FORMULATION AND EVALUATION OF LOADED SOLID LIPID NANOPARTICLES OF ENZALUTAMIDE

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ABSTRACT: ***Nanoparticles as an innovative formulation of enzalutamide with improved therapeutic efficacy. Enzalutamide solid lipid nanoparticles were prepared by emulsification solvent evaporation technique by applying ultrasonic energy through Sonicator, Enzalutamide has low solubility and permeability which give rise to limited and variable bioavailability; its low stability makes it difficult to develop stable aqueous liquid formulations, this study aimed to investigate the effectiveness of a strategy based on the development of solid lipid The different formulations with various ratios of drug-lipid and surfactant were evaluated and optimized. The method used for the formulation of Enzalutamide containing soya lecithin solid lipid nanoparticles was the solvent evaporation method followed by sonication to reduce the particle size. The prepared nanosuspensions were characterized for particle size, surface morphology by SEM, drug excipient compatibility by FTIR, and in-vitro drug release studies. Formulation (F-8) showed the highest encapsulation efficiency. In this research, a drug encapsulation efficiency as high as 98.12% has been achieved. It was found that as the concentration of soy lecithin increased, the encapsulation efficiency was also increased. The present study revealed that the solvent evaporation technique followed by sonication can be used as an effective tool for the preparation of Enzalutamide solid lipid nanoparticles.***
Keywords: Enzalutamide drug, solid lipid Nano Particles, Solvent Evaporation, in-vitro drug release, Stability studies.

I. INTRODUCTION

The field of Novel Drug Delivery systems is emerging at an exponential rate with the deep understanding gained in diversified fields of Biotechnology, Biomedical Engineering and Nanotechnology[1]. Many of the recent formulation approaches utilize Nanotechnology that is the preparation of Nanosized structures containing the API[2]. Nanotechnology, as defined by the National Nanotechnology Initiative (NNI), is the study and use of structures roughly in the size range of 1 to 100 nm[3]. The overall goal of nanotechnology is the same as that of medicine: to diagnose as accurately and early as possible and to treat as effectively as possible without any side effects using controlled and targeted drug delivery approach[4]. Some of the important Drug Delivery System developed using Nanotechnology principles are- Nanoparticles, Solid Lipid Nanoparticles, Nanosuspension, Nano emulsion, Nanocrystals[5]. In this article the main focus is on Solid Lipid Nanoparticles (SLNs). SLNs introduced in 1991 represent an alternative and better carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles[6]. Nanoparticles made from solid lipids are attracting major attention as novel colloidal drug carrier for intravenous applications as they have been proposed as an alternative particulate carrier system[7]. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water-soluble drugs[8].The Enzalutamide has low solubility and permeability which give rise to limited and variable bioavailability; and its low stability makes it difficult to develop stable aqueous liquid formulations Enzalutamide is poorly water-soluble drug with poor oral bioavailability due to extensive first-pass metabolism[9], The aim of this study was to investigate the effectiveness of a strategy based on the development of solid lipid Nanoparticles as an innovative formulation of enzalutamide with improved therapeutic efficacy. So, to increase the bioavailability enzalutamide is prepared by using solid lipid nano particles. Enzalutamide is clinically effective in the treatment of metastatic castration - resistant prostate cancer. An up-to 89% decrease in serum prostate specific antigen (PSA) levels have been reported after a month taking it. It can be used as an Anti - androgen in feminizing hormone therapy for transgender women10.

II. MATERIALS AND METHOD

2.1 MATERIALS

Enzalutamide was collected as a gift sample from Aurobindo labs, Hyd, polymers and other excipients were

purchased from AR Chemicals, Hyd.

2.2 Methododology

Compatibility studies:

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study[11].

Fourier Transform Infrared Spectroscopy (FTIR)

A proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Compatibility must be established between the active ingredient and other excipients to produce a stable, efficacious, attractive and safe product. So before producing the actual formulation, compatibility of Enzalutamide with polymers and other excipients were tested using the Fourier Transform Infrared Spectroscopy (FT-IR). For this study, potassium bromide (KBr) pellet method was employed. The samples were thoroughly mixed with dry powdered potassium bromide. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded. The application of infra-red spectroscopy lies more in the qualitative identification of substances either in pure form or in the mixtures and as a tool in establishment of the structure. Since I.R. is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds[12].

