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Formulation, optimization and *In vitro & In vivo* biological evaluation of Eudragit-S-100 nanoparticles loaded with Clofarabine as potential antitumor drug delivery system

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Abstract

Clofarabine is a second-generation purine nucleoside analogue with antineoplastic activity. The objective of this study was to prepare Eudragit-S-100 nanoparticles loaded with Clofarabine as potential antitumor drug delivery system. Nano-suspension of CLOFARABINE was prepared by Nano-precipitation method. Five Nano -suspension sample formulas were prepared using Nano-precipitation technique. The prepared formulations were characterized by parameters such as average diameter of Nanoparticles in suspension form, poly- dispersity index, zeta-potential and entrapment efficiency (EE) and in-vitro drug release studies. Furthermore, the cytotoxicity and cellular uptake of conjugated nanoparticles in to cancer cell linings were investigated.

Keywords: Nanoparticles, Clofarabine, Nano-precipitation method, encapsulation efficiency

1. Introduction

Clofarabine is a second-generation purine nucleoside analog with antineoplastic activity[1-3]. It is metabolized intracellular to the active 5'-triphosphate metabolite, which inhibits DNA synthesis is and so stops the growth of cancer cells. Clofarabine is used as an antimetabolite antineoplastic

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agent in the treatment of relapsed or refractory acute lymphoblastic[4, 5] leukemia. It has a role as an antineoplastic agent and an antimetabolite. After extensive survey of literature, for the first time the authors are optimizing and developing Eudragit-S-100 Nanoparticles loaded with Clofarabine as potential antitumor drug delivery system. To the best of our knowledge, the ability to formulate a multicomponent structure encapsulating Clofarabine, which can be reconstituted without loss of activity that enhanced the oral bioavailability of clofarabine, is the novelty in this study. Nano-suspension of CLOFARABINE was prepared by Nano-precipitation method. Five Nano -suspension sample formulas were prepared using Nano-precipitation technique. The nanoparticles were further characterized using, electron microscope and transmission electron microscope (TEM). Subsequently, drug loading (DL) and encapsulation efficiency (EE) were carried out to determine the capability of clofarabine to be entrapped in the formulation system before conducting in vitro release study using MC7 cancer cell linings. All formulations were subjected to solubility study and stability evaluation to observe sustained release profiles in Cancer cell linings.

2. Experimental

2.1. Materials

Clofarabine was kindly gifted by Mylan Pharmaceutical Co., Ltd. (Hyderabad India). Eudragit S 100, PEG 400 and Acetone were supplied by Central Drug House (p) Ltd (India). Tween 80 was procured from Loba Chemie (India) and Pluronic F-68 was provided by Himedia (Mumbai; Maharashtra). MCF-7 cell line, received as gift sample from KIMS, Hyderabad). RPMI-1640 and calf serum were purchased from Jena biosciences. MTT was obtained from Hymedia laboratory. Dimethyl sulfoxide was received from Merck (India). Injections of streptomycin sulfate (1 million units) and injection of sodium penicillin (800,000 units) were purchased from Local market in Hyderabad. All other reagents used were of analytical grade or purer and water was double distilled.

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2.2. Pre-Formulation Study

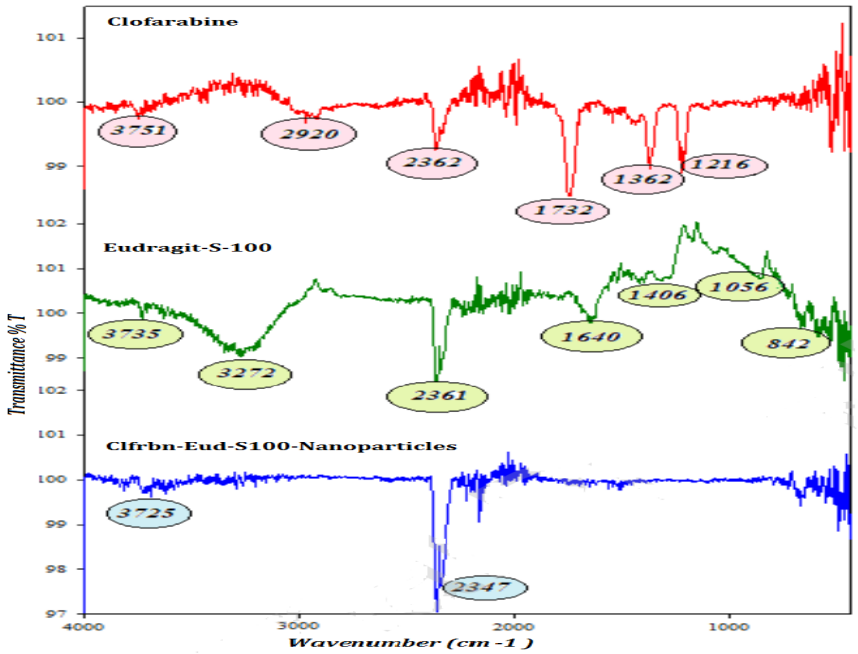
X-Ray Diffraction (Powder X-Ray Diffraction)

Crystallinity of the pure CLOFARABINE drug was evaluated by powder X-ay diffraction (PXRD) measurements.

