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DESIGN, PREPARE AND IN VITRO EVALUATION OF NANOEMULSION LOADED DEXAMETHASONE

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ABSTRACT
The objective of the present study was to develop and characterize an optimal stable nanoemulsion formulation of Dexamethasone with an aim to increase its bioavailability. The components for the formulation of nanoemulsion were polymers. Optimized formulation was selected for in vitro study on the basis of higher drug release, optimum globule size, minimum polydispersity value, lower viscosity, and overall lower surfactant concentration and co-surfactant. The diffusion of drug from nanoemulsion was compared with marketed formulation and we obtained better result from it. Thus nanoemulsion could be used effectively to improve the bioavailability of poorly water soluble drugs to improve their bioavailability. The nanoemulsion were optimized optical transparency, viscosity measurement, phase separation determination of pH, measurement of globule size, zeta potential, drug content, in vitro diffusion study, stability study.

KEYWORDS: Dexamethasone, Nano emulsions FTIR Studies, In Vitro Drug Release Studies.

INTRODUCTION
Nano emulsions/Sub-micron emulsions (SMEs)/Mini-emulsions are thermodynamically stable transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a globule size of less than 100 nm.[1] Nano emulsions are formed spontaneously and readily and sometimes generally without high-energy input. In many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase.[2] Nano emulsions are colloidal dispersions composed of an oil phase, aqueous phase, surfactant and cosurfactants at appropriate ratios. Unlike coarse emulsions micronized with external energy Nano emulsions are based on low interfacial tension.[3] This is achieved by adding cosurfactants, which leads to spontaneous formation of a thermodynamically stable Nano emulsion. Corticosteroids binding to the glucocorticoid receptor mediates changes in gene expression that lead to multiple downstream effects over hours to days.[4] Dexamethasone provides relief for inflamed areas of the body. It is used to treat a number of different conditions, such as inflammation (swelling), severe allergies, adrenal problems, arthritis, asthma, blood or bone marrow problems, kidney problems, skin conditions, and flare-ups of multiple sclerosis.[5]

MATERIALS
Dexamethasone was obtained from Alkem Pvt Mumbai, Span 80, Ethyl cellulose and Eudragit RLPO procured from SD fine chemicals Mumbai. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY
Fourier Transform Infrared Spectroscopy
Fourier transform IR spectra were obtained on Shimadzu FT-IR spectrometer. Samples were prepared in KBr disks (2mg sample in 200mg KBr). The scanning range was 450-4000 cm^{-1} and the resolution was 4 cm^{-1}.[6]

Formulation Development

Table 1: Composition of Dexamethasone Nano Emulsion.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Dexamethasone	2	2	2	2	2	2	2	2
Eudragit RLPO	100	200	300	400	-	-	-	-
Ethyl cellulose	-	-	-	-	100	200	300	400
Span 80	2	2	2	2	2	2	2	2
Methanol	50	50	50	50	50	50	50	50
PVP k30	2	2	2	2	2	2	2	2

Preparation Method of Nano emulsion

The drug used to be molten in methanol to prepare an organic solution and fixed Amount. The polymers and surfactant are dissolved in a mentioned quantity of water it is the aqueous phase. The aqueous water is kept under a high- pressure homogenizer (Remi RQ-127) at room temperature. The organic solution is added drop-wise through a syringe to the aqueous solution. Under process of high-pressure homogenizer at a rotation speed of 100 Rpm upto 8 hours. Nano emulsion was formed, Spectacular organic solution used to be gaseous at temperature.[7]

EVALUATION PARAMETERS

Particle Size

All the prepared batches of nanoemulsion were viewed under microscope to study their size. Size of Nano emulsion from each batch was measured at different location on slide by taking a small drop of nanoemulsion dispersion on it and average size of nanoemulsion were determined.[8]

Zeta Potential (ZP) Analysis

The ZP is a measure of the electric charge at the surface of the particles indicating the physical stability of colloidal systems. The ZP values being higher than |30 mV| indicate electrostatic long-term stability of aqueous dispersions. In this study, the ZP values were assessed by determining the particle electrophoretic mobility using ZP equipment (Brookhaven Instruments, Holtsville, NY). The Nano emulsion samples for ZP values analysis were added to the small sample dispersion unit. Three observations were recorded for each sample.[9]

