DEVELOPMENT AND IN VITRO EVALUATION OF ZINC OXIDE NANOPARTICLES LOADED WITH PACLITAXEL

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ABSTRACT

This study aimed to investigate the effectiveness of a strategy based on the development of Zinc oxide Nanoparticles as an innovative formulation of paclitaxel with improved therapeutic efficacy. Paclitaxel Zinc oxide Nanoparticles were prepared by sol to gel method. FTIR studies indicated no interaction between drug and Zinc nitrate. Zinc oxide nanoparticles were characterized for particle size, zeta potential, entrapment efficiency and surface morphology. *In vitro* drug release studies were performed in phosphate buffer of pH 7.4 using dialysis bag diffusion technique. The F8 batch had shown maximum entrapment up to 90.22% and sustained drug release for 8 h. The scanning electron microscopy and zeta potential study showed formation of good zinc oxide nanoparticles dispersion. *In vitro* release profiles were biphasic in nature and followed Higuchi model of release kinetics. The stability study showed successful formation of stable zno nanoparticles.

Keywords: Paclitaxel, Zno Nano Particles, sol to gel technique, FTIR, in-vitro drug release.

INTRODUCTION

Nanotechnology deals with the manufacturing of materials with nanometer dimensions and their uses in different fields around us.[1] Nanoparticles (NPs) have attracted the attention of scientists in recent years because of their high efficacy and safety. Among all NPs, zinc oxide nanoparticles (ZnO NPs) are one of the most exploited candidates in drug delivery, cancer diagnosis, and treatment due to their unique physical and chemical properties.[2] ZnO NPs are not only used in fighting cancer, but also proved to be very efficient in fighting many other diseases and in a variety of other sectors such as cosmetics, electronics, and the textile industry as well.[3] ZnO NPs have a high biocompatibility, allowing it to be used in a therapeutic environment for antibacterial, antifungal, antiviral, and anticancer properties.[4] Paclitaxel (PX), isolated from the bark of Pacific Yew. It is one of the most effective chemotherapeutic drugs and is mainly used to treat lung, ovarian, and breast cancer, etc. The mechanism of action of PX is to promote and stabilize microtubules and inhibit late G2 or M phases of cell cycle, thereby causing the cell death.[5]

MATERIALS

Paclitaxel was obtained from Hetero Labs, Zinc nitrate, Eudragit and Carbopol 934 procured from Synpharma Research Labs, HYD. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY

Compatibility study (IR spectroscopy)[6]

Fourier Transform Infrared Spectroscopy (FTIR) is an important technique that provides an easy way to identify the presence of certain functional groups in an organic molecule. Functional groups have vibration frequencies that are characteristic of that functional group. These vibration frequencies fall with the infrared (IR) frequency range. As such, passing an IR signal through the organic compound causes the functional groups to vibrate at specific frequencies. In other words, an infrared signal that passes through an organic compound will be absorbed at these characteristic frequencies, which can be transformed into a unique spectrum.

The sample is placed in a holder in the path of the IR source. A detector reads the analog signal and converts the signal to a spectrum. A computer is used to analyze the signals and identify the peaks. An IR beam goes through a partially silvered mirror, which splits the beam into two beams of equal intensity.

Formulation development[7]

Sol-to-gel method

Two beakers containing 50ml of deionized water in each of them. Let's mark these beakers as 1 and 2. In the next step, we will add zinc nitrate with polymers (Ethyl cellulose and Eudragit) in beaker 1 and will place a magnetic pellet within the solution and the beaker 1 is then placed on the magnetic stirrer. Solution 1 in beaker 1 is thus stirred for around 5 minutes. Similarly, we will add sodium hydroxide and Paclitaxel (drug) to beaker 2 with a magnetic pellet in it and will place it on the magnetic stirrer. Solution 2 is also stirred for around 5 minutes. The solution placed in the beaker gets stirred fast due to the rotating magnetic field generated. Solution 2 consisting of sodium hydroxide is then added to solution 1 consisting of zinc nitrate hexahydrate. While adding solution 2 into solution 1, we should add it dropwise using a syringe that allowing the solutions to mix properly and hence initiate the mechanism or reaction. Also, the entire process of stirring takes place at normal room temperature. On adding the solution 2 dropwise using a syringe, we will be able to see a slight difference in the color of the mixture. The mixture will be turning into white color slowly as we add the solution 2 dropwise. This

