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SELECTION-EVALUATION OF DIFFERENT EXCIPIENTS FOR PREPARATION OF LEFLUNOMIDE TABLETS

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ABSTRACT

Disease Modifying Anti Rheumatic Drug(DMARDs) reduces the rate of damage to the bone and cartilage and prevent bone and joint damage from occurring secondary to the uncontrolled inflammation. DMARDs have been found to produce durable remission and delay or half disease progression. Chemically synthesize DMARDs are Azathioprine, Cyclosporine, Hydroxychoroquine, d-penicillamine, Leflunomide. Leflunomide is recently introduced immonumodulator inhabits proliferation of activated lymphocytes in patients with Rheumatoid arthritis. Arthritis symptoms are suppressed and radiological progression of disease is retarded. The study was under taken with an aim of Selection-Evaluation of Excipients (Binders) for preparation of Leflunomide tablets will be carried out. Preformulation study was done on pure drugs and granules and results directed for further course of formulation. Based on preformulation studies different batches of Lefiunomide were formulated using different excipients. Granules were evaluated for Loss of drying, Angle of repose, Bulk density, Tapped density, Carrs index and Hausner"s Ratio. Tablets were tested for weight variation, thickness, hardness, disintegration time. In-vitro drug release studies as per specifications. The formulation that has been found to posses ideal characteristics required for Leflunomide 10mg, 20mg Tablets, so it was concluded as the final formula for Leflunomide 10mg and 20mg tablets are 150mg, 200mg respectively made by co-precipitation of drug with Hydroxypropylmethylcellulose K100M, Polyvinylpyrrolidine K30, Gelatin, Guar gum. The drug release profile of Leflunomide compared with the market sample. From the studied it was concluded that preparation of Leflunomide tablets containing Lactose monohydrate, Starch, Magnesium streate, Dry starch taken as ideal or optimized preparation of tablets.

KEYWORDS: Azathioprine, Cyclosporine, Hydroxychoroquine, d-penicillamine.

Drug Profile of Lufifiunomide

Brand name : Arava.

Synonyms : HWA486

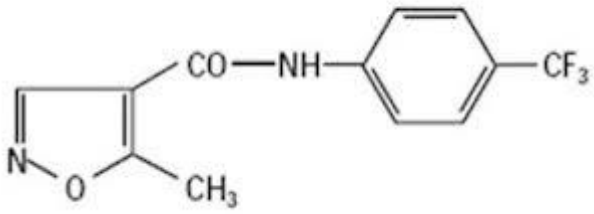
Chemical/ IUPAC name: N – (- 4 – trifluoromethyl Phenyl) – 5 – methylisoxazole – 4 – Carboxamide

Description

Appearance : Leflunomide is a white or almost white powder.

Solubility : Leflunomide is freely soluble in acetone, in Methanol in alcohol, in 2 – propanol, in ethyl acetate, in acetonitrile and in chloroform. Practically in soluble in water.

Chemical structure



Molecular formula : C_{12} H_9 F_3 N_2 O_2

Melting point : 165-166oC

Molecular weight : 270.2

Purity : move than 99%

Half life : Approximately 2week

Storage : Store in well closed light resistant container

PHARMACOLOGY

Leflunomide is an isoxazole derivative. It exact mode of action is unclear. The active metabolite of l (A777726) binds to dihydro- orotate dehydrogenase, an enzyme involved in the synthesis of pyrimidines. As consequence there is reduction in uridine tri-phosphate levels and pyrimidine synthesis by Lymphocytes and other rapidly dividing cells. The action of the enzyme triosinekinase is also reduced. These effect result in changes in DNA and RNA synthesis and T – and B – cell proliferation in addition to suppression of immunoglobulin in

production and inference with cell adhesion. There is also suggestion that its anti-inflammatory activity is due to its ability to inhibit histamine release and cycloxygenase-2 in-vitro.

DRUG INTERACTION

Cholestyramine significantly reduces the plasma concentration of leflunomide metabolite and the two should not be co-administered (unless as part of the washout procedure) Administration with other DMARD's or other hepator haimatotoxic drugs may inercase the rise of toxicity it switching from leflunomide to another DMARD a washout procedure should be considered. Alcohol should be avoided with Leflunomide. Causion should be used if given concomitantly with phenytoin, warfarin, tobutamide or rifampicin.

Patient should not be given live vaccination whilst taking leflunomide for some time after wards to its long half life

ADVERSE EFFECT

The most common effect with leflunomide are gastrointestinal symptom's (excluding diarrhea, nausea, vomiting, abdominal pain and oral ulceration), allergic reaction (eg- – rash, pruritus and rarely anaphylaxis) Anorexia, weight loss, heat ache, hypertension and dizziness has been reported.

Dose : 10mg, 20mg/day,

It is not to be used in children and Pregnant / Lactating women.

Use : leflunomide usually and in the treatment of rheumatoid arthritis.

Pharmacokinetic and Pharmacodynamic

Bioavailability : 80% bioavailability after oral administration (Gonzalez et at 1994)

Protein binding : Leflunomide is highly bound of plasma protein (>99%)

Volume of distribution: 25L

Elimination half life : approximately 2 week

Metabolism : Leflunomide is metabolized to major metabolite A771726, a malanonitrilamide, plasma concentration of A771726 appears linear across the different dosages range (in 5 – 25 mg daily)

Elimination : Biliary, renal.

5. MATERIAL AND METHODS

Table 5.1 List Of Ingredients Used

SL.No	*Ingredients*	*Company name*
1	Leflunomide	Aventis pharma limited, bmfay
2	HPMC K100M	Stadmed Pvt. Ltd, Kolkata
3	Magnesium Stearate	Merck Specialities private Limited
4	Lactose monohydrates	Merck specialties Private Limited
5	Guar gum	Merck specialties Private Limited
6	Polyvinyl pyrrolidone K30	Merck specialties Private Limited
7	Starch	Merck specialties Private Limited
8	Acetone	Merck specialties Private Limited
9	Athanol	Merck specialties Private Limited
10	Sodium hydroxide	Merck specialties Private Limited

5.2 List of Instruments Used

SI. NO.	**Instruments**	**Company name**
1	Electronic balance	Denver instruments, Germany
2	Digital Ph meter	Sartorius PB-11, USA
3	Tablet compression machine	Hindustan machineries, Kolkata
4	Sieves (18mesh)	Testing instruments, kolkata
5	UV- Visible Spectrophotometer	Shimadzu- 1700, Japan
6	Hot air oven	Testing instruments, Kolkota
7	Dissolution apparatus	Digital dissolution apparatus, Testing instruments, kolkata
8	Digital friability test apparatus	
9	Monsanto hardness tester	Testing instruments, kolkata
10	Disintegration apparatus	Testing instruments, kolkata
11	FTIR Spectrometer	Perkin Elmer, spectrum-100,UK

5.3. SPECTROPHOTMERIC METHOD FOR LEFLUNOMIDE

The UV spectrophotometry method was developed for the analysis of the drug using double beam shimadzu 1700 spectrophotometer.

A) Determinatinatio maximum wavelenth of Leflunomide in Phospate Buffer(Ph 7.4)

Leflunomide was accurately weighed and dissolved in 10m. Mol phosphate buffer of pH 7.4 to prepare a stock solution of 1mg/ml.the stock solution was futher diluted to 10mcg/ml with dilutent (10m.Mol phosphate buffer of Ph 7.4).then the diluted solution was scanned for maximum absorbance in UV double beam spectrophotometer (shimandzu1700) in the range of 190 to 400 nm, using 10m.Mol phosphate buffer of pH 7.4 as blank. The λmax of the was found to be 250 nm.

B). PREPARATION OF STANDARD CURVE OF LEFLUNOMIDE LEFLUNOMIDE

100 mg of Leflunomide was accurately weighed and dissolved in 100 ml of m.Mol phosphate buffer of Ph 7.4 to prepare stock solution. The adequate amount of stock solution was further diluted with 10m. mol phosphate buffer of ph 7.4 to get 1ug to 10ug of drug per ml of the final solution. Then the absorbance was measured in a UV spectrophotometer at 250nm against 10m.mol phosphate buffer of Ph 7.4 as blank. The results obtained were as shown and the average absorbance Vs conc. Curve (standard curve) plotted was shown in figure.

5.4. PREFORMULATION STUDIES

5.4.1. Drug – Polymers Interaction study

A). Demonstrated Scanning Chromotography(DSC) of the Leflunomide and Excipients to study the interaction between components.

In this technique the difference in energy inputs into a substance and reference material is measured as a function of temperature as the specimens are subjected to controlled temperature program. The samples were prepared by physical mixture of drug and excipients (1:1) using a clean dried glass mortar and pestle. Samples (3mg) were accurately weighed in aluminum pan and hermetically sealed in aluminum lid. Thermograms were obtained using Parkin Elmer, Jada (Pyris 6.0), USA, instrument, heating at a constant rate of 10° C/min, over a temperature range of 50 – 200°C. to maintain on inert atmosphere nitrogen gas was purged at a rate of 20 ml/min.

B) Fourier Transform Infared(FTIR) spectroscopy of the Leflunomide and Excipients to study the interaction between components.

There are two types of fundamental vibrations in a molecule (Dyer, 1984).

Stretching, in which the distance between two atoms increased or decrease, but atoms remain to in same bond axis.

Bending in which the position the atom changes relative to the original bond axis.

The various stretching and bending vibrations of bond occur at certain frequencies. When infrared light of same frequency is incident on the molecule, energy is absorbed and the amplitude of the vibration increased. Most groups such as C – H, C = O and C = N give rise to infrared absorption band which ultimately characterizes a compound. For a non – linear molecule, which contains ‘n’, atoms have 3n-6 possible fundamental absorption bands.

Sometimes an additional (non-fundamental) absorption band occurs because of the presence of overtones.

