Formulation and Evaluation of Topical Leflunomide Hydrogel for Rheumatoid Arthritis

Naman Jain[*], Kratika Daniel
Oriental College of Pharmacy, Oriental University, Indore

ABSTRACT: Aim: To formulate and evaluate leflunomide hydrogel for the treatment of rheumatoid arthritis.
Materials and Methods: Leflunomide drug was used in this research. The gel was prepared by simple method. The gel was prepared in nine batches F-1 to F-9.
Results and Discussion: It was found thatF-4 was the best formulation. All the evaluation parameters were successfully evaluated.Formulation F4 was found to have better results in all the Evaluation parameters. In formulation F4 the noted thing was the drug was highly in nature hence best suited for the preparation of topical Leflunomide hydrogel formulation. This formulation was the best formulation for the topical administration and to easy penetration through the skin. This is concluded that the hydrogel has been proved to be more beneficial, much convenient to the patients and shows less side effects with total care of patient. It is clear from above discussion that F4 were best among all the prepared formulations.

I.   INTRODUCTION:

Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. The main advantage of topical delivery system is to bypass first pass metabolism. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time are other advantage of topical preparations. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solution, and even medicated adhesive systems are in use. Topical drug delivery systems include a large variety of pharmaceutical dosage form like semisolids, liquid preparation, sprays and solid powders. Most widely used semisolid preparation for topical drug delivery includes gels, creams and ointment.
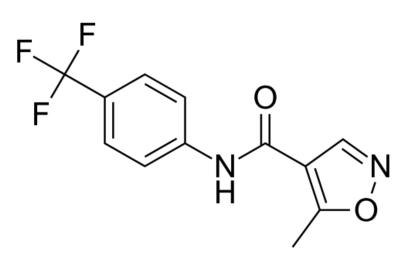

Figure 1: Structure of Leflunomide

II. MATERIALS AND METHODS

2.1 Materials: Leflunomide drug sample was a generous gift byCadila Pharmaceuticals Limited. (Indore). All other chemicals used were of analytical grade and procured from local market.

A Shimadzu-1700 UV-Visible spectrophotometer with 1 cm matched silica cells were used for spectrophotometric analysis.

2.2 Estimation of Leflunomide

2.2.1 UV spectrophotometric analysis of leflunomide:

UV-visible spectrophotometer was used to determine the maximum absorbance (λ-max) ofLeflunomide using a digital double - beam recording spectrophotometer with scanning range of 200-400 nm. Solution of Leflunomide was prepared using double distilled water. Shimadzu UV visible spectrophotometer (Perkin Elmer, Lambda 35 Model) 1800 with spectral bandwidth of 1 nm ± 0.3 nm wavelength accuracy and 10 mm pair of quartz cells were used to record the spectral and absorbance readings**.**

2.2.2 Preparation of calibration curve of leflunomide in 0.1 N HCl: The UV absorbance of Leflunomide standard solutions in the range of 2-10 µg/ml of drug in buffer pH 6.4 showed linearityatλmax261nm. Theabsorbancevaluesandstandardcurvepresentedinfigur**e**2.

2.2.3. Partition coefficient-Partition coefficient is defined as the ratio of un-ionised drug distributed between the organic and aqueous phase at equilibrium.Partition coefficient was determined the following procedure. 50mg of drug was dissolved in 50ml of water which was taken in separating funnel and 50ml of octanol was added to it. Funnel was shaked for 30 min and stand for 5 min aqueous layer was separated and centrifuged for 1hour at 2000 rpm.1ml of this taken and diluted up to 10ml. The aqueous phase is assayed before (Σc) and after partitioning (C_w) [the aqueous concentration] by using UV-visible spectrophotometer and $K_{o/w}$ calculated by using formula

2.3 Solubility studies: Equilibrium solubility of the drug in various selected solvents was determined Like water, methanol, ethanol and DMSO. Excessive quantity of drug was dissolved in 5.0ml of solvents in volumetric flask and kept in a shaker for the period of 5 hours. The resulting saturated solutions were kept aside for 24 hours, filtered through sintered glass filter and was analyzed by UV spectrophotometer.

Table- 1. Solubility data of Leflunomide in different solvent systems

S. No.	Solvent systems	Solubility (mg/ml)
1.	N, N-Dimethylformamide	Freelysoluble
2.	Ethanol,Chloroform,Methanol,Aceticacid	Slightlysoluble
3.	Water,Petroleumether	Practicallyinsoluble

2.4. Formulation and evaluation of optimized batch

2.4.1. Preparation of Leflunomide Hydrogel

Leflunomide, methyl paraben, Propyl paraben, propylene glycol, triethanolamine. Accuratelyweighed Quantity of Carbopol 940 and Paraben were dissolving incold distilled water in beaker and kept aside for overnight at room temperature. Drug wasdissolve separately in Propylene Glycol and this solution was added to above Carbopol 940polymer solution.

