

FORMULATION AND EVALUATION OF POLYMERIC NANOPARTICLE BY EMULSION SOLVENT EVAPORATION METHOD

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Abstract- The present work was aimed to formulate and evaluate Polymeric nanoparticles by Emulsion solvent evaporation method. Polymeric nanoparticles are the colloidal drug delivery system with a particle size of 10 – 1000 nm that potentially delivers the therapeutic agent in the systemic circulation in a controlled manner. Benidipine is Biopharmaceutical Classification System (BCS) class-II drug having low solubility and high permeability. Polymeric nanoparticles were prepared using Eudragit S 100 as polymer, poloxamer 188 as surfactant and dichloromethane as organic phase. Drug and polymer compatibility study was analysed by FTIR. The prepared formulation was characterized for melting point, particle size, polydispersity index, entrapment efficiency, zeta potential, surface morphology, *in-vitro* drug release and kinetic studies.

Keywords: Drug delivery, Polymeric nanoparticles, Benidipine hydrochloride, Solubility, Emulsion solvent evaporation method.

INTRODUCTION:

Drug delivery is an intriguing field of research that has captured the interest of researchers because delivering a medicine to its site of therapeutic action is one of the main limitations of pharmaceutical and biotechnology industries.¹ Drug delivery systems (DDS) have been utilized to treat a variety of ailments in the past. To treat diseases, all medications rely on pharmacologic active metabolites (drugs). Some medications are created as an inactive precursor, but when they are transformed by the body, they become active.²

During last two decades, considerable attention has been given to the development of Novel Drug Delivery System (NDDS). The rationale for novel controlled drug delivery is to alter the pharmacokinetics and pharmacodynamics of a drug substance in order to improve the therapeutic efficacy and safety through the use of novel drug delivery system.³

The emergence of nanotechnology is likely to have a significant impact on the drug-delivery sector and nanoparticles (NPs) are at the leading edge, with many potential applications in clinical medicine and research. NPs can be correctly envisioned as the future of drug-delivery technology as they have the potential to become useful therapeutic and diagnostic tools in the near future. The fundamental component of nanotechnology is the nanoparticles.⁴

Nanosystems may enhance oral absorption by increasing the gastric residence time through mucosal adhesion or by increasing cell or tissue entry.⁵ The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.⁶ The input of today's nanotechnology is that it allows real progress to achieve temporal and spatial site-specific delivery.⁷

Polymeric nanoparticles (PNPs) are particles obtained from natural, semi-synthetic or synthetic polymers. Polymeric NPs are small particles with a diameter of 1 to 1000 nm that can be loaded with active chemicals or surface-adsorbed onto the polymeric nucleus. Polymeric NPs have showed considerable promise in the delivery of pharmaceuticals to specific locations for the treatment of a variety of ailments.

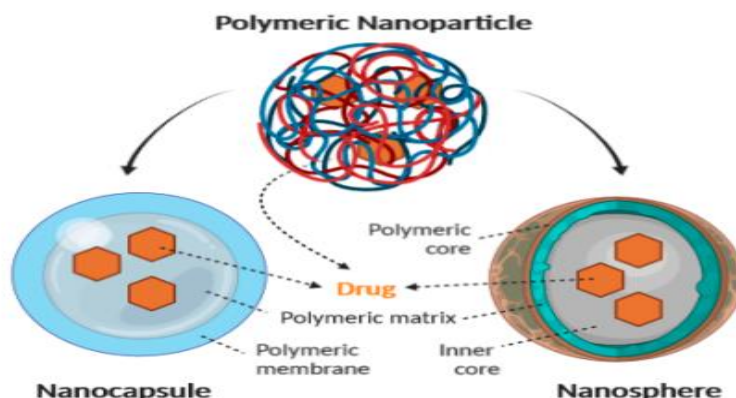


Fig. 01: Schematic representation of the structure of nanocapsules and nanospheres (arrow stands for the presence of drug/bioactive within the nanoparticles)

Polymeric NPs are employed in drug administration for a variety of applications, including medicine conjugation and entanglement, prodrugs, stimuli sensitive systems, imaging modalities, and theranostics. Polymeric NP is recognized as one of the most ideal drug delivery techniques to solve drug delivery issues such as low solubility, permeability, and bioavailability of BCS class II and III drugs.⁸

Hypertension is one of the most common disorders throughout the world. Managing hypertension continues to be challenging with the currently available drugs, since they have poor bioavailability by oral route and toxicity due to higher doses. Benidipine is a novel calcium channel blocker (CCB) drug which blocks three calcium channels. Benidipine is an orally active drug for the treatment of hypertension and angina pectoris and has become one of the three best-selling CCBs and is highly useful as a potent, long-lasting antihypertensive and antidiabetic agent.⁹

ADVANTAGES OF POLYMERIC NANOPARTICLES:¹⁰

- Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
- Delivers a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

MATERIALS AND METHODS:

Table 01: List of ingredients used

SL. NO	CHEMICALS	COMPANY
1	Benidipine hydrochloride	Yarrow chem products, Mumbai
2	Eudragit S 100	Yarrow chem products, Mumbai
3	Pluronic F 68	HiMedia
4	Dichloromethane	HiMedia
5	Ethanol	Yarrow chem products, Mumbai
6	Distilled Water	Local Market

Preformulation studies of drug and polymers:

1. Identification of pure drug:

a) **Determination of melting point:** The melting point of the drug was determined by an open capillary tube method. The one end closed capillary tube was taken and the drug was filled into the capillary tube by repeated tapping's. Then the capillary tube was placed in the melting point apparatus. The temperature at which the drug started melting was recorded.¹¹

2. Physicochemical parameters:

a) **Organoleptic properties:** The physical appearance of drug will be observed and compared with the pharmacopeial specifications.¹²

b) **Solubility measurement studies:** To determine solubility, each solvent (water, 0.1 N HCl, phosphate buffer pH 6.8, dichloromethane, ethanol) was mixed with excess of drug and the mixtures were equilibrated for 24 h on a mechanical shaker. An aliquot was filtered and diluted suitably and analysed by UV-spectrophotometer.¹³

c) Screening of the absorbance-maxima of Benidipine hydrochloride:¹⁴

➤ **Determination of absorbance maxima of Benidipine hydrochloride:** 50 mg of Benidipine hydrochloride drug was transferred to a 100 ml of volumetric flask by dissolving in methanol and made up to volume with phosphate buffer pH 6.8 (500 µg/ml). 4 ml was pipetted out into a separate 100 ml flask and made up to volume with phosphate buffer (20 µg/ml). 1 ml was taken and made upto 10ml using phosphate buffer (2 µg/ml). The absorbance of the resulting solutions was measured between 200-400 nm using a UV spectrophotometer.

