulation code	Viscosity (cps)
F1	8819 ± 2
F2	8850 ± 1.5
F3	8980 ± 1.5
F4	9142 ± 2.5
F5	9236 ± 1.5
F6	9344 ± 2
F7	9589 ±2
F8	9636 ± 1.5
F9	8819 ± 2
F10	8850 ± 1.5

4.3. Drug Content:

For assay of the drug in gels, all the processes are followed as discussed earlier. Hence it is observed that the concentration of the drug was estimated from the calibration curve and compiled in the following tabulated (Table 8) format.

Table 8: Drug Content Determination of Formulations

Formulation code	Drug content
F1	98.5 ± 0.2
F2	98.7 ± 0.2
F3	99.2 ± 0.1
F4	99.7 ± 0.1
F5	99 ± 0.4
F6	99.8 ± 0.2
F7	99.7 ± 0.1
F8	99.6 ± 0.3
F9	98.5 ± 0.2
F10	98.7 ± 0.2

4.4. Swelling studies:

Dried hydrogels are placed in distilled water for the specified time of 1,2,3,5,10,20,60,100 min and their swelling properties were determined. The water which is not absorbed was removed from the sample by the process of filtration. After 70mins average swelling equilibrium was attended.

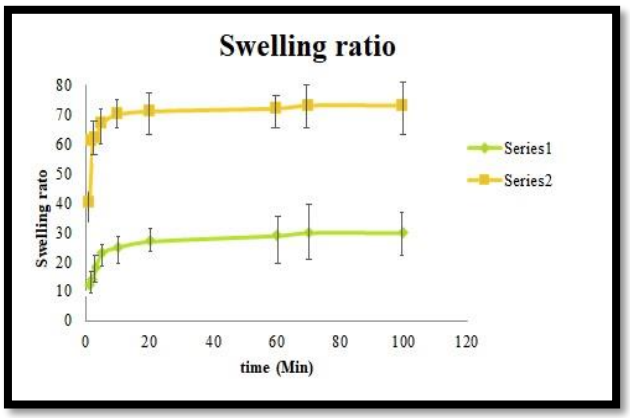

Fig5: Swelling ratio (Series1: 1% and series 2: 2%)

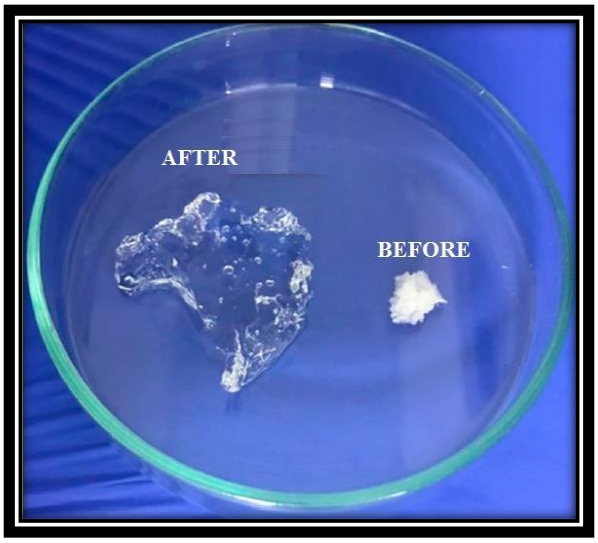

Fig 6: The swelling ability

Table 10: Swelling ratio

Sr. no.	Time	Swelling ratio	
		1%	2%
1	1	12±0.9	40±0.9
2	2	14±0.7	61±0.7
3	3	18±0.9	68±1.5
4	5	23±0.8	69±0.9
5	10	27±0.9	72±1.1
6	20	29±1	73±0.8
7	60	30±0.9	74±2.8
8	70	30±1.2	74±2.6
9	100	30±0.9	74±1.9

4.5. Thermal properties characterization:

Thermogravimetric analysis is used to evaluate the stability of the gel. The temperature at which the first weight loss is there is known as thermal degradation temperature. The gel began to lose its weight at

222-282^{0}C which indicated that the gel is having relatively high thermal stability. The thermogravimetric analysis showed higher phase change temperatures for gels with higher degrees of PLA polymerization, specifically 222°C for standard and 280°C for test hydrogel.