The dried powder sample of 100 mg was taken with 10mg of KBr and grounded together to make it fine. They were scanned over a wave number range of 4000 cm^{-1} to 400 cm^{-1} using FTIR (Perin Elmer, USA, Model: Spectrum one, Version A).

5.4.2. Scanning Electron Microscopy (SEM)

Conventional light microscopes use as series of glass lenses to bend light waves and create a magnitude image. The Scanning Electron Microscope creates the magnified image by using electrons instead of light waves. The SEM shows very details 3- dimensional images at much higher magnification that is possible with a light microscope. The image created without light waves black and white.

Samples have to prepared carefully to withstand the vacuum inside the microscope. Our SEM samples are coated with a very thin layer of gold by a machine called a sputter coater. The sample is placed inside the microscope's vacuum column through an air-tight door. After the air is pumped out of the column, and electron gun (at the top) emits a beam of high energy electrons. This beam travels downwards through a series of magnetic lenses designed to focus the electrons to a very fine spot. Near the bottom, a set of scanning coils moves the focused beam back and forth across the specimen, row by row. As the electron beam hits each spot on the sample, secondary electrons are knocked loose from its surface. A dectector counts electrons and seds the signals to an amplifer.

The final image is build up from the number of electrons emitted from each spot on the sample. The Scanning Electon Microscope is revealing new levels of detais and complexity in the amazing world of miniature structures.

5.5. METHOD OF PREPARATION OF LEFLUNOMIDE TABLET

Tablets containing 20 mg of Leflunomide were prepared by wet granulation method. In the wet granulation method the drug Leflunomide were, polymer HPMC K100M, Dry starch, guar gum or PVP K30, lactose monohydrates, gelatin were accurately weighed (as mentioned in table) and mixed homogeneously in geometrical proportion. Then prepared sufficient amount of binder (10% HPMC K100M dissolved in 20ml of hot water, stirred and cooled, 10% of guar gum dissolved in 20ml of hot water, stirred and cooled, 10% starch dissolved in

20ml of hot water stirred cooled, 10% PVP K30 dissolved in 20ml cold water stirred well and10% Gelatin dissolved in 20ml of hot water, stirred well) and added to the mixture of drug, polymer, dry starch and lactose, then mixed these to get a cohesive mass and granulated though sieve no.16. wet granules were dried in a hot air oven at 40oC for 4 hrs. The dried granules were passed through sieve No.18 and blended with magnesium stearate. The homogeneous blend was then compressed into tablets using 8 mm circular flat punches in 10 station compression machine.

5.6 GENERAL DESIGN OF EXPERIMENTS

Design of experiments

The purpose of the "Design of Experiments" is to ensure that the experimenter Obtains data relevant to his hypothesis as comical a way as possible. The economic aspect of experimentation cannot be emphasized too strongly. The experimenter is always in the position of being able too, or wishing to, spent only a certain amount of time, labour, money etc, on his investigation, and it is certain that there are were and efficient way of using these resources. However every statement drawn from experimental data is subject to so me error, whose probability can be made with the help of mathematical statistics.

5.6.1 FOR MULATION STUDIES

TABLE 5.3. Composition all trial batch of formulation of leflunomide tablet.

Ingredients	F1(mg)	F2(mg)	F3(mg)	F4(mg)	F5(mg)
Leflunomide	100	100	100	100	100
Lactose mono hydrate	1200	1200	1200	1200	1200
Dry starch	0.7	0.7	0.7	0.7	0.7
Mg. stearate	0.2	0.2	0.2	0.2	0.2
Bender	qs	qs	qs	qs	qs
Bending agents	PVP10%	Guar gum10%	Starch paste 10%	H PMC K100M10%	Gelatin10%

*Every batch is estimated at least performing 5 batches of products.

Abbreviation:

HPMC : Hydroxy propyl methyl cellulose

PVP : Polyvinyl pyrrolidenone

Mg.stearate : Magnesium stearate

Wt : weight

Tablet- enlists the composition of different trial formulation prepared using varying amount of hydorxy propylmethyl cellulous (HPMC) as release controlling polymer and polyvinyl pyrrolidinone (PVPK30), guar gum, starch paste,gelatin as binder along with fixed quantity of magnesium stearate as lubricant and lactose monohydrate as filler or diluents.

5.7. Experimental Evaluations

Granulation is the key process in the production of table dosage form involving the sustained release of a drug from different type particles. Physical properties of such as specific surface area, shape hardness, surface characteristice and size can significantly affect the rate of dissolution of drug.

Micromeritic properties

5.7.1 Angle of Repose

Angle of repose of granules was measured by fixed funnel standing method. The accurately weighed granules were taken in a funnel, the height of the funnel was adjusted in such a way that the tip of the funnel just touch the apex of the heap of the granules. The granules were allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured. Angle of repose was calculated using the following equation (cooper and Gunn, 1986):

$\theta = \tan^{-1} h/r$ (4.1)

Where

θ = angle of repose,

r = the radius of the base the pile,

h = height of the pile.

5.7.2 Bulk density of powder blend for Leflunomide tablet

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. A quantity of 2 gm of powder from each trial formulation, previously lightly shaken to break any agglomerates formed, was introduced into a 10-ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from the height of 2.5 cm at 2-second intervals. The tapping was continued until no further change in volume was noted. LBD and TBD were calculated using the following formulas (shah et al., 1997)

TBD= weight of the powder/ tapped volume of the packing---------- (4.2)

LBD= weight of the powder / volume of the packing ---------- (4.3)

5.7.3 Compressibility index of powder blend for Leflunomide tablet

The **Carr's index (CI)** is an indication of the compressibility of a powder. It is calculated by the formula, where is the freely settled volume of a given mass of powder, and V_T is the tapped volume of the same mass of powder.

The Carr index is frequently used in pharmaceutics as an indication of the flow ability of a powder. A Carr index greater then 25 is considered to be an indication of poor flow ability, and below 15, of good flow ability [mark Gibson et al 2001]. But Carr index, 20 to 40 shows reasonable flow property.

Carr's index (%) = $[(1-V_T/V_B) \times 100]$ ----------4.4

It also expressed as,

Carr's index (%) = $[(1-\rho_B/\rho_T) \times 100]$ ----------4.5

Were, ρ_B is loose bulk density and ρ_T is tapped bulk density.

5.7.4 Hausner ratio of powder blend for Leflunomide tablet

Hausner ratio was determined by using the ρ_B is loose bulk density and pt is tapped bulk density. Hausner ratio is greater than 1.25 is considered to be an indication of poor flow ability.

Hausner ratio = ρ_T / ρ_B ---------- (4.6)

5.8. Evaluation of Leflunomide Tablets

5.8.1 Measurement of thickness of Leflunomide tablet

The thickness of tablet was measured by using screw gauge. The thickness variation should be with in ±5% limit. All the thickness was measured by screw gauge. In this screw gauge, main scale reading (MSR) = 2mm

Circular scale reading (CSR) = 64mm

Least count (LC) = 0.01

Error = 6 x 0.01 =0.06

Total thickness = [{MSR + (CSR x LC)} +0.06] (4.6)

5.8.2 Tablet Hardness (lee et al., 1999, Lachman et al., 1987)

In this experiment Monsanto hardness tester determined hardness of tablets. It has a graduated scale, which gives the reading in kg / sq cm. the tablet to be tested was placed

between the spindle and anvil. The desired pressure needed to hold the tablet in position was moved so that the indicator was fixed zero. The pressure was then applied till the tablet broken. The reading was noted, which indicate the pressure which was needed to break the tablet.

5.8.3 Measurement of tablet friability of Leflunomide tablet

The friability of tablets was determined by Roche friabilator. This device subjected a number of tablets to combined effects of abrasion and shock by utilizing a plastic chamber that revolved at 25 rpm, dropping the tablets a distance of six inches with each revolution. 20 tablets were weighed and placed in the friabilator; it was operated for 100 revolutions. The tablets were then dusted and reweighed.

For the calculation,

%weight loss = initial weight of tablets (w^1) – final weight (w^2) / w1 x 100 ---(4.8) conventional compressed tablets loss less then 0.5 to 1.0 % of their weight are generally acceptable.

5.8.4 Weight variation test of Leflunomide tablets

For each batch 20 tablets were selected randomly and their average weight was determined. Weight of the individual tablet was also determined. The tablets meet the weight variation test if not more than of the individual weights deviate from the average weight by more than the percentage shown in table 4.4 below and none deviates by more than twice that percentage.

Table 5.4. official specification of weight variation

Average weight of tablet	Percentage deviation
80 mg or less	10
More than 80 mg but less than 250 mg	7.5
250 mg or more	5

5.8.5. Content uniformity test of Leflunomide tablets

The Weight variation test is clearly not sufficient to assure uniform potency of tablets of moderate or low dose drugs, in which excipients make up the bulk of the tablet weight. To assure uniform potency for tablets of low dose drugs, a content uniformity test is applied. 30 tablets are randomly selected for sample, and at least 10 tablets are assayed individually spectrophotomerically. Nine of the 10 tablets must contain not less than 85% or more than

115%of the labeled drug content. The 10th tablet may not contain not less than 75% or more then 125% of the labeled drug content. If these conditions are not met, the tablets remaining from the 30 must be assayed individually, and none may fall outside of 85 to 115%.

The content uniformity test of Leflunomide tablets was performed (as per IP, 1996) by following way

Weighed and powered 20 tablets, weighed accurately a quantity of the powder equivalent to 0.5 g of leflunomide extracted with 60 ml of acetone for 15 minutes and filter. Washed the residue with three quantities, each of 10 ml, of acetone and gently evaporated the filtrate just to dryness in a current air. Dissolved the residue in 100 ml of ethanol (95%), previously neutralized to phenolphthalein solution, and titrated with O.IM sodium hydroxide using phenolphthalein solution as indicator.