Method of preparation of Enzalutamide loaded nanoparticles:

Enzalutamide-loaded SLN were prepared by solvent emulsification/evaporation method. The composition of all the formulations 20 mg of drug was dissolved in 10 mL methanol, and Phosphatidylcholine was dissolved in 20 mL chloroform separately; drug and lipid solutions were mixed together. The organic solvent mixture was completely evaporated at 70°C using a rotary evaporator to remove the organic solvent. Drug embedded lipid layer was then poured into 100 mL of an aqueous solution containing poloxamer 407 surfactants and the mixture was Sonicated for 15 minutes by using Sonicator followed by homogenized for 15 minutes at different homogenization speed using a high-speed homogenizer. The suspension was then allowed to cool at room temperature. The suspension was filtered through a membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and nanoparticles were collected.[13]

Table -:1 composition of Enzalutamide for preparation of solid lipid nanoparticles

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Enzalutamide	20	20	20	20	20	20	20	20
Phosphatidylcholine	50	75	100	125	50	75	100	125
Chitosan	20	30	40	50	-	-	-	-
Poloxamer 407	-	-	-	-	20	30	40	50
Solvent (Methanol)	10	10	10	10	10	10	10	10
Chloroform	20	20	20	20	20	20	20	20

Evaluation of Enzalutamide loaded nanoparticles:

Particle size

All the prepared batches of nanoparticles were viewed under a microscope to study their size. The size of Nanoparticles from each batch was measured at a different location on a slide by taking a small drop of nanoparticle dispersion on it and the average size of nanoparticles was determined[14].

SEM analysis

The morphology of NPs was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the NPS was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA[15].

Drug encapsulation efficiency:

Lyophilized nanoparticles 50mg were dissolved in 100ml of phosphate buffer and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Enzalutamide in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the Enzalutamide nanoparticles was expressed as loading capacity[16].

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

In-vitro drug release studies:

The release studies were carried out by a Franz diffusion cell. It contains 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 ml) was placed in a 10 ml of the beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37 ± 5^0C. A dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Enzalutamide dispersion was filled in the dialysis membrane and another 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 the same quantity of fresh buffer medium[17].

The percentage of drug release was determined using the following formula.

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

Release kinetics[18,19]

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 shown high 'R' value was considered as the best fit on the release data[18].

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

1. Zero Order Release Equation:

The equation for zero-order release is

$$Q_t = Q_o + K_o t$$

re,

Q_o = Initial amount of drug

Q_t = Cumulative amount of drug release at time "t"

K_o= Zero-order release constant

T= Time in hours

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

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

Where,

Q_o = Initial amount of drug

Q_t = Cumulative amount of drug release at time "t"

K= First order release constant

T= Time in hours

Here, 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

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

Where ,

Q = Cumulative amount of drug release at time "t"

K_H = Higuchi constant

T = Time in hrs

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 intragranular openings created by the porosity of the formulation. A graph is plotted between the square root of time taken on the x-axis and the cumulative percentage of drug release on the y-axis.

4. Korsmeyer -Peppas Release Equation:

Korsmeyer –Peppas equation is

$$F=M_t/M = K_m t^n$$

Were,

F = fraction of drug released at time 't'

M_t = amount of drug released at time 't'

M = total amount of drug in dosage form

K_m= kinetic constant

n = diffusion or release exponent

t = time in hrs

'n' = Linear regression of log (M_t / M) versus log t

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 analyzed every month for period of three months.
2. 30^{0}C/75% RH analyzed every month for period of three months.
3. 40^{0}C/75% RH analyzed every month for period of three months.

3.RESULTS AND DISCUSSION

In the present study, 8 formulations with variable concentrations of lipid were prepared and evaluated for physic-chemical parameters, in-vitro release studies and stability studies.

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluate dosing FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals.