➤ Determination of calibration curve of Benidipine hydrochloride using phosphate buffer pH 6.8:

Stock A (500 µg/ml): 50 mg of drug was transferred to a 100 ml of volumetric flask and made up to volume with phosphate buffer pH 6.8.

Stock B (20 µg/ml): 4 ml was pipetted out into a separate 100 ml flask and made up to volume with phosphate buffer.

Preparation of the working standards: From stock B 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, and 9ml were taken in 10 ml volumetric flask and made up to 10 ml using phosphate buffer to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml, 14µg/ml, 16µg/ml, and 18µg/ml concentrations respectively.

The absorbance of the resulting solutions was measured at screened wavelength by UV spectrophotometer. The calibration curve was plotted against concentration versus absorbance.

3. **Drug polymer interaction study using FTIR spectroscopy:** The pure drug, pure polymer, drug and polymer and physical mixture of drug, polymer and other excipients were prepared and scanned from 4000-400cm⁻¹ in FTIR spectrophotometer.¹⁵

Preparation of polymeric nanoparticles:¹⁶

Polymeric Nanoparticles were prepared by Emulsion solvent evaporation method. Required quantity of polymer and drug (2:1) were weighed & dissolved in 10ml of dichloromethane and ethanol. Quantity of poloxamer 188 was mixed with 40ml of water & this solution was kept in another beaker. Solution containing drug and polymer were added drop wise to aqueous phase (using

microneedle syringe) under continuous stirring. The formed nanoparticle suspension was homogenized at 18000 rpm for 30 min. The suspension was centrifuged at 9,000 rpm for 15 min. After centrifugation, the supernatant was removed and formed NPs washed 3 times using water and dried at room temperature in desiccator.

Ingredients	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Benidipine hydrochloride (mg)	10	10	10	10	10	10	10	10	10
Eudragit S 100 (mg)	200	100	300	300	100	200	200	100	300
Pluronic F 68 (%)	2	1	1.5	1	1.5	1	1.5	2	2
Dichloromethane (ml)	10	10	10	10	10	10	10	10	10
Ethanol (ml)	5	5	5	5	5	5	5	5	5
Distilled water (ml)	40	40	40	40	40	40	40	40	40

Table 02: Composition of excipients used for the formulation

Characterization of polymeric nanoparticles:

1) **Percentage yield:** The prepared drug loaded polymeric nanoparticles were collected and weighed. The weight obtained is noted as practical yield. The percentage yield was calculated by following formula,¹⁷

% yield = $\frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$

2) **Particle Size Analysis:** Nanoparticles formulation was characterized for particle size using Malvern 2000. Double distilled water was used as a dispersant medium.¹⁶

3) **Polydispersity Index (PDI):** Polydispersity index is a parameter to define the particle size distribution of nanoparticles obtained from photon correlation spectroscopic analysis. It is a dimensionless number extrapolated from the autocorrelation function and ranges from a value of 0.01 for mono dispersed particles and up to values of 0.5-0.7. Samples with very broad size distribution have polydispersity index values > 0.7.¹⁸

4) **Entrapment Efficiency:** The amount of drug loaded polymeric nanoparticles (entrapped drug) were separated from the aqueous medium by centrifugation method. Then, the supernatant layer was taken and further diluted with the help of buffer solution. The concentration of free drug present in the supernatant layer were determined by UV spectrophotometer. The % entrapment efficiency (EE) was calculated by using following equation,¹³

EE (%): $\frac{\text{Total amount of drug taken} - \text{unentrapped drug}}{\text{Total amount of drug taken}} \times 100$

5) **In-vitro drug release studies:** Analysis of the *in-vitro* drug release study of drug loaded polymeric nanoparticles were done by a dialysis bag diffusion method. Samples were suspended in 5 ml phosphate buffer solution (pH 6.8) and it was kept in a dialysis bag and tied at both ends. It was immersed in a receptor compartment containing 100 ml of phosphate buffer (pH 6.8) stirred at 50 rpm and maintaining temperature $37 \pm 1^\circ\text{C}$. 2 ml of the aliquots were withdrawn at various time intervals and replaced with a fresh volume of phosphate buffer, diluted appropriately. The concentration of the drug was measured by UV-Visible spectrophotometer.¹⁹

6) **Morphology by Optical Microscopy:** This study was performed by optical microscope for structural attributes such as lamellarity, uniformity of size, shape and physical stability characteristics i.e., aggregation and/or irregularity.¹³

7) **Zeta Potential:** The surface charge (Zeta potential) were determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern instrument). The ideal zeta potential value must be in the range of above +30 to -30mV and this range prevent the aggregation of particle.²⁰

8) **Study of drug release kinetics:** In order to understand the kinetics and mechanism of drug release, results of *in vitro* drug release study of the prepared PNPs were fitted into various kinetic equations like zero order (cumulative % remaining vs. time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), Peppas (log % drug release vs. log time). Rate constant (K) and regression coefficient (R^2) values were calculated for the linear curve obtained by regression analysis of the above plots.²¹

9) **Stability Studies as per ICH guidelines:** Stability studies were carried out as per the modified ICH guidelines. Stability studies were conducted under accelerated ($40 \pm 2^\circ\text{C}$, $75\% \pm 5\%$ relative humidity (RH)) condition for over a period of 3 months. The samples were withdrawn at different intervals (0, 1, and 3 months) and evaluated for physical appearance, entrapment efficiency and % cumulative drug release studies and were analyzed according to the related substances and assay methods reported.¹⁵

RESULTS AND DISCUSSION:

A. **Pre-formulation study of the drug:** Pre-formulation studies of Benidipine hydrochloride determined that it was pale yellow coloured amorphous powder with bitter in taste having a melting point of 212°C .

1. **Solubility study of Benidipine hydrochloride:** Benidipine hydrochloride was found to be practically insoluble in water (0.0021 ± 0.0004 mg/ml), slightly soluble in 0.1 N HCl (8.05 ± 0.12 mg/ml) and phosphate buffer pH 6.8 (7.93 ± 0.05 mg/ml),

soluble in dichloromethane (27.2 ± 0.41 mg/ml) and methanol (79.6 ± 0.3 mg/ml), and sparingly soluble in ethanol (14.27 ± 0.06 mg/ml).

2. **Screening of the absorbance-maxima of Benidipine hydrochloride:** The UV spectrum of Benidipine hydrochloride shows prominent absorbance maxima (λ_{\max}) at wavelength 238.5 nm when scanned between 200-400nm using phosphate buffer of pH 6.8. The peak obtained is shown in Fig. 02.