5.8.6 Disintegration test of Leflunomide tablets

The drug release process from tablets often includes a step at which the tablets disintegrate into smaller fragments. In our experiment the disintegration test was done by using USP disintegration test apparatus (excel, India).

To test the disintegration test three tablets of each batch was placed in 3glass tube and the basket rack was positioned in the beaker containing 900 ml of phosphate buffer pH 7.4 solution maintained at 37 ±2˚c. The disintegration time was recorded using mobile phone stop watch.

5.8.7. Dissolution studies of Leflunomide tablets

The drug delivery from the dosage form is studies by using the in vitro release test. The purpose of an in vitro release study is to provide a fast, easily performed and inexpensive method that correlates with the performance of a dosage form in human subjects. Numerous advantages can be exemplified on treating in vitro data in kinetic fusion.

In vitro release profile of Leflunomide from the preparations was examined in pH 7.4, 10 m.Mol phosphate buffer from 0 to 2hrs using USP-II dissolution rate test apparatus (rotating paddle type) at 100 rpm. One tablet accurately weighed, was placed in the 900 ml of dissolution medium and maintained at 37 ± 0.5˚c. 1ml aliquot of was withdrawn from dissolution media periodically at intervals of one hr by manual sampling, and same volume of fresh medium (10 m. mol phosphate buffer pH 7.4) was replenished immediately to maintain

the sink condition automatically. Withdrawn samples were further diluted properly with fresh medium. The diluted samples were analyzed spectrophotometrically at the wavelength of maximum absorbance (λ max) 250 nm. Concentration of drug in solution was, than determined from the calibration curve and cumulative percent release was calculated.

5.9. Study of release kinetics on in vitro release profile of Leflunomide tablets.

Different kinetic equation (zero order, first order, higuchi's equation) were applied to interpret the release rate from matrix system using linear regression. From the release kinetic the mechanism of drug release may also be known.

Zero- order release kinetics

According to this model, under stander condition of temperature and agitation the dissolution medium, the dissolution rate model can be described by the equation:

dq/ dt = ko---- (4.9)

Or, in integrated form,

q= kot -------- (4.10)

Where, q= cumulative % release,

ko = zero order release rate constant

t = time

A plot of q vs t gives a straight line staring from the origin.

First order release kinetics (Noyes Whitney's equation)

According to Noyes Whitney, under standard condition of agitation and temperature, the dissolution rate process for solids can be described by the equation: dq/dt = k□ (Cs-Ct) (4.11)

Under sink conditions, i.e., when Ct <0.15cs the equation becomes,

Dq/dt=k□Cs (4.12)

Or, in an integrated form,

In qt = Inqo+k□t (4.13)

Where, q=amount of drug released per unit surface area,

k1= first order release rate constant,

Qo = initial amount

Cs = saturation solubility

Ct = concentration at time t

A plot of log cumulative% release vs time gives a straight line with a negative slope.

Higuchi square root equation

For coated type dosage form, the dissolution medium enters the dosage form in order to the drug to be released and diffused into the solution. In such cases, often the dissolution follows the equation proposed by higuchi:

Q = [D€ (2a -€Cs) CST / τ] 0.5 (4.14)

Or Q= k нgt 0.5 (4.15)

Or, Mт/m∞= Kнgt1/2 (4.16)

Where, Q = amount of drug released per unit surface area

€ = Porosity of the matrix

τ = Tortuosity of the matrix

Kнg = Higuchi release rate constant

Cs = saturation of the drug in the surrounding liquid

A = concentration of the drug in the matrix, t = time.

A plot of cumulative % release v/s square root of the time is plotted to represent a Higuchi release kinetics (higuchi et al., 1963)

Korsmeyer- peppas model (power law) (koresmeyer et al., 1983)

The power law describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in following equation:

$Mt/m\infty = k\ t^n$ (4.17)

Log [mt/m∞] = log k+n log t (4.18)

Where, Mt and m∞ are cumulative amounts of drug release at time t and infinite time. K is the constant, and n is a diffusional release exponent indicative of the mechanism of drug release for dissolution.

A plot of log [Mt/M∞] Vs log t will be liner with slope of n and intercept gives the value of log k.

Antilog of log k gives the value of k. Peppas used the n value in order to characterize different release mechanisms as shown in result and discussion.

Table 5.5. Diffusion exponent and solute release mechanism.

	Fickian diffusion
0.45<n<0.89	Anomalous(non firkin)diffusion
0.89	Case –II transport
n>0.89	Super case- II transport

5.10. Accelerated stability studies of prepared Leflunomide tablets

The tablets from the selected Bach are kept at 4.5.c temperature and 7.5% relative humidity for a period of 3 months for accelerated stability study. The selected batches are kept in the humidity chamber (Thermo lab. India) maintaining the required temperature and humidity. After 3 months samples are taken for in vitro dissolution analysis.

6. RESULTS AND DISCUSSIONS

6.1 Determination of λmax of Leflunomode

Determination of λmax of Leflunomide chapter -4 (section -5.3.A.) scan report is shown in figure -6.1. It was found that leflunomide had maximum UV absorption at 250 nm in phosphate buffer (pH 7.4) respectively.

Figure 6.1. λmax of leflunomide in phosphate buffer (pH7.4).

6.2. Preparation of standard curve of Leflunomide

Standard curve of Leflunomide was prepared by taking different known serial concentration of Leflunomide solution and measuring the absorbance as per described in the chapter -4. The result in given below in the table no -6.2. The carve is given in the following page.

Table 6.2. Standard Curve of Leflunomide in phosphate buffer(pH7.4).

Serial No.	Concentration (mcg/ml)	Absorbance
1	5	0.143
2	10	0.342
3	15	0.5961
4	20	0.787
5	25	0.9064

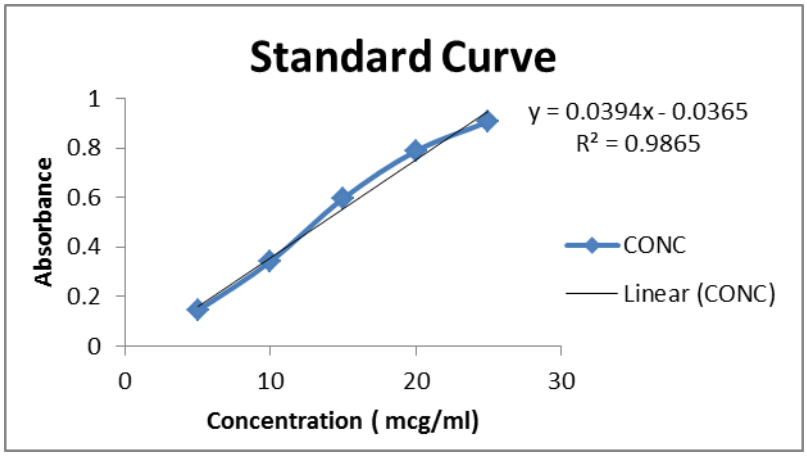


Figure 6.2. Standard curve for Leflunomide in phosphate buffer (pH 7.4) solution.

The standard carve figure -6.2 drawn as per table -6.2, has shown acceptable regression value (**R^2 =0.986**).

6.3. Compatibility study of Leflunomide with Excipients.

A) DSC for compatibility study of Leflunomide

DSC provides information about the physical properties of samples as crystalline or amorphous nature and demonstrates a possible interaction between drug and polymer information. According to the thermograms, Leflunomide presented a sharp endothermic peak at 166.69˚C and Leflunomide, Guar gum and HPMC presented a sharp peak at 165.56˚C corresponding to the melting points of the drug in the amorphous forms. The was detected for the interaction study further accomplices with FTIR study. The thermo gram as shown figure -6.3(a,b&c).

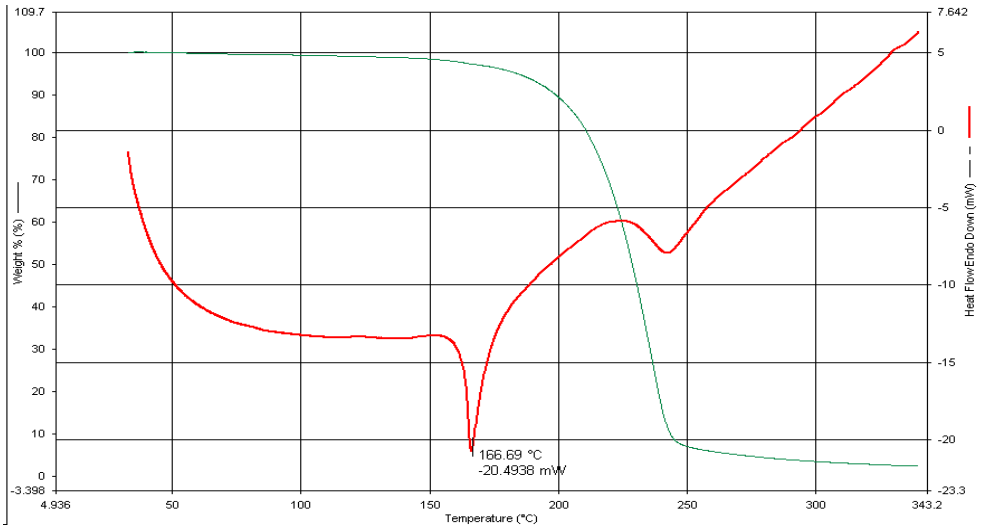


Figure 6.3(a) DSC Spectra of Leflunomide.