Table-2. Formulation composition of different batches of Leflunomide

S. no.	Formula tion	Leflunom ide	Carbopo l 940	Propyleneglyco l	Methyl paraben	Propyl paraben	Triethan ol -amine	Distilled water
1.	F1	1%	0.5 %	1.5 ml	0.18 %	0.02%	q.s	q.s
2.	F2	1%	1 %	1.5 ml	0.18 %	0.02%	q.s	q.s
3.	F3	1%	1.5 %	1.5 ml	0.18 %	0.02%	q.s	q.s
4.	F4	1%	1.25 %	1.5 ml	0.18 %	0.02%	q.s	q.s
5.	F5	1%	1.5 %	1.5 ml	0.18 %	0.02%	q.s	q.s
6.	F6	2%	1.75 %	1.5 ml	0.18 %	0.02%	q.s	q.s
7.	F7	2%	2 %	1.5 ml	0.18 %	0.02%	q.s	q.s
8.	F8	2%	2.5 %	1.5 ml	0.18 %	0.02%	q.s	q.s
9.	F9	2%	3 %	1.5 ml	0.18 %	0.02%	q.s	q.s

III.   RESULTS AND DISCUSSION:
3.1.Preformulation studies
3.1.1. Calibration curve of Leflunomide
The UV absorbance of Leflunomide standard solutions in the range of 2-10 µg/ml of drug in buffer pH 6.4 showed linearityatλmax261nm.
Theabsorbancevaluesandstandardcurvepresented in table no. and figure no..

Table-3: Absorbance data of Leflunomide in Phosphate buffer pH 6.4

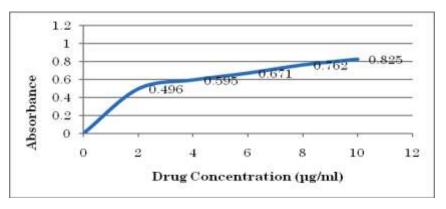| ConcentrationofDrug(µg/ml)	Absorbanceat261nm
0	0
2	0.496
4	0.595
6	0.671
8	0.762
10	0.825

Figure.2: Calibration curve of Leflunomide

3.2Evaluations of hydrogel
3.2.1 Appearance-All the gels were formulated for inflammation were tested for their appearance and colour by visual inspection and the results were noted.The appearance of the formulated hydrogel batches were given in table no.4.

Table 4: Appearance of Hydrogel

S.No	Formulation	Appearance
1	F1	Translucent,Creamish
2	F2	Translucent,Creamish
3	F3	TranslucentCreamish
4	F4	TranslucentCreamish
5	F5	TranslucentCreamish
6	F6	TranslucentCreamish
7	F7	TranslucentCreamish
8	F8	TranslucentCreamish
9	F9	TranslucentCreamish

3.2.2 pH

5 gm of each gel formulation were transferred in 45ml of distilled water and measured it by using the digital pH meter.The pH of the formulated hydrogel batches were given in table no.5.

Table 5: pH of different batches of hydrogel

S.No	Formulation	pH
1	F1	6.57 ± 0.21
2	F2	6.57 ± 0.78
3	F3	6.60 ± 0.48
4	F4	6.64 ± 0.55
5	F5	6.64 ± 0.37
6	F6	6.67 ± 0.15
7	F7	6.71 ± 0.35
8	F8	6.72 ± 0.16
9	F9	6.73 ± 0.54

3.2.3Spreadability

Spreadability was determined by using the apparatus which is made up of a wooden block. It is measured on the basis of slip and drag property of gels. An excess of gel (2.5gm) was placed on the ground slide. The gel was sandwiched between the two slides. 80 gm weight was placed at the top of the slide and gel started spread, time required to cover atleast 7.5 cm of distance was noted. Then spreadability was calculated by using the following formula

$S = M \times L / T$

The spreadibility of different formulation batches were given in table no.6.

