DEVELOPMENT AND CHARACTERIZATION OF FENOFIBRATE-LOADED NANOPARTICLES

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ABSTRACT : Fenofibrate is an anti-hyperlipidaemic drug belonging to Biopharmaceutical classification systems -II (low solubility, high permeability). Nanoparticle compounds present a promising solution to the insolubility of BCS class II drugs. The main objective of the study was to development and characterisation of fenofibrate loaded nanoparticles, fenofibrate nanoparticles to improve the therapeutic efficacy of fenofibrate by loading in nanoparticle drug delivery system. Fenofibrate belongs to a group of drugs known as fibrates, it helps reduce cholesterol and triglycerides in the blood Nanoparticles had been prepared from different polymer which extent the therapeutic effect as well as reduces side effect., This research discussed about preparation of fenofibrate by solvent diffusion method, solvent diffusion method was successfully employed to Fenofibrate nanoparticles to improve the dissolution as well as bioavailability. characterization techniques, such as Fourier-transform infrared spectroscopy, particle size, drug entrapment efficiency, In-vitro drug release, Drug release kinetics, Scanning electron microscopy, zeta potential and stability studies. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. Examination of the preparation, characterization and in-vitro drug delivery of the nanoparticles was carried out. The different formulations of with different concentration of drug-polymer and surfactant were inspected and finalized. Formulation (F-6) showed the highest encapsulation efficiency. In this research, a drug encapsulation efficiency as high as 87%has been achieved.

Key words: Fenofibrate, solvent diffusion method, Nanoparticles, Zeta potential, Drug entrapment efficiency.

I.INTRODUCTION

Colloidal drug carrier is one of the most important entities essentially required for successful transport of loaded drugs. Colloidal drug carriers such as liposomes and nanoparticles are able to modify the distribution of an associated substance[1]. They can therefore be used to improve the therapeutic index of drugs by increasing their efficacy and/or reducing their toxicity. Nanotechnology is the science of the small; the very small. it is the use and manipulation of matter at a tiny scale. at this size, atoms and molecules work differently, and provide a variety of surprising and interesting uses. Nanotechnology and nanoscience studies have emerged rapidly during the past years in a broad range of product domains. it provides opportunities for the development of materials, including those for medical applications, where conventional techniques may reach their limits. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix[2].Nanoparticle research is currently an area of intense scientific interest. The reason behind nanoparticles are attractive is based on their unique and important features, such as their surface to mass ratio, which is much larger than that of other particles and materials, their ability to absorb and carry other compounds such as drugs, probes and proteins as well as permitting the catalytic promotion of reactions[3].

II. MATERIALS AND METHODS

2.1. Materials

Fenofibrate was collected as a gift sample from Lupin, and various polymers like Chitosan, tragacanth and other excipients were purchased from Vijaya enterprises, Hyderabad.

2.2. Methodology

Drug and excipient compatibility studies[4]

IR spectra of pure drug individually and physical mixture of drug were recorded by KBr method using Fourier Transform Infrared Spectrophotometer. A base line correction was made using dried potassium bromide pellet. The potassium bromide-and sample individual pellet of approximately l mm diameter was prepared by grinding 3-5 mg of physical mixture of drug excipients with 100-150 mg of potassium bromide in pressure compression machine. The sample pellet was mounted in IR compartment and scanned at wavelengths 4000 cm^{-1} to 400 $cm.^{-1}$

Preparation of nanoparticles[5]

Method

Fenofibrate were prepared by a solvent diffusion method in an acidic aqueous system in order to improve the recovery of the method. The drug and polymer were dissolved in acetone and ethanol at 50^0C in water bath, the resultant organic solution was poured into an acidic aqueous (pH 1.10) containing 1% polyvinyl alcohol (PVA) under mechanical agitation at room temperature. The filtrate was centrifuged (1000 rpm for 10 minutes) and supernatant was collected. Further the ultra-centrifugation (3200 rpm for 1 hour) was carried for supernatants. Following ultracentrifugation, the drug loaded nanoparticles was quickly produced with an aggregation state and easily separated by centrifugation allowing the formation of a turbid nanoparticulate suspension. The suspension was filtered through membrane filter. The compositions of the nanoparticles is shown in Table:1

Table-:1 Composition of the Nanoparticles

Ingredients (mg)	Batch no							
	F1	F2	F3	F4	F5	F6	F7	F8
Eudragit RL	50	100	150	200	-	-	-	-
Eudragit RLPO	-	-	-	-	50	100	150	200
Fenofibrate	50	50	50	50	50	50	50	50

Evaluation of nanoparticles:

Scanning electron Microscopy (SEM)[6]: A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons.