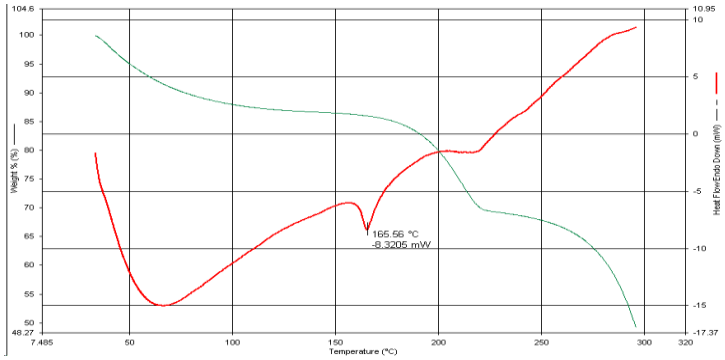


Figure 6.3(b) DSC Spectra of Leflunomide and Guar gum.

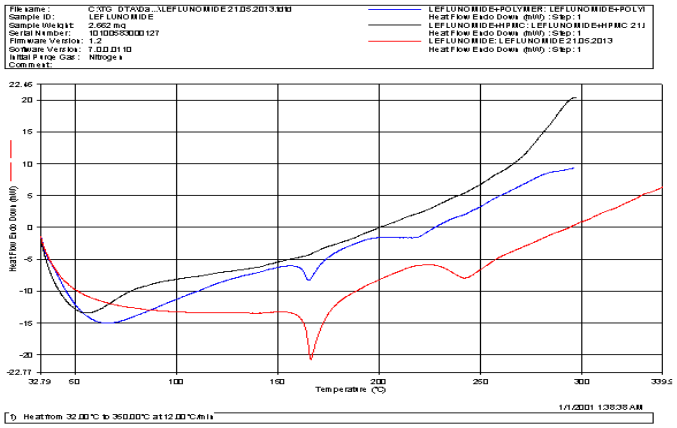


Figure 6.3(c) DSC Spectra of Leflunomide, Guar gum and HPMC.

B) FTIR spectroscopy for compatibility study of Leflunomide

The drug polymer compatibility was ascertained by subjecting the drug with HPMC K100M, Guar gum to Infrared Spectrophotometer study. The spectrum is given in the figure--. It has been observed that the absorption bend of both pure drug and drug with polymer mixture was same.So it was concluded that no such interaction between the active medicament and polymer was it was concluded that no such interaction between the active medicament and polymer.

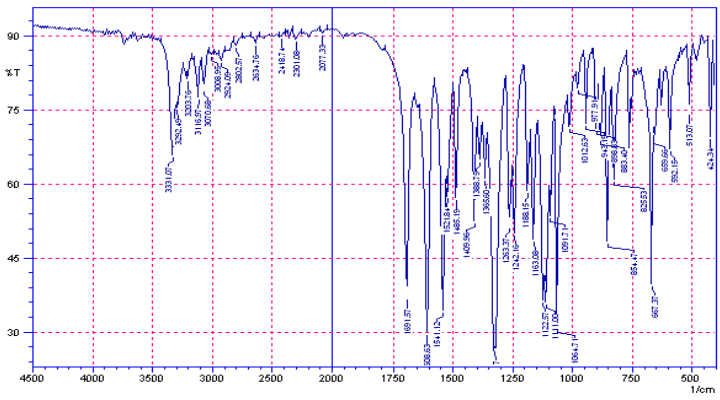


Figure 6.4(a) FTIR Spectra of Leflunomide.

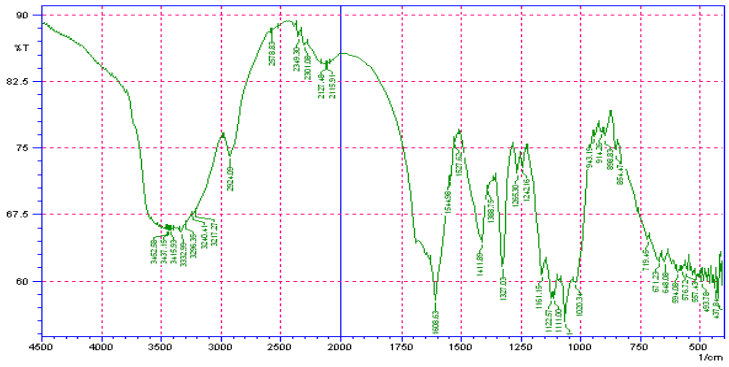


Figure 6.4(b) FTIR Spectra of Leflunomide and HPMC.

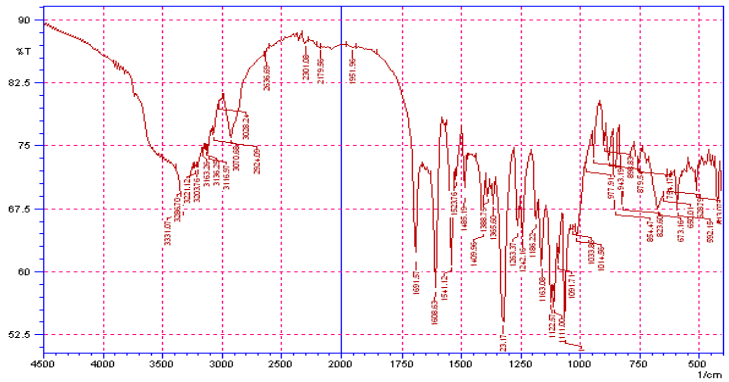


Figure 6.4(c) FTIR Spectra of Leflunomide Guar gum.

6.4. Experimental Evaluations

6.4.1. Evaluation of Micromeritic properties of Leflunomide granules

To maintain nature of granules under control the physical properties were evaluated. To prevent the adhesion of the granules, it kept in dry trays and moisture content was found to be 22° - 32°, it show that the flow ability was good and further to describe the granules the bulk density, tapped density, Carr's index, It answer ratio were calculated (table -6.4.).

Granules of different formulation were evaluated for angle of repose. The results of angle of repose (<30°) indicates good flow properties of granules (Martin, 2001). It was further supported by compressibility (or Carr's index) values given in table -6.4. Carr's index value up to 15% results in good to excellent flow properties (Aulton satisfactory flow properties and compressibility.

Table 6.3. Micromeritic properties of Leflunomide granules.

Batch code	Angle of repose (degree) mean= ±S.D	Bulk density (gm/ml) mean= ±S.D	Tapped Density (gm/ml) mean= ±S.D	Carr's Index(%)	Hauser ratio mean= ±S.D
F1	29.23±0.5	0.46±0.03	0.53±0.04	13.6±1.32	1.15±0.06
F2	27.44±0.06	0.45±0.08	0.56±0.07	19.69±1.87	1.24±0.02
F3	29.26±0.03	0.47±0.04	0.57±0.04	17.54±1.21	1.21±0.02
F4	28.71±0.4	0.47±0.02	0.55±0.05	15.34±1.62	1.17±0.04
F5	29.57±0.03	0.42±0.02	0.51±0.04	18.73±2.21	1.21±0.03

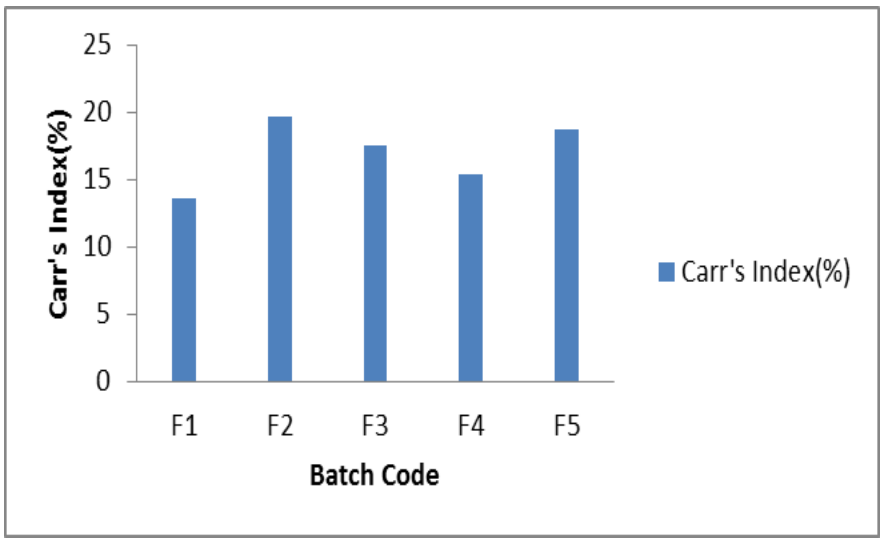


Figure 6.5. Micromeritic properties of Leflunomide granules.

6.4.2. Thickness of Luflunomide tables.

The thickness was measured by using slide calipers. The thickness should within ± 5% variation of standard value. All thickness was measured by venire caliper (Asahi, India).

Table 6.4.Results of thickness measurement of Leflunomide tablets.

Batch code	Average thickness (mm)	S.D (n=5)
F1	3.57	0.01
F2	3.52	0.02
F3	3.70	0.05
F4	3.66	0.02
F5	3.85	0.02

Average thickness of each batch of Leflunomide tablets were measured and the results of each set are within ± 5% deviation range. These results are satisfactory.

6.4.3. Hardness of Leflunomide tablets

Hardness of tablets is **3.8 to7.1kg/sq.cm** in all batches. Hardness of tablets in all force required to break a tablet in the diametric compression test. This can affect the drug dissolution and consequently the drug release. Hardness of a tablet depends up on compression force, proportion of binder etc.

Tablet 6.5. Results of Hardness of Leflunomide tablets.