Fourier Transform Infrared (FTIR) Study

The polymeric interactions with active pharmaceutical ingredient were studied by FT-IR-spectrophotometer. 2 %(w/w) of the prepared sample, with respect to potassium bromide was mixed with dry KBR. Each potassium-bromide pellet was scanned 16 times at 2mm/size at a resolution of 4 cm^{-1} using Carson apodization. The peaks of potential functional groups were recorded. Eudragit S 100 and P.F. 127 were conjugated with CLOFARABINE Hence, FTIR analysis was done to find out the chemical properties of clofarabine conjugated nanoparticles with polymer and its mode of chemical bonding with nano-suspension was also characterized.

Figure 1- FTIR Studies of polymer and active pharmaceutical ingredient



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2.3. Method

Nano-suspension of CLOFARABINE was prepared by Nano-precipitation method. Eudragit S100 (100mg) and CLOFARABINE (10mg) were dissolved in 5ml of acetone in a beaker with the help of a bath sonicator for five minutes by covering the beaker with the help of aluminium foil in order to prevent the evaporation of acetone during sonication and to form a uniform organic solution. The prepared organic solution was then injected slowly drop wise into the external aqueous phase (20ml) containing Pluronic F-68 0.5% (w/v) & Tween 80 (0.02%) with the help of a syringe. The drug to polymer ratio of the formulation was (1:10). The resultant Nano-suspension was kept on magnetic stirring (500 rpm) for about 4h in order to evaporate the organic solvent. Finally the prepared Nano suspension was subjected to rota-evaporation under reduced pressure at 60°C for removal of the traces of acetone present in it .The parameters such as drug to polymer ratio, agitation time and concentration of containing Pluronic F-68 were varied for various formulations based on the formulation design

2.4. Fabrication of Nano-suspension Particles

Five Nano -suspension sample formulas were prepared using Nano-precipitation technique. The prepared formulations were characterized by parameters such as average diameter of Nanoparticles in suspension form, poly- dispersity index, zeta-potential and entrapment efficiency (EE).

Table-1: Various Formulations Nanoparticles loaded with Eudragit for Clofarabine

Components	CLFRBN-1	CLFRBN-2	CLFRBN-3	CLFRBN-4	CLFRBN-5
Clofarabine	10 mg	10 mg	10 mg	10 mg	10 mg
Eudragit-S-100	100 mg	200 mg	300 mg	400 mg	500 mg
Pluronic-F-68	0.5 % (w/v)	1 % (w/v)	1.5 % (w/v)	2 % (w/v)	2.5 % (w/v)
Tween 80 % (w/v)	0.02	0.02	0.02	0.02	0.02
Acetone	5	8	10	10	12
Aqueous Phase	20 mL	25 mL	25 mL	30 mL	40 mL

Previously, a number of attempts were made to optimize the Eudragit S 100 en-coated Nano-suspension of Clofarabine, before optimizing the original formulation design.

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2.5. Characterization of Nano-Suspension Particles-

2.5.1. Particle size determination and distribution:

Preliminary Particle Size Determination was performed by using Master-Sizer. The nano-suspension size was determined by photon correlation spectroscopy by master sizer (Master-sizer 2000; Malvern instruments Corp, U.K.). The particle size analysis was conducted at scattering-angle of 90° at ambient temperature (25°C) using suitably diluted sample solution with distilled & deionized (DD) water. The final optimized formulas prepared after trials were analysed under Particle Size Analysis by master sizer for distribution width (DV), Mean-Particle-Size (MPS), Surface Area (SA) (m^2 /g), Span and Uniformity. Among all the formulas (CLFRBN1-to-CLFRBN5) CLFRBN1, CLFRBN2 and CLFRBN3 were found to give better results in the above listed parameters. The particle size analysis report of Master sizer was found to be varying with the formulations (CLFRBN1-CLFRBN5). The formulations CLFRBN1, CLFRBN2, CLFRBN3 which demonstrated the Nano range in the master sizer reports were further subjected to analysis by Delsa Nano^{TM}C. Before going to analysis by the TM zeta sizer (Delsa Nano^{TM} C) the formulations CLFRBN1, CLFRBN2 and CLFRBN3 were subjected to probe sonication for 3 minutes with an impulse of 5 seconds. The probe sonication leads to the reduction of particle size as well as improving the stability of the formulation. The results after the analysis by Delsa Nano^{TM} C revealed that the formulation CLFRBN1 can be declared as best formulation in terms of size with a value of 873±118.

2.5.2. Particle Size Analysis by Delsa Nano TM Common:

The best formulations of Eudragit S 100 group (CLFRBN1-CLFRBN5) were subjected to particle size analysis by Beckman Coulter. The formulation CLFRBN1, CLFRBN2 and CLFRBN3 were found to be better in achieving the Nano range in master sizer. Among the three Eudragit S 100 formulations (CLFRBN1, CLFRBN2 and CLFRBN3) the formulation CLFRBN1 was found to be the best in terms of size (416.4 nm) which was the lowest size of all the Eudragit formulations.

