FORMULATION AND EVALUATION OF EZETIMIBE LOADED PLGA NANOPARTICLES FOR IMPROVED DYSLIPIDEMIA ACTIVITY

Vemula Sumanth[1], Rekha Devi Allagadda[*1], Pasupuleti Prakash[2], Veluru Hari[3], Naveen Kumar Gandupally[4], Samyuktha Chagalammari[5]

[1]Department of Pharmaceutics, Seven Hills College of Pharmacy(Autonomous), Tirupati-517561.

[*1]Associate Professor, Department of Pharmaceutics, Seven Hills College of Pharmacy(Autonomous), Tirupati-517561, Tirupathi District, Andhra Pradesh.

[2]Associate Professor, Department of Pharmaceutics, Sri Padamavathi School of Pharmacy (Autonomous), Tiruchanoor-517503, Tirupathi District, Andhra Pradesh.

[3]Associate Professor, Department of Pharmacology, MB School of Pharmaceutical Sciences, Mohan Babu University, Sri Sainath Nagar, A. Rangampeta, Chandragiri Mandal, Tirupati-517102, Tirupathi District, Andhra Pradesh.

[4]Assistant Professor in Pharmacology, Sri Venkateswara College of Pharmacy (Autonomous), RVS Nagar, Tirupathi Road, Chittoor-517127, Chittor District, Andhra Pradesh.

[5]Department of Pharmaceutics, Seven Hills College of Pharmacy, Tirupati-517561, Tirupati District, Andhra Pradesh.

ABSTRACT

A promising method of regulated and targeted drug release is represented by nanoparticles. They are specifically made to release the drugs close to the targeted tissue. This study's objective was to formulate and evaluate PLGA nanoparticles with ezetimibe in various drug-to-polymer ratios. Nanoparticles have a distinct spherical shape, according to SEM. The stability of the medication and the polymer did not interact chemically, according to FTIR measurements. It was discovered that all of the drug-loaded batches exhibited Higuchi release in vitro, which offered continuous release for 12 hours. For every formulation, the Zeta potential falls between -12.11 mV and -28.25 mV. The new formulation may be beneficial in terms of enhanced bioavailability of ezetimibe and overcomes and mitigates the limitations and downsides of ezetimibe sustained release formulations.

Keywords: Nanoparticles, Ezetimibe, biodegradable.

1. INTRODUCTION

Small colloidal particles called nanoparticles are composed of both biodegradable and non-biodegradable polymers. They range in diameter from 1 to 1000 nm. Nanospheres, which are matrix systems, and Nano capsules, which are reservoir systems made of a

polymer membrane enclosing an aqueous or oily core, are the two different forms of nanoparticles. The resulting particles were easily freeze-dried and stable. These factors led to the development of biodegradable polymer nanoparticles for medication delivery. In fact, numerous medications (antibiotics, cystostatics, peptides and proteins, nucleic acids, etc.) could successfully target tissue with nanoparticles. Both active and passive drugs targeting are accomplished with nanoparticles. Because only small amounts of material may be enclosed, these devices' comparatively modest size restricts their usage. There are other kinds of (non-biodegradable) nanoparticle systems, such as colloidal gold and sulphur. As a diagnostic tool, colloidal sulphur (designated with 99mTC) is employed. Usually, gelations are added as a polymeric stabiliser to prevent it from aggregating. Additionally, colloidal gold (198Au) is employed as a medicinal agent and a diagnostic tool.

2.METHODOLOGY

2.1 PRFORMULATION STUDY

2.1.1. Drug-Excipient compatibility studies: FTIR, and XRD studies

(IR Spectroscopy) FTIR Studies:

Using a Fourier transform infrared spectrophotometer, the non-thermal examination of drug-excipient compatibility (a binary mixture of drug and excipient 1:1 ratio) was investigated. FTIR spectra from the Bruker FTIR were used to determine whether the pure medicine and excipients were compatible. The 550–4000 cm-1 range was used to record each sample's spectra. The yellow crystals composed of Zn Se was directly exposed to the powder sample. The spectra were obtained over the wave number range of 4000 to 550cm^{-1}.

2.1.2. X-Ray Diffraction(XRD) Studies:

The X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ. A goniometer is the device that rotates the sample and maintains the angle. Data is gathered at 2θ from around 5° to 70°, which are predefined angles in the X-ray scan, for common particle patterns.