Batch code	Average Hardness (kg/sq.cm)	S.D (n= 3)
F1	6.8	0.1
F2	7.61	0.15
F3	6.9	0.07
F4	7.2	0.05
F5	7.1	0.05

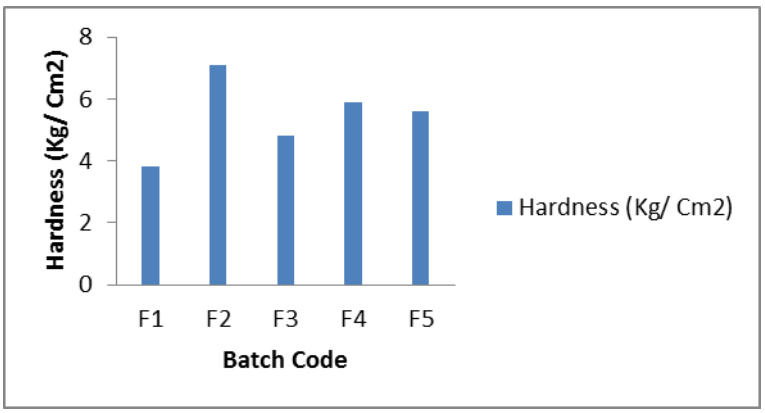


Figure 6.6. Hardness of Leflunomide tablet.

6.4.4. Weight Variation of Leflunomide tablets

For each batch 6 tablets were selected randomly and their average weight was determined weight of the individual tablet was also determined. The details method of weight variation test is given the chapter -4.

Tablet 6.5. Results of Hardness of Leflunomide tablets.

Batch code	Average weights (mg)	±SD (n=6)
F1	290.93±1.58	±1.25
F2	300.28±2.1	±2.04
F3	290.29±1.49	±1070
F4	310.00±1.96	±2.10
F5	300.42±1.06	±1.67

The above table shows the result of the weight variation test of each batch of formulation. The weight variation test, pharmacopoeia limit for the each percentage deviation for tablets of

more than 250 mg is ± 5%. The results shows that the weight variation comes within the official specification.

6.4.5. Friability of Leflunomide tablets

The friability of tablets was determined by Roche friability. The details methods as described in the chapter -4 and the results are shown in the table -6.7.

Table 6.7. Results of friability analysis of Leflunomide tablets.

Batch code	Initial weight (gm)	Final weight (gm)	Weight loss (gm)	Percentage weight loss
F1	1.863	1.852	0.011	0.593
F2	1.905	1.891	0.014	0.740
F3	1.882	1.872	0.010	0.534
F4	1.885	1.871	0.014	0.748
F5	1.856	1.841	0.015	0.814

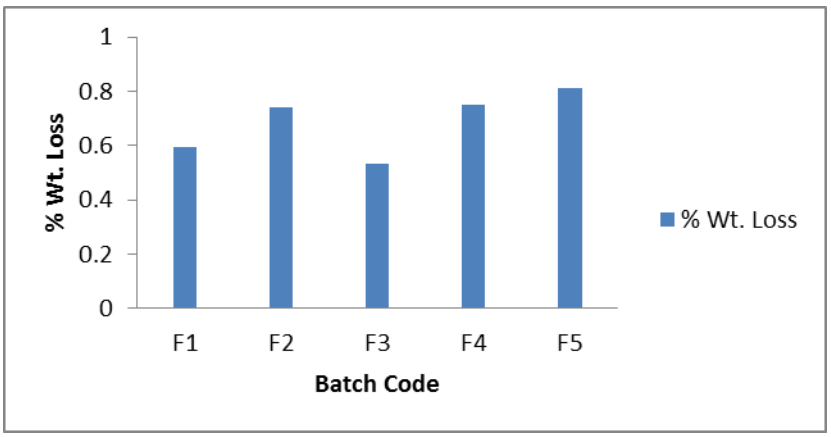


Figure 6.7 Friability analysis of Leflunomide tablets.

The friability(0.534to0.814) of all formulation are complaint with official friability specification which allow not more than 1% of mass on 6 tablets. Convention compressed tablet that loss less than 1% of their weight are generally considered acceptable. In the present study, the percentage friability for all the tablets for all the formulations are below 1%, indicating that the friability is within the prescribed limits.

6.4.6. Disintegration Profil of Leflunomide tablets.

In vitro disintegration time (DT) was determined using a disintegration test apparatus (Excel, India). This test was carried out at 37°c ± 2°c in 900 ml of phosphate buffer (pH 7.4) solution.

Table 6.8.Results of Desintegration profile of Leflunomide tablets.

Batch code	Average DT (Minutes)	SD (n=3)
F1	34	1.50
F2	70	1.62
F3	36	1.15
F4	57	2
F5	54	0.57

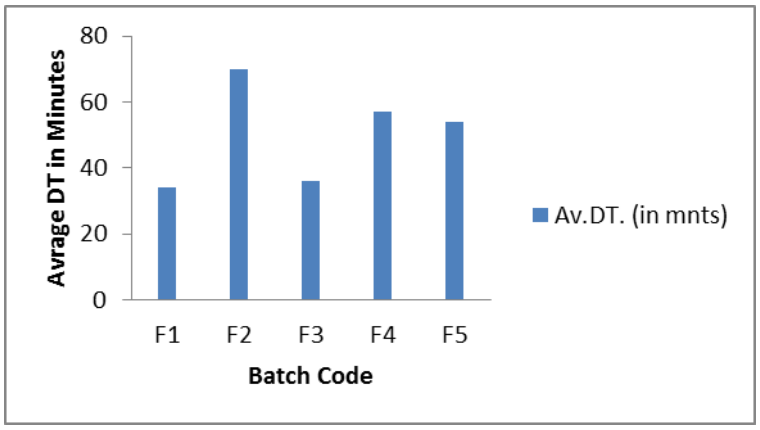


Figure 6.8 Desintegration profile of Leflunomide tablets.

The above table show that the result of the disintegration test of tablets of each batch of formulation. The disintegration time of the tablets varied from -34 to -70minutes.

6.4.7. Content Uniformity of Leflunomide tablets

All batch of formulation are assayed for content uniformity as per the method described in chapter -4. The results of the test are stated in table TABLE-6.11 Results of Leflunomide content in each batch of formulation.

Table 6.9. Results for uniformity of Leflunomide tablets.

Batch code	Average Drug content (mg)	Theoretical Drug content %	±SD (n= 4)
F1	20	99.52	±0.54
F2	20	99.80	±0.76
F3	20	99.95	±0.63
F4	20	100.02	±1.36
F5	20	99.77	±0.97

The above table shows the result of content uniformity test of tablets of each set formulation. These are determined following IP 1996 (vol. II). The quantity of drug in each batch of tablet should not be less than 98% and more than 101%. All drug content in formulation remain between this limit.

6.5. SCANNING ELECTRON MICROSCOPY (SEM)

6.5.1. SEM of drug and Polymer

The surface morphology changes after dissolution were studied using SEM, and the instrumental conditions were described in the chapter-4. The samples were collected from dissolution studies at specific time interval and scanned under SEM. From the observation of all samples, the erosion was held on the samples surfaces which is directly proportional to time of sampling. A gel formation also found. All these these observations were recorded as photograph at 500X and X100K magnification. showing-6.9.(a,b,c,d).

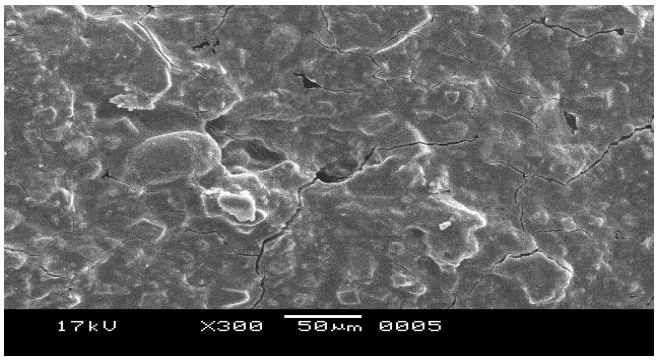


Figure 6.9.(a) The surface morphology of sample at 0 minutes.

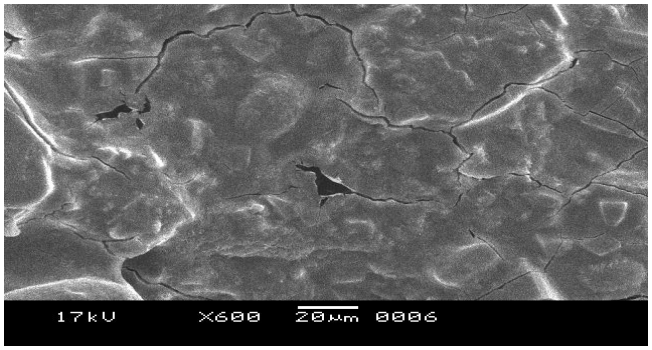


Figure 6.9.(b) The surface morphology of sample at 10 minutes.

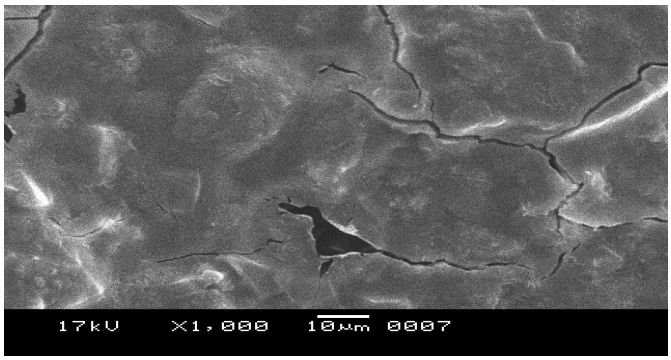


Figure 6.9.(c) The surface morphology of sample at20 minutes.

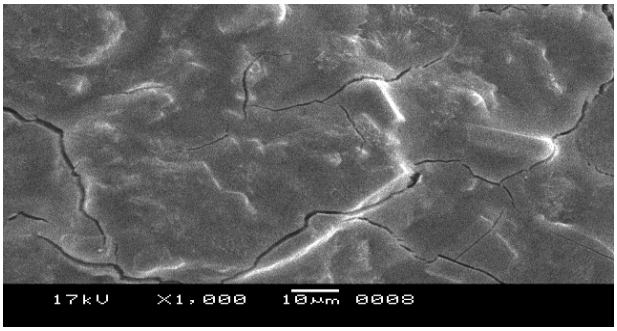


Figure 6.9.(d) The surface morphology of sample at 30 minutes.