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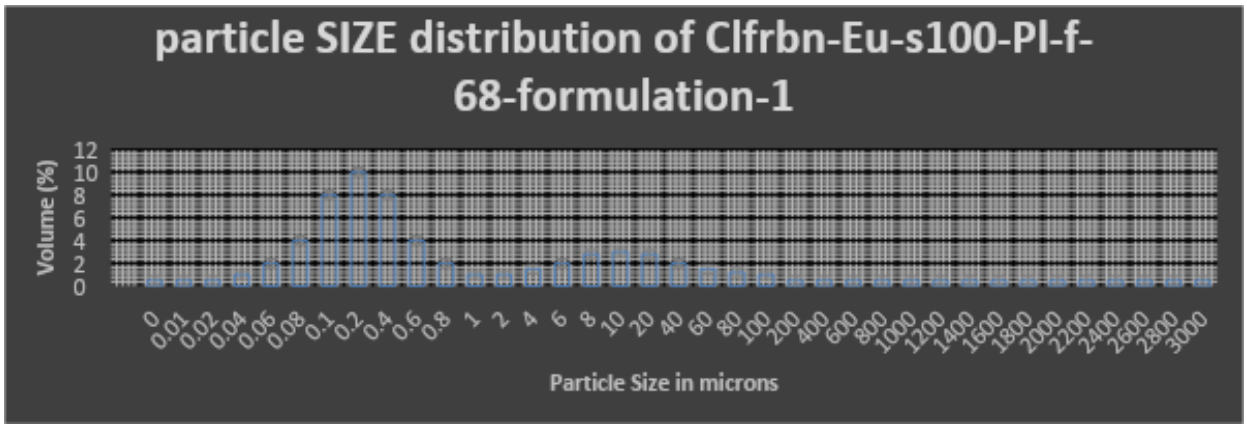
Table 2: Particle Size analysis and distribution by Master-sizer and Delsa Nano

Particle Size Analysis of CFRBN-EUD-S-100-P.L.F-68-NPs by Malvern Master Sizer							
Formulation Code Number	Distribution width in (nm)			MPS	SA (m^2/g)	Span	Uniformity
	Dv-10	Dv-50	Dv-90				
CFRBN-1	11.1	24.5	2435.7	823.77	27.7	98.963	32.7
CFRBN-2	12.8	482.2	6198.3	2231.1	22.5	12.827	4.31
CFRBN-3	11.8	46.3	5624.4	1894.16	24.2	120.96	32.5
CFRBN-4	444.7	985.4	2191.8	1207.3	0.865	1.772	0.84
CFRBN-5	379.2	847.5	1821	1015.9	0.982	1.701	2.02
Particle Size Analysis of CFRBN-EUD-S-100-P.L.F-68-NPs by Delsa-Nano-™							
Formulation Code Number	Distribution width in (nm)			MPS±SD	Diameter in nm		PDI
	Dv-10	Dv-50	Dv-90				
CFRBN-1	164.2	542.6	1912.2	873±118	485.9		0.45
CFRBN-2	375.4	874.4	2358.5	1235±14	986.6		0.87
CFRBN-3	14.52	563	10826.5	2764±09	911.9		0.68

Figure 2. Particle Size Distribution of Eudragit Nanoparticle Formulations loaded with Clofarabine



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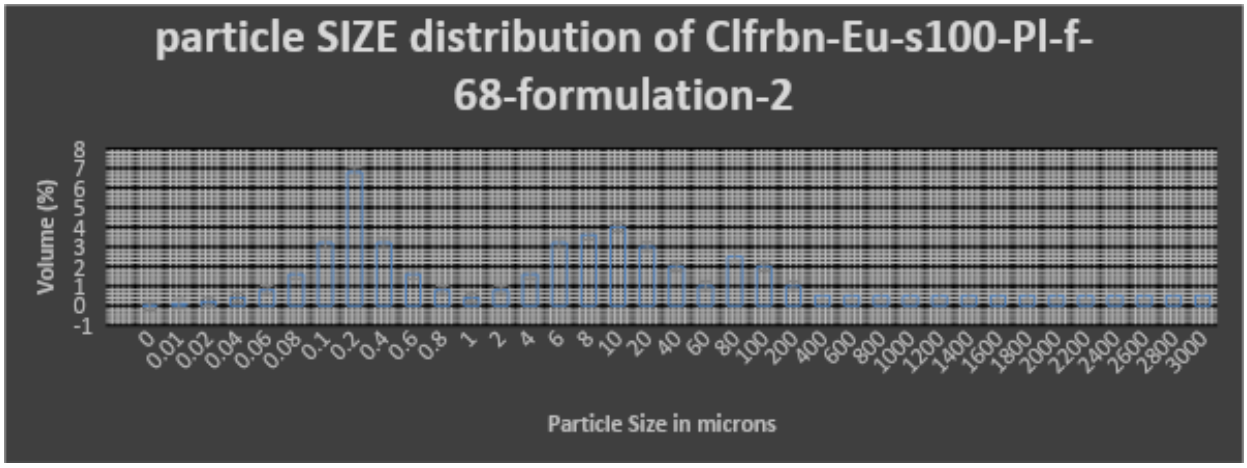