Zeta Potential[7]:

The analysis was performed by using the Malvern Zeta sizer ver. 6.12 (Malvern instrument, UK) the electrophoretic mobility was converted to the zeta potential. To determine the zeta potential, nanoparticle samples were diluted with KCl (0.1 mM) and placed in electrophoretic cell where an electrical field of 15.2 V/cm was applied. All measurement was performed in triplicates.

Particlesize[8]:

All the prepared formulations of nanoparticles were viewed under microscope to study their size. Size of nanoparticles from each batch was measured at different location on slide by taking a small drop of nanoparticle dispersion on it and average size of nanoparticles were determined.

Drug entrapment efficiency[9]:

Nanoparticles50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined at 256nm by using UV spectrophotometer. The encapsulation efficiency was determined as the mass ratio of entrapped Fenofibrate in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the Fenofibrate nanoparticles was expressed as loading capacity.

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies[10]:

The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10 ml) was placed in a 10 ml of beaker. The beaker was assembled on a magnetic stirrer and the medium

was equilibrated at 37±5^{0}C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non entrapped Fenofibrate dispersion was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug in the patch
Da = The amount of drug released

1. Release kinetics[11]

Drug release mechanisms and kinetics are the two important characteristics of a drug delivery system in describing drug dissolution profile. Mathematical models are used to evaluate the kinetics and mechanism of drug release from the tablets. The model that best fits the release data was selected based on the correlation coefficient(R) value in various models. The models that have show high 'R' value was considered as the best fit on the release data.

% drug release =concentration × no. of dilutions × volume of dissolution fluid/1000

Various mathematical models are:

1. Zero Order Release Equation:

The equation for zero order release is

$$Q_t = Q_o + K_o t$$

The zero-order kinetics describes the systems in which the drug release rate is independent of its concentration of the dissolved substance. A graph was plotted between the time taken on x-axis and the cumulative percentage of drug release on y-axis.

2. First Order Release Equation:

The first order release equation is

$$\text{Log } Q_t = \text{Log } Q_o + K_t /2.303$$

The drug release rate depends on its concentration. The first order kinetics describes the systems in which the drug release rate is concentration dependent.

3. Higuchi Release Equation

The Higuchi release equation is

$$\mathbf{Q_t = K_H \sqrt{t}}$$

Higuchi described the release of drug from an insoluble matrix as square root of time dependent process. The Higuchi square root model also gives the drug release from a planar surface of an insoluble heterogeneous matrix by diffusion through the intra granular openings created by porosity of the formulation. A graph is plotted between the square root of time taken on x-axis and the cumulative percentage of drug release on y-axis.

4. Korsmeyer -Peppas Release Equation:

Korsmeyer –Peppas equation is

$$\mathbf{F = M_t / M = K_m t^n}$$

In case of Korsmeyer-Peppas model, the drug release from such devices having constant geometry will be observed till the polymer chains rearrange to equilibrium state. A graph is plotted between the log time taken on x-axis and the log percentage of drug release on y-axis.

Stability studies:

Selected Formulation was subjected to stability studies as per ICH guidelines.
Following conditions were used for Stability Testing.
1. 25^{0}C/60% RH analysed every month for period of three months.
2. 30^{0}C/75% RH analysed every month for period of three months.
3. 40^{0}C/75% RH analysed every month for period of three months.

III.RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The studies are performed to check the compatibility of drug and excipients used in the formulation in order to prevent degradation by interaction. FT-IR spectra for the drug and various physical mixtures are obtained in a FT-IR spectroscopy in the transmission mode with a wave number region 4000 – 400 cm-1. KBr pellets are prepared gently by mixing 1mg sample powder with 100 mg KBr. (Fig-1 and Fig-2).