SEM Analysis

Morphological evaluation of Dexamethasone Nano emulsion was conducted by transmission electron microscopy. The samples were placed over a copper grid coated with carbon film and air-dried, and then were stained with 0.1% phosphotungstic acid. Finally, the samples were air dried and then observed with an H-7650 transmission electron microscope (Hitachi Ltd, Tokyo, Japan).[10]

Drug Encapsulation Efficiency

Precisely weighed 100mg of Dexamethasone Nano emulsion were suspended in 100ml of phosphate buffer (pH 7.4) and kept in sonication for 2hrs. Then the samples were centrifuged at 1000rpm for 20mins to remove the supernatant layer, if any. The samples were filtered. From this filtered solution 1 ml of sample was withdrawn and diluted to 100 ml with phosphate buffer (pH 7.4). Then it was analyzed spectrophotometrically at 260nm.[11]

Dissolution study

In vitro release of Dexamethasone Nano emulsion was conducted by a Franz diffusion cell apparatus. The dialysis membrane having a pore size of 2.4 mm with 10 ml of pH 7.4 phosphate buffer at 37°C. Briefly in a 10 ml beaker 10 ml of pH 7.4 phosphate buffer was taken. A 2 ml of formulation was taken into a dialysis bag and dipped into the buffer solution. The flask was kept on a magnetic stirrer. Stirring was maintained at 300 rpm and the temperature of the buffer was maintained at 37°C. Sampling was done by withdrawing 1 ml of aliquots from a beaker. Immediately 1 ml of new buffer was added to keep the sink condition. Samples were analyzed after sufficiently diluting with buffer by using a UV/Spectrophotometer at a wavelength of 280 nm. Each test was conducted thrice and average value taken for the calculation.[12]

Drug Release Kinetics[13]

The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and Korsmeyer Peppas model (equation 4).

i) Zero Order Kinetics:

$R = K_o t$ -- (1)

R=cumulative percent drug

Ko=zero order rate constant

ii) First Order Kinetics

$\log C = \log C_o - K_1 t / 2.303$ -- (2)

Where C = cumulative percent drug

K_1= first order rate constant

iii) Higuchi Model

$R = K_H t^{0.5}$ -- (3)

Where R = cumulative percent drug

K_H = higuchi model rate constant

iv) Korsmeyer Peppas Model

$M t / M \alpha = K_k t^n$

$\log M t / M \alpha = \log K_k + n \log t$ -- (4)

Where K_k = Korsmeyer Peppas rate constant

'M t / M α' is the fractional drug, n = diffusional exponent, which characterizes the mechanism of drug.

The obtained regression co-efficient (which neared 0.999) was used to understand the pattern of the drug from the Nano emulsions.

Stability Study[14]

Storage stability was studied by storing the lyophilized Nano emulsion samples at 4°C and room temperature for 3 months. Periodically, samples were removed, and the particle size and ZP were measured. In addition, Dexamethasone stability in the Nano emulsion was examined by determining the amount of parent drug remaining after specific storage periods.

RESULTS AND DISCUSSION

FTIR Studies

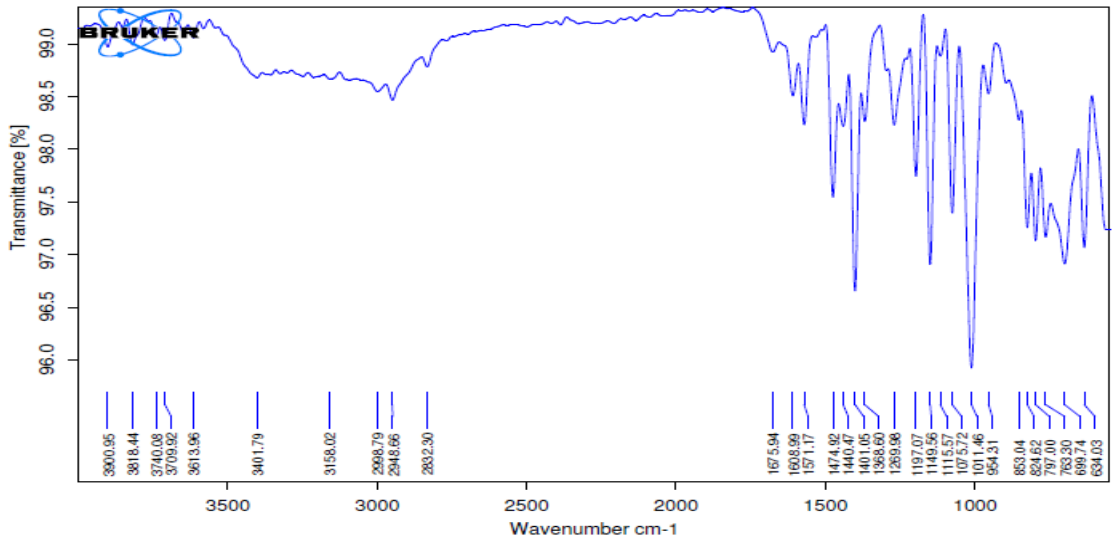

Fig-1: FT-IR Graph for Pure Drug.