2.2 EZETIMIBE LOADED NANOPARTICLES PREPARATION METHOD:

2.2.1 Dispersion of solvents (nanoprecipitation):

The medication is dissolved in an organic phase together with the poly (lactic-co-glycolic acid) polymer (PLGA) to create the nanoparticles, which are then added to an aqueous solution that contains TPGS (d-alpha-tocopheryl polyethylene glycol 1000 succinate), an emulsifier. Drop by drop, the organic phase solution was added to the aqueous phase while being homogenized at 11,000 rpm. For four hours at room temperature, the dispersion was stirred magnetically. For two to three minutes, the solution is maintained at a lower pressure. Drug-loaded nanoparticles are created using this approach.

Table 1: Composition of the Nanoparticles

Ingredients	**Formulation Code**							
	E1	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**
Ezetimibe (mg)	15	15	15	15	15	15	15	15
PLGA (mg)	20	45	70	95	120	145	170	195
TPGS (mg)	5	10	15	20	25	30	35	40
Acetone (ml)	6	6	6	6	6	6	6	6
Water (ml)	10	10	10	10	10	10	10	10

2.2.2. LYOPHILIZATION:

After centrifuging, the resulting samples were lyophilised and kept between 2 and 80°C. To achieve stability, the samples underwent lyophilization. The lyophilized powder is used to measure the other parameters.

2.3 EVALUATION OF EZETIMIBE LOADED NANOPARTICLES:

2.3.1. %Yield

2.3.2. Mean Particle size

2.3.3 Entrapment efficiency (%)

2.3.4 In vitro drug release

2.3.5 Drug Release Kinetics to Optimised formulation

2.3.6 SEM for Optimised Formulation

2.3.1 Percentage Yield

For drug-containing nanoparticles, the weight ratio of the dried nanoparticles to the loading amount of the drug and polymer was used to calculate the production yield. Using equation, the production yield was determined, and the results are shown in the table.

$$\%\ Yield = \frac{Overall\ mass\ of particles}{Overall\ mass\ of\ the\ drug\ and\ polymer} X100$$

2.3.2 Determination of the average particle size:

The particle size was assessed using Particle Size Analyzer (Malvern Zetasizer). The results of particle size are displayed in the table.

2.3.3 Drug Encapsulation Efficiency:

Lyophilized nanoparticles 3mg were dissolved in 1ml of diluents and the drug amount was determined by UV analysis. The encapsulation efficiency was determined as the mass ratio of entrapped Ezetimibe in nanoparticles to the theoretical amount of the drug used in the preparation. The entrapment of the Ezetimibe PLGA nanoparticles was expressed as loading capacity.

Overall mass of the drug and polymer.

$$\%\ Entrapment\ Efficiency = \frac{Amount\ entrapped}{Total\ drug\ loaded} X100$$

***2.3.4 In-vitro* Ezetimibe drug release:**

2.3.5 Drug Release Kinetics to Optimised formulation

Drug Release Kinetics to Optimised formulation Drug equivalent of 10 mg of freeze-dried Ezetimibe-loaded nanoparticles powder was transferred to a dialysis bag dispersed in 3 ml of PBS, then suspended in 100 ml of isotonic pH 7.4 Phosphate Buffer Solution (PBS). The bag was kept at 37 ± 0.5°C in a water bath with magnetic stirring. 5 ml of samples were removed at predetermined intervals, and new buffer was added. To ascertain the drug release, UV analysis was performed on the obtained solution.

Zero Order Equation:

This formula characterises the systems in which the release rate is unaffected by the dissolved species concentration. The equation of zero order is fitted to the dissolution data:

$$Q = Q_0 . K_0 t$$

First Order Equation:

The release from systems where the dissolution rate depends on the concentration of the dissolving species is described by the first order equation. In general, release behaviour adheres to the first-order release equation as follows:

$$\ln M = \ln M_0 - K_1 t$$

Higuchi Square Root Law:

The classical Higuchi equation provides physically meaningful kinetics, aiding device optimization and understanding drug release mechanisms. A form of the Higuchi Square Root Law is given by equation:

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

Korsemeyer Peppas Equation:

The linear line representing the log cumulative percentage of drug release was the source of Korsmeyer's equation. Vs. log time curve is

$$M_t / M_\infty = Kt^n$$

2.3.6 Scanning Electron Microsocopy (SEM):

SEM is known for its high magnification, detailed topography, and ability to provide information about the sample's surface morphology and composition. Non-conductive samples are coated with a thin layer of metal, such as gold. Images are then generated by scanning a focused beam of high-energy electrons across the sample.

2.4 Stability Studies:

An optimized formulation sample was kept between 2 and 6°C and 25°C, which are refrigerator and room temperatures respectively, where the optimized formulation may be kept while being transported from the producer to the final consumer. An optimized formulation was kept in standard glass vials (USP type 1) that were sealed with rubber. After 30, 90, and 120 days, the samples were taken out and measured for particle size and entrapment efficiency.

