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Preparation, characterization and in vitro evaluation of oxaliplatin solid lipid nanoparticles

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ABSTRACT

Conventional chemotherapy has limitations such as site non-specificity, inability of the drug to penetrate inside the tumor, adverse effects there by reducing the clinical application. So, in this study oxaliplatin solid lipid nanoparticles were prepared by emulsification solvent evaporation technique, 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 oxaliplatin with improved therapeutic efficacy. The Oxaliplatin solid lipid nanoparticles were prepared by emulsification solvent evaporation technique by applying ultrasonic energy through Sonicator, The different formulations with various ratios of drug-lipid and surfactant were evaluated and optimised. The method used for the formulation of Oxaliplatin containing soya lecithin solid lipid nanoparticles was solvent evaporation method followed by sonication to reduce the particle size. The prepared nanosuspensions were characterised for particle size, surface morphology by SEM, drug excipient compatibility by FTIR and *in-vitro* drug release studies. Formulation (F-4) showed the highest encapsulation efficiency. In this research, a drug encapsulation efficiency as high as 93.56 % has been achieved. It was found that as the concentration of soya lecithin increased, the encapsulation efficiency was also increased. The present study revealed that solvent evaporation technique followed by sonication can be used as an effective tool for preparation of Oxaliplatin solid lipid nanoparticles. In conclusion, we demonstrate a preparation and characterization of oxaliplatin immuno nanoparticles for receptor mediated targeting of the drug.

Keywords: Oxaliplatin drug, solid lipid Nano Particles, Solvent Evaporation, lipid, FTIR, invitro drug release.

INTRODUCTION

Nanotechnology-based approach is one of the promising approach in the treatment of cancer due to their ability to enhance the drug delivery inside the tumor imparting intended therapeutic effect and decrease in the adverse effects to healthy cells.[1] Solid Lipid Nanoparticles (SLN) mainly comprise lipids that are in solid phase at the room temperature and surfactants for emulsification, the mean diameters of which range from 50 to 1000nm for colloid drug delivery applications[2] Advantages of SLN are the use of physiological lipids, the avoidance of organic solvents in the preparation process, and a wide potential application spectrum (dermal, oral and intravenous). Additionally, improved bioavailability, protection of sensitive drug molecules from the environment (water, light) and controlled and/or targeted drug release,[2,3] improved stability of pharmaceuticals, feasibilities of carrying both lipophilic and hydrophilic drugs and most lipids being biodegradable.[4] SLNs possess a better stability and ease of upgradability to production scale as compared to liposomes. This property may be very important for many modes of targeting. [5] Oxaliplatin (OP) (oxalate (translate-1,2-diaminocyclohexane) belongs to third-generation anti-tumor compound and is one of the compounds at present used for the first-line chemotherapy along with 5-fluorouracil for the treatment of colorectal cancer in advanced stages.[6]

MATERIALS AND METHODS

Materials

Oxaliplatin was obtained from Arudavis labs private limited (Tamilnadu, India). Phosphotidyl choline and Poloxamer 407 were procured from Vijaya chemicals, Hyderabad and other chemicals and reagents used were of analytical grade.

Methods

Compatibility study (IR spectroscopy)[7]

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

Method of preparation of Oxaliplatin loaded nanoparticles[8]

Oxaliplatin 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 rotary evaporator to remove the organic solvent. Drug embedded lipid layer was then poured into 100 mL of aqueous solution containing poloxomer 407 surfactant and the mixture was Sonicated for 15 minutes by using Sonicator followed by homogenized for 15 minutes at different homogenization speed using high speed homogenizer. The suspension was then allowed to cool at room temperature. The suspension was filtered through membrane filter. The filtrate was centrifuged (1000 rpm for 10 minutes) and nano particles was collected

Table 1: composition of Oxaliplatin for preparation of solid lipid nanoparticles

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Oxaliplatin	50	50	50	50	50	50	50	50
Phosphatidylcholine	50	75	100	125	150	175	200	225
Poloxamer 407	20	30	40	50	-	-	-	-
Tween 20	-	-	-	-	20	30	40	50
Methanol	10	10	10	10	10	10	10	10
Chloroform	20	20	20	20	20	20	20	20

Evaluation of Oxaliplatin loaded nanoparticles:[9,10,11]

Particlesize

All the prepared batches of nanoparticles were viewed under microscope to study their size. Size of Nano particles 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.