transition of the solution from transparent to opaque shows the initiation of the chemical reaction. Thus, after the complete addition of solution 2 into solution 1 we can notice that the mixture appears cloudy. Thus, the resultant solution 3 is stirred for another 2 hours on the magnetic stirrer. After stirring it for 2 hours the solution 3 is transferred into the centrifuge tube. Solution 3 thus undergoes a process called centrifugation where the precipitate is removed or filtered out and we obtain the gel at the end of the process. Around 3 rounds of centrifugation is done. After the first round, the centrifuge tubes are filled with deionized water up to the marking and then is placed in the centrifuge machine again. After doing two rounds of centrifugation using deionized water, we will now use ethanol and centrifuge it. Finally, we will obtain the gel as the residue. Here also the entire process of centrifugation takes place at the normal room temperature i.e 28 °C. The gel obtained as the residue of the centrifugation process is then spinned by keeping on a spinner. This is done to displace the gel stuck at the bottom of the centrifuge tube. Also while doing this a small amount of ethanol is added. Thus, the gel is then finally taken into a petridish and is broken using a spatula. The petridish containing the gel is then covered using an aluminum foil and tiny perforations are made over this aluminium foil covering the petridish. The petridish containing the gel is placed overnight to dry. Once dried the petridish is placed in a hot air oven for 10 minutes and is then placed in the muffle furnace at a temperature of 500°C for 3 hours. This process of heating is called calcination. After undergoing calcination the particles on the petridish are transferred to a mortar and are finely grinded into a powder form. The finally obtained powdered particles are then transferred into tubes and stored. To determine if the synthesized material is ZnO nanoparticles we will have to use various characterization methods.

Table-1: Formulation development

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Paclitaxel	200	200	200	200	200	200	200	200
zinc nitrate	100	100	100	100	100	100	100	100
Carbopol 934	50	100	150	200	-	-	-	-
Eudragit	-	-	-	-	50	100	150	200
Sodium hydroxide	50	50	50	50	50	50	50	50
Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of Paclitaxel loaded Zno nanoparticles:

Particle size:

All the prepared batches of Zno 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 Zno nanoparticles were determined.[8]

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.[9]

Drug encapsulation efficiency:

Lyophilized Zno 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 Paclitaxel in Zno nanoparticles to the theoretical amount of the drug used in the preparation .The entrapment of the Paclitaxel Zno nanoparticles was expressed as loading capacity.[10]

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

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 ± 5^0C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped Paclitaxel 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.[11]

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

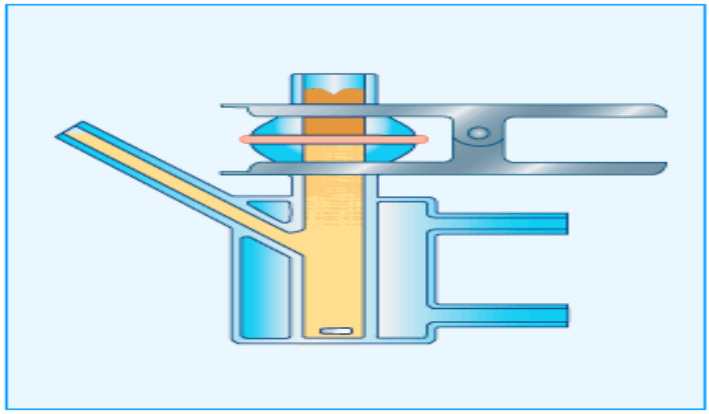

Fig-5: Franz diffusion cell

Drug release kinetics:[12]

The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and KoresmeyerPeppas 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) korsermeyer peppas model:

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

$$\log M t / M \alpha = \log K_k + n \log t \quad \text{-- (4)}$$

where

K_k = korsermeyerpeppas 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 Zno nanoparticles.

Stability studies:[13]

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 AND 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.