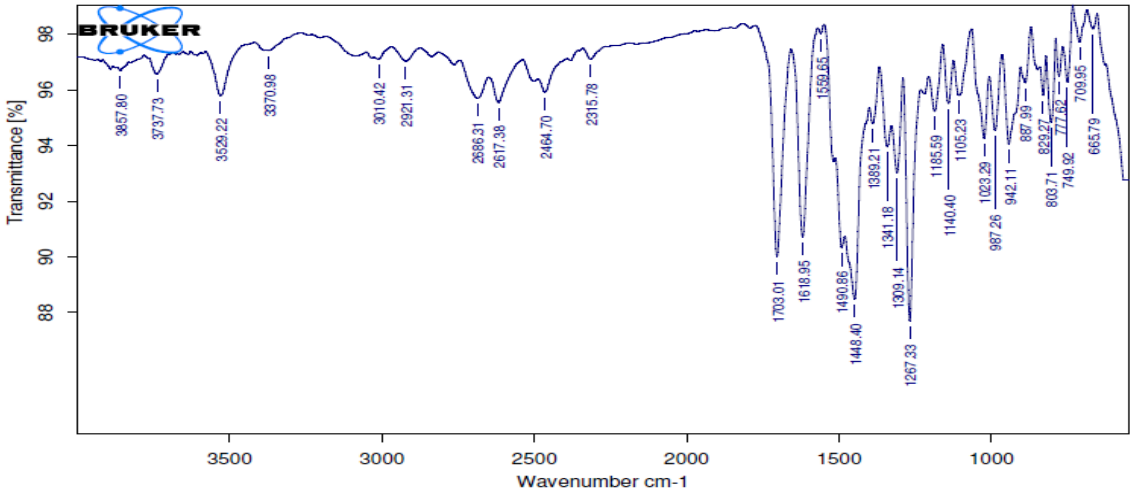

Fig.1. FT-IR Sample for Enzalutamide

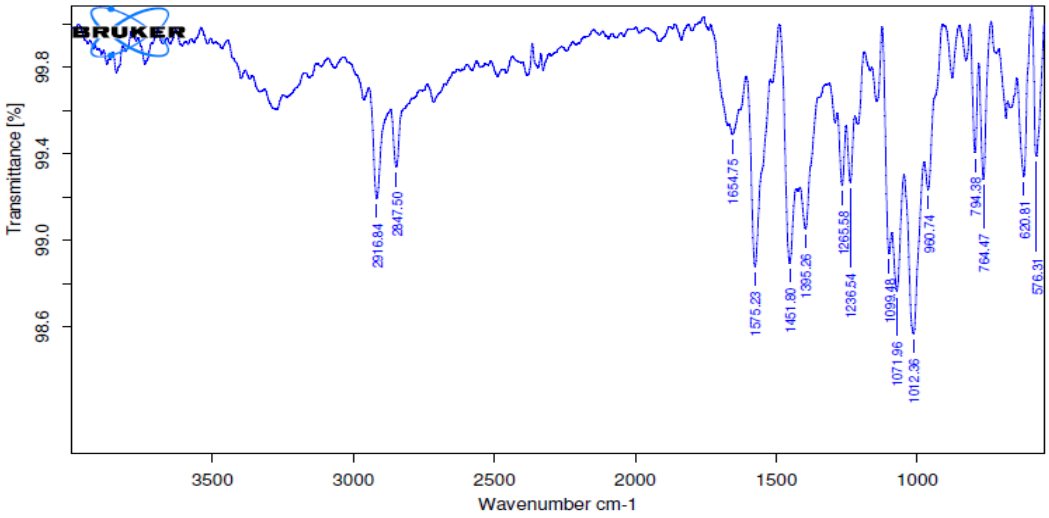

Fig.2. FT-IR Sample for Optimized Formulation

III. EVALUATION PARAMETERS

The solid lipid nanoparticles prepared were evaluated as per the following parameters-

- Particle size and SEM analysis
- Entrapment efficiency
- In vitro release study
- Stability studies

Particle size:

The particle size increased with increasing of lipid concentration. Based on particle size distribution and entrapment efficiency.

Surface morphology:

Scanning electron microscopy (SEM) SEM revealed that the MTX solid lipid nanoparticles were smooth and spherical without any aggregation.