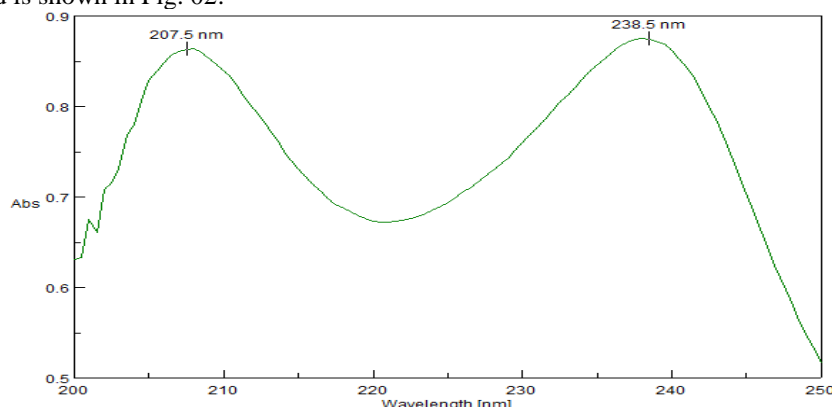


Fig. 02: λ_{\max} of Benidipine hydrochloride in pH 6.8 phosphate buffer

3. **Calibration curve for Benidipine hydrochloride:** The calibration curve of Benidipine hydrochloride with slope, intercept and regression co-efficient were determined and shown in Fig 02. The absorbance value remained linear and obeyed Beer's Lambert's Law in the range of 2-18 $\mu\text{g/ml}$ with the R^2 value of 0.9992.

Table 03: Calibration data of Benidipine hydrochloride in phosphate buffer pH 6.8

	Concentration ($\mu\text{g/ml}$)	Absorbance at 238.5 nm*
1	0	0.00 ± 0.00
2	2	0.1121 ± 0.0045
3	4	0.2234 ± 0.004
4	6	0.3232 ± 0.0092
5	8	0.4239 ± 0.008
6	10	0.5197 ± 0.0065
7	12	0.6203 ± 0.0075
8	14	0.7184 ± 0.009
9	16	0.8111 ± 0.0052
10	18	0.9042 ± 0.0087

(*Data represented as mean \pm standard deviation and $n=3$)

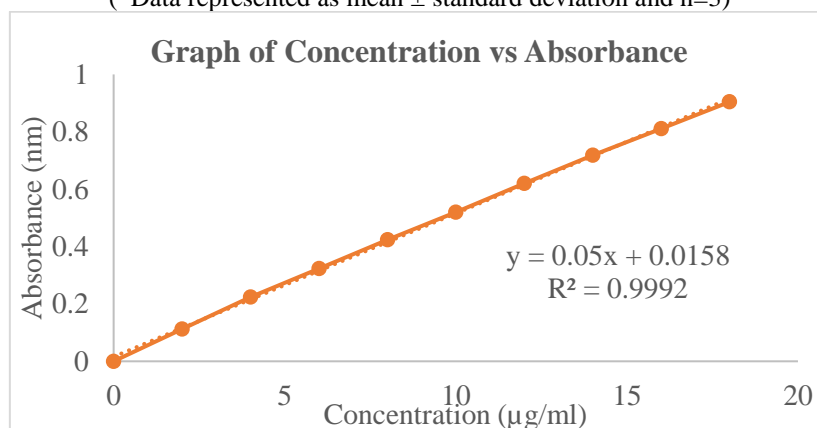


Fig. 03: Calibration curve of Benidipine hydrochloride in phosphate buffer pH 6.8

4. **Drug polymer interaction study using FTIR spectroscopy:** The compatibility between the drug and polymers was carried out using the FT-IR peak matching method.

The FT-IR spectra of pure Benidipine hydrochloride showed absorption at 2889 cm^{-1} , 1663 cm^{-1} , 1522 cm^{-1} , 1498 cm^{-1} , 3352 cm^{-1} and 3102 cm^{-1} for C-H, C=O, NO_2 , C=C aromatic, N-H and H-Cl stretching. Eudragit S100 showed the band at 1147 cm^{-1} , 1453 cm^{-1} , 1727 cm^{-1} and 2955 cm^{-1} for C-OH, CH_3 bending, C=O and O-H stretching. The spectra of Poloxamer 188 showed absorption at 2889.5 cm^{-1} , 1342.1 cm^{-1} , 1100.6 cm^{-1} and 843.25 cm^{-1} for C-H, O-H bending, C-O and C-C-O stretching. The physical mixture showed bands at 3325.98 cm^{-1} , 1634.88 cm^{-1} , 1045.11 cm^{-1} , 1099 cm^{-1} and 2390 cm^{-1} for C-H, C=O, C-OH, C-O and O-H stretching. All the characteristics of IR peaks related to pure drug Benidipine hydrochloride, Eudragit S100, and Poloxamer 188 have also appeared in the FT-IR spectrum of physical mixture with small shifting indicating the compatibility and uniformity of polymers with the drug without any chemical modification of the drug.