6.5.2 Surface Topography of tablet by SEM

The both surfaces of the tablet (outer surface & inner surface) were coated with gold-palladium alloy using fine coat ion- sputter(Hitachi, E-1010) and examined at 17.0KvX1000 &17.0KvX3,000 &17.0KVX600&17.0KvX330 and with little edge of 45degree centigrade. The coated samples were subsequently analysed under emission. SEM(Hitachi,S-3400N). From the observation, it was that the tablets were well compacted, intact less porous and fractured surface contained less of loose particle, shown figure-6.10(a,b,c,d).

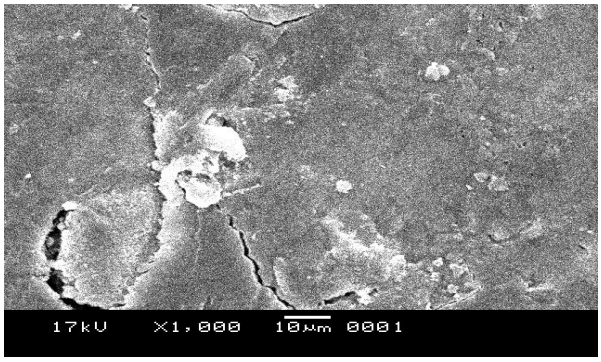


Figure 6.10.(a) The outer surface of tablet.

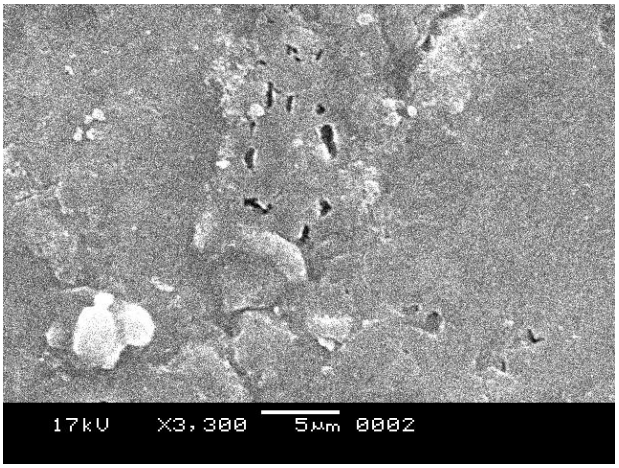


Figure 6.10.(b) At 17 Kv X 3,300 magnification.

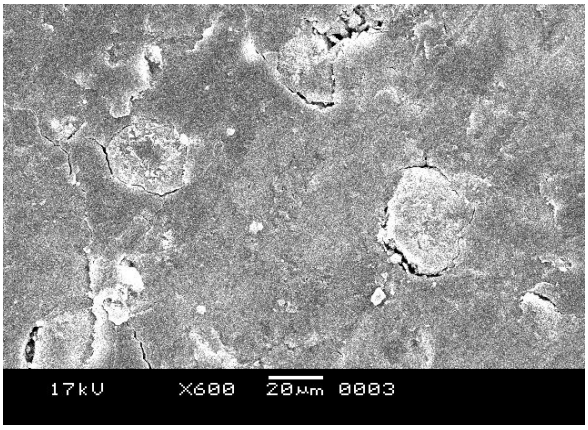
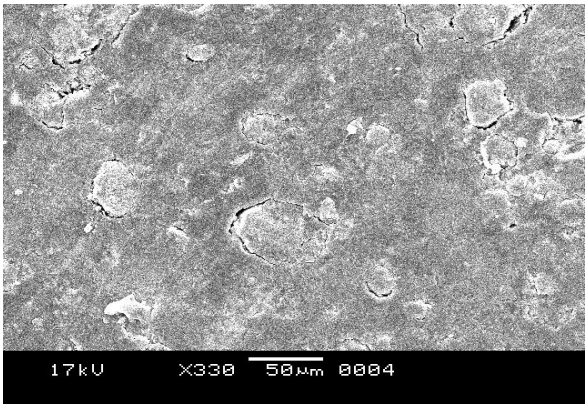


Figure 6.10.(c) The inner surface of tablet.



Figuer 6.10.(d) The fractured surface of tablet.

6.6. Distribution of granules

Table 6.10. Results of distribution of granules in different meshes.

Batch code	←Cumulative% of granules in different batches→			
	Mesh 20	Mesh 25	Mesh 30	Mesh 40
F1	45	25	80	12
F2	30	45	10	15
F3	42	28	20	10
F4	12	18	30	40
F5	40	35	15	10

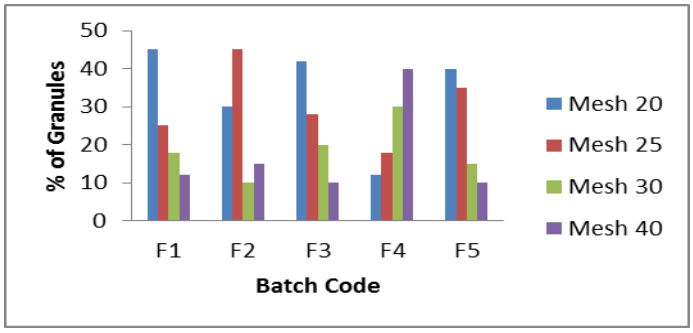


Figure 6.11. Distribution of granules in different meshes.

6.6.1. Friability of Leflunomide tablets

The friability of tablets was determined by Roche friability. The details methods as described in the chapter -4 and the results are shown in the table -6.9.

Table 6.11. Results of friability analysis of Leflunomide tablets.

Batch code	Initial weight (gm)	Final weight (gm)	Weight loss (gm)	Percentage weight loss
F1	1.863	1.852	0.011	0.593
F2	1.905	1.891	0.014	0.740
F3	1.882	1.872	0.010	0.534
F4	1.885	1.871	0.014	0.748
F5	1.856	1.841	0.015	0.814

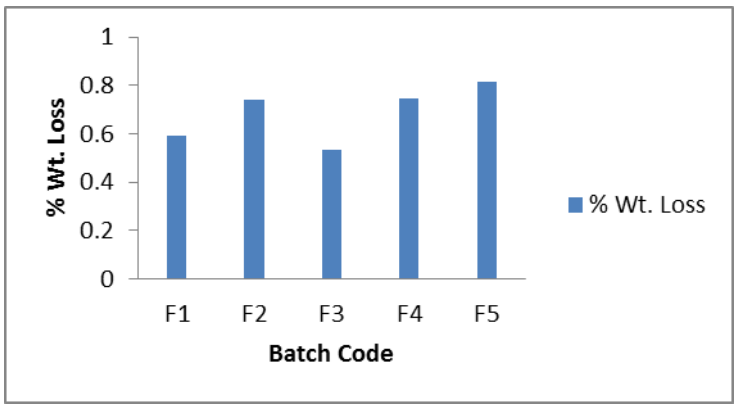


Figure 6.12. Friability analysis of Leflunomide tablets.

The friability (0.534 to 0.814) of all formulation are complaint with official friability specification which allow not more than 1% of mass on 6 tablets. Convention compressed tablet that loss less than 1% of their weight are generally considered acceptable. In the present study, the percentage friability for all the tablets for all the formulations are below 1%, indicating that the friability is within the prescribed limits.

6.6.2. Disintegration Profil of Leflunomide tablets.

In vitro disintegration time (DT) was determined using a disintegration test apparatus (Excel, India). This test was carried out at 37°c ± 2°c in 900 ml of phosphate buffer (pH 7.4) solution.

Table 6.12. Results of Desintegration profile of Leflunomide tablets.

Batch code	Average DT (Minutes)	SD (n=3)
F1	34	1.50
F2	70	1.62
F3	36	1.15
F4	57	2
F5	54	0.57

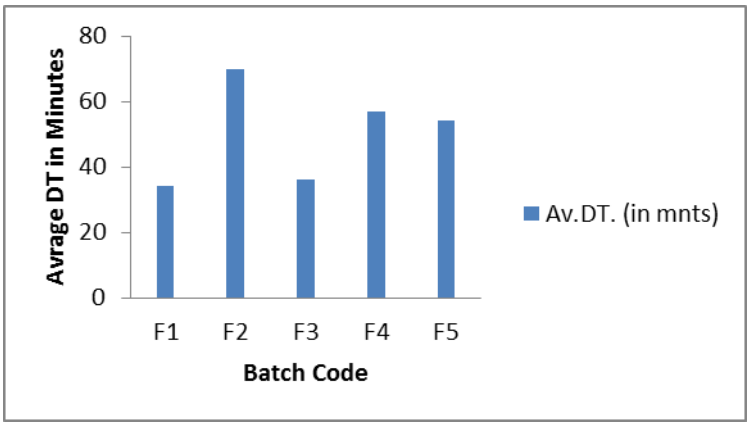


Figure 6.13 Desintegration profile of Leflunomide tablets.

The above table show that the result of the disintegration test of tablets of each batch of formulation. The disintegration time of the tablets varied from -34 to -70minutes.

6.6.3. Content Uniformity of Leflunomide tablets

All batch of formulation are assayed for content uniformity as per the method described in chapter -4. The results of the test are stated in table TABLE-6.11 Results of Leflunomide content in each batch of formulation.

Table 6.13. Results for uniformity of Leflunomide tablets.

Batch code	Average Drug content (mg)	Theoretical Drug content %	±SD (n= 4)
F1	20	99.52	±0.54
F2	20	99.80	±0.76
F3	20	99.95	±0.63
F4	20	100.02	±1.36
F5	20	99.77	±0.97

The above table shows the result of content uniformity test of tablets of each set formulation. These are determined following IP 1996 (vol. II). The quantity of drug in each batch of tablet should not be less than 98% and more than 101%. All drug content in formulation remain between this limit.