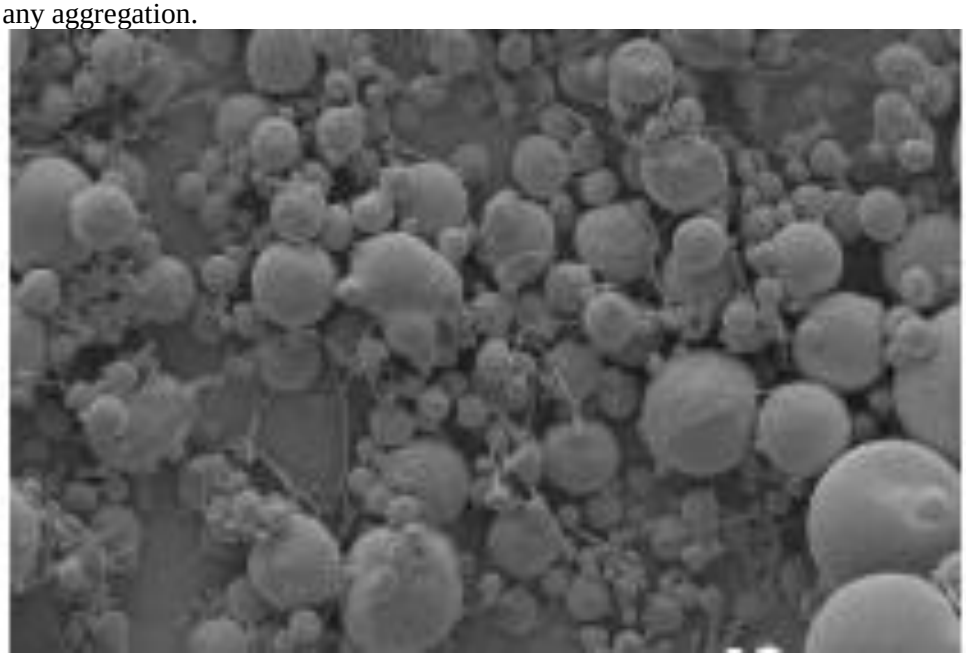

Fig.3. SEM analysis of Optimized solid lipid nanoparticle

Drug entrapment efficiency:

The first part of the plan of work was to optimize the concentration of Lipid to be used in the formulation of solid lipid nanoparticles. The optimization of lipid concentration was done on the basis of particle size and entrapment efficiency of solid lipid nanoparticles obtained.

Table-: 2 Evaluation Studies of Prepared solid lipid nanoparticles: Entrapment Efficiency and Particle size

Batch No	Particle size (nm)	Entrapment Efficiency (%)
F1	224	75.56
F2	236	77.24
F3	242	79.56
F4	249	80.96
F5	253	82.3
F6	268	84.1
F7	279	86.5
F8	284	92.68

In vitro drug release studies

The in vitro drug release results revealed that the prepared Enzalutamide solid lipid nanoparticles would be able to control drug release for extended period of time.

Table-: 3 Drug release study profiles for all formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	**0**	**0**	0	0	0	0	0	0
1	19.56	18.95	12.9	13.1	15.8	17.7	20.2	22.8
2	32.12	35.62	23	20.3	24.9	25.7	34.2	36.4
3	39.65	40.32	31.5	29.1	37	37.1	40.4	48.1
4	47.65	49.56	38.4	40.2	51.6	41.1	56	56.1
5	58.25	58.65	47.6	51.3	62.1	52.7	60.7	61.4
6	64.63	68.92	56.3	59.2	75.7	64.5	75.8	75.3
7	72.53	73.25	69.6	67.6	87.2	73.4	80.1	81.1
8	83.32	85.31	86.19	88.12	91.56	93.52	94.64	98.12

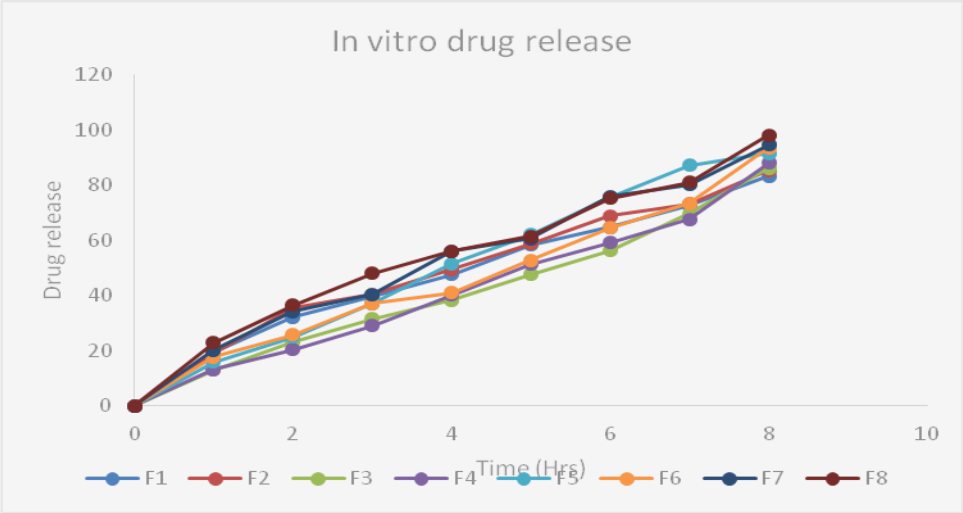

Fig.4. In vitro drug release studies for all formulations

The in vitro diffusion studies were performed in pH 7.4 buffer using Dialysis membrane for 8 hours. Initially the

release of drug from all the batches was found to be about 25-30% in 8 hours. This was due to the release of adsorbed drug from the surface of solid lipid nanoparticles. Later on, a constant and slow drug release was observed for 8hrs. F8 formulation which had lipid ratio was decided to be the optimized formulation.