3. RESULTS AND DISCUSSION:

3.1 Preformulation Studies:

The melting point of the drug was determined by using melting point apparatus and was found to be melt at 165 ℃ and completely melted at 166 ℃ which is within the specified temperature.

3.2 Studies of drug-excipient compatibility

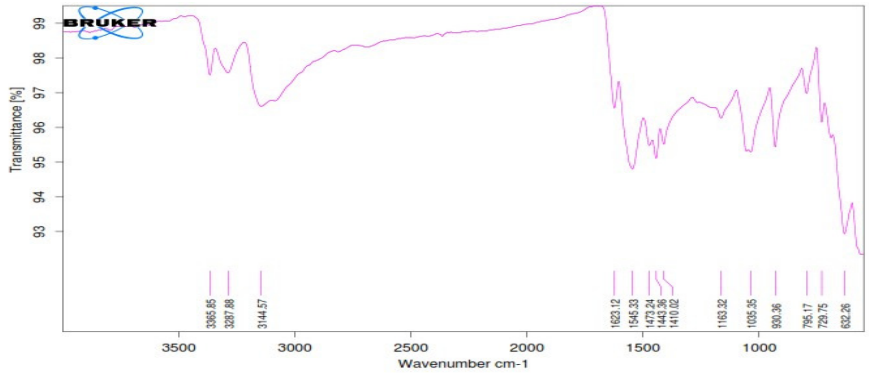


Figure 1: FTIR Spectrum of Ezetimibe pure drug.

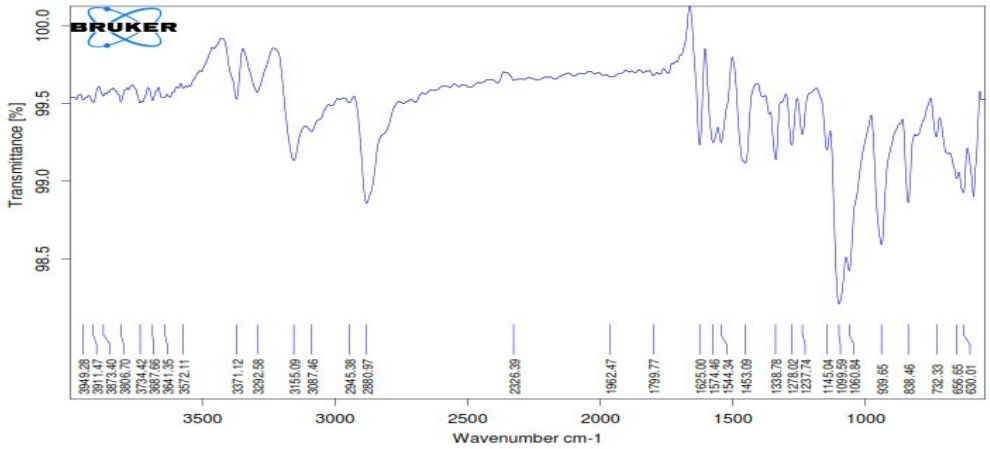


Figure 2: FT-IR Spectrum of Optimized Formulation

The Ezetimibe drug consisting of functional groups C-H Stretching(2950-2840), =C-OH Stretching(1200-1020) and -C-OH Stretching, the peaks are observed at 3147.57, 1035.35, 3365.85 for pure drug and 2945.38, 1099.59 and 3371.12 for formulation, indicating that there is no any drug-excipient interaction and have compatibility of drug with excipients.