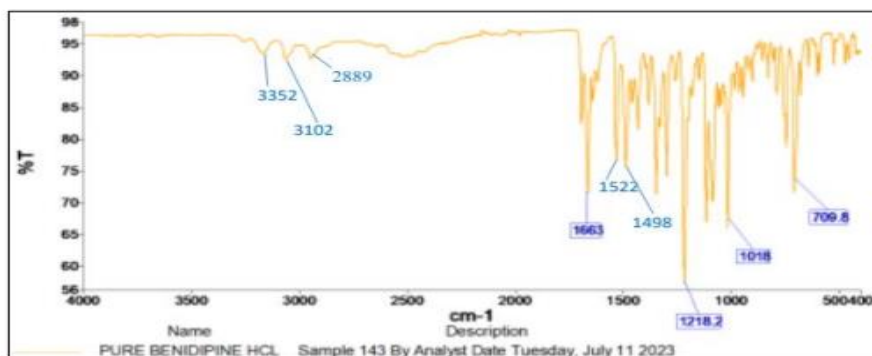


Fig. 04: FTIR spectra of pure drug Benidipine hydrochloride

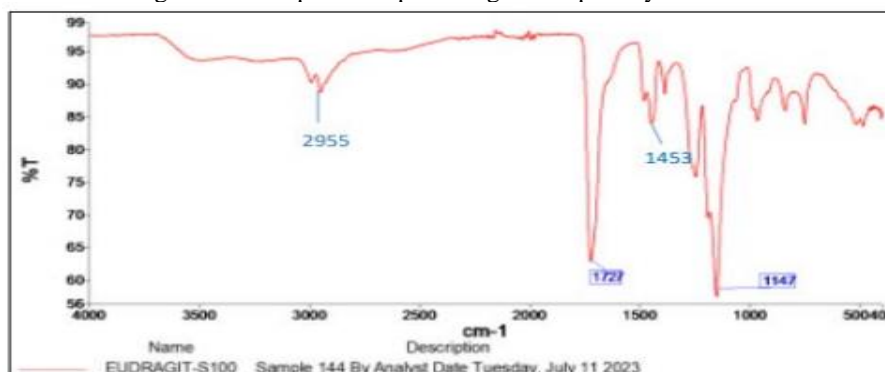


Fig. 05: FTIR spectra of Eudragit S100

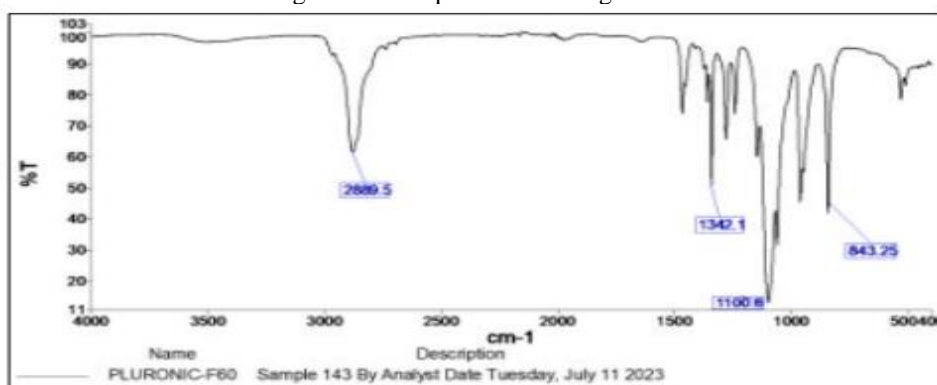


Fig. 06: FTIR spectra of Poloxamer 188

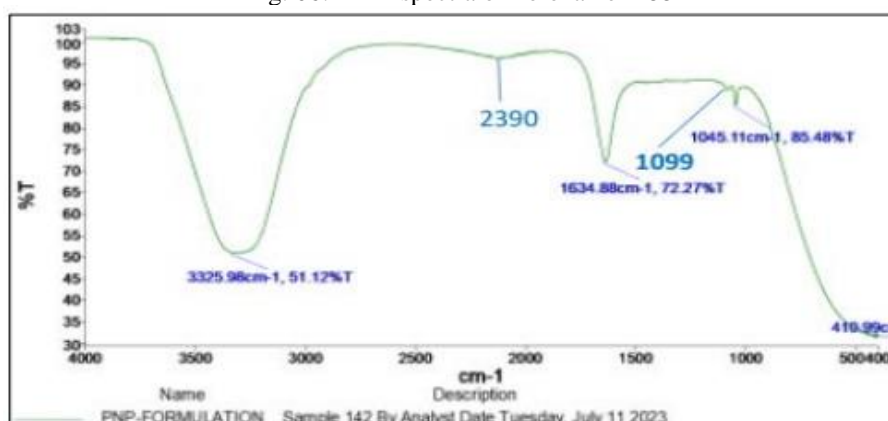


Fig. 07: FTIR spectra of optimized polymeric nanoparticle (PNP) formulation

B. Formulation of benidipine hydrochloride loaded polymeric nanoparticles by emulsion solvent evaporation method: Benidipine hydrochloride loaded Polymeric nanoparticles were successfully prepared using the emulsion solvent evaporation method. Prepared polymeric nanoparticle suspension was whitish in color as shown in Fig. 08.



Fig. 08: Formulation batches prepared based on Central Composite Design

1. **Percentage Yield:** Percentage yield was found to be 39.37 ± 0.19 to 95.23 ± 0.1 % for formulation F1 to F9. Percentage yield depends on the concentration of polymer added, as the concentration of polymer increases, there is increase in the percentage yield. The results of the study were shown in Table 04 and Fig. 09.
2. **Particle size analysis:** The mean particle size of prepared Polymeric nanoparticles (F1 to F9) ranged from 456.1nm to 940.5nm (Table 04). Both the polymer (Eudragit S 100) and stabilizing agent (poloxamer 188) exhibit significant effect on particle size. It was observed that mean particle size increases with the increase in the polymer concentration upto a level. Formulation F8 showed average particle size of 456.1 nm which was considered as the best formulation. Fig. 10 (a) to 10 (i) showed the particle size of formulations F1 to F9.
3. **Polydispersity Index (PDI):** PDI is a measure of homogeneity of particle size within the dispersed systems and ranges from 0 to 1. Homogeneous dispersion has PDI value close to zero while PDI values greater than 0.5 suggest high heterogeneity. The PDI of the optimised batch (F8) was found to be 0.398. From this observation it was found that presence of surfactant has led to smaller particles, with a satisfactory PDI and this may be attributed to the fact that surfactant ensures a good emulsification process and, therefore, leading to the formation of smaller particles with uniform size distribution.
4. **Entrapment Efficiency:** The drug entrapment efficiency (EE) of formulations F1 to F9 were in the range of 37.60% to 85.68% as shown in Table 04. So F8 was considered as the optimised best formulation with the EE of 37.6%. From these results, it was clear that EE increases with increase in polymer concentration.

Table 04: Percentage yield, particle size, PDI and entrapment efficiency of Polymeric Nanoparticles

Formulation code	Percentage yield (%)	Particle size (nm)	Polydispersity Index	Entrapment efficiency (%)
F1	64.28	736.4	0.665	69.22
F2	79.09	484.7	0.465	39.44
F3	71.99	940.5	0.729	85.68
F4	72.90	922.5	0.651	77.76
F5	39.37	581.6	0.613	49.44
F6	95.23	791.8	0.645	75.78
F7	60.95	788.3	0.350	74.48
F8	45.45	456.1	0.398	37.6
F9	60.64	909.1	0.582	76.24

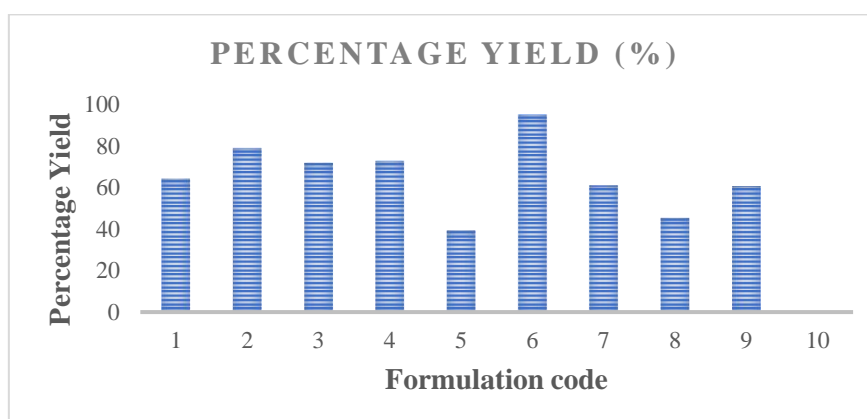


Fig. 09: Percentage yield of Polymeric Nanoparticles of F1-F9 formulations

Results

	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 736.4	Peak 1: 1451	87.6	581.7
Pdl: 0.665	Peak 2: 125.7	10.8	45.25
Intercept: 0.739	Peak 3: 30.76	1.6	7.628
Result quality : Refer to quality report			