Kinetic studies:

Zero order plot:

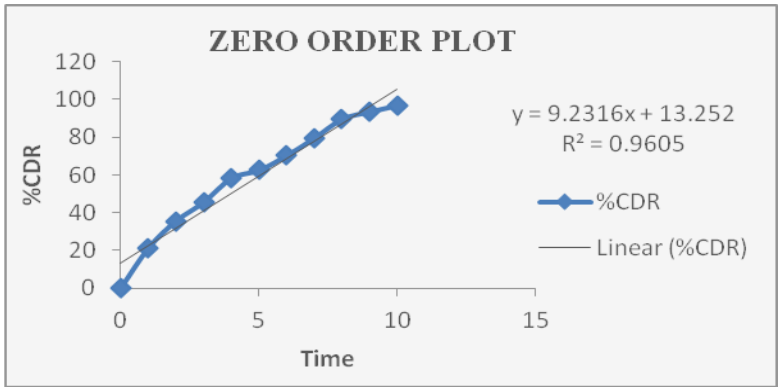

Fig. 5. showing picture of zero order reaction

First order plot:

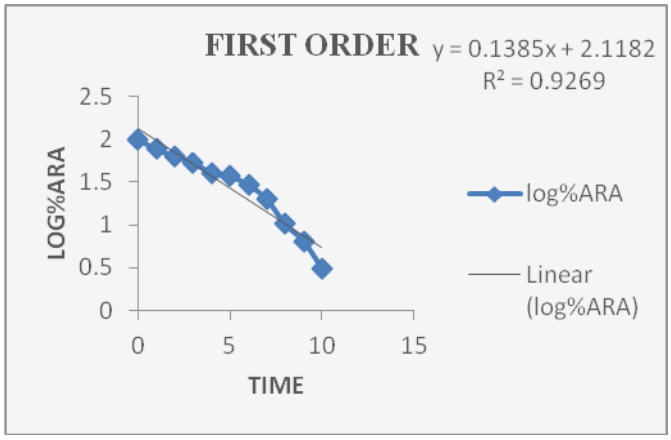

Fig.6. Showing picture of First order Reaction

Higuchi plot:

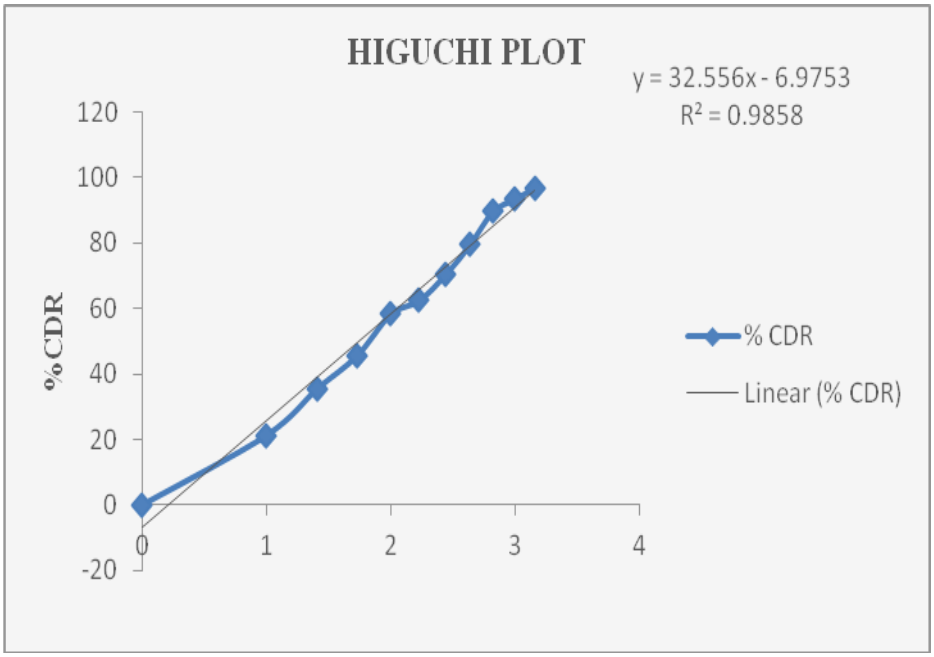

Fig.7. showing picture of Higuchi

Korsmeyer Peppas plot:

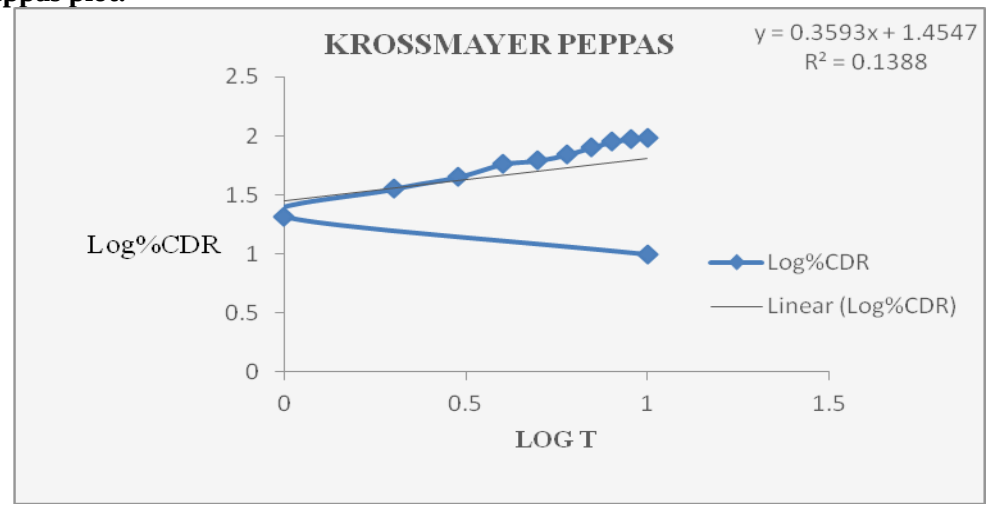

Fig.8. showing picture of Korsmeyer Peppas

Indicated that majority of formulations were governed by Peppas model and mechanism of drug release was anomalous mediated. Regression analysis of the *in vitro* permeation curves was carried out. The slope of the curve obtained after plotting the mean cumulative amount released per vs. time was taken as the *in vitro* release for Enzalutamide. Formulation F8 showed maximum release in 8 h, follows Peppas model) and mechanism of drug release was Anomalous mediated. It can be concluded that rate of diffusion of drug from solid lipid nanoparticles depends on the particle size of nano particle; if the particle size is small the rate of diffusion of drug is fast and vice-versa.

Stability studies:

There was no significant change in physical and chemical properties of the nanoparticles of formulation F-8 after 3 months. Parameters quantified at various time intervals were shown

Table-: 4 Results of stability studies of optimized formulation F-8

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-8	25^0C/60%RH % Release	98.12	98.06	97.96	97.64	Not less than 85 %
F-8	30^0C/75% RH % Release	98.12	98.02	97.94	97.54	Not less than 85 %
F-8	40^0C/75% RH % Release	98.12	98.01	97.65	97.52	Not less than 85 %

IV. CONCLUSION

The present research proposed a novel formulation Enzalutamide solid lipid nanoparticles for controlled release. Investigation of the preparation, characterization and in-vitro release of the solid lipid nanoparticles was carried out. The different formulations of with various ratios of drug-lipid and surfactant were evaluated and optimized. In this research, a drug encapsulation efficiency as high as 98.12 % has been achieved. The method used for the formulation of Enzalutamide containing soya lecithin solid lipid nanoparticles was solvent evaporation method followed by sonication to reduce the particle size.

solid lipid nanoparticles formulations showed good results in terms of 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-8) showed the highest encapsulation efficiency. It was found that as the concentration of soya lecithin increased, the % of encapsulation efficiency was also increased. Permeation studies with dialysis membrane were carried out as per the method reported. The formulations showed good drug release from the lipid, the *in vitro* drug release profiles of all the formulations showed an initial burst effect, and followed by a slow drug release. majority of formulations were governed by Peppas model and mechanism of drug release was anomalous mediated. Regression analysis of the *in vitro* permeation curves was carried out. The Enzalutamide release was faster for those solid lipid nanoparticles with higher drug content.

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