3.3 XRD

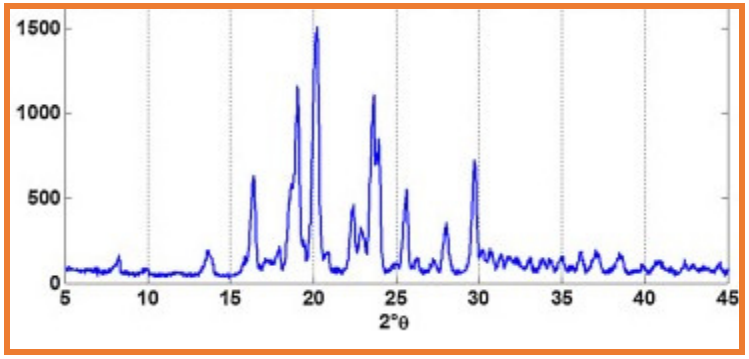


Figure 3: Ezetimibe Pure drug

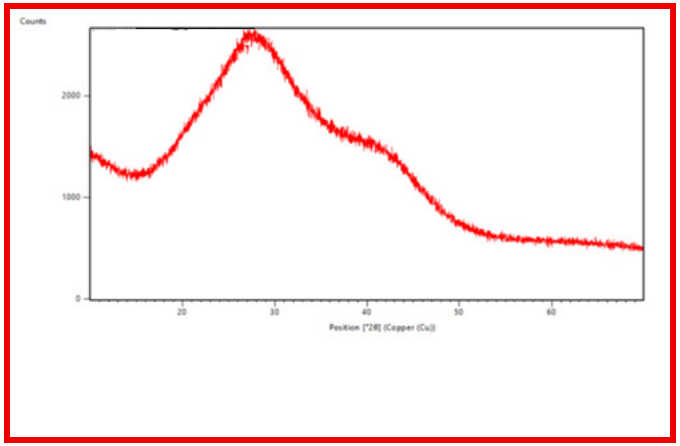


Figure 4: Ezetimibe E4 Optimised formulation

The pure drug shows a sharp peak whereas after formulation into PLGA nanoparticles, it shows a broad peak indicating a crystalline to amorphous form which may also increase solubility.

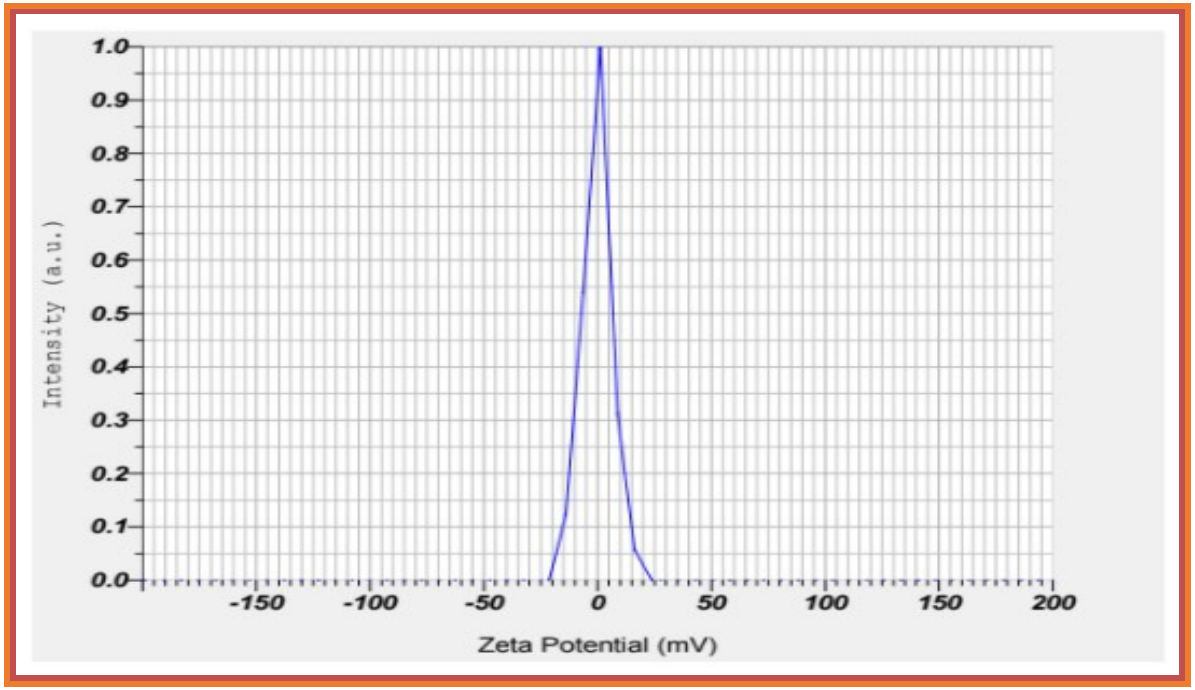
3.4 EVALUATION OF EZETIMIBE PLGA NANOPARTICLES:

Table 2: Evaluation of Nanoparticles

Formulation Code	Mean Particle size(nm)	%Yield	%Entrapment	PDI	Zeta Potential (mV)
E1	96.07 ±0.45	90.36±1.82	90.91±1.24	0.668±0.24	-26.12±0.83
E2	100.92±0.22	93.51±2.55	92.35±1.67	1.268±0.58	-24.81±0.43
E3	121.07±1.54	95.28±1.69	94.17±1.83	1.153±0.41	-23.52±0.27
E4	153.09±0.89	97.10±1.28	97.76±1.86	0.168±0.26	-28.25±0.33
E5	100.24±1.27	88.35±1.92	86.42±1.43	0.277±0.12	-16.55±0.82
E6	145.21±1.44	91.51±1.33	90.30±2.58	0.309±0.08	-20.83±0.61
E7	160.64±0.78	94.62±1.47	92.91±1.88	0.698±0.31	-22.59±0.47
E8	171.06±0.77	94.02±0.63	95.35±1.63	0.385±0.68	-12.11±0.52

The percentage yield of formulations E1 through E8 by drug variation was calculated and is shown in the table 3. The E4 formulation had the highest drug concentration and the highest entrapment efficiency. PDI, which is 0.168 in the E4 formulation, was noted. For formulations, the Zeta potential falls between -12.11 mV and -28.25 mV.