SEM analysis

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.

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 Oxaliplatin in nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Oxaliplatin nanoparticles was expressed as loading capacity.

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

In-vitro drug release studies

The release studies were carried out by franz diffusion cell. It containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (100 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 Oxaliplatin 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.

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

Where, Dt = Total amount of the drug

Da = The amount of drug released

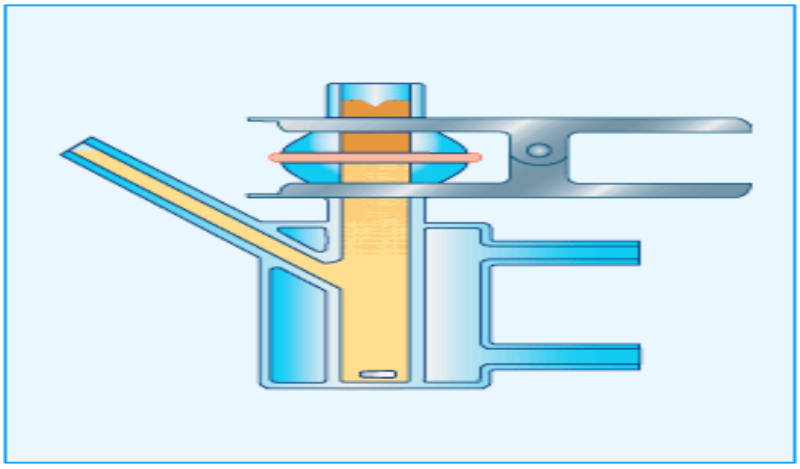

Fig1: Franz diffusion cell

Release kinetics

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 model
2. First order release model
3. Higuchi release model
4. Korsmeyer – Peppas release model

Zero Order Release Equation

The equation for zero order release is

$$Q_t = Q_o + K_o t$$

Where ,

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.

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.

Higuchi Release Equation

The Higuchi release equation is

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

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 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.

Korsmeyer -Peppas Release Equation

Korsmeyer –Peppas equation is

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

Where,
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. 25^0C/60% RH analyzed every month for period of three months.
2. 30^0C/75% RH analyzed every month for period of three months.
3. 40^0C/75% RH analyzed every month for period of three months.

RESULTS & DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using 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.