Table 6: Spreadability of different batches of hydrogel

S.No	Formulation	Spreadability(gm.cm/sec)
1	F1	75.422 ± 0.121
2	F2	66.149 ± 0.049
3	F3	60.079 ± 0.027
4	F4	58.465 ± 0.153
5	F5	57.457 ± 0.141
6	F6	54.627 ± 0.223
7	F7	48.839 ± 0.068
8	F8	54.627 ± 0.003
9	F9	43.021 ± 0.018

3.2.4 Drug Content
The drug content of different formulation batches were given in table no.7.

Table 7: Drug Content ofdfferent batches of hydrogel

S.No	Formulation	Drugcontent
1	F1	98.1 ±0.3
2	F2	98.3±0.3
3	F3	97.3±0.3
4	F4	99.4±0.3
5	F5	96.6±0.3
6	F6	96.2 ±0.3
7	F7	96.1±0.3
8	F8	95 ±0.3
9	F9	94.2 ±0.3

3.2.5 Viscosity
Viscosity of prepared gel was determined by using Brookfield viscometer. For the viscosity measurement 50g of hydrogel was filled in 100 ml beaker. T shaped spindle was selected (spindle no. 4), then rotated at 6 rpm. The viscosity of different formulated batches were given in the table no.

Table 8: Viscosity of Hydrogel

S.No	Formulation	Viscosity(cps)
1	F1	31724.1 ± 0.32
2	F2	32665.6 ± 0.27
3	F3	32229.2 ± 0.45
4	F4	32187.4 ± 0.23
5	F5	33417.1 ± 0.55
6	F6	33724.1 ± 0.16
7	F7	33865.6 ± 0.44
8	F8	33982.2 ± 0.98
9	F9	34417.5 ± 0.54

3.2.6 Drug Release
In vitro Permeation In vitro permeation was carried out on jacketed vertical glass Franz diffusion cells (with the 15 ml of acceptor compartment volume) using dialysis membrane (mol. wt. 12000-14000) with effective surface area of 3.14 cm2.

The mounting of the membrane was done by placing circular rubber above the permeation barrier and in between the acceptor and donor compartment and supported with clips at the rim of the compartments to avoid leakage of the test sample. The temperature of the receiver compartment having phosphate buffer solution maintained at 37 ± 1°C under continuous stirring with teflon coated magnetic bar at constant rate, in such a way that the dialysis membrane surface just flushes the phosphate buffer solution. Membrane was allowed to stabilize in both the compartment for 15 minutes with continuous stirring on magnetic stirrer. After 15 min; stabilized nanosponges (equivalent of 10 mg griseofulvin) have added in donor compartment. Samples are withdrawn at predetermined time intervals from sampling port of acceptor compartment and replaced with the fresh buffer media. The aliquots were diluted suitably and analyzed using validated UV method and concentration of drug permeated was determined. Studies have carried out for 24 hr (every hr till 12 hrs then at 24 hrs directly).The drug release of hydrogel were given in table no**.**

Table 9: Drug Release of Hydrogel

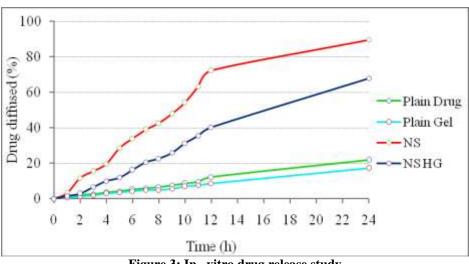| S.No	Formulation	Drug release(6hr.)
1	F1	99%
2	F2	97%
3	F3	98%
4	F4	99%
5	F5	97%
6	F6	97%
7	F7	96%
8	F8	97%
9	F9	93%

Figure.3: In –vitro drug release study

IV.   SUMMARY AND CONCLUSION:

The hydrogel for topical application was formulated using Leflunomide and Carbopol 940 and evaluation tests were performed. Proper selection of Polymers and their proportions is a prerequisite for designing and developing a transdermal drug delivery system. The formulated gels showed good homogeneity, good stability and better drug release rates. The study has demonstrated various aspects and from the results obtained, following conclusions are drawn. The preformulation parameters i.e. melting point, and solubility of the drug were evaluated and satisfactory and all the values obtained comply within pharmacopoeial standards.

There was no possible interaction between pure drug Leflunomide and excipients used in the preparation of Leflunomide hydrogel. Absorbance maximum of pure drug Leflunomide was determined using UV- spectroscopy and drug showed maximum absorbance at 261 nm.

Leflunomide hydrogels were evaluated for pH, spreadability, viscosity. The values obtained were found to be Satisfactory and complies with standard range. In-vitro drug release studies were carried out for 12 hours using Franz diffusion cell. The results revealed that Leflunomide hydrogel prolonged the drug release for prolonged period of time and was significantly prolonged than plain gel.