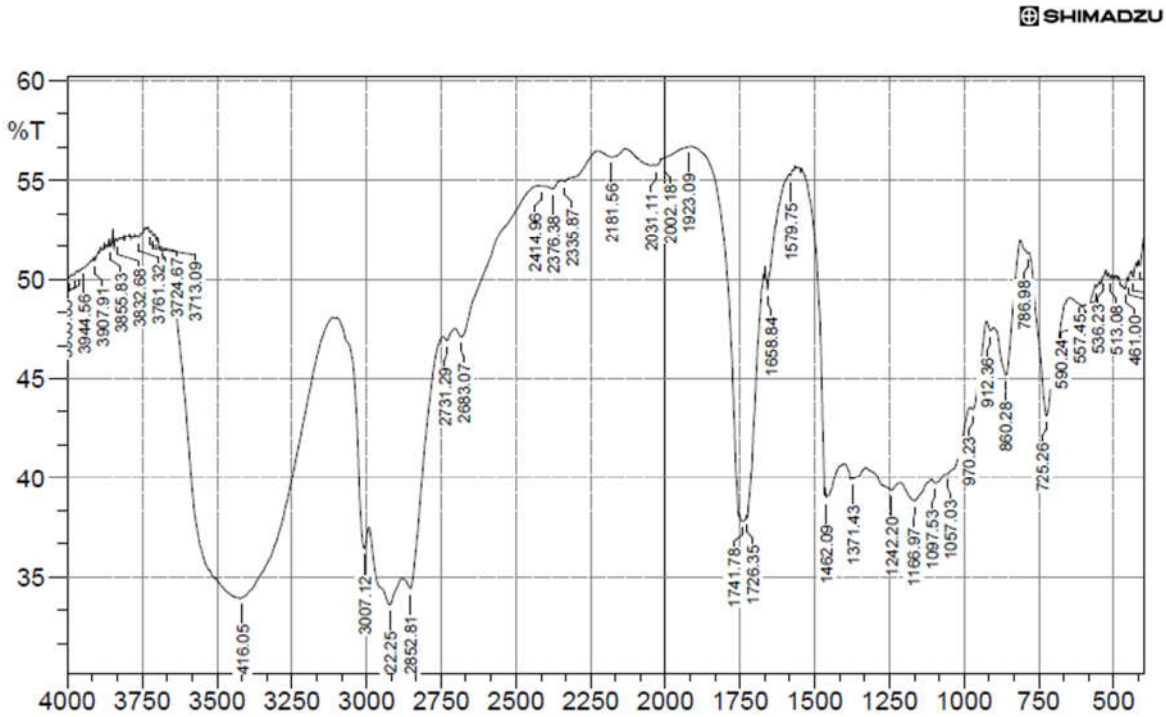

Fig-1: FTIR Studies of Paclitaxel

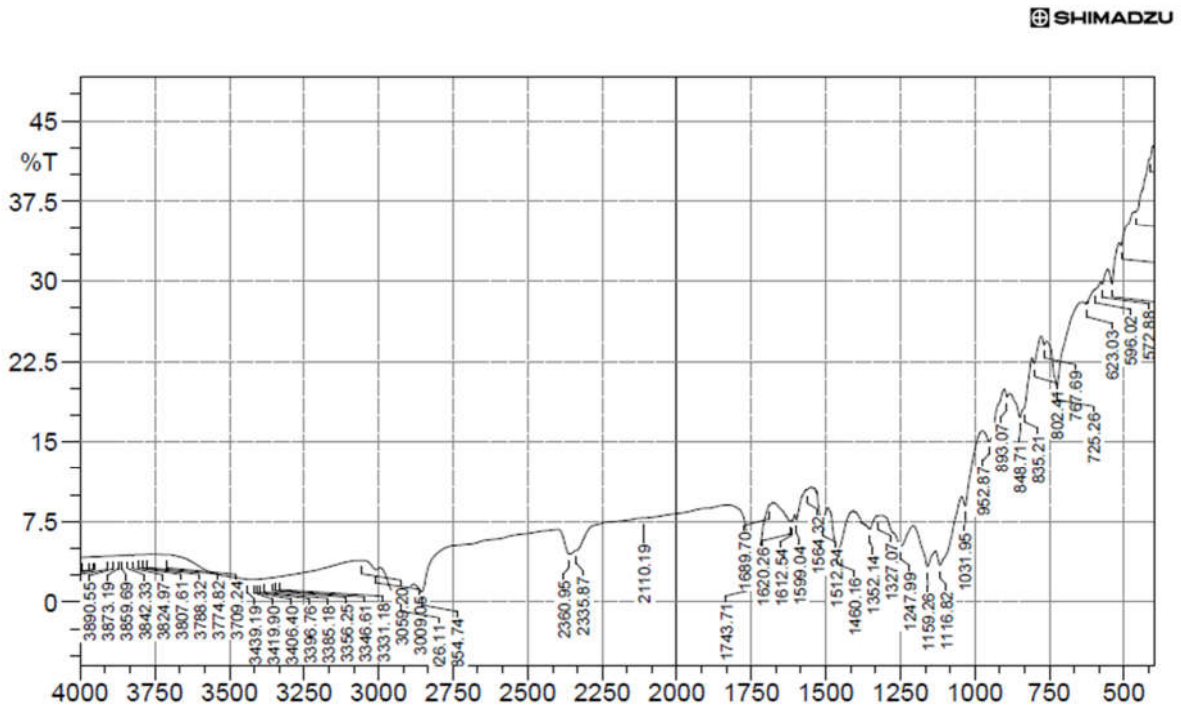

Fig-2: FTIR Studies of optimized formulation

Scanning Electron Microscopy:

The surface characteristic of prepared crystal was studied by SEM (ZEISS Electron Microscope, EVO MA 15). Powder samples was mounted onto aluminum stub using double sided adhesive tape and sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode.

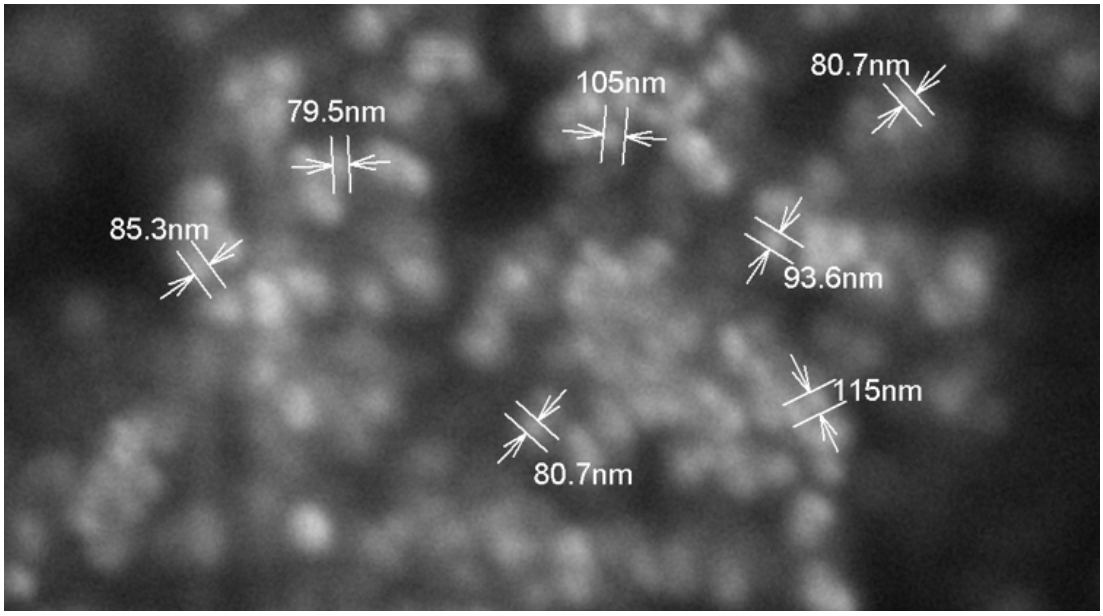

Fig-3: SEM Analysis of Zno nanoparticle

Determination of Zeta potential:

Zeta potential is a measure of charge present on the vesicle surface. It was determined by using phase analysis light scattering with Malvern zetasizer at field strength of 20V/cm in distilled water and based on electrophoretic mobility of charged particles present in the nanocarrier system. Charged particles were attracted to the electrode with the opposite charge when an electric field is applied.