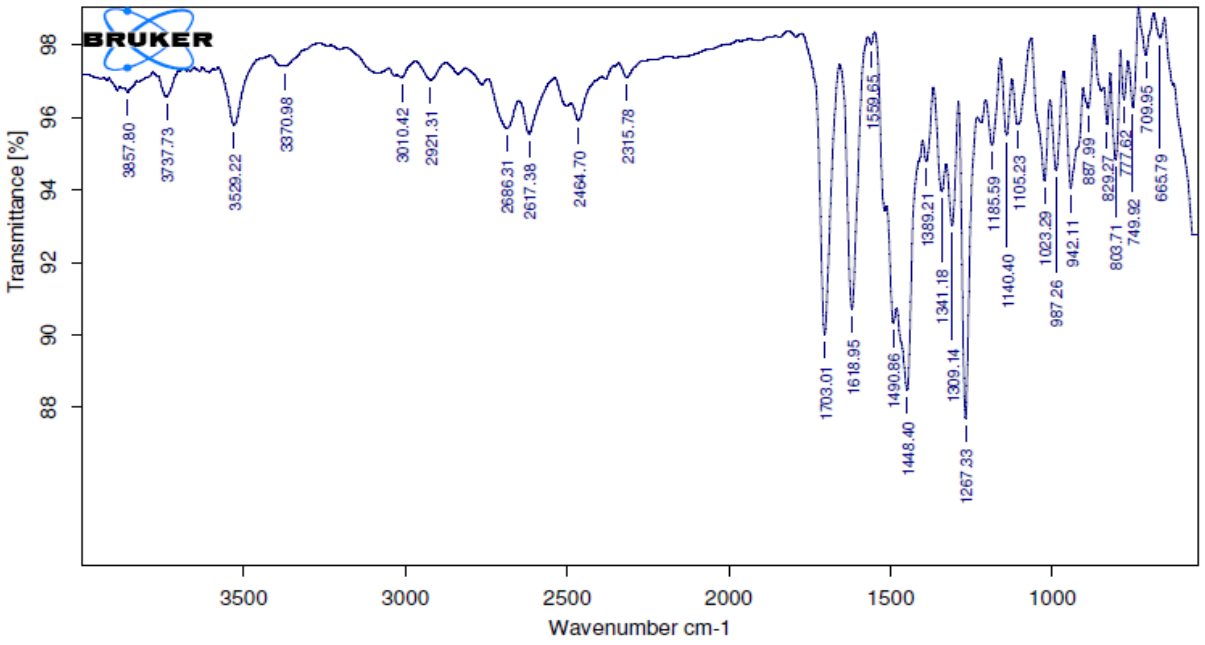

Fig-2: FT-IR Sample for Oxaliplatin

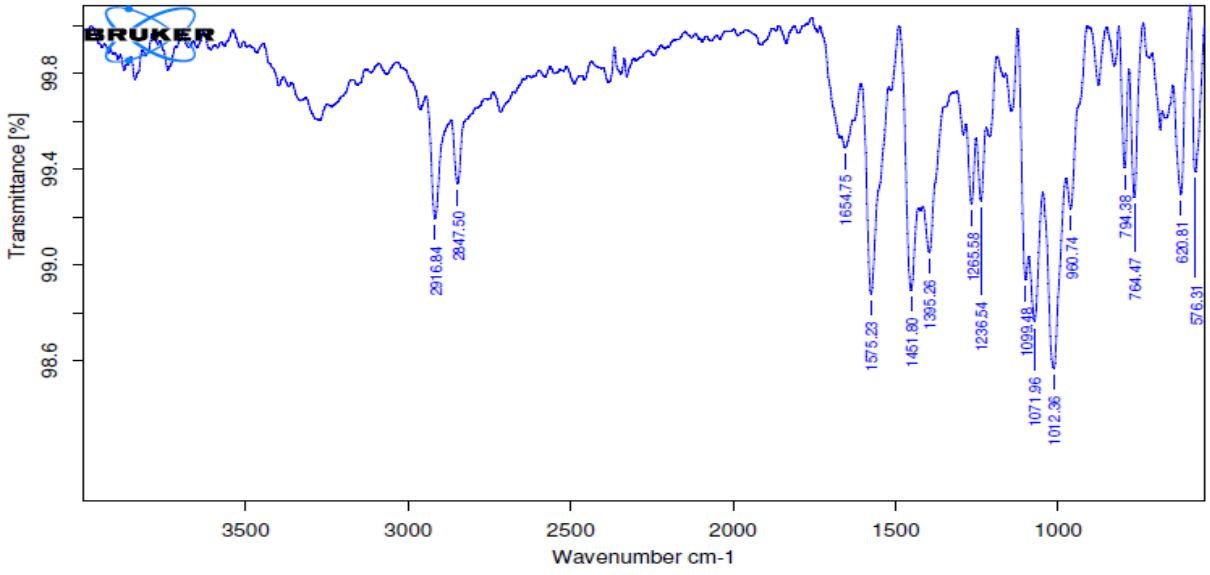

Fig 3: FT-IR Sample for Optimized Formulation

EVALUATION PARAMETERS

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.