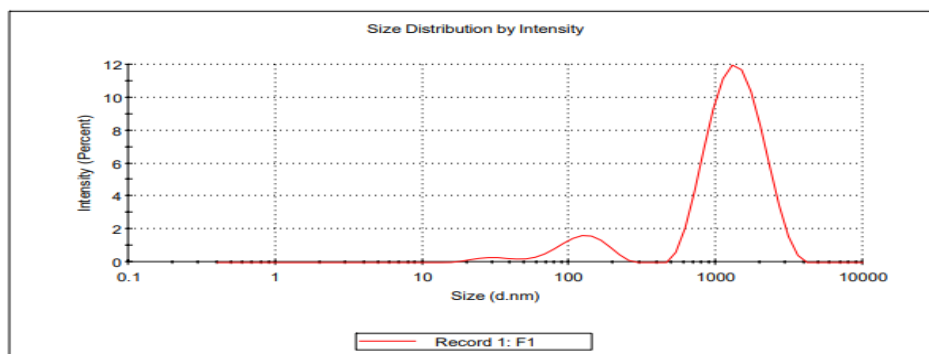


Fig. 10 (a): Particle size of F1 formulation

Results

	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 484.7	Peak 1: 636.5	87.5	416.5
Pdl: 0.465	Peak 2: 4034	12.5	1060
Intercept: 0.920	Peak 3: 0.000	0.0	0.000
Result quality : Good			

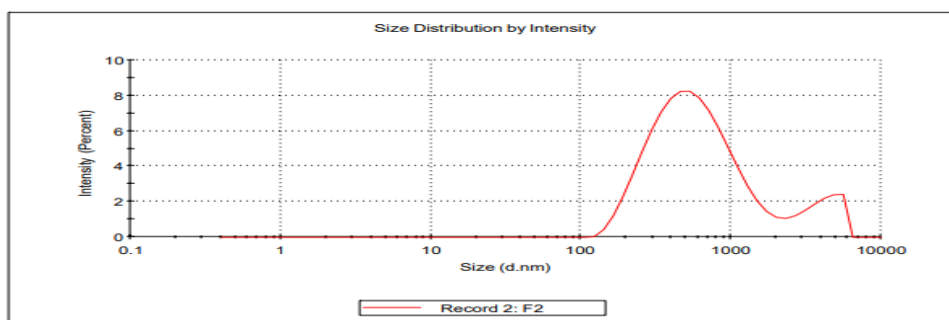


Fig. 10 (b): Particle size of F2 formulation

Results

	Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 940.5	Peak 1: 1871	37.9	600.9
Pdl: 0.729	Peak 2: 4655	35.7	834.7
Intercept: 0.726	Peak 3: 278.1	14.8	71.72
Result quality : Refer to quality report			

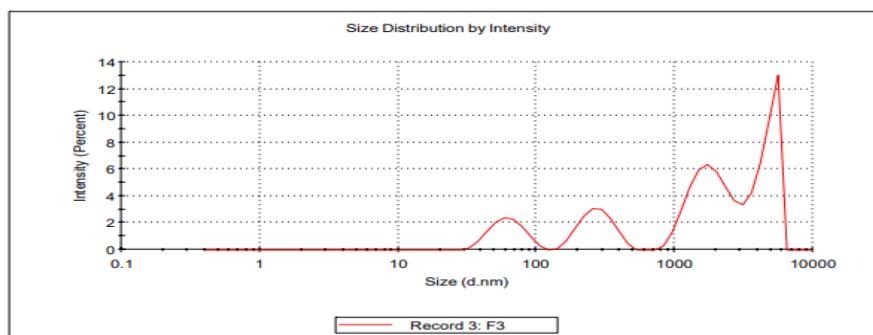


Fig. 10 (c): Particle size of F3 formulation

Results

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 922.5	Peak 1: 3994	68.0	1142
Pdl: 0.651	Peak 2: 127.0	18.9	84.71
Intercept: 0.726	Peak 3: 791.6	13.1	319.7
Result quality : Refer to quality report			

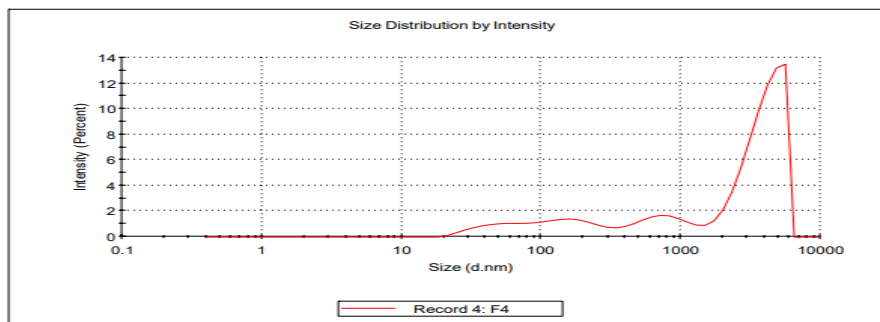


Fig. 10 (d): Particle size of F4 formulation

Results

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 581.6	Peak 1: 421.5	100.0	97.25
Pdl: 0.613	Peak 2: 0.000	0.0	0.000
Intercept: 0.939	Peak 3: 0.000	0.0	0.000
Result quality : Refer to quality report			

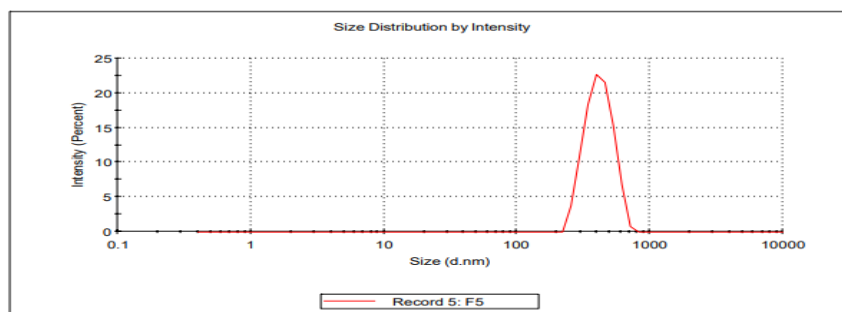


Fig. 10 (e): Particle size of F5 formulation

Results

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 791.8	Peak 1: 1824	85.7	823.9
Pdl: 0.645	Peak 2: 155.6	11.2	59.66
Intercept: 0.732	Peak 3: 38.46	3.1	9.401
Result quality : Refer to quality report			