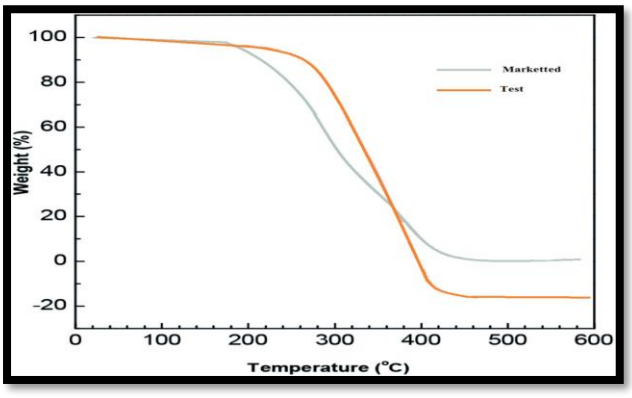

Fig 7: Thermogravimetric Analysis of Hydrogel

Results of DSC analysis show the relatively high thermal stability levels of the gel. As indicated in the data gel is showing two physical transitions-

1. Glass transition temperature T_g which is close to 180^{o}C.
2. Crystal melting at a temperature T_m which is near 280^{o}C

The HA and SC crystallites show successful stereocomplexation due to which crystal melting peak was observed. As the degree of polymerization of HA increases the T_m value also increased gradually. The glass transition temperature did not affect much from 180^{0}C as the HA polymerization degree was increased, indicating the little effect of the stereocomplex on the movement of the segments in the blend. Optimized formulation (F6) was considered for evaluation.

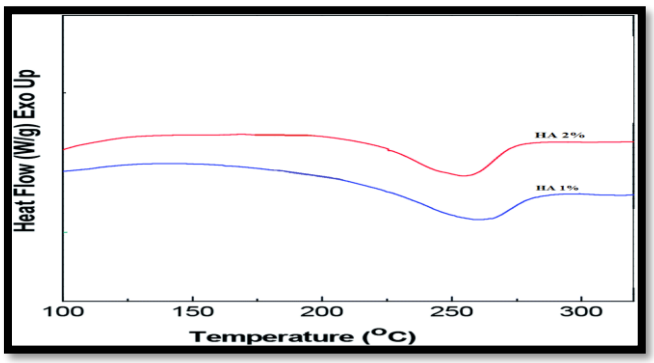

Fig 8: DSC of gels

Table 11: In-vitro Drug Release

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
0	0	0	0	0	0	0	0	0	0	0
2	6	7	11	10	10.5	12	9	9	7	8
4	8	15	22	19	19	25	24	13	12	13
6	19	30	29	24	28	38	30	25	25	28
12	26	41	41	39	41	49	42	38	54	38
16	44	49	49	52	55	62	53	52	59	52
18	55	55	57	54	63	74	61	70	62	56
20	62	60	62	59	72	84	69	79	74	62
22	69	61	69	62	76	89	75	82	79	68
24	69	66	76	75	82	93	87	88	86	71

Table 12: Average and standard deviation chart at the investigated time interval

AVG	SD
8.95	2.335614
17	5.925402
27.6	6.510624
40.9	9.152272
52.7	9.149207
60.7	11.06822
68.3	13.07679
73	13.62556
79.3	14.52631

Drug release for the formulation F6 was found to be consistent over the period of test conditions. In comparison with the release pattern of other formulations, F6 may be an optimized one. The predicted and actual validation experiments have shown similar values and hence it may also conclude that the design space suggested by the software was optimum and best fitted.