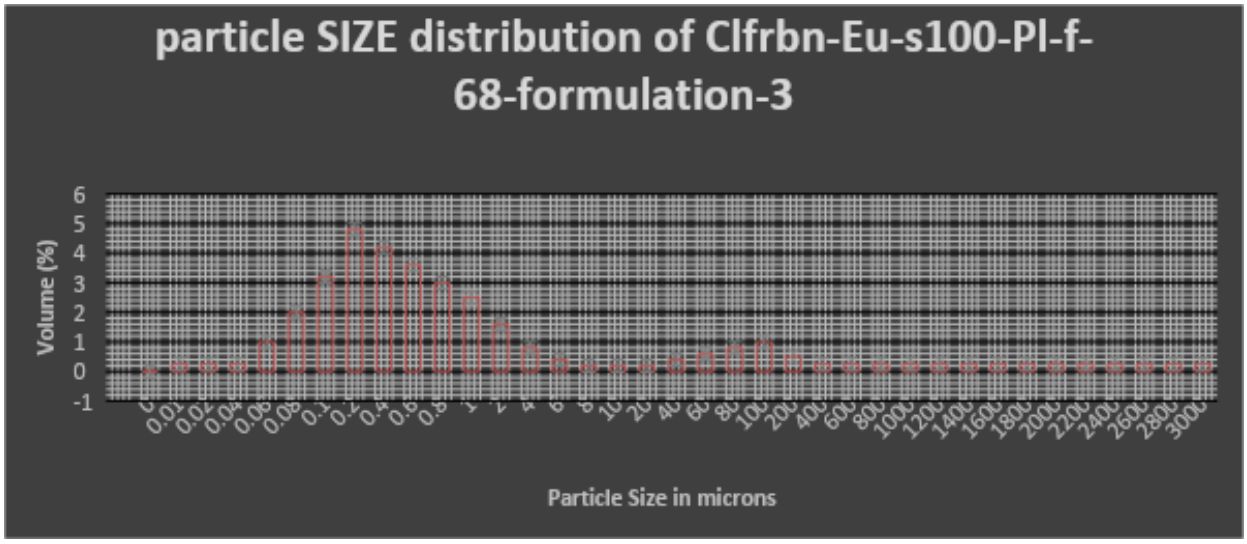

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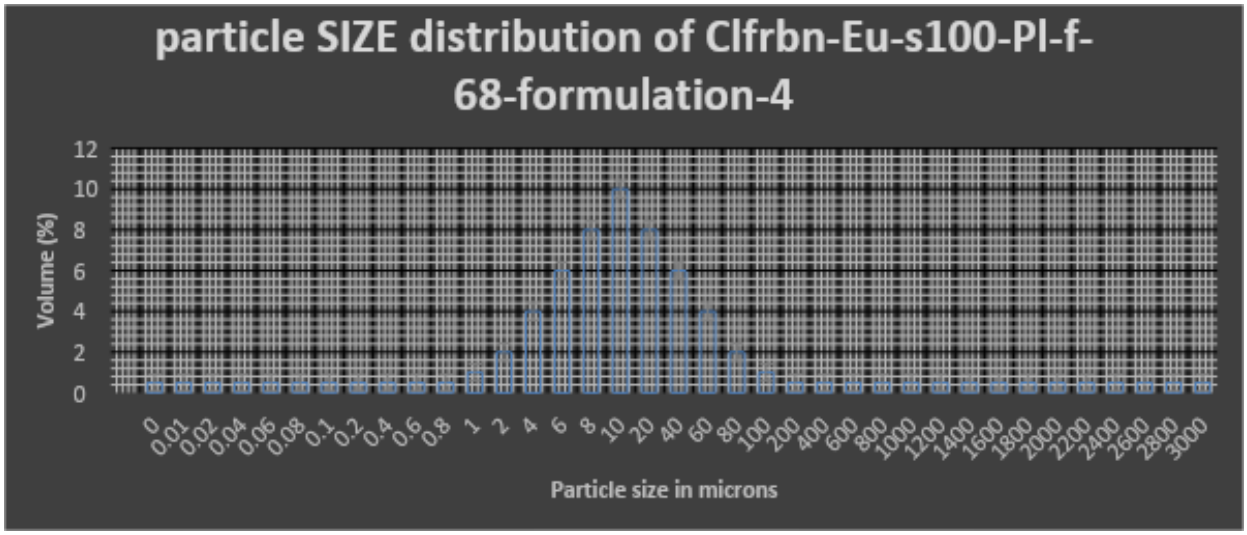