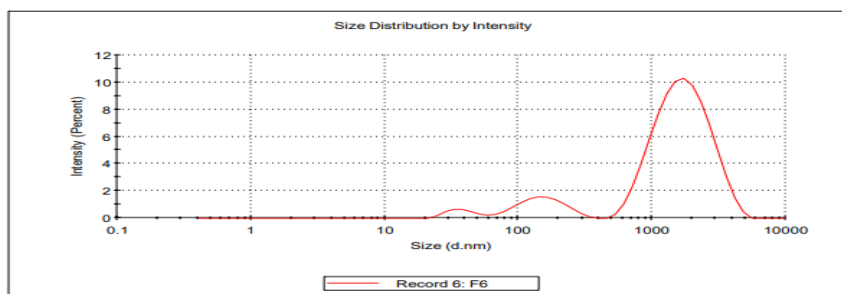


Fig. 10 (f): Particle size of F6 formulation

Results

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 788.3	Peak 1: 1191	93.5	481.2
Pdl: 0.350	Peak 2: 58.50	6.5	10.47
Intercept: 0.749	Peak 3: 0.000	0.0	0.000
Result quality : Good			

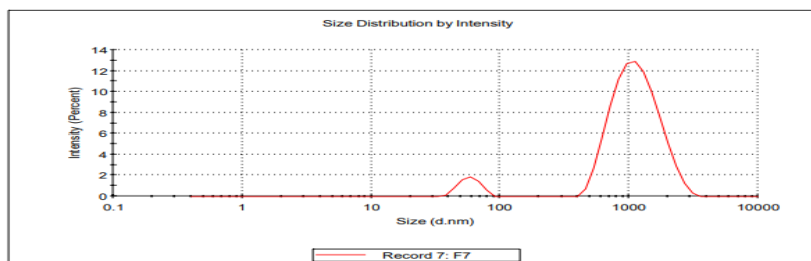


Fig. 10 (g): Particle size of F7 formulation

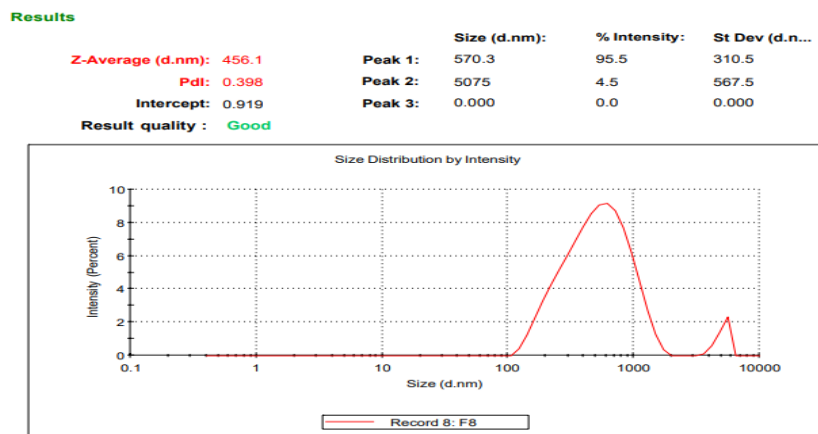


Fig. 10 (h): Particle size of F8 formulation

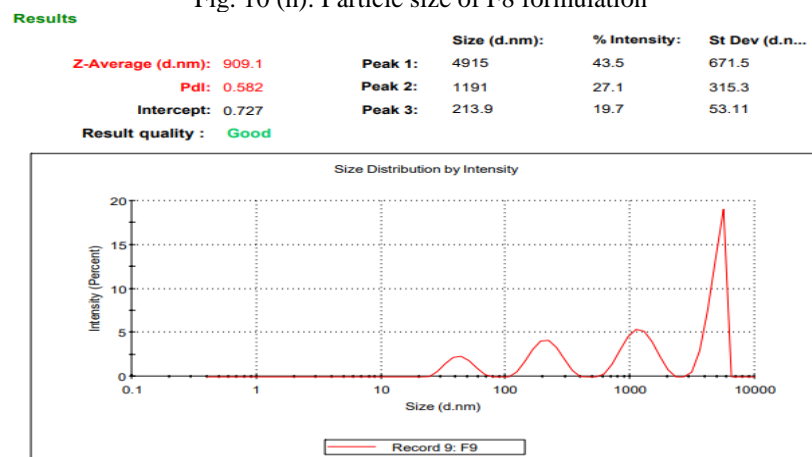


Fig. 10 (i): Particle size of F9 formulation

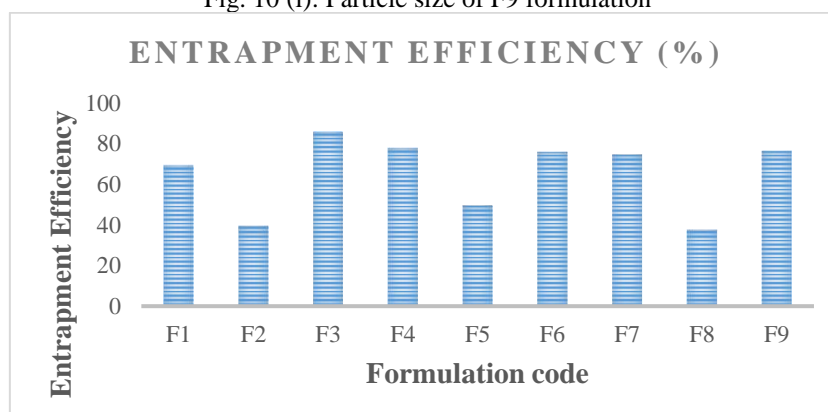


Fig. 11: Entrapment Efficiency of Polymeric Nanoparticles of F1-F9 formulations

5. **In-vitro drug release studies:** The % cumulative drug release for all the formulations (F1 to F9) were shown in Fig. 12. The drug-polymer composition influences the *in-vitro* drug release rate from polymeric nanoparticles. The formulations showed a biphasic release profile, an initial rapid release phase upto 4 hours, followed by a controlled release phase over 8 hours. The initial rapid release may be due to presence of drug on the surface of nanoparticles, free drug in the solution. It was observed that, with increase in concentration of polymer, the release rate was retarded. Therefore, formulation F8 showed highest drug release of 85.09% after 8 hours which was considered as the best formulation.