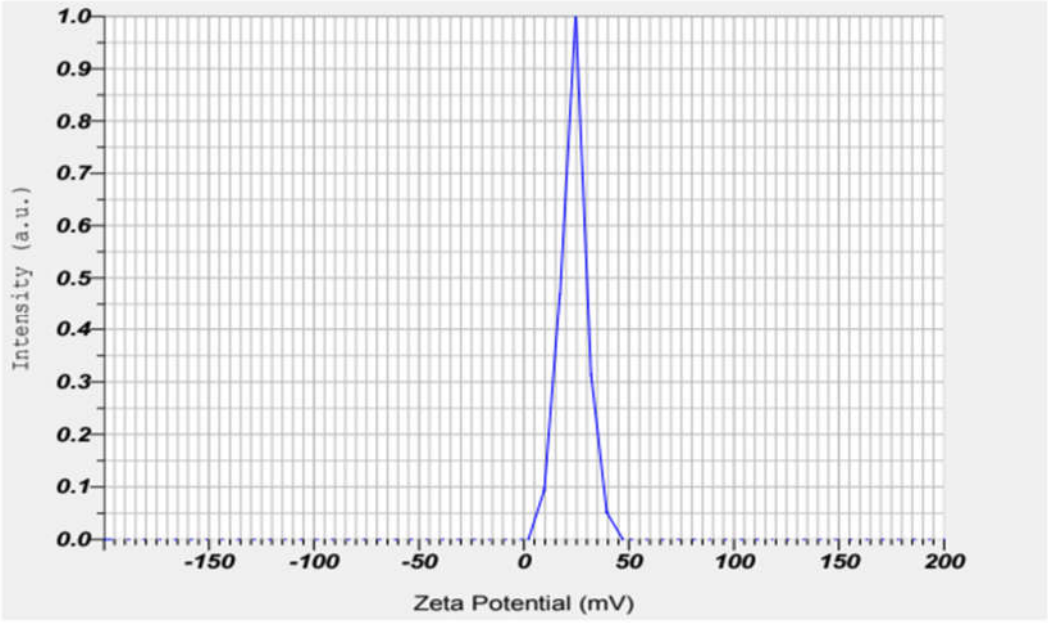

Fig-4: Zeta potential of Optimized formulation

Zeta potential

The addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized Paclitaxel Zno nanoparticles formulation was measured and found to -38 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.

Particle size

In general, particle size was with a diameter of < 92.68 nm. The surfaces of the Zno nanoparticles were smooth.

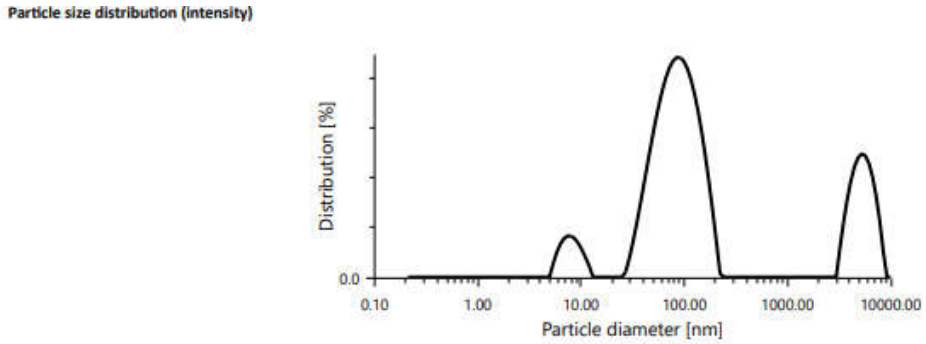

Fig-5: Particle size of optimized formulations

Characterization of Zno nanoparticles of Paclitaxel

Table-2: Evaluation Studies of particle size Zno nanoparticles

F. no	Particle size (nm)	Entrapment Efficiency (%)	Zeta Potential(mV)
F1	100.2	80.24	-22.40
F2	96.85	81.53	-30.16
F3	93.48	84.62	-38.37
F4	90.74	83.25	-38.52
F5	99.65	90.23	-40.21
F6	89.69	92.27	-42.10
F7	92.68	82.65	-32.25
F8	102.13	90.22	-38.90

Entrapment efficiency

The drug entrapment efficiency of all 8 formulations was evaluated. From the F6 formulation showed maximum drug entrapment efficiency 90.22% compared to other formulations. The zeta potential or the change on the surface of colloidal particles in Paclitaxel Zno nanoparticles was measured by electrophoretic light scattering mode using zetasizer Nano ZS. The particle charge of Paclitaxel Zno nanoparticles were quantified at 25^{o} C. The samples were diluted approximately with the deionized water for the measurements of particle size.