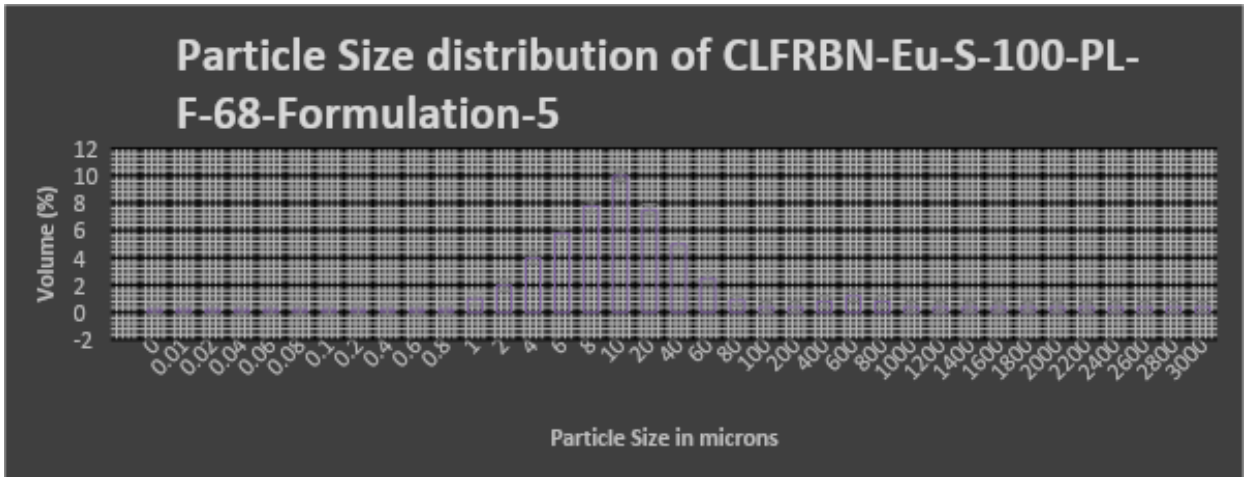

2.5.3. Zeta Potential Measurement

Zeta potential of the proposed formulations was determined by using Beckman coulter. The best formulations identified by the master seizer and Beckman coulter counter were also further subjected to zeta potential measurements. The zeta potential of the Nano suspension is vital for the finding the amount of Surface charge on Nano-suspension particles. The particle surface charge parameters are related to the stability of Nano-suspension particles at the pilot scale manufacturing level. The zeta potential values are commonly calculated by determining the

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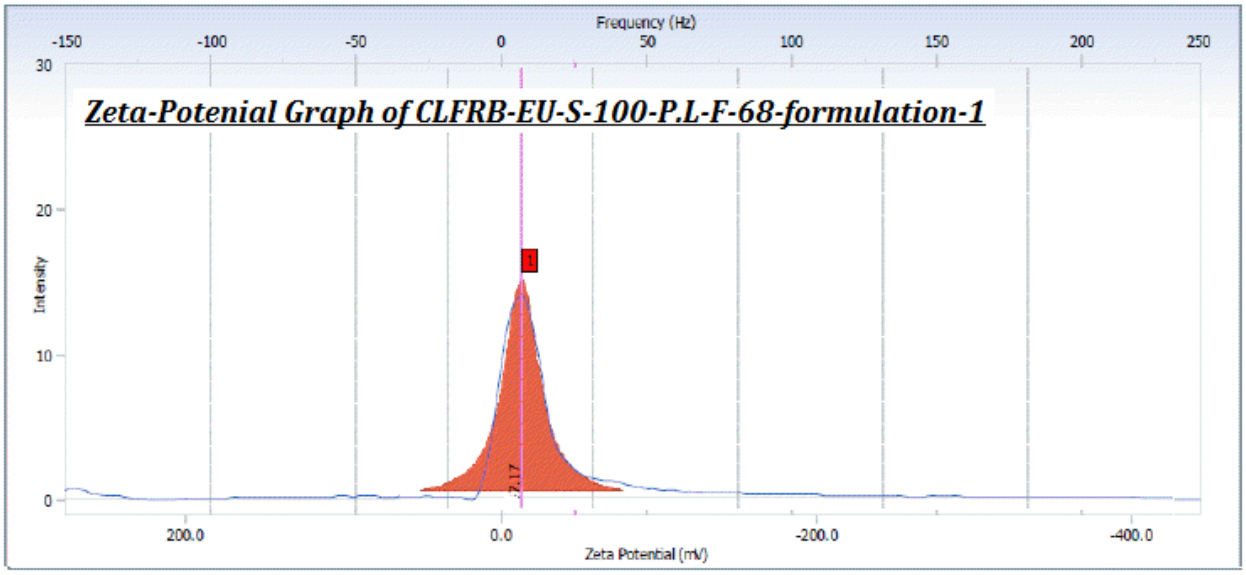
particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential. The zeta potential of the Eudragit S 100 preparation was found to be -7.17mV

Figure 3: Zeta Potential Graph



2.5.4. Entrapment Efficiency of Clofarabine:

The 10 ml of Clofarabine Nano-dispersion was subjected to drug entrapment efficiency test to check the percentage of incorporated CLOFARABINE. Entrapment efficiency was determined spectrophotometrically at 293.6nm using UV-Visible spectrophotometer (Systronics, 2201; India). Initially, 20ml of Nano-suspension was prepared and subjected to centrifugation at 5000 RPM for 45 minutes. The amount of free drug was detected in supernatant and the amount of loaded drug was determined as a result. Entrapment efficiency was calculated by using equation:

$$\frac{\text{Total Quantity of drug- Quanitity of Unbound Drug}}{\text{Total amount of drug}} \text{ X } 100$$

The percent entrapment efficiency was determined by using suitable procedures. The % entrapment efficiency was found to be the lowest in CLFRBN5 which is (37.5±1.23) and the

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highest was found in CLFRBN1 (82.5±3.56) which is the highest drug entrapment efficiency found in Eudragit group. This revealed that lowest particle size encouraged the highest entrapment efficiency as described by the many researches. The other formulations also demonstrated the entrapment efficiency of 65±2.64, 55±1.75 and 42.5±1.09 for CLFRBN2, CLFRBN3 and CLFRBN4 respectively.