Figure 5: Zeta Potential of E4 Formulation



3.5 In vitro Drug release studies:

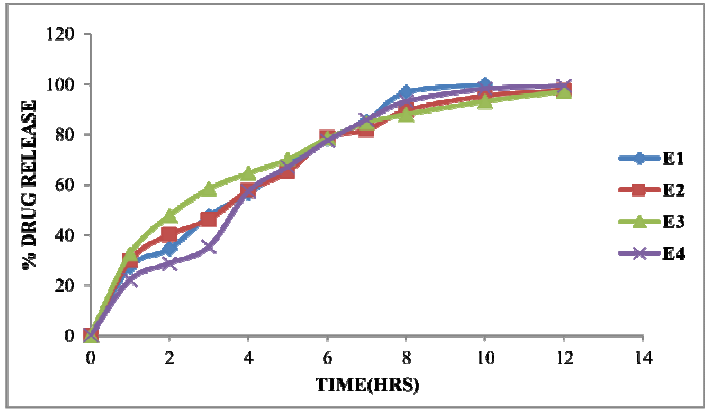


Figure 6: Dissolution study of Ezetimibe Nanoparticles E1-E4

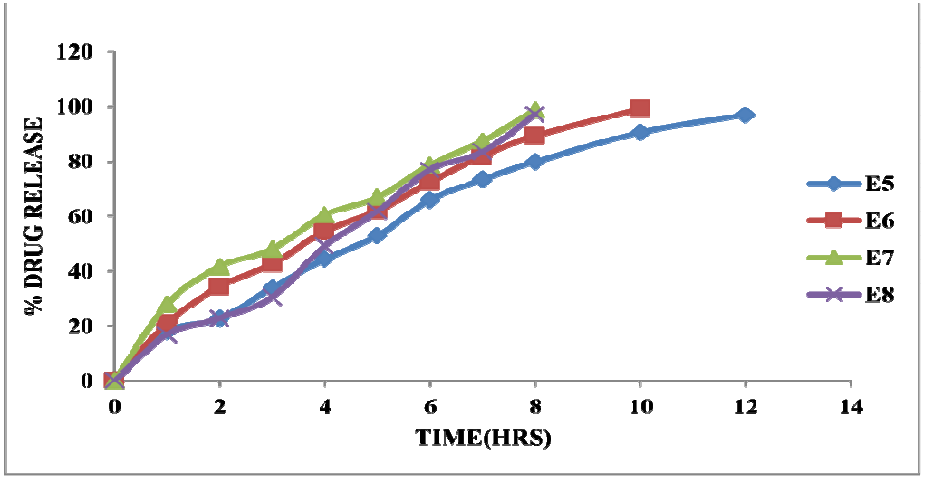


Figure 7: Dissolution study of Ezetimibe Nanoparticles E5-E8

Therefore, according to the dissolution results of eight formulations, the E4 PLGA (50:50) (95 mg) formulation demonstrated superior release (99.37%) for up to twelve hours. Thus, the E4 formulation is the most optimized one.

3.6 Release Rate Kinetics to Dissolution Data

The release kinetics of drug release from nanoparticles were explained by fitting data from in vitro release experiments of formulations that demonstrated improved drug release into several equations. The results are displayed in the table 5, which corresponds to zero order kinetics. The data was fitted into a number of kinetic models, including zero, first order kinetics, Higuchi, and Korsmeyer peppa's processes.

Table 3: Release kinetics data for optimized formulation (E4)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM % RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
22.26	1	1.000	1.348	0.000	1.891	22.260	0.0449	-0.652	77.74	4.642	4.268	0.374
28.78	2	1.414	1.459	0.301	1.853	14.390	0.0347	-0.541	71.22	4.642	4.145	0.496
35.36	3	1.732	1.549	0.477	1.811	11.787	0.0283	-0.451	64.64	4.642	4.013	0.628
57.23	4	2.000	1.758	0.602	1.631	14.308	0.0175	-0.242	42.77	4.642	3.497	1.144
66.98	5	2.236	1.826	0.699	1.519	13.396	0.0149	-0.174	33.02	4.642	3.208	1.433
77.46	6	2.449	1.889	0.778	1.353	12.910	0.0129	-0.111	22.54	4.642	2.825	1.817
85.68	7	2.646	1.933	0.845	1.156	12.240	0.0117	-0.067	14.32	4.642	2.428	2.213
93.14	8	2.828	1.969	0.903	0.836	11.643	0.0107	-0.031	6.86	4.642	1.900	2.741