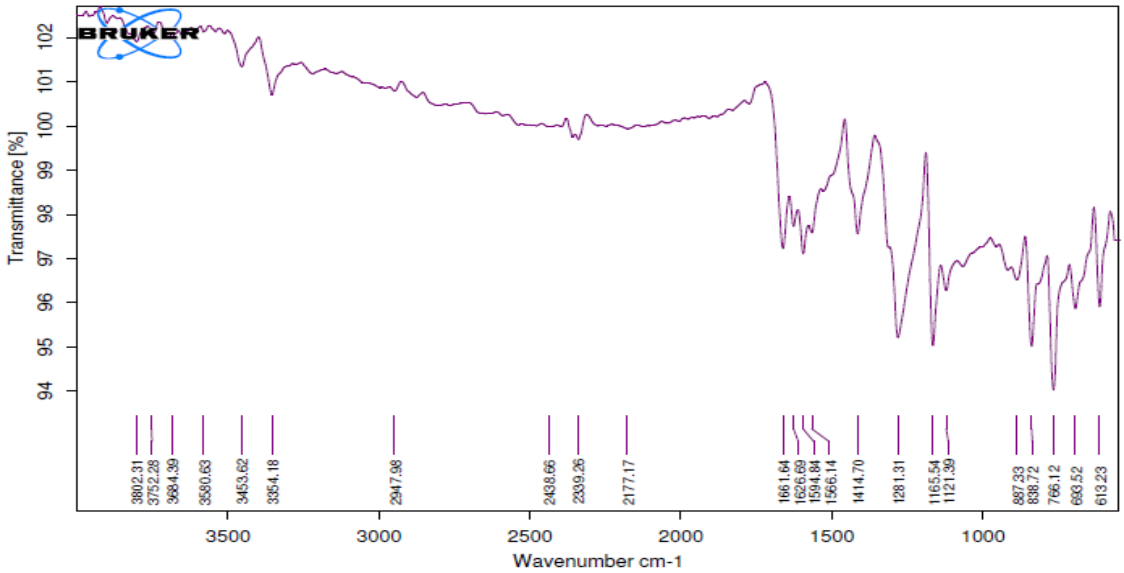

Fig-2: FT-IR Graph for Optimised Formulation.

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks was obtained as above and as they were in official limits (±100 cm^{-1}) the drug is compatible with excipients. The compatibility between the drug and the selected polymer and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-polymer mixture, which confirmed the absence of any chemical interaction between the drug, polymer and other ingredients used.

EVALUATION PARAMETERS

Entrapment Efficiency

Separation of unentrapped drug from Nano emulsion was done by exhaustive dialysis method. A measured quantity of Nano emulsion was placed in a dialysis tube to which osmotic cellulose membrane was attached securely on one side and the dialysis tube was suspended in 100ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer. Through the osmotic cellulose membrane the unentrapped drug was separated into the medium. For every one hour the whole medium was replaced with same quantity of fresh medium and continued for about 9 to 12hrs till the absorbance of collected medium reaches a constant reading indicating complete separation of unentrapped drug. The Nano emulsion in the dialysis tube was further lysed with propane–1–ol and the entrapped drug was estimated with the help of double beam UV spectrophotometer at 315 nm. The entrapment efficiency was measured in % with the help of following equation,

$$\%\text{Entrapment efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} X\ 100$$

Table 2: Evaluation of Entrapment Efficiency.

F. No.	Drug Entrapment Efficiency
F1	75.89
F2	74.51
F3	79.86
F4	80.34
F5	76.89
F6	73.54
F7	79.35
F8	78.22

Determination of Vesicle Morphology and Size

Sample was coated with gold and allowed the SEM to capture the images at a temperature of - 120^0c and voltage of 5kV.