In vitro drug release studies

Table-3: In vitro drug release studies of all formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	12.36	11.20	11.23	10.24	11.22	12.39	13.10	14.22
2	23.50	25.62	24.32	30.26	22.37	26.68	29.50	28.63
3	42.68	40.39	38.92	37.39	35.90	41.55	45.20	44.30
4	53.16	52.35	50.16	49.20	48.37	52.13	55.92	54.28
6	60.21	62.39	63.91	62.33	54.92	58.30	57.95	55.23
8	72.35	75.68	73.24	72.56	70.15	72.50	71.16	70.15
10	86.92	85.34	84.32	81.20	81.22	81.35	87.39	88.40

12	95.30	94.31	93.67	95.12	94.68	94.99	96.30	97.19

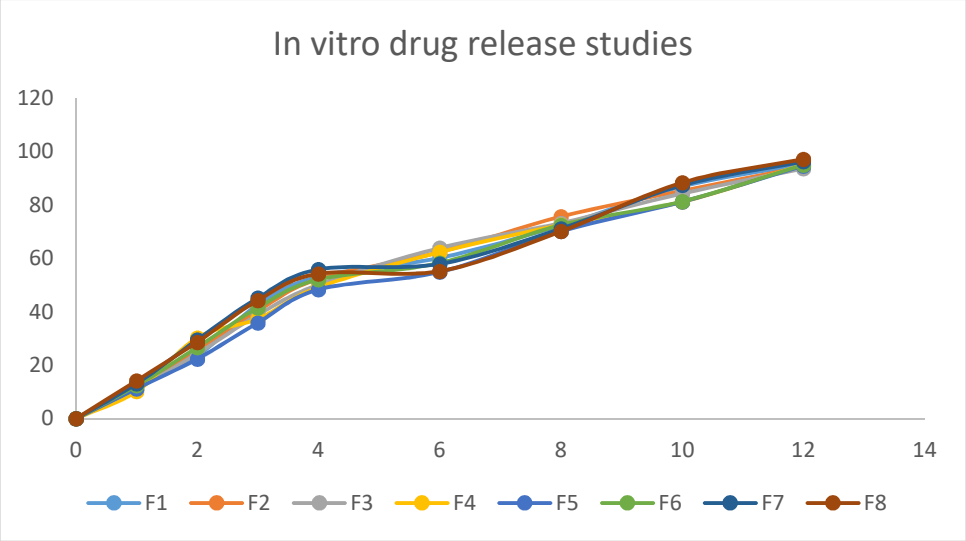

Fig-6: In vitro drug release studies of (F1-F8) formulations

The drug release studies of all formulations of Paclitaxel Zno nanoparticles were conducted by means of diffusion apparatus for a time period of 8 hrs. From the drug release studies as depicted in Figure, the results showed that 8 formulation showed maximum drug release rate of 97.19 % within 12 hrs.

Drug release kinetics

The release kinetics for all the prepared Zno nanoparticles was evaluated to determine the release behaviour of Paclitaxel from the prepared Zno nanoparticles. The release data were analyzed with zero-order kinetic, first-order kinetic, and Korsmeyer–Peppas kinetic models, as well as the Higuchi kinetic model. It was revealed that the release data from Zno nanoparticles fit to Higuchi kinetic model with the highest (r) value, while for free Paclitaxel Zno nanoparticles, the release data fit the zero order kinetic model.