98.13	10	3.162	1.992	1.000	0.272	9.813	0.0102	-0.008	1.87	4.642	1.232	3.410
99.37	12	3.464	1.997	1.079	-0.201	8.281	0.0101	-0.003	0.63	4.642	0.857	3.784

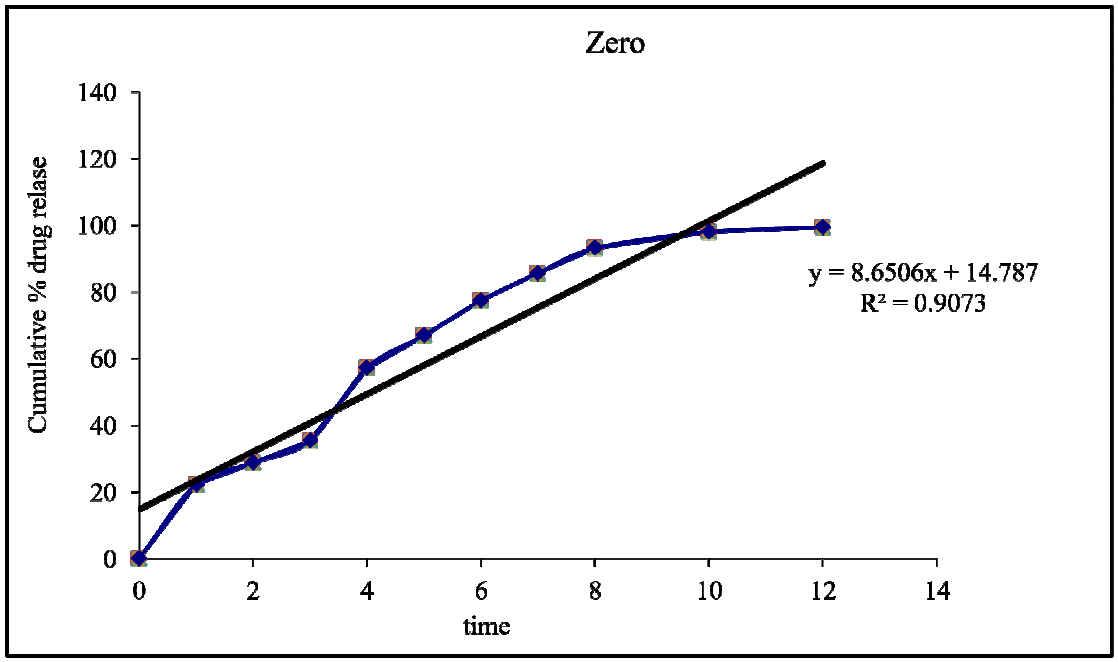


Figure 8: Graph of Zero order

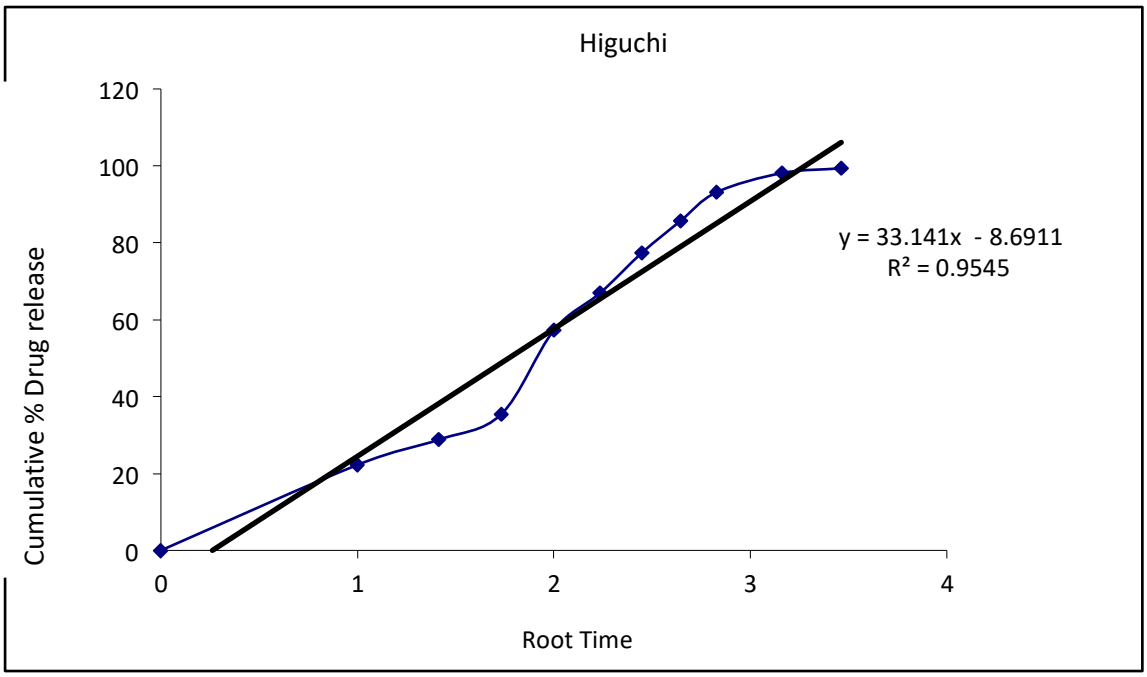


Figure 9: Graph of Higuchi

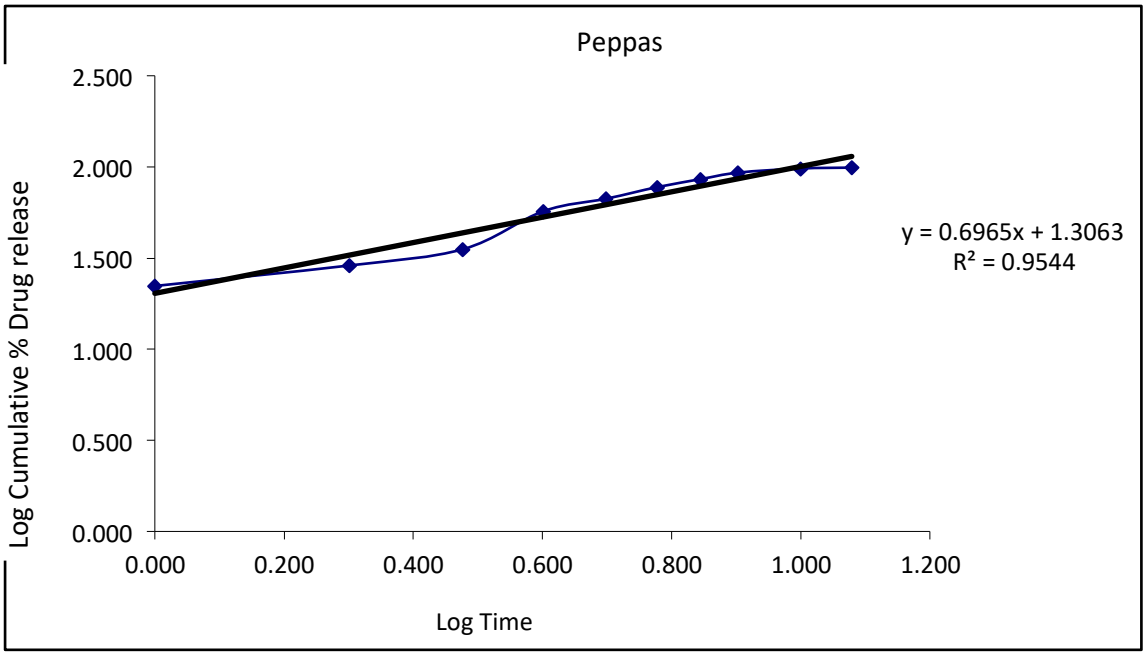


Figure 10: Graph of Korsmeyer Peppas

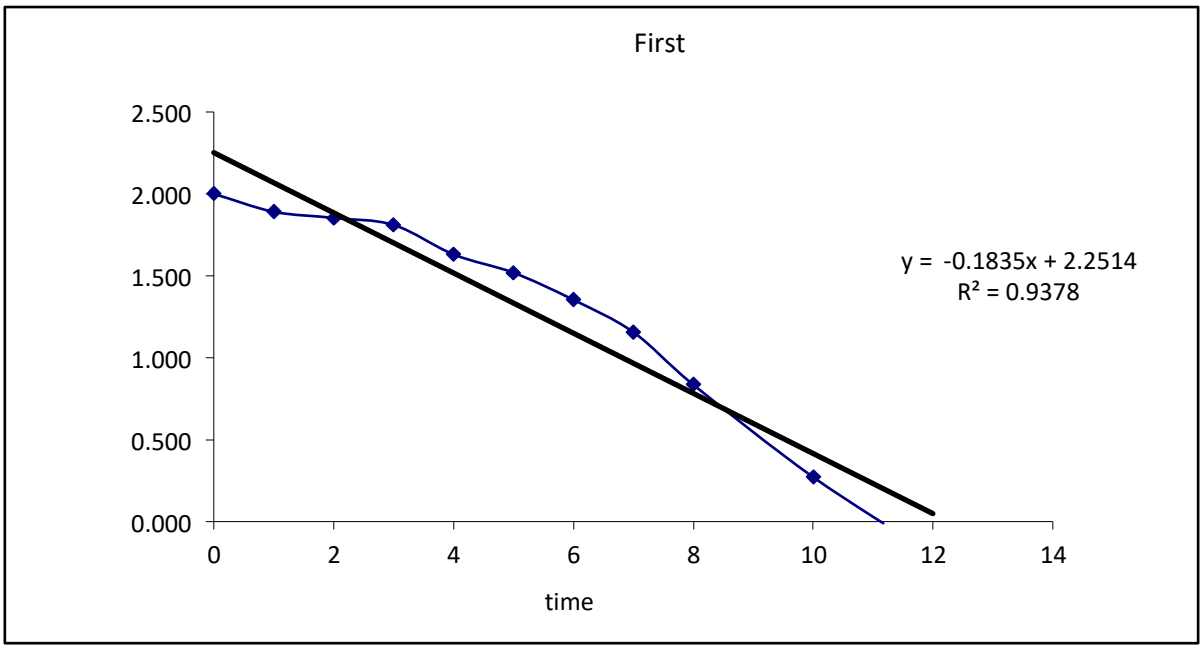


Figure 11: Graph of First Order

The Optimised formulation adhered to Higuchi release kinetics based on the data and results above.

SEM

According to SEM analyses, the ezetimibe-loaded nanoparticles had a rough surface and a spherical shape, as seen in Figure 5.

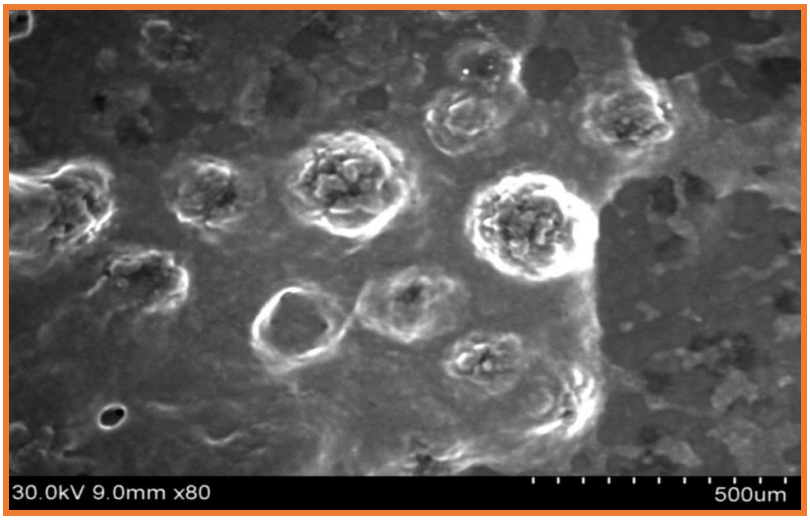


Figure 12: SEM graph of optimized formulation