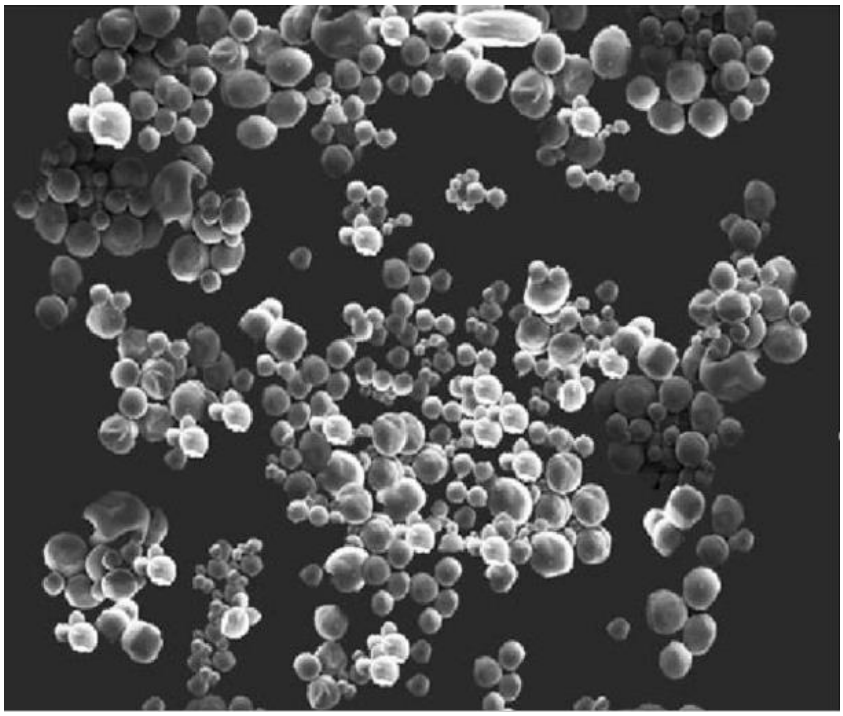

Fig 4: SEM analysis of Optimized solid lipid nanoparticle

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	243	81.32
F2	258	83.61
F3	269	84.12
F4	274	93.56
F5	234	79.38
F6	248	80.14
F7	268	85.36
F8	274	90.32

In vitro drug release studies

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

Table3: In vitro drug release studies of 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
	26.55	25.45	28.55	27.55	24.14	22.32	26.36	25.69
2	33.25	31.26	34.6	33.52	32.56	30.27	31.36	34.65

3	42.82	36.74	41.56	47.21	40.28	44.36	39.49	46.04
4	53.52	54.46	56.57	57.65	52.89	54.32	50.26	54.76
5	62.28	64.85	66.58	67.55	62.28	63.24	68.58	64.68
6	68.25	71.85	73.92	74.42	72.02	74.24	75.18	79.54
7	76.52	82.34	85.12	88.75	84.36	86.32	83.54	83.26
8	89.56	91.55	92.29	97.55	91.26	90.24	93.36	94.56