Table 05: *In-vitro* drug release profiles of Polymeric Nanoparticles

Time (hours)	% Cumulative drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
1	9.41 ± 0.04	13.02 ± 0.122	8.55 ± 0.124	8.64 ± 0.17	11.13 ± 0.085	10.53 ± 0.065	9.31 ± 0.08	15.82 ± 0.065	7.15 ± 0.055
2	11.57 ± 0.056	15.58 ± 0.13	12.63 ± 0.125	10.29 ± 0.088	18.36 ± 0.081	12.14 ± 0.065	11.47 ± 0.091	20.65 ± 0.08	13.19 ± 0.206

3	29.6 ± 0.251	24.28 ± 0.21	16.37 ± 0.126	23.94 ± 0.655	22.67 ± 0.08	20.11 ± 0.06	23.78 ± 0.13	21.41 ± 0.045	23.91 ± 0.135
4	34.26 ± 0.415	36.44 ± 0.202	22.57 ± 0.086	27.29 ± 0.065	36.01 ± 0.04	20.55 ± 0.077	30.11 ± 0.06	40.06 ± 0.081	30.15 ± 0.065
5	37.28 ± 0.051	42.82 ± 0.065	34.01 ± 0.061	42.11 ± 0.08	36.34 ± 0.14	46.59 ± 0.04	43.78 ± 0.045	44.36 ± 0.066	43.83 ± 0.025
6	66.56 ± 0.065	70.08 ± 0.06	51.33 ± 0.036	56.24 ± 0.484	57.59 ± 0.055	62.8 ± 0.212	63.81 ± 0.247	76.95 ± 0.135	52.86 ± 0.12
7	74.26 ± 0.07	80.14 ± 0.065	58.16 ± 0.117	60.79 ± 0.097	73.55 ± 0.095	69.47 ± 0.07	69.12 ± 0.072	83.25 ± 0.452	60.28 ± 0.228
8	76.79 ± 0.225	84.29 ± 0.368	61.68 ± 0.145	64.12 ± 0.436	78.2 ± 0.362	74.55 ± 0.275	72.82 ± 0.201	85.09 ± 0.36	64.25 ± 0.176

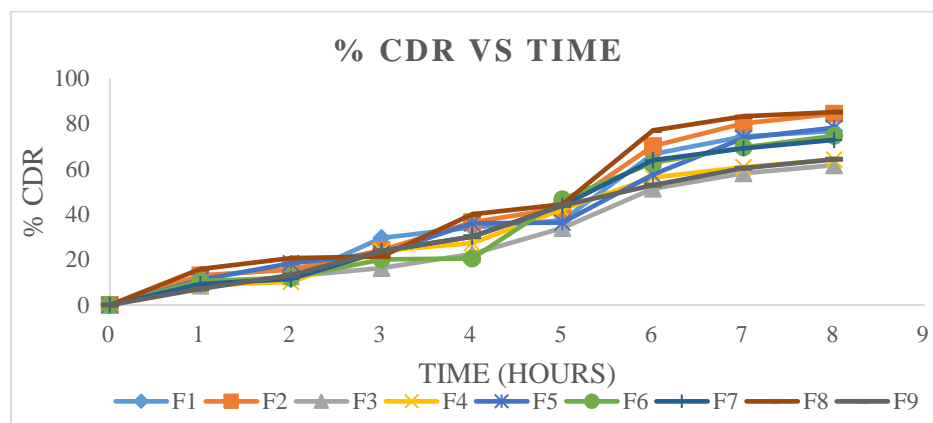


Fig. 12: % Cumulative drug release of Polymeric Nanoparticles of F1-F9 formulations

6. **Morphology by Optical Microscopy:** The surface morphology of polymeric nanoparticles was studied by using the optical microscope. Fig. 13 demonstrates the surface morphology of polymeric nanoparticle formulation (F8). It illustrated that the polymeric nanoparticles were observed as smooth spherical surfaced particles.

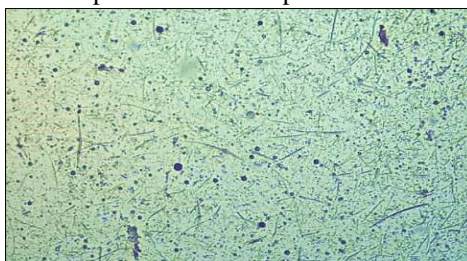


Fig. 13: Surface morphology of optimized polymeric nanoparticle formulation

7. **Zeta Potential:** Zeta potential is an important physico-chemical parameter that influences stability of the polymeric nanoparticle suspension. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy dispersion. Zeta potential of the optimized batch of polymeric nanoparticle formulation (F8) was found to be -6.07 mv (Fig. 14) which indicated a stable formulation. Negative potential was due to the polymer and drug anionic nature.

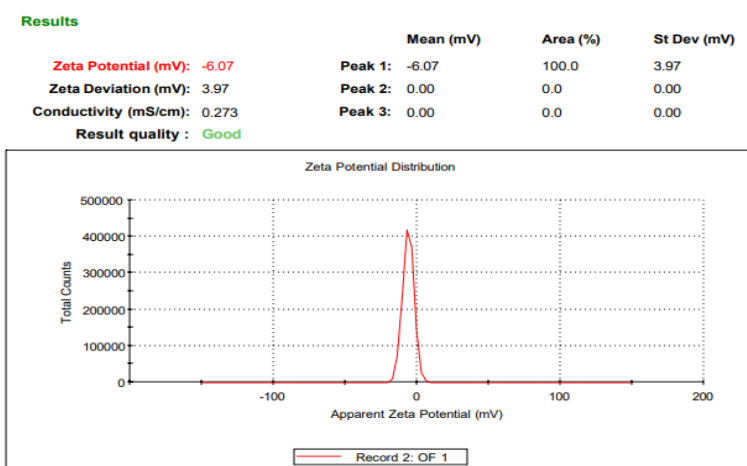


Fig. 14: Zeta potential of optimized polymeric nanoparticle formulation

8. **Drug release kinetics:** In order to study the exact mechanism of drug release from optimized batch of polymeric nanoparticles, *in-vitro* drug release data were fitted into various mathematical models such as Zero order, First order, Higuchi, and Korsmeyer-Peppas models. The data were processed for regression analysis using MS-EXCEL statistical function. The release constants were calculated from the slope of appropriate plots, and the regression coefficient (R^2) was determined. It was found that *in-vitro* drug release of polymeric nanoparticle was best explained by the Korsmeyer-Peppas model as the plot shows the highest linearity (Fig. 18). Regression coefficient (R^2) was found to be 0.8844 (Table 06) with an 'n' value of 0.909 which showed that formulated polymeric nanoparticles followed case II transport mechanism indicating zero order release of the drug.