2.5.5. Transmission Electron Microscopy (TEM)

TEM was used to characterize the microstructure of CLOFARABINE loaded nanoparticles. For TEM analysis, the prepared Nano-suspension was diluted with distilled water (HPLC Grade) and sonicated in bath sonicator for 2 minutes and from this a drop was taken and was placed on the carbon coated copper grid and superfluous was wiped out with Whattman filter paper and TEM images were obtained using (HITACHI Japan. Model H7500 ID). Transmission Electron Microscopy (TEM) analysis of prepared Eudragit nanoparticles of CLFRBN1 formulation was done by using advanced technique such as TEM. The particles were found to be spherical in shape with uniform Size and also demonstrated that the particles appear in the uniform range which were depicted in the images taken at different magnification 80000X-200000X.The particle size determination in the TEM images was found to be 44.4nm, 40.6 nm and 30.9 nm revealed that particles were having small span with particle size-distribution range is within narrow region. Hence the Nano precipitation method of preparation for the nanoparticles was appropriately validated by this analysis.

Figure 4: TEM Images of Eudragit Nanoparticles loaded with Clofarabine

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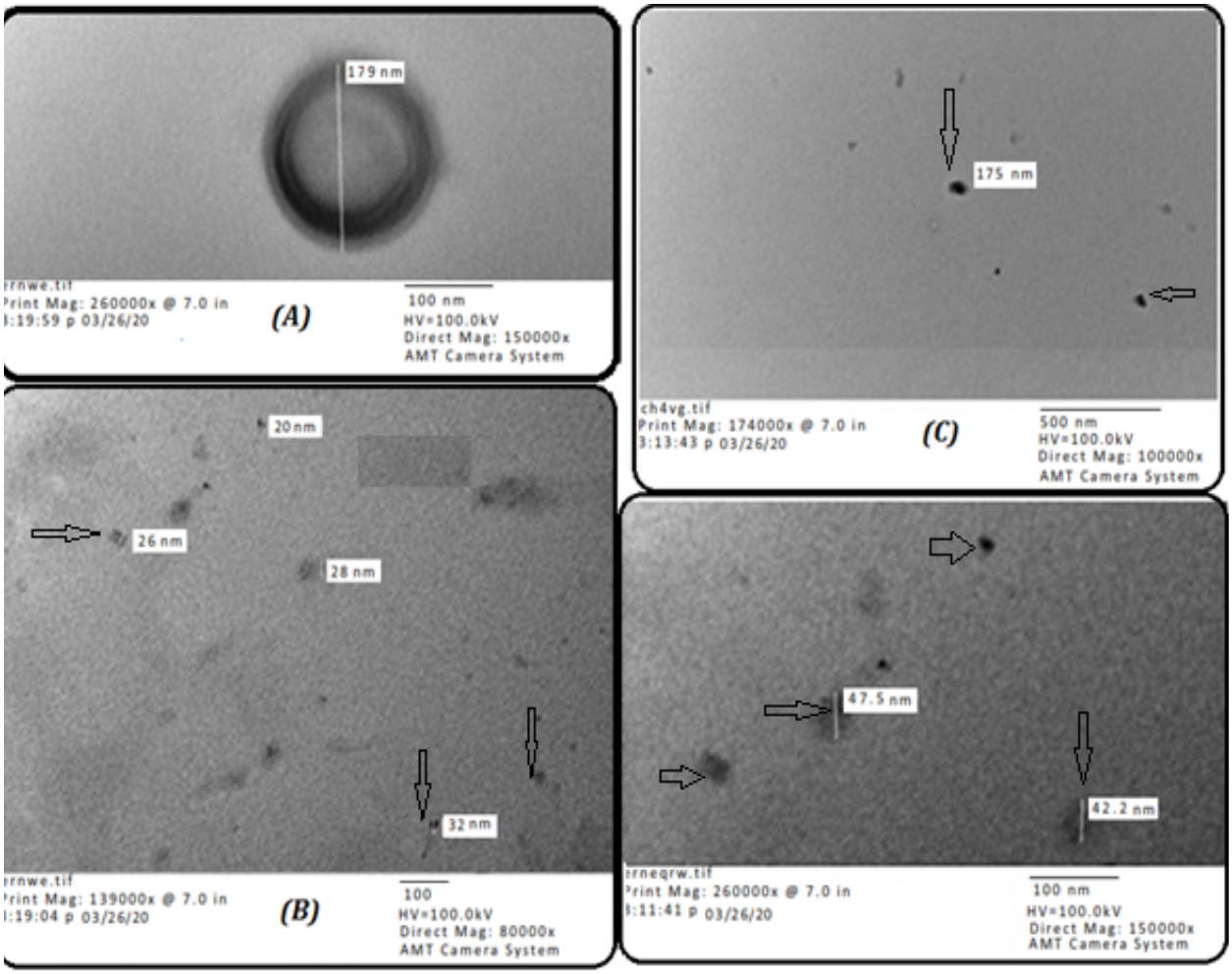