6.7. In- Vitro Drug Release Profile

Leflunomide tablets was determined using USP standard dissolution apparatus (paddle type) as describe in chapter -4. The cumulative percentage of drug release time were tabulated in table -6.14. and the graph of release profile was shown figure 6.14.

Table 6.14. In vitro-release of Leflunomide tablet against time.

Time in minutes	←----------Cumulative % of drug release----------→					
	F1	F2	F3	F4	F5	M
0	0	0	0	0	0	0
10	47.01	60.23	72.33	69.51	68.55	65.91
20	53.37	65.22	74.44	77.91	78.51	75.58
30	60.99	88.37	89.31	94.48	94.88	88.05

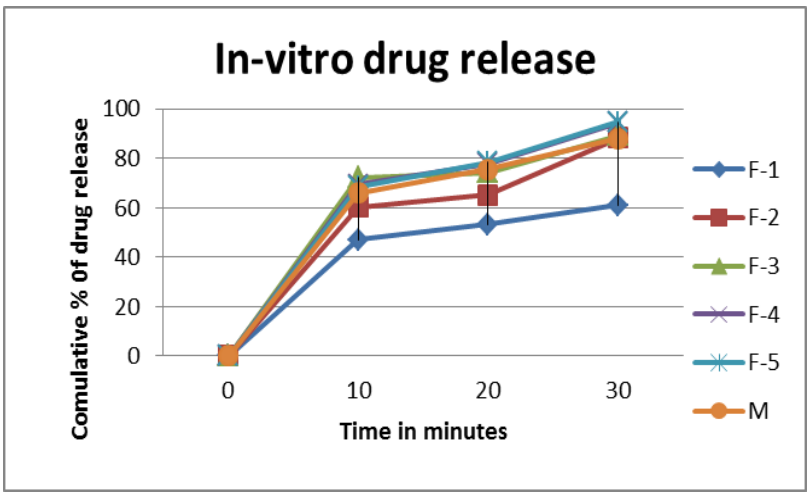


Figure 6.14 In vitro-release of Leflunomide tablet against time.

6.7.1. Drug release kinetics of Leflunomide tablet formulation

To study the mechanism of release of Leflunomide forms matrix tablet, the release kinetic has to be followed. Here four types of release kinetic for Leflunomide tablet are considered to find out the best fit kinetic of release. These are zero order, first order, Higuchi model and Poppas model. The release constant was calculated from the stop of the appropriate plots and the regression co-efficient / correlation coefficient (R^2) was determined (table -6.14.).

The zero – order rate (equation q=kot, where "q" is cumulative % release, "k0" is zero-order release rate constant, "t" is time) describe the system where the drug release rate is independent of its concentration. Figure-6.14 show the cumulative amount of drug release Vs time for zero-order kinetics.

Table 6.1 Zero-order release of Leflunomide tablets against time.

Time in minutes	←----------Cumulative % of drug release----------→					
	F1	F2	F3	F4	F5	M
0	0	0	0	0	0	0
10	47.01	60.23	72.33	69.51	68.55	65.91
20	53.37	65.22	74.44	77.91	78.51	75.58
30	60.99	88.37	89.31	94.48	94.88	88.05

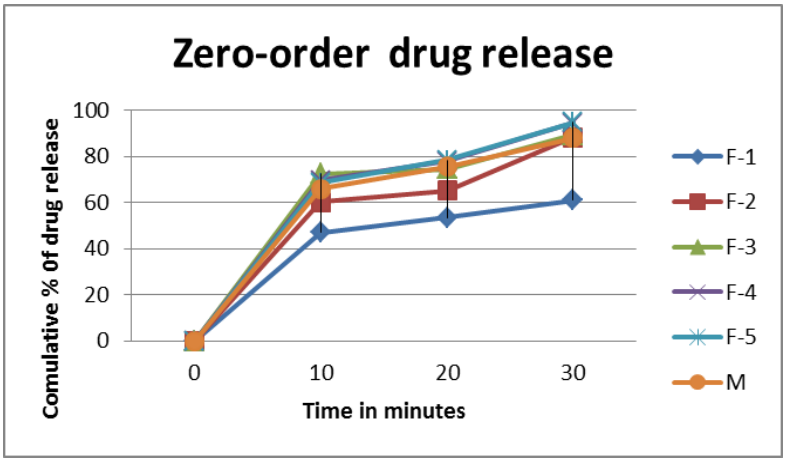


Figure 6.15 In-vitro release of Leflunomide against time.

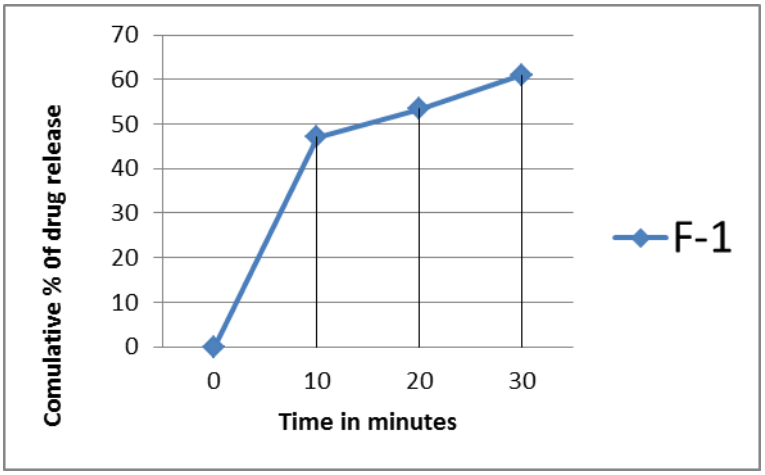


Figure 6.15(a) Zero-order release kinetics of formulation F-1.

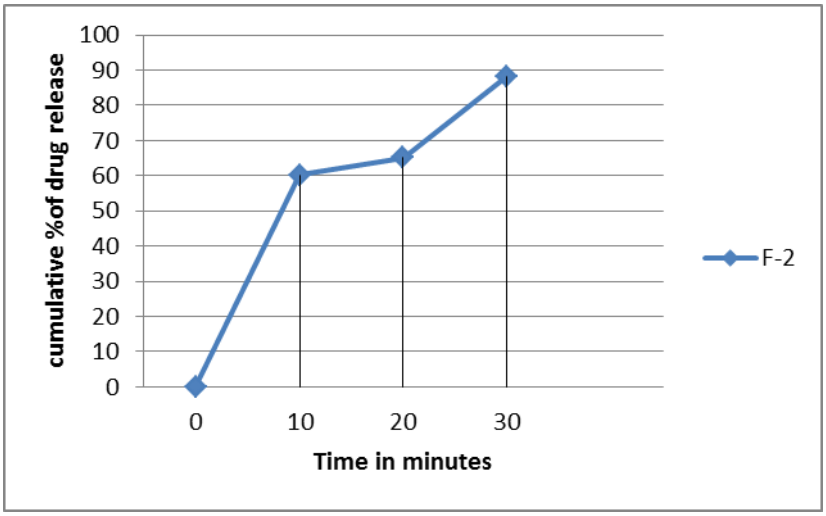


Figure 6.15(b) Zero-order release kinetics of formulation F-2.

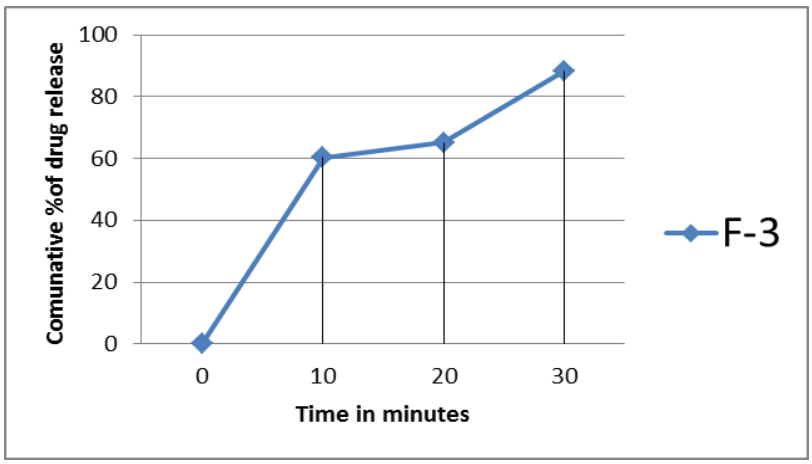


Figure 6.15(c) Zero-order release kinetics of formulation F-3.

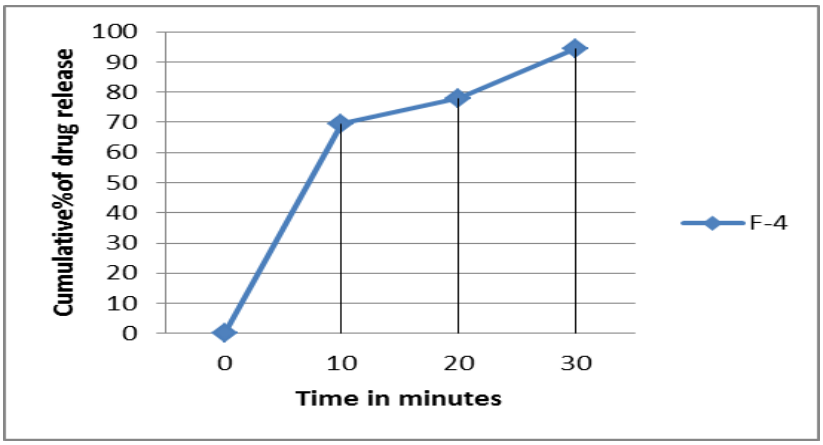


Figure 6.15(d) Zero-order release kinetics of formulation F-4.