Formulation F4 was found to have better results in all the Evaluation parameters. In formulation F4 the noted thing was the drug was highly in nature hence best suited for the preparation of topical Leflunomide hydrogel formulation. This formulation was the best formulation for the topical administration and to easy penetration through the skin. This is concluded that the hydrogel has been proved to be more beneficial, much convenient to the patients and shows less side effects with total care of patient. It is clear from above discussion that F4 were best among all the prepared formulations.

Here, the stability studies were performed and checked that the formulations showed no signs of instability.

REFERENCES:

[1]. S.P. Vyas, R.K. Khar. Targeted and Controlled Drug Delivery Novel Carrier Systems: Molecular Basis of Targeted DrugDelivery.CBSPublishersandDistribut ors;NewDelhi, 2012;38-40.

[2]. N.K.Jain.Introductiontonoveldrugdelivery system.VallabhPrakashan,Delhi.2017.

[3]. TripathiKD.Essentialsofmedicalpharmacol ogy.NewDelhi:JaypeeBrothersMedicalPub lishers(P)Ltd.2003;5:261-270.

[4]. Chien YW. Concepts and system design for rate controlled drug delivery in novel drug delivery systems. New York: MarcelDekkerInc., 1992;2:1-42.

[5]. ShastrulagariShivani and Kranthi Kumar Poladi. Nanosponges a novel emerging drug delivery system, International journalofpharmaceutical scienceandresearch.Vol. 6(2):529-540.

[6]. Rahul SampatTambe, Pratik WamanBattase, et al. Nanosponges as a targeted drug delivery system. American journal ofpharmatechresearch, 2015;5(1).

[7]. Priyanka D, Sindhu S. Design and development of Ibuprofen loaded nanosponges. International journal of chemtech research,2018;11.

[8]. Dr. PratimaSriniwas, Sreeja K, et.al. Formulation and Evaluation of Voriconazole Loaded Nanosponges for Oral and TopicalDelivery.Int. J.DrugDev. &Res.,2013;5(1):55-69.

[9]. G. Jilsha and VidhyaVishwanad. Nanosponges loaded hydrogel of cephalexin for topical drug delivery. International journalofpharmaceutical scienceandresearch.IJPSR, 2015;Vol. 6(7):2781-2789.

[10]. Renuka S., Kamla P., Polymeric Nanosponges as an alternative carrier for improved retention of econazole nitrate onto theskinthroughtopical Hydrogelformulation. PharmaceuticalDevelopmentandTechnolo gy,2011;16(4):367–376

[11]. "Production of Lactic Acid from Cheese Whey by Immobilized Cell Reactor of Strain Lactococcuslactissubsplactis SP1Adsorbed OntoPozzolanaBed",InternationalJournalo fAppliedandNaturalSciences(IJANS),Vol. 3,Issue2,pp.21-34

[12]. H.V. Gangadharappa, M. Sarat Chandra Prasad et.al. Formulation, in vitro and in vivo evaluation of celecoxibnanospongeshydrogelfortopicala pplication. JournalofDrugDeliveryScienceandTechnol ogy. 2017;41:488-501.

[13]. Jyotipandey, amandeepsinghet.al. Formulation and evaluation of nanosponges based controlled release gel preparation ofketoconazole.International Journalof PharmacyandPharmaceutical Research. 2018;12(3):367-382.

[14]. "Synthesis and Characterization of In2s3 Nanoparticles by Electrochemical Method: Evaluation of its Performance in Photo-AssistedDegradationofIndigoCarmineDye, AntibacterialandAntimitoticActivityStudie s",InternationalJournalofNanotechnologya ndApplication(IJNA),Vol. 7, Issue3, pp. 1-16

[15]. Dr. P. Srinivas, K. Sreeja. Research on formulation and evaluation of vericonazole loaded nanosponges for oral or topicaldrugdelivery.International Journal ofDrugDevelopment andResearch.2012;5(1):55-69.

[16]. "EnhancedTargeting ofPolyethylenimine(PEI)-BasedTumor-SpecificAntisense Nano-Oligonucleotides(TANOL)inCancer Therapy",TJPRC:JournalofMedicineand PharmaceuticalScience(TJPRC:JMPS),Vo l.2,Issue2,pp.1-8

[17]. Hanietal.Preparationandevaluationofclotri mazoleloaded nanospongesgel for vaginaldrugdelivery.Internationaljournalof pharmaceutical scienceandresearch, 2014;Vol. 5(1):220-227.

[18]. M.Rao,R.C.Bhingole.Nanosponge-basedpediatric-controlledreleasedrysuspensionofGabapen tinforreconstitution.DrugDevelopment andIndustrialPharmacy.2015; 41(12):1-8.