3.7 Stability studies:

Table 5: Stability Studies

Temp (ºC)	30 Days		90 Days		120 Days	
	Particle size	%EE	Particle size	%EE	Particle size	%EE
2-6ºC	15.96±5.34	97.65±1.123	158.73±6.12	97.13±1.25	159.12±4.72	96.89±1.08
25±2ºC	182.61±6.32	91.46±2.31	188.74±5.92	90.86±1.88	190.39±5.84	90.64±1.05

The Optimised formulation was divided into two samples and stored at two temperatures 2 ºC to 6ºC and 25 ºC respectively, to study the storage conditions at 30days, 60days, and 90days. The results showed that there are no significant changes in particle size and % entrapment efficiency at 2 ºC to 6 ºC. There is a significant changes of particle size as pronounced effect at 25±2 ºC. Hence, nanoparticles are more stable at 2 ºC to 6 ºC.

CONCLUSION

Ezetimibe nanoparticles have been created in our present research. A popular, easy, quick, and repeatable technique for creating nanospheres is nanoprecipitation. Getting small particles and a narrow size distribution is its greatest advantage. With a nano size range of 153.09±0.89 nm and a high drug release of 99.37%±0.83, the Optimised Ezetimibe loaded PLGA nanoparticle formulations (E4) demonstrated a homogeneous, stability study showed that E4 formulation was stable at 2-6^{0}c and effective. Thus, the Optimised E4 formulation may increases the solubility, bioavailability, reduces dose, dose frequency and side effects of Ezetimibe leads to patient affordable formulation.

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