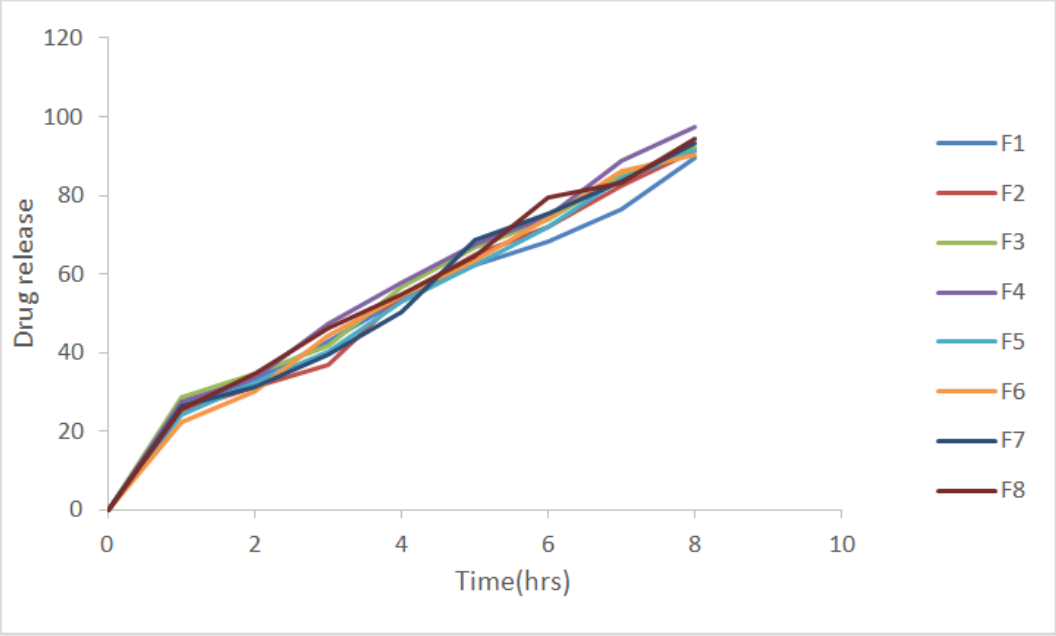

Fig 5: In vitro drug release studies for all formulations

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 three 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 solid lipid nanoparticles. Later on a constant and slow drug release was observed for 8hrs. F4 formulation which had lipid and surfactant ratio was decided to be the optimized formulation.

Release kinetic

Kinetics and mechanism of drug release from all formulation was evaluated on the basis of zero order, Higuchi equation and Pappas model. Correlation coefficient (r^2) and slop value for each equation was calculated from Microsoft excel. Zero order plot for all formulations were found to be linear in both dissolution medium. That indicates it may follow zero order mechanism. Higuchi plot was found to be linear, which indicates diffusion may be the mechanism of drug release for each formulation. Peppas plot was found good linear, n > 0.5 for all formulations, indicated that drug release may follow anomalous diffusion. Zero order plot for F4 formulation was found to be linear in both dissolution medium, it considered as a best fit for drug release.