In vitro drug release kinetics

Zero order kinetics

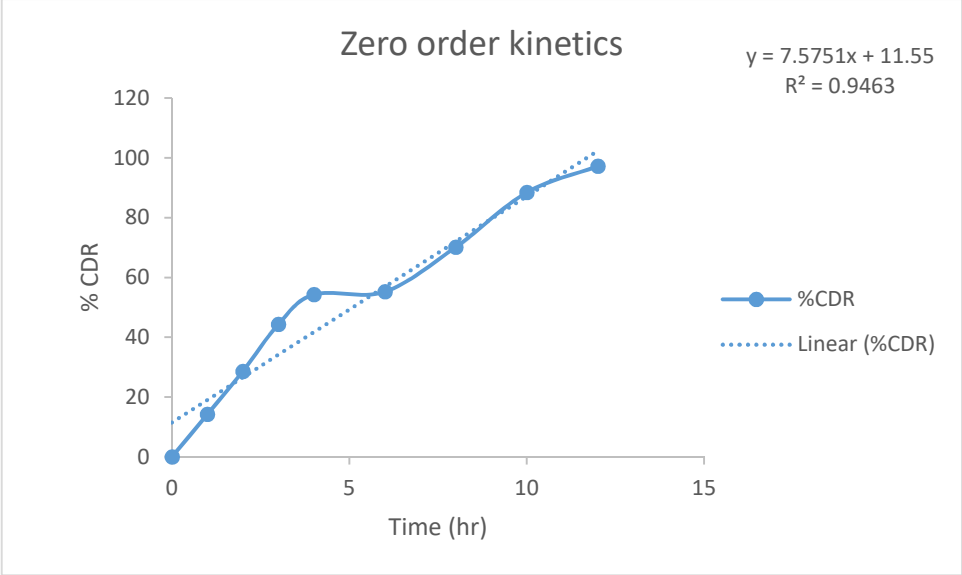

Fig-7: Zero order kinetics of optimized formulation

First order kinetics

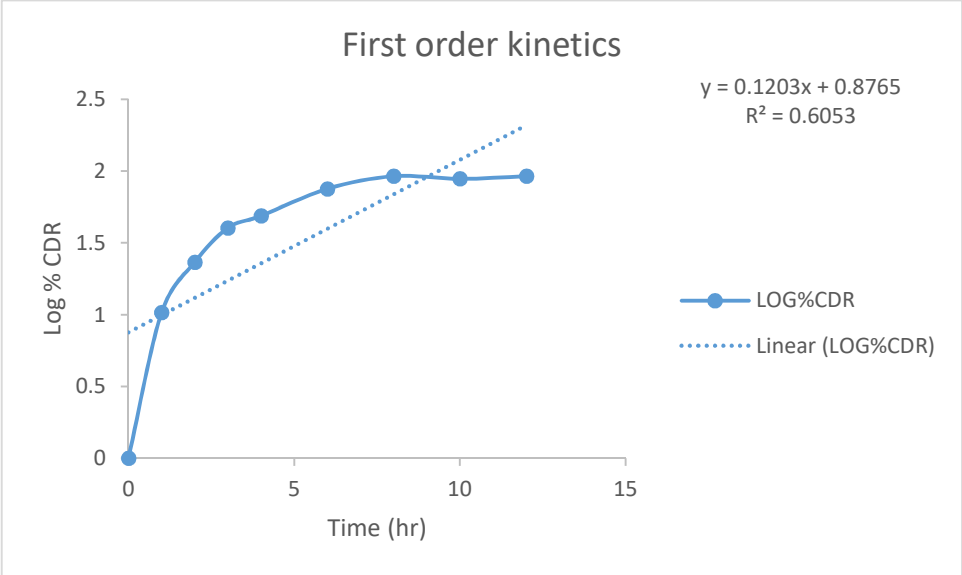

Fig-8: First order kinetics of optimized formulation

Higuchi model

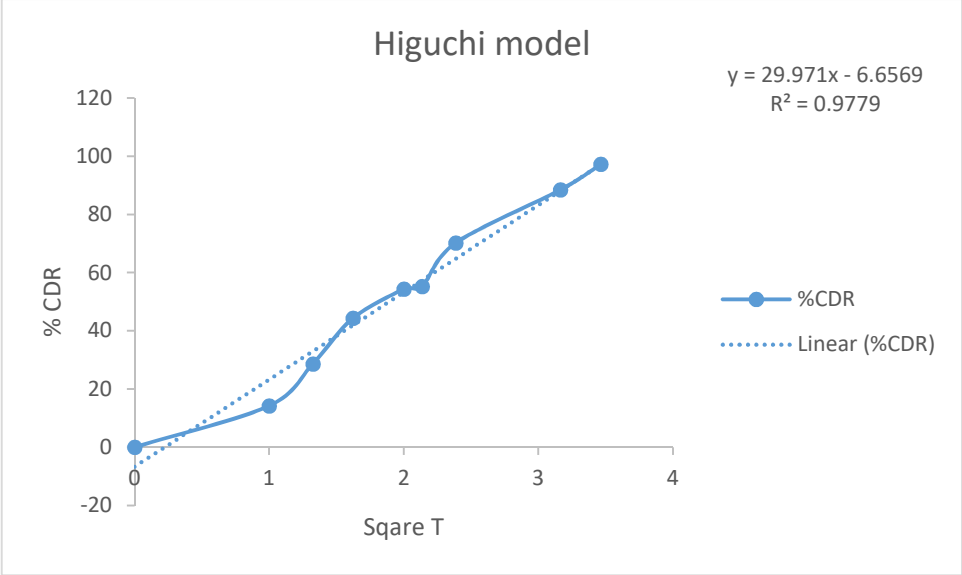

Fig-9: Higuchi model of optimized formulation

Kormeyer peppas

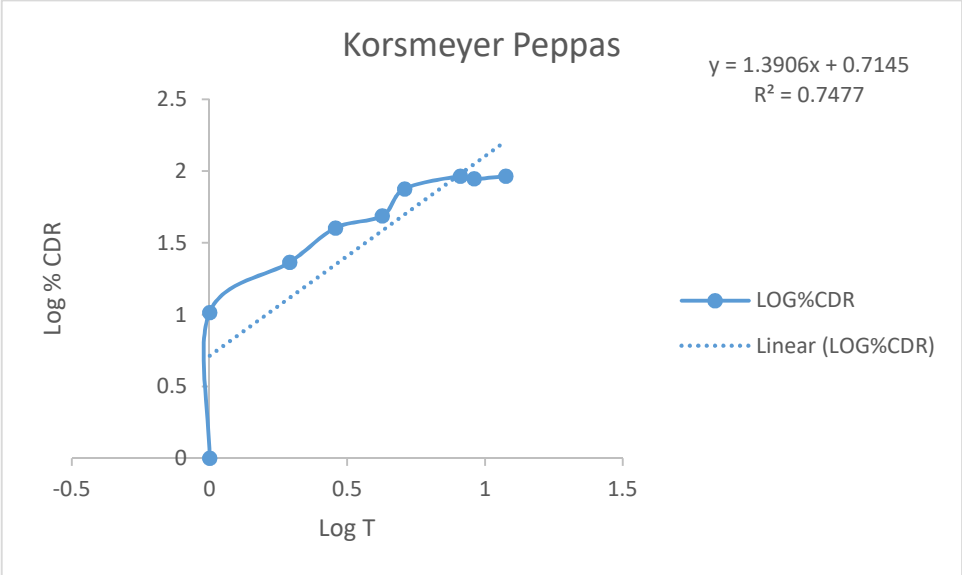

Fig-10: Korsmeyer peppas of optimized formulation

Stability studies

There was no significant change in physical and chemical properties of the Zno nanoparticles of formulation F-8 after 90 days. Parameters quantified at various time intervals were shown.

Table-4: Stability studies of all formulations

F.no	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-8	25^0C/60%RH % Release	97.19	96.89	95.93	94.90	Not less than 85 %
F-8	30^0C/75% RH % Release	97.19	96.53	95.81	94.65	Not less than 85 %
F-8	40^0C/75% RH % Release	97.19	96.42	95.75	94.53	Not less than 85 %

CONCLUSION

The current study suggested a unique Paclitaxel polymeric zno nanoparticle formulation for regulated release. A drug encapsulation effectiveness of up to 90.22 % has been attained in this study. Paclitaxel polymeric zno nanoparticles containing zinc nitrate and synthetic polymers were created using the sol to gel method, then the particle size was decreased by sonication formulation using polymeric zno nanoparticles performed well in terms of medication content and encapsulation effectiveness. This shows that the formulation procedure was suitable and reproducible in nature, and it provided a good yield. The formulation with the best encapsulation efficiency was (F-8). It was discovered that the percentage of encapsulation efficiency along with the soy lecithin concentration. According to the method described, permeation studies with dialysis membrane were conducted. The in vitro drug release profiles of all the formulations indicated an initial burst effect, followed by a gradual drug release. The formulations demonstrated good drug release from the polymer. These polymeric zno nanoparticles contained more Paclitaxel and released it more quickly.

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