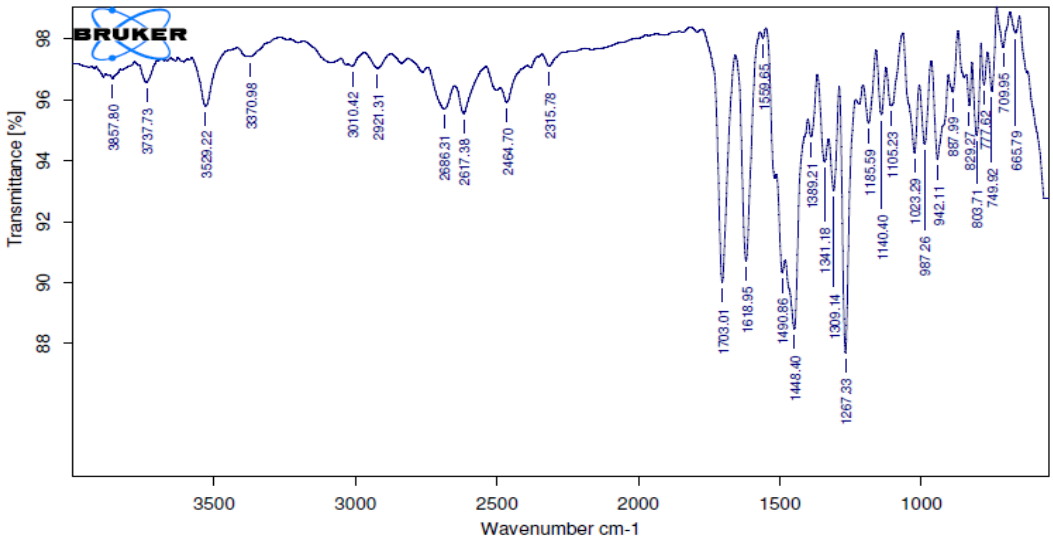

Fig.1. FT-IR Sample for Fenofibrate

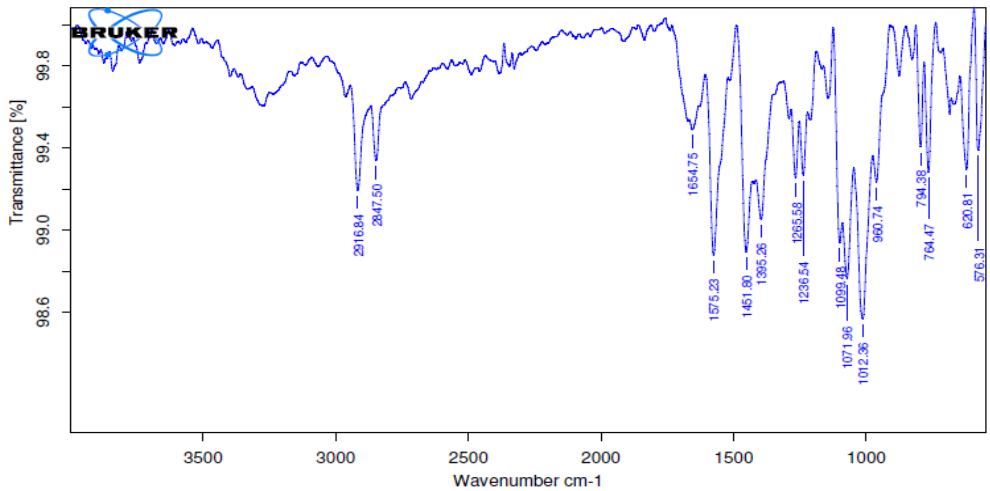

Fig.2. FT-IR Sample for Optimized Formulation

Particle size

Vesicle shape: Vesicle shape of the prepared formulation was found to be spherical from the SEM (scanning electron microscope) analysis at 1500kV. Scanning electron microscopy (SEM) SEM revealed that the Fenofibrate nanoparticles (Fig-3).