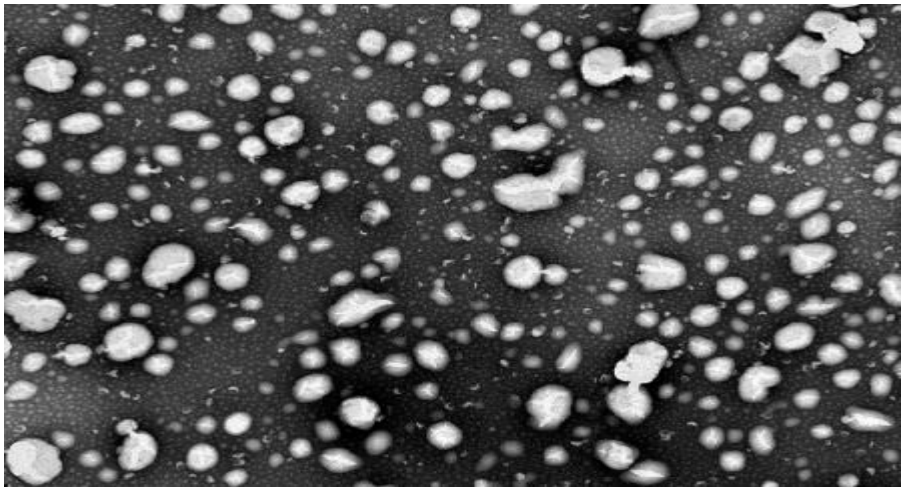

Fig-3: SEM Analysis of Optimized Nano Emulsion.

The SEM photomicrographs of the nanoparticles are shown in Figures, the morphology of the prepared different types of Nano emulsion was found to be almost spherical in shape and have rough surface. The mean particle size of the different formulations of the prepared Nano emulsion was between 185 to 198 nm, it was observed that the particle size increase with increasing in the concentration of polymer and surfactant ratio as shown in the formulations that contain the highest ratio of polymers.

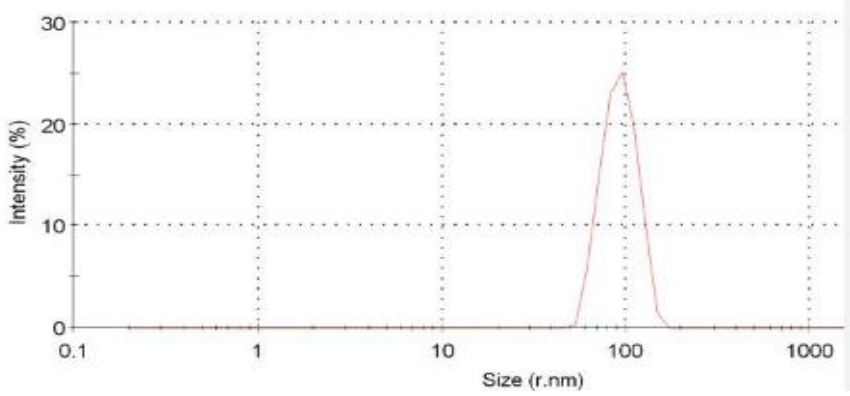

Fig-4: Particle Size Analysis of Optimized Nano Emulsion.

Zeta Potential

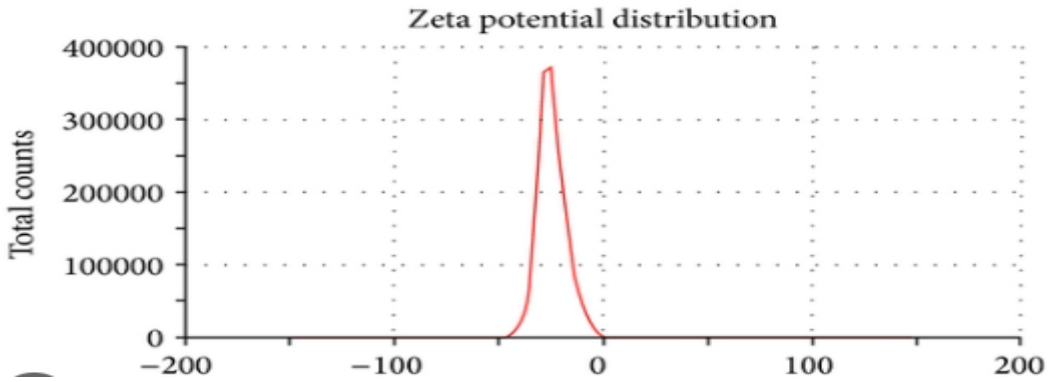

Fig-5: Zeta Potential of Dexamethasone Nanoemulsion.