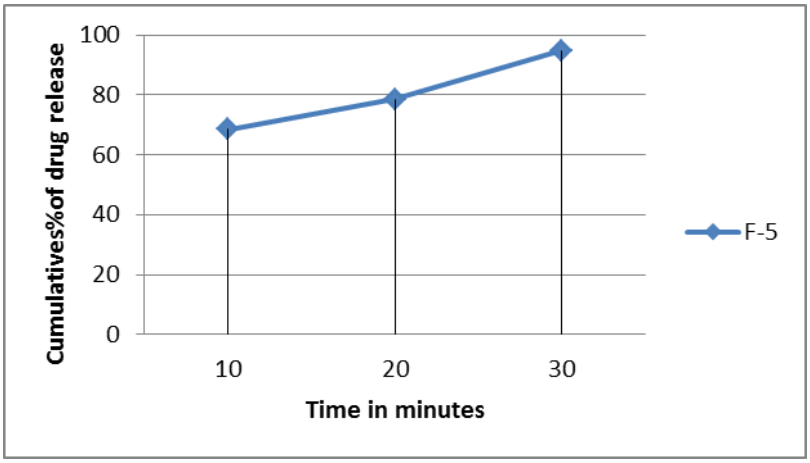


Figure 6.15(e) Zero-order release kinetics of formulation F-5.

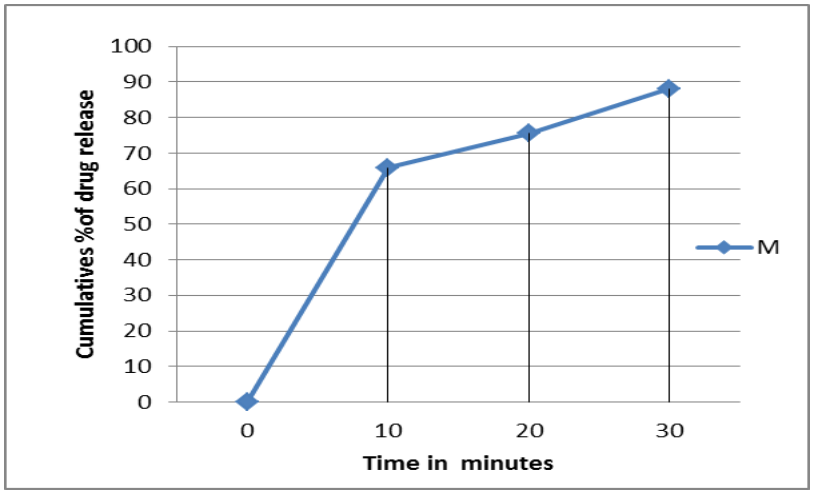


Figure 6.15(f) Zero-order release kinetics of formulation M.

The first order (In $q_o / q_2 = k_1 t$) rate describes the release from system where the release rate is independent of its concentration figure -6.15. show the log cumulative % release Vs time.

Table 6.15. The first order release kinetic of Leflunomide tablet.

1st order	← Log cumulative % of drug Release →				
Time (mnts)	F1	F2	F3	F4	F5
0	0	0	0	0	0
10	1.356	1.220	1.263	1.321	1.332
20	1.554	1.532	1.607	1.532	1.564
30	1.754	1.704	1.767	1.689	1.714
40	1.900	1.804	1.890	1.839	1.786
50	1.950	1.895	1.914	1.932	1.890
60	1.995	1.937	1.950	1.991	1.994

Figure 6.15 In vitro-release of Leflunomide tablets against time.

Higuchis model ($Q = K_{HG}t$ ½, k is constant reflecting the design veriable of the system and t is the time in hours) described the release of drugs from an insolute matrix as a square root of a time dependent process based on Fickian diffusion. Figure - Table-6.13. Describes the Higuchis square root kinetics, showing the cumutative % release Vs square root of time.

Table 6.16. Higuchi's square root of Leflunomide tablets.

Sq. RT	←Cumulative % of drug release→				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
3.16	22.72	16.60	18.35	20.97	21.50
4.47	35.83	39.09	40.49	34.09	36.71
5.47	56.81	50.69	58.56	48.95	51.57
6.32	79.54	63.81	77.79	69.05	61.18
7.07	89.16	78.67	82.16	85.66	77.79
7.74	98.95	86.53	89.16	98.07	98.77

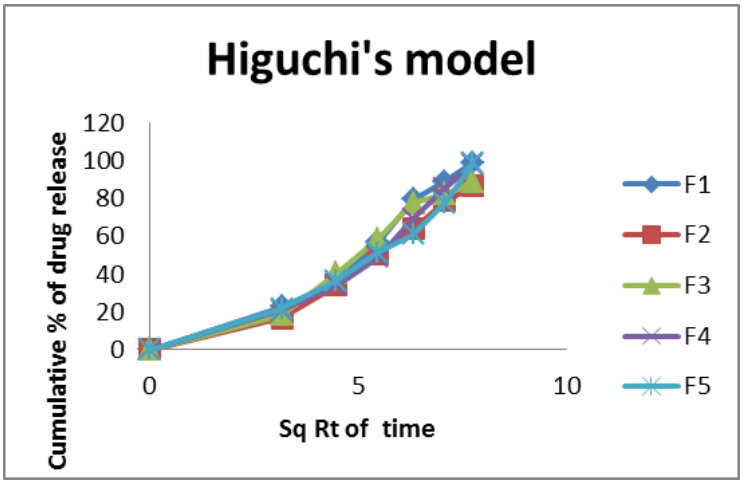


Figure 6.16. Higuchi's square root of Leflunomide tablets.

Korsmeyer- peppas model (power law) (Kors – meyer et. Al. 1983)

The power law descrines the drug release from the polymeric system in which release deviatis from Fickian diffusion as expressed in following equation

$M_t / M\infty = Kt^n$

$\text{Log } [M_t / M\infty] = \text{Log } K + n \text{ Log } t$

When M_t and $M\infty$ are cumulative amount of drug release at time "t" and infinite time is the constant and "n" is a diffusion release exponent indicative of the mechanism of drug release dissolution Figure—and Table:6.14.

Describe the korsmeyer – peppas model (power law), showing log cumulative % release Vs Log of time.

Log of time in minutes	←Log Cumulative % of drug release→				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	1.356	1.220	1.263	1.321	1.332
1.30	1.554	1.532	1.607	1.532	1.564
1.47	1.754	1.704	1.767	1.689	1.712
1.60	1.900	1.804	1.890	1.839	1.786
1.69	1.950	1.895	1.914	1.932	1.890
1.77	1.995	1.937	1.950	1.991	1.994

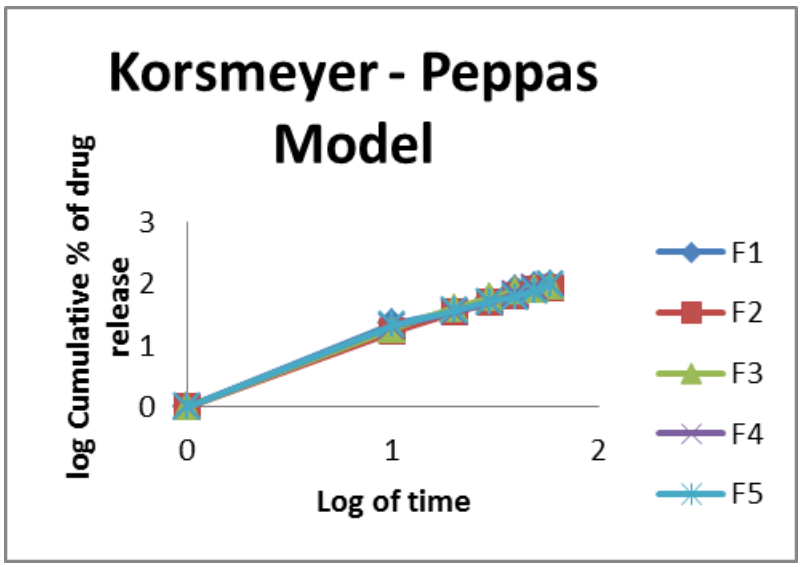


Figure 6.17. Korsmeyer-Peppas model of Leflunomide tablets.

6.7.2. Correlation Coefficient Value (R^2) for different in vitro release kinetic for Leflunomide from formulation

Table 6.18

Batch code	←R^2 value of different rate kinetics→			
	Zero order	1st order	Higuchi	Korsmer – peppas
F1	0.983	0.685	0.938	0.987
F2	0.990	0.708	0.942	0.994
F3	0.958	0.677	0.951	0.988
F4	0.996	0.703	0.923	0.990
F5	0.999	0.683	0.928	0.986

The release kineties of 6 batches of formulation was studied and plotted as describe above. The correlation co-efficient (R^2) for different in-vitro release kinetic is tabulated above. For the above said data, it is observed that the correlation co-efficient (R^2) values are ranged from **0.958 to 0.996**, **0.677 to 0.708**,**0.928 to 0.923** and **0.951,0.986 to 0.994** zero order, first order, Higuchi's and korsmer peppas respectively.

6.7.3. Accelerated stability study of tablet containing Leflunomide tablet

The accelerated stability study of the final tablets was performed according to the procedure describe in chapter -4. The results are shown in table -6.16. It is revalued that the formulation remained stable at 40°c / 75% RH upto three months.

Table 6.19. Results of stability study for of Leflunomide tablets.

Sl. No.	Parameters	Standards	Initial	40°c & 75% RH			R.T
				1 month	2 months	3months	3 months
1	Description	Round, biconvex, white color tablet	Complies	Complies	Complies	Complies	Complies
2	Average wt. (mg)	300.00	229.89	300.33	300.90	229.54	229.53
3	Dissolution						
	Leflunomide	10 – 30% in 1st hour	19.73%	18.98%	18.77%	19.01%	19.58%
4	Assay						
	Leflunomide	90 – 110% of label claim	99.90%	99.22%	98.70%	98.95%	98.75%

Each value represent the mean of 6 results.

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