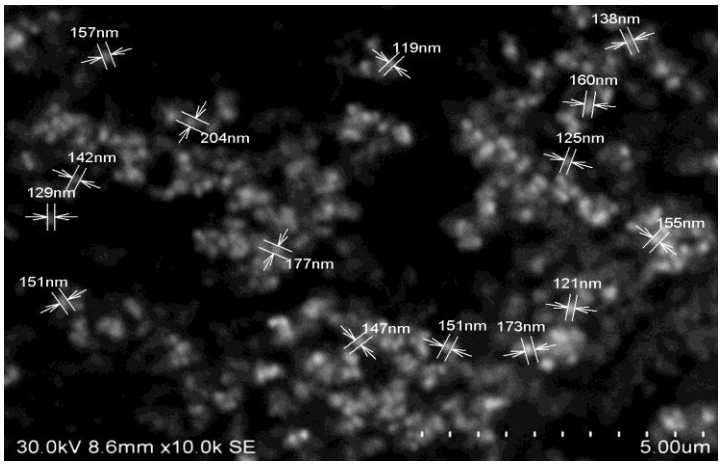

Fig.3. SEM Analysis of Optimized Nanoparticles

Determination of Zeta potential:

Zeta potential the addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized Fenofibrate nanoparticles formulation was measured and found to -3.8 mv. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable. Which is shown in fig-4. Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency, Particle size is shown in Table-2.

Table:2 Evaluation Studies of Prepared Nanoparticles: Entrapment Efficiency, Particle size.

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	142	48
F2	151	52
F3	147	64
F4	160	66
F5	151	56
F6	204	87
F7	157	72
F8	173	69

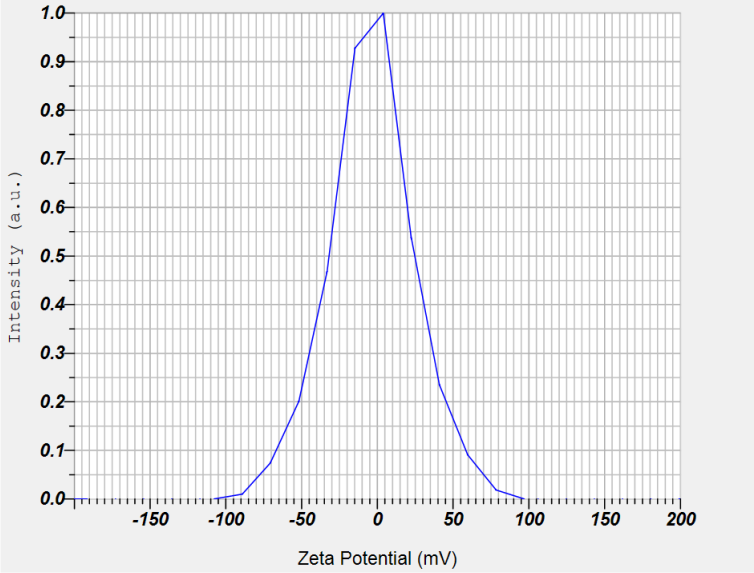

Fig.4. Zeta potential of Optimized formulation

Drug release studies:

Diffusion study profiles for all formulations was shown in the table-3.The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 8 hours(Fig-5). F6 formulation which had drug polymer Eudragit RLPO as optimized formulation.

Table-:3 Diffusion study profiles for all formulations

Time (hrs)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
0	0	0	0	0	0	0	0	0
1	29.43	24.29	28.82	33.62	31.50	32.10	29.94	35.32
2	32.51	30.78	31.28	40.19	40.19	39.50	31.52	41.71
3	41.78	40.76	39.61	49.28	47.30	52.69	45.21	50.69
4	50.7	52.32	49.20	62.62	61.50	61.19	51.87	66.65
5	59.2	66.49	55.81	70.74	69.50	71.40	61.71	75.37
6	65.3	78.77	71.76	78.56	75.47	82.91	70.86	85.78
7	79.2	85.84	83.63	92.68	89.71	92.90	88.82	90.90
8	81.3	87.24	89.32	94.82	91.80	98.62	90.12	94.21