2.6. Determination of CLOFARABINE Solubility

CLOFARABINE solubility experiments were done utilizing PEG 400. The abundance quantity of API was included various dilutions of PEG 400. The PEG 400 and water were blended in various fixations (0.5:4.5), (1:4), (1.5:3.5), (2:3) and (3:2). The medication CLOFARABINE exhibited the better dissolution pattern in 2:3 proportion of PEG and water. Already five test tubes were taken containing various proportions of PEG and water as portrayed before. The 1Mg medication was weighed precisely and brought into each test tube and was blended for few moments. After the total dissolution of CLOFARABINE again 1mg of CLOFARABINE was

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added to the above test tubes and again the test tubes were shaken for few moments. These operations were replicated for multiple times until the measure of the medication became 5 mg in all the test tubes. At that point these test tubes were again shaken for 1 h persistently so as to accomplish greatest dissolvability. At that point the test tubes were placed aside as such for 24 h and after 24 h the medication solvency in the test tubes were outwardly assessed. The test tube with no medication appears at the base was in the 2:3 proportion of solvent mixture and the other test tubes were containing the medication at the base which was undissolved.

3. Results and Discussion

3.1. Standard Calibration curve of Clofarabine

The medication solvencies with 1mg/ml without any traces at the base was exposed to the UV examination by planning working standard dilution of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/ml and were checked in UV-Spectrophotometer between the range 200-600 nm. The maximum absorbance was seen at 295 nm and 448 nm. As the working dilutions of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/ml were seen as straight-line relations ship, hence these ranges of concentrations were chosen and calibration curve was set up in triplicate and the mean was taken.

3.2. UV Analysis of Clofarabine

In the UV analysis a primary standard solution of CLOFARABINE (1mg/1ml) was set up by dissolving precisely measured amount of CLOFARABINE in water and PEG 400 (3:2) and the working standard solutions were set up by further diluting the standard solution with PEG 400 and water to make the final working standard solutions. The absorbance of resulting testing solutions was recorded in a 10mm quartz cell of an UV-Visible Spectrophotometer (Systronics, India). The calibration plot of the medication and the absorbance maxima, as per Beer-lamberts' law were recorded. A similar methodology was completed utilizing the 0.1 N HCl, 6.8 pH phosphate and 7.4 pH buffer solutions as the definition of nanoparticles implied for sustained and controlled release at specified site. The absorbance maxima were chosen at 295 nm.

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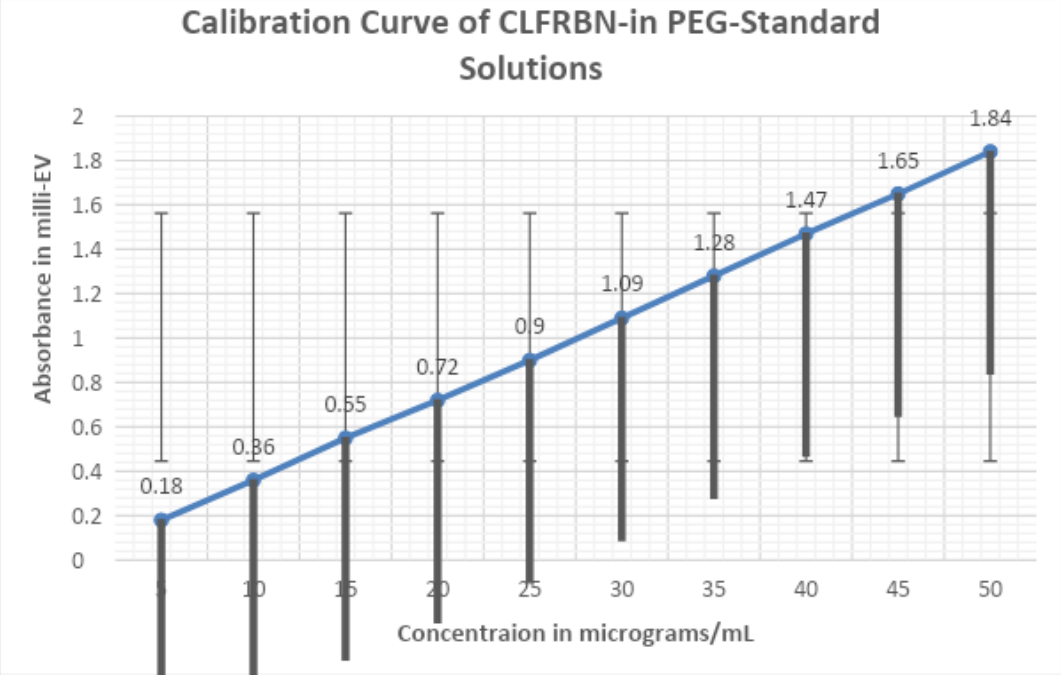
Figure 5: Calibration Curve of Clofarabine in PEG-Standard solutions



3.3. Drug-Release studies of Clofarabine from Nano suspended particles

The medication release profile from arranged Nano-suspension was controlled by utilizing dialysis pack (Himedia labs, cut-off weight 12000-11000 Da) strategy for more than 24 h. In this technique, the dialysis packs were soaked in two-fold refined water for 12 hours before use. At that point the dialysis sacks were removed from the water and attached with the assistance of a string from one end. Clofarabine Nano-suspended particles were then brought into the dialysis sack by methods for a pipette. Dialysis pack held the nanoparticles and permitted the free medication in dissolution media with a cut-off of 12000 Dalton. The 10mg equivalent quantity of Clofarabine containing Nano-suspension particles was filled in the dialysis sack and the opposite end was fixed solidly by methods for strings. The sack was set in the measuring glass containing 100ml of receiving medium as a media. The framework was held under attractive mixing conditions at 100 rpm. The samples were collected at predetermined time frames, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hrs. Testing solutions were collected from the dissolution media and were

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renewed with the same quantity of fresh new dissolution media so as to keep up the sink conditions. The testing dilutions were investigated spectrophotometrically utilizing UV spectrophotometer against the blank or placebo. All the perceptions were completed in triplicate.