Table 3: Evaluation Studies of Dexamethasone Nano emulsion Particle Size and Zeta Potential.

F. No.	Particle Size (nm)	Zeta Potential
F1	256	-25
F2	301	-35
F3	279	-28
F4	246	-32
F5	254	-35
F6	263	-38
F7	270	-29
F8	284	-36

In Vitro Drug Release Studies

Table 4: Results of Dexamethasone Nano Emulsion of all Formulations.

Time (hours)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	13.65	15.52	14.89	15.10	14.97	13.69	16.35	14.98
2	25.96	23.86	26.87	21.83	20.17	23.45	25.17	24.56
3	32.15	30.45	32.95	30.45	32.94	33.82	34.91	32.19
4	43.96	42.85	43.72	42.85	40.27	42.18	43.75	40.30
5	52.18	54.94	56.90	55.16	53.86	53.49	55.48	52.48
6	60.79	63.89	67.82	66.12	61.15	63.48	69.41	70.15
7	75.16	78.90	81.95	79.18	77.58	79.27	80.18	79.18
8	89.68	90.12	92.35	93.47	91.59	92.14	90.88	91.47

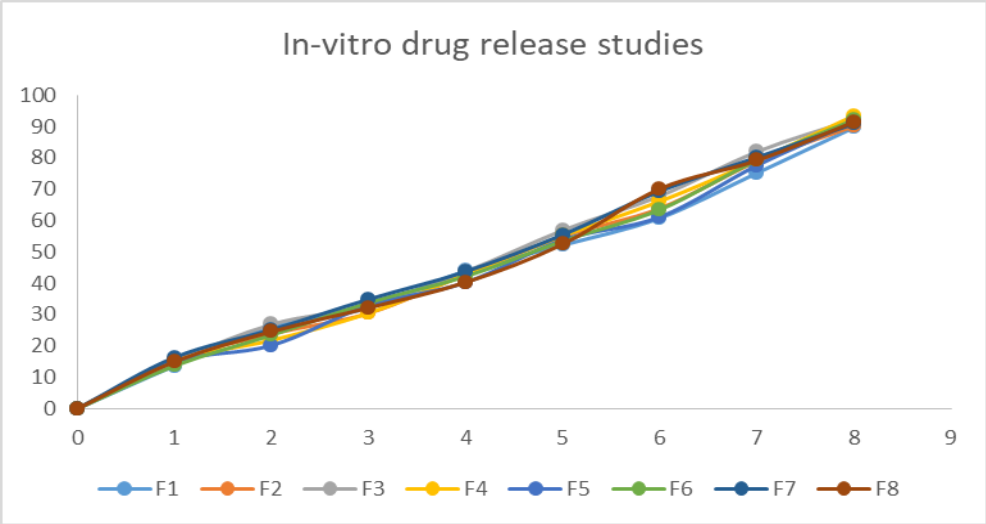

Fig-6: Drug Release Studies of all Formulation.

Among the four types of nanoemulsion highest amount of release percentage i.e., 93.47 % was found for Dexamethasone nanoemulsion.

Drug Release Kinetics

Table 5: Drug Release Kinetics of Formulation F4.

TIME	%CDR	SQARE T	LOG T	LOG%CDR	ARA	LOG%ARA
0	0	0	0	0	0	0
1	15.1	1	0	1.17897695	84.9	1.92890769
2	21.83	1.41421356	0.30103	1.33905374	78.17	1.89304011
3	30.45	1.73205081	0.47712	1.4835873	69.55	1.84229713
4	42.85	2	0.60206	1.63195083	57.15	1.75701623
5	55.16	2.23606798	0.69897	1.74162426	44.84	1.6516656
6	66.12	2.44948974	0.77815	1.82033284	33.88	1.5299434
7	79.18	2.64575131	0.8451	1.8986155	20.82	1.31848073
8	93.47	2.82842712	0.90309	1.97067224	6.53	0.81491318