Zero order kinetics

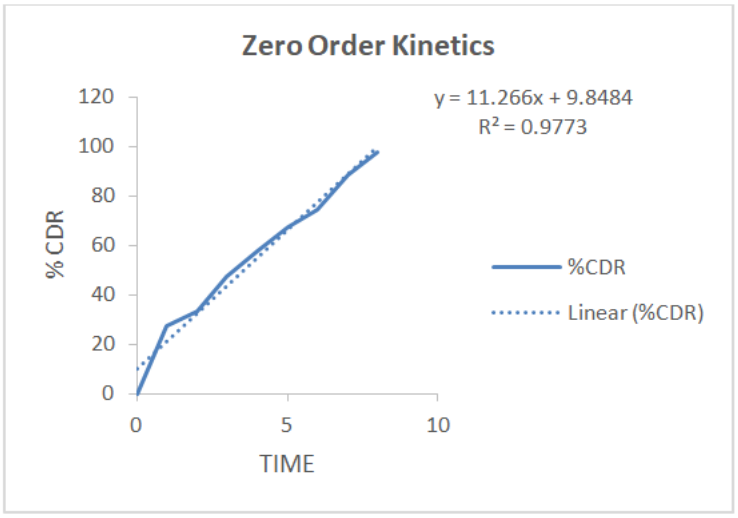

Fig 6: Zero order kinetics

First order kinetics

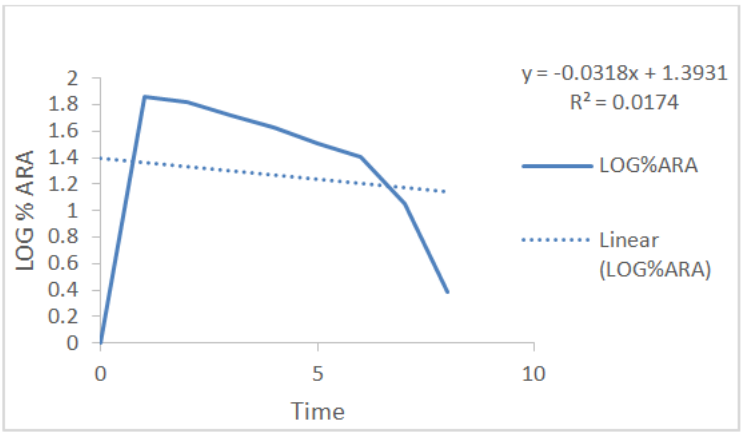

Fig 7: First order kinetics

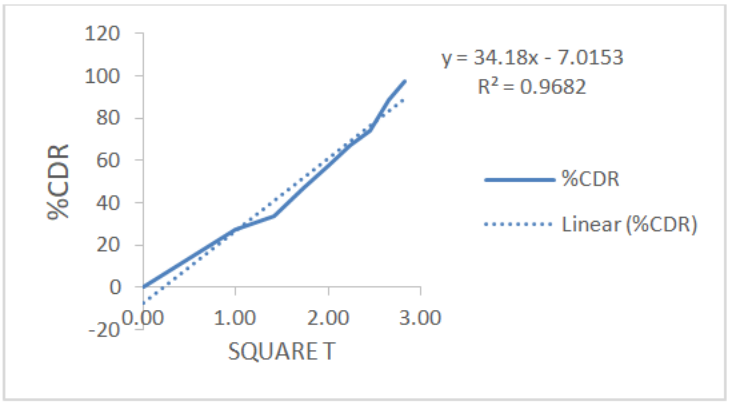

Fig 8: Higuchi model

Krossmayer peppas

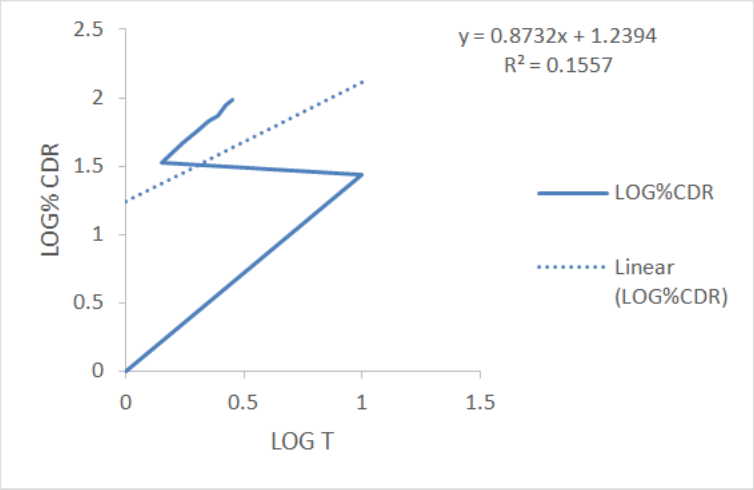

Fig 9: krossmayer peppas

Stability studies

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

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

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	25^0C/60%RH % Release	97.55	96.41	95.38	94.34	Not less than 85 %
F-4	30^0C/75% RH % Release	97.55	96.45	95.37	94.32	Not less than 85 %
F-4	40^0C/75% RH % Release	97.55	96.50	95.35	94.30	Not less than 85 %

CONCLUSION

present research proposed a novel formulation Oxaliplatin 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 optimised. In this research, a drug encapsulation efficiency as high as 94.82% has been achieved. The method used for the formulation of Oxaliplatin 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-4) 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. The Oxaliplatin release was faster for those solid lipid nanoparticles with higher drug content.

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