Table 06: Regression values of kinetic study of Benidipine hydrochloride loaded polymeric nanoparticle formulation

Formulation	Zero order model	First order model	Higuchi model	Korsmeyer-Peppas model	
	R^2	R^2	R^2	R^2	n
Polymeric nanoparticle	0.9455	0.879	0.8311	0.8844	0.909

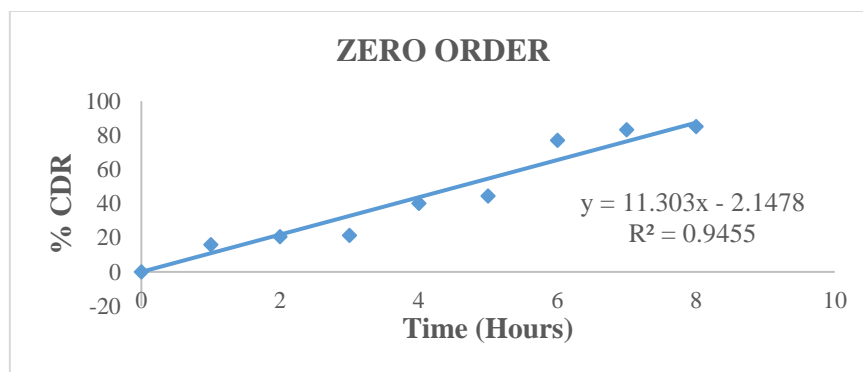


Fig. 15: Plot of % CDR Vs Time

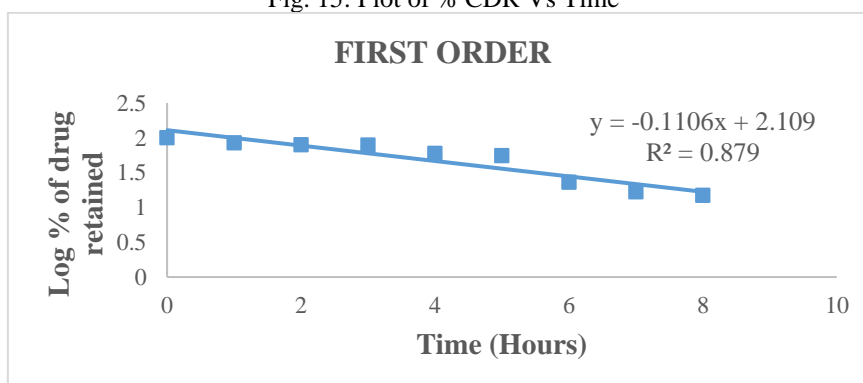


Fig. 16: Plot of Log % of drug retained Vs Time

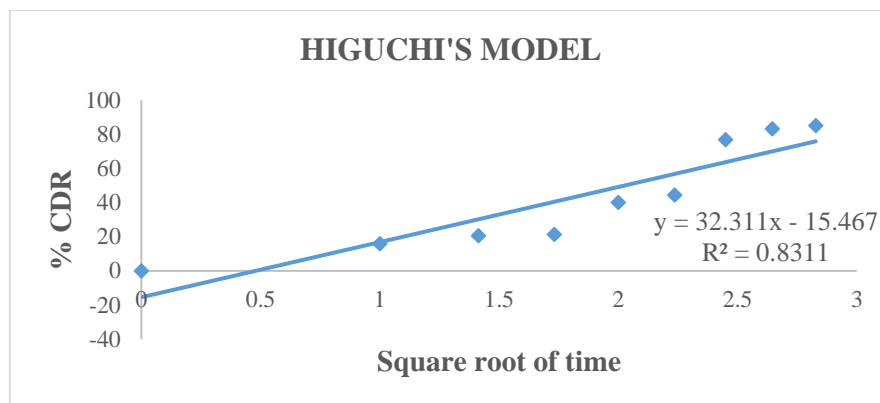


Fig. 17: Plot of % CDR Vs square root of time

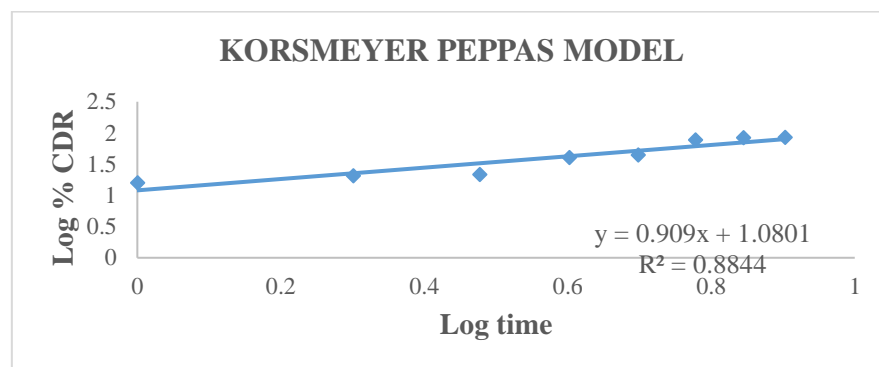


Fig. 18: Plot of Log % CDR Vs Log time

9. **Stability Studies as per ICH guidelines:** The stability study was carried out for optimized polymeric nanoparticles at accelerated conditions ($40 \pm 2^\circ\text{C}$ & $75 \pm 5\%$ RH) for 3 months (30, 60, 90 days) as per ICH guidelines. It was confirmed from the stability studies that the evaluated formulation remained stable at $40 \pm 2^\circ\text{C}$; $75 \pm 5\%$ RH, for a period of 3 months. The data showed that there were no significant changes in physical appearance, entrapment efficiency and % cumulative drug release over 8 hours before and after 3 months of stability study as depicted in Table 07.

Table 07: Stability studies of optimized formulation (F8)

Evaluation parameter	Time (days) Accelerated condition: $40 \pm 2^\circ\text{C}$; $75\% \pm 5\%$ RH			
	0 day	30 days	60 days	90 days
Physical appearance	Milky white	Milky white	Milky white	Milky white
Entrapment efficiency (%)	37.6 ± 0.251	37.25 ± 0.19	36.94 ± 0.09	36.81 ± 0.07
% Cumulative drug release (after 8 h)	85.09 ± 0.237	85.01 ± 0.125	84.93 ± 0.158	83.77 ± 0.162

CONCLUSION:

The research undertaken establishes the successful development of Polymeric nanoparticles by emulsion solvent evaporation method. From all the observations and results obtained, it can be concluded that the prepared formulation batches showed satisfactory organoleptic properties. A characterization of the drug and excipient was performed and no immeasurable peaks were observed in FT-IR analysis, so characterization confirmed that there was no interaction between the drug and polymers. All results were compared to the standard, which concluded that the drug and excipient were of pure and standard quality. The particle size and PDI of the polymeric nanoparticles (F8) was found to be 456.1 nm and 0.398 respectively. Entrapment efficiency was in the range of 37.60% to 85.68%. The *in-vitro* drug release study confirmed the maximum drug release of 85.09% over 8 hours. The surface morphology study showed the presence of smooth spherical surfaced particles. The zeta potential value of -6.07 mV revealed the better physical stability of the optimized formulation (F8). The formulation was analysed for drug release kinetics from the results of *in vitro* drug release, and it was best explained by Korsmeyer-Peppas model and followed case II transport mechanism indicating zero order release of the drug. Accelerated stability study ($40 \pm 2^\circ\text{C}$ & $75 \pm 5\%$ RH) of the optimized batch showed no

significant changes in the visual appearance, entrapment efficiency and % cumulative drug release during the period of the study. Hence it was proved that prepared formulation was found to be stable. From this overall study hence it can be concluded that the above formulation is more effective than conventional tablets used in treatment of hypertension.

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