Zero Order Kinetics

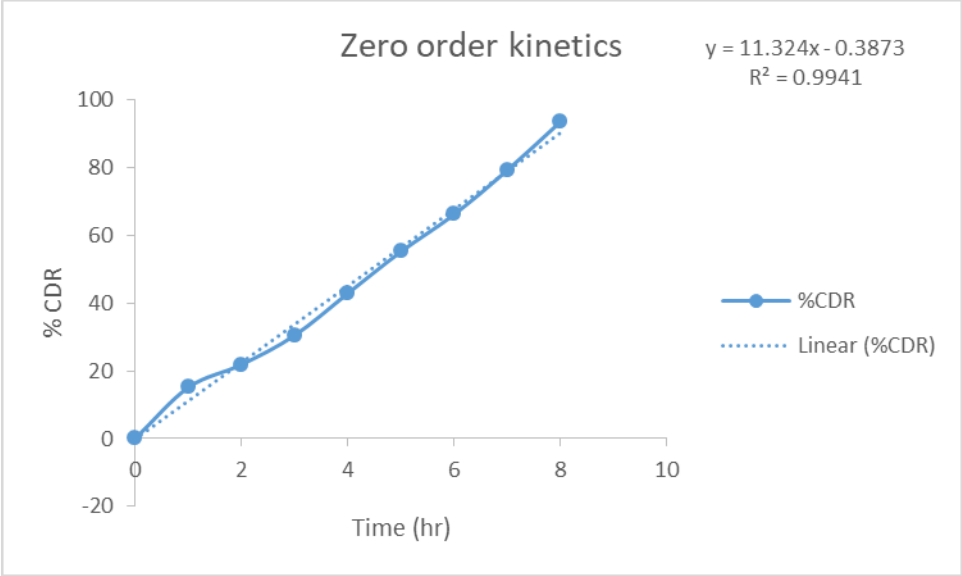

Fig-7: Zero Order Kinetics of Optimized Formulation.

First Order Kinetics

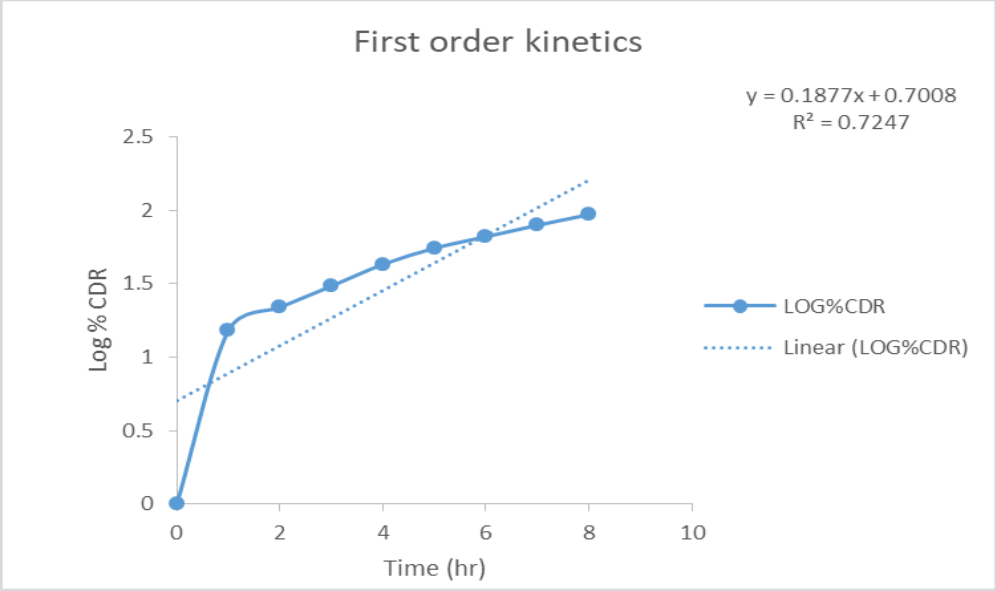

Fig-8: First Order Kinetics of Optimized Formulation.