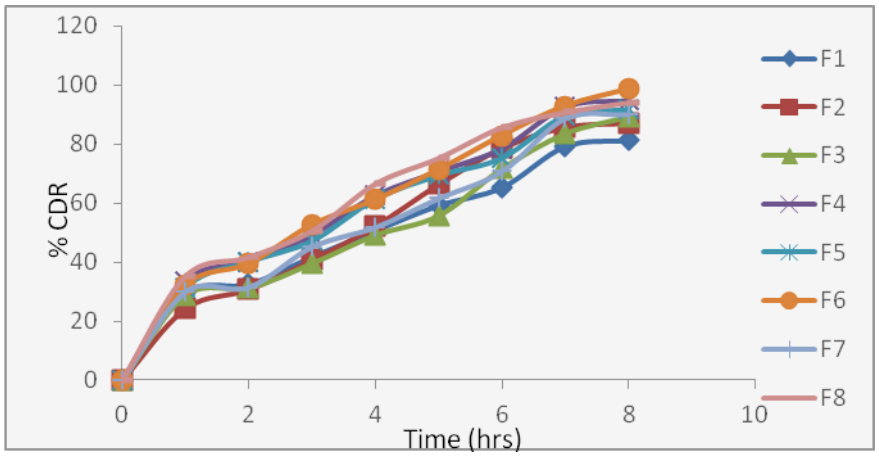

Fig.5. In vitro drug release studies for all formulation

Kinetic modelling of drug release

All the eight formulations of prepared of Fenofibrate nanoparticles were subjected to in vitro release studies these studies were carried out using diffusion apparatus.

The drug release from the Nanoparticles was found to follow Zero order release based on the "r" value obtained for Zero order (0.961) for F6 formulation (Fig-6,7,8,9).