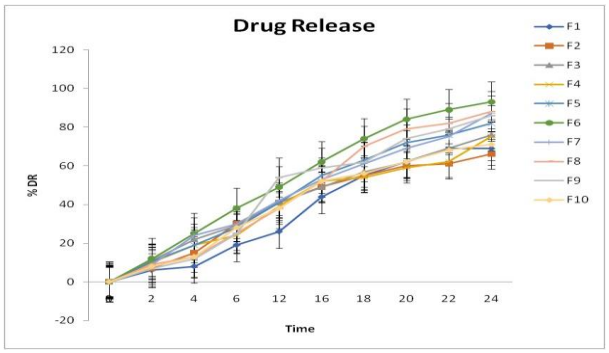

Fig 9: In-vitro Drug Release

4.6. Stability Studies [27,28]:

During the stability study specifications like physical appearance, drug content, spreadability, and viscosity were studied. The stability study revealed that the formulation was stable during the stability study. These parameters were tested timely and as per the ICH guidelines during the study. Data at the end of 90 days [29,30] (temperature 40 ± 20°C and relative humidity 75 ± 5%.) are shown in the following table.

Table 13: stability study at the end of 90 days (temperature 40 ± 20°C and relative humidity 75± 5%)

Formulation Code	Visual Appearance	Drug Content	Viscocity (CPS)	Spreadability
F1	Thick, Translucent	98.2 ± 0.2	881 ± 2.1	++
F2	Thick, Translucent	97.7±0.2	885 ± 1.2	++
F3	Thick, Translucent	98.2±0.1	898 ± 1.2	++
F4	Thick, Translucent	97.7 ± 0.1	914 ± 2.4	++
F5	Thick, Translucent	98 ± 0.3	923 ± 1.2	++
F6	Thick, translucent	99.1 ± 0.2	934 ± 1	++
F7	Thick, Translucent	99.1 ± 0.1	959 ±3	++
F8	Thick, Translucent	99.2 ± 0.3	966 ± 1.1	++
F9	Thick, Translucent	98.2± 0.1	881 ± 3	++
F10	Thick, Translucent	98.1 ± 0.3	885 ± 1.1	++

5.

6. Conclusions:

The Preformulation study was performed successfully. During the preformulation study parameters like organoleptic properties, appearance, FTIR, thermal analysis, UV spectroscopy and calibration studies were conducted.

From the preformulation study, it may be concluded that the procured powder of API, Teriflunomide has got similar organoleptic characteristics as that reported in the drug data bank, and hence preliminary identification was done to confirm the identity of Teriflunomide. A solubility study revealed that the Teriflunomide was practically insoluble in water but freely soluble in DMSO. Solubility study has also shown that the Teriflunomide was soluble in alkaline pH rather than acidic.

A melting point study was done which revealed that the procured sample of TRF and HBV both have comparative melting points as that of standard references obtained during the literature review. UV characterization has given the absorption maxima at 292 nm. Melting point and solubility analysis may be used to confirm the identity of the drug which will be used to formulate in aimed dosage formulation. An incompatibility study done with the FTIR confirmed that there was not any significant drug interaction with the excipients to be used during dosage form development as all the peaks observed in FTIR spectra of the physical mixture were present in the FTIR spectrum of pure TRF.

Dosage form development of hydrogel containing TRF was done successfully using DoE. The design space suggested by the software was validated by conducting a validating experiment which confirms the fitness of the DoE used. Independent variables, X1, percent of HA (Product variable), and X2, Temperature (process variable) were used as factors at two levels and percent of drug release was used as a dependant variable. During the optimization, it was predicted that the F6 could be the optimized formulation. Drug release for the same formulation was found to be maximum and consistent over the duration of the release study. Evaluation parameters like swelling study, spreadability, viscosity, and drug content were also performed for the formulation developed. All the evaluation parameters were found to be optimum and comply with all standards which are essential for parenteral hydrogel preparation. Short-term stability

studies show that the solution was stable in phosphate buffer.

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Author Contributions- Both the authors are contributed to this work

Ethics Approval- This paper does not have any animal study.

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