Higuchi Model

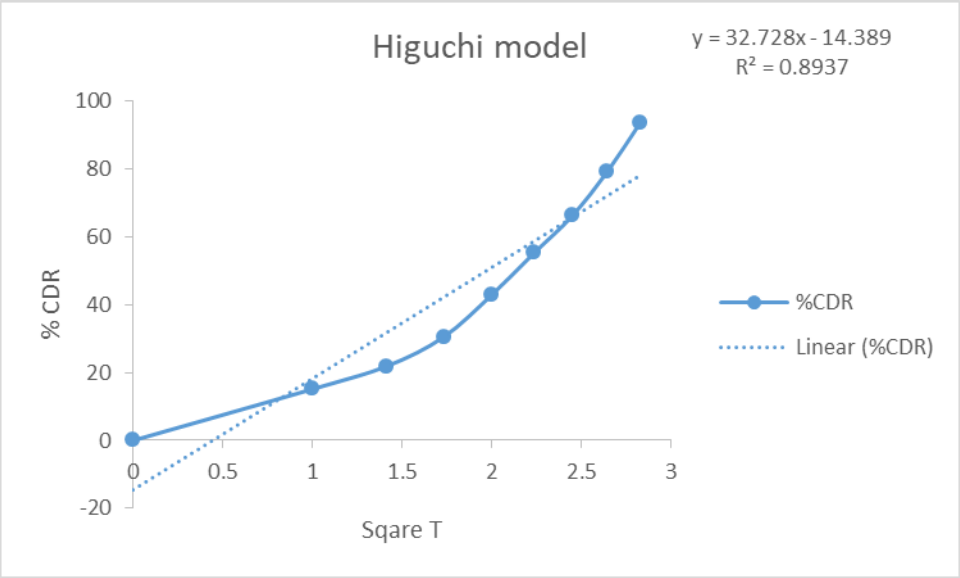

Fig-9: Higuchi Model of Optimized Formulation.

Korsmeyer Peppas Model

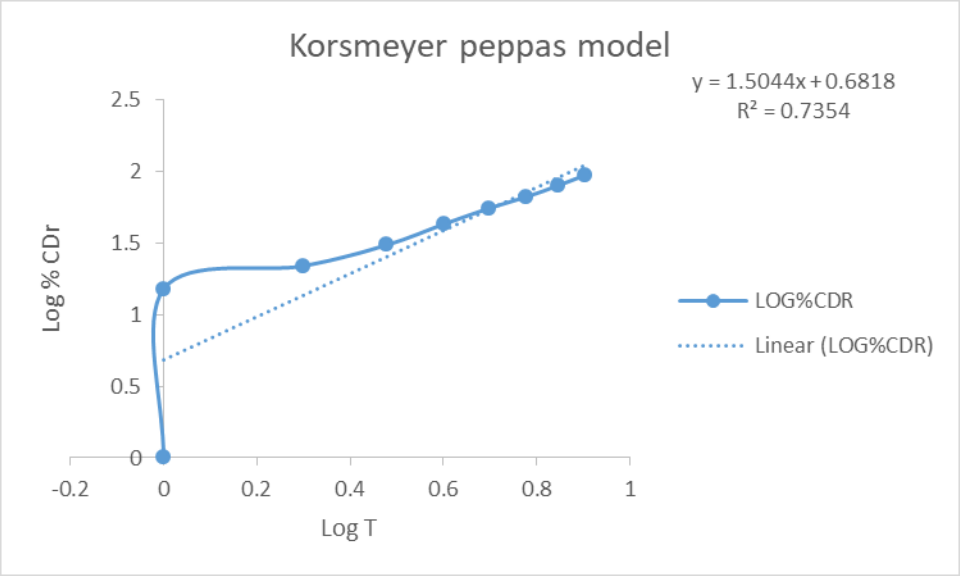

Fig-10: Korsmeyer Peppas of Optimized formulation.

Stability Studies

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

Table-6: Stability Studies of Optimized Formulations.

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	25^0C/60%RH % Release	93.47	92.68	91.69	90.85	Not less than 85 %
F-4	30^0C/75% RH % Release	93.47	92.58	91.58	90.32	Not less than 85 %
F-4	40^0C/75% RH % Release	93.47	92.46	91.40	90.20	Not less than 85 %

CONCLUSION

The present in vitro study revealed that the dexamethasone Nano emulsion can be a useful means for the targeted delivery of drugs. This work suggests the dexamethasone Nano emulsion of the size ranging from 246 nm -301 nm and their solutions are stable at neutral pH. The prepared Nano emulsion found to be stable without any tendency of aggregation and shown higher entrapment efficiency. The dissolution data indicates that the release of dexamethasone Nano emulsion with controlled manner is directly proportional with the size. Therefore, nanosuspension release increased with smaller size of particles. In this study, the prepared dexamethasone Nano emulsion exhibited prolonged intestinal absorption, and prevents gastric release, avoid gastric erosion side effects and thus improve patient compliance.

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