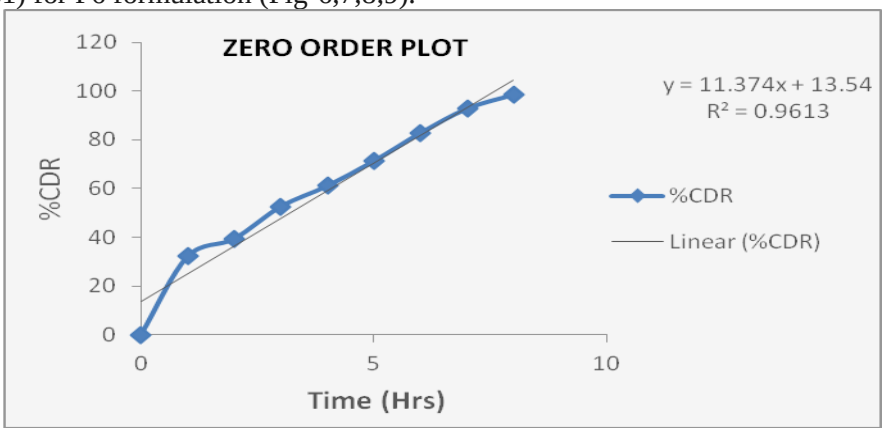

Fig.6. zero order plot for optimized formula

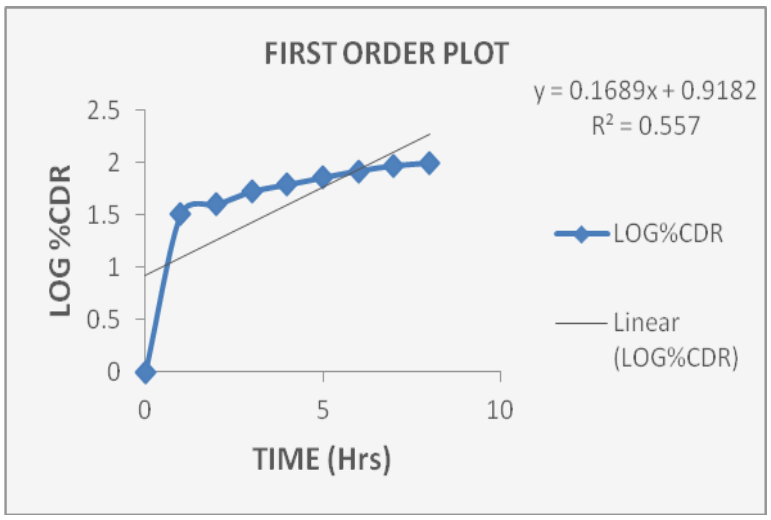

Fig.7. First order for optimized formula

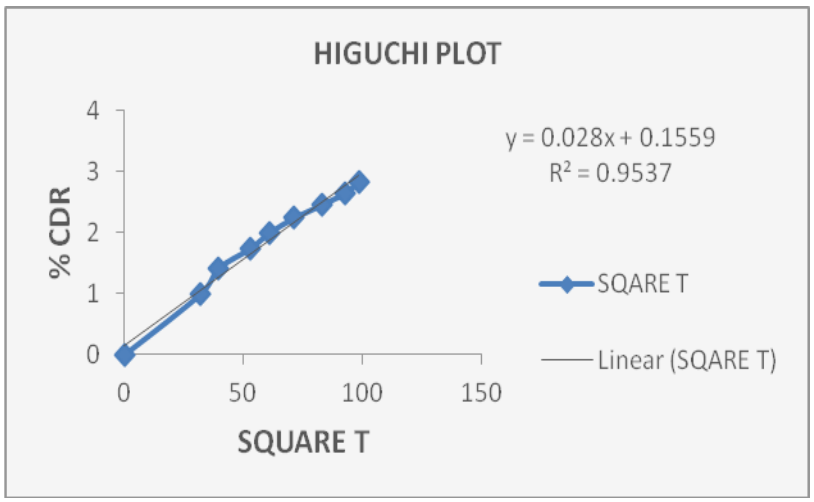

Fig.8. Higuchi plot for optimized formula

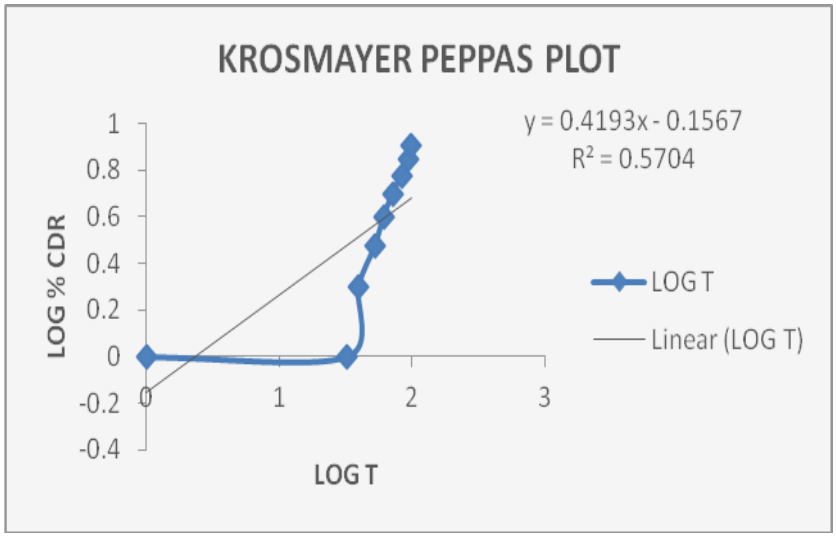

Fig.9. Korsmayer peppas plot for optimized formula

Stability studies

There was no significant change in physical and chemical properties of the nanoparticles of formulation F-6 after 3 months. Parameters quantified at various time intervals were shown (Table-4).

Table- :4 Results of stability studies of optimized formulation F-6

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-6	25^0C/60%RH % Release	98.62	97.12	96.44	95.34	Not less than 85 %
F-6	30^0C/75% RH % Release	98.62	97.68	96.46	95.25	Not less than 85 %
F-6	40^0C/75% RH % Release	98.62	96.56	95.35	95.12	Not less than 85 %

IV. CONCLUSION

The present research proposed a novel formulation Fenofibrate Nanoparticles for controlled release. Investigation of the preparation, characterization and in-vitro release of the Nanoparticles was carried out. The different formulations of with various ratios of drug-polymer were evaluated and optimised. In this research, a drug encapsulation efficiency as high as 87% has been achieved. The method used for the formulation of Fenofibrate containing Eudragit RLPO nanoparticles was solvent diffusion method Nanoparticles formulations showed good results in terms of the assayed drug content and encapsulation efficiency. This indicates that the method used for the formulation produced good yield and it was suitable and reproducible in nature. Formulation (F-6) showed the highest encapsulation efficiency.

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