3.4. In vitro Drug Release Study.

In vitro drug release pattern was performed by utilizing the various buffer solutions, for example, 0.1N HCl (1.2 pH), 6.8 pH potassium dihydrogen phosphate buffer system and 7.4 pH phosphate solution to simulate the Gastro intestinal tract situation of stomach, digestive system and colon separately. All the prepared formulation samples (CLFRBN1-CLFRBN5) were studied to find out the drug-release fashion with reference to time profile analysis.

The medication release studies were performed up to 24 h. The testing dilutions were taken in different time spans. During the acidic conditions (1.2pH) the sample dilutions were collected for each thirty minutes up to 1.5 h. At that point during the intestinal conditions 6.8 pH the testing dilutions were withdrawn for each 1 h up to 4 h followed by the colonic conditions 7.4 pH the testing dilutions were collected for 1 h up to 8 h and then final two dilutions were taken in 12 h and 24 h. The examples pulled back from the framework were renewed with new media. The plan CLFRBN1 was seen as best as it discharged the medication up to 24 h.

During the acidic condition the detailing CLFRBN1-formulaiton was released around 9% of the medication only at 1.5 h. Being a sustained released dosage form containing the nanoparticles, it is suggested that the medication release should be minimum as per the protocol to be least in the upper GI tract and it was acquired in the prepared eudragit nanoparticles of CLFRBN1. After the acidic condition the medication release profile was gradually extended up to 12 hrs and it was found that as 52.928±1.22. The further sample was collected at 24 h and the medication release was seen as 85.506±2.13. The other formulation is also showed the controlled release profile however the medication release pattern was highly variable may be due to different proposition of polymer in each developed formulation. The CLFRBN2-formulaiton and

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CLFRBN3-formulation discharged the medication measure of 44.092±1.26 and 46.122±1.19 individually toward the end of 12 hrs. The medication discharge from these details was 79.126 ±2.15 and 73.225±2.931respectively for complete 24 hrs. The rest of the details CLFRBN4 and CLFRBN5 showed the futile drug release patterns of 60.017±3.33 and 64.165±3.11 individually. In any case, these dosage forms were at that point discarded from the class of best formulation dependent on the results of study. Hence it was demonstrated that the best detailing CLFRBN1 discharged the medication in a deferred discharge design with a foreordained medication discharge profile. Relative measure of medication released from polymeric nanoparticles of Eudragit S 100 (CLFRBN1-CLFRBN5) are represented in the Figure.

Figure 6: Drug Release from Polymeric Nanoparticles

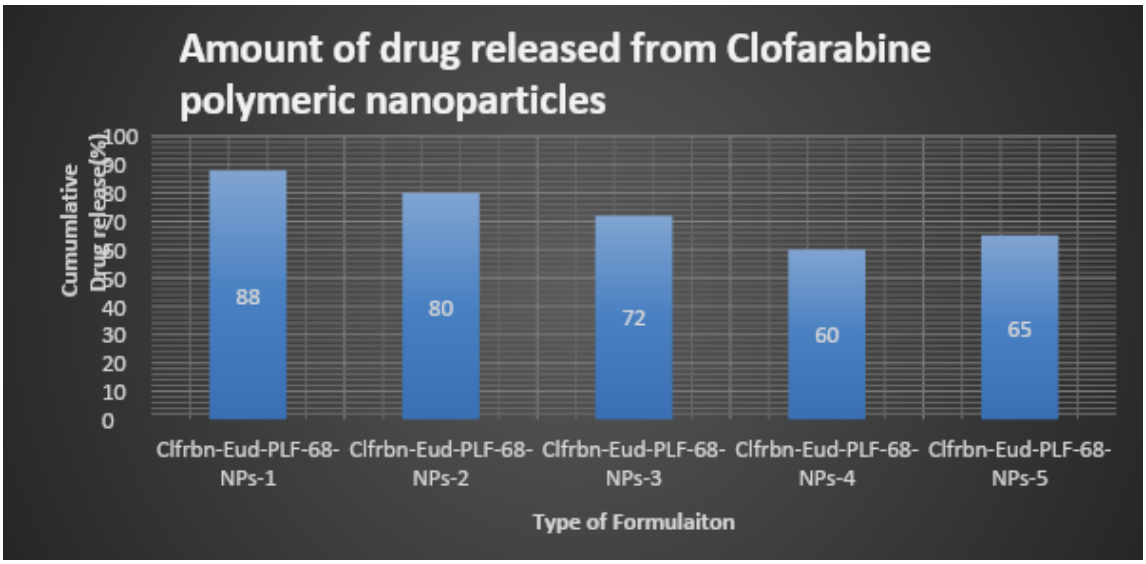

Figure 7: Drug Release Profile-Comparative amount of drug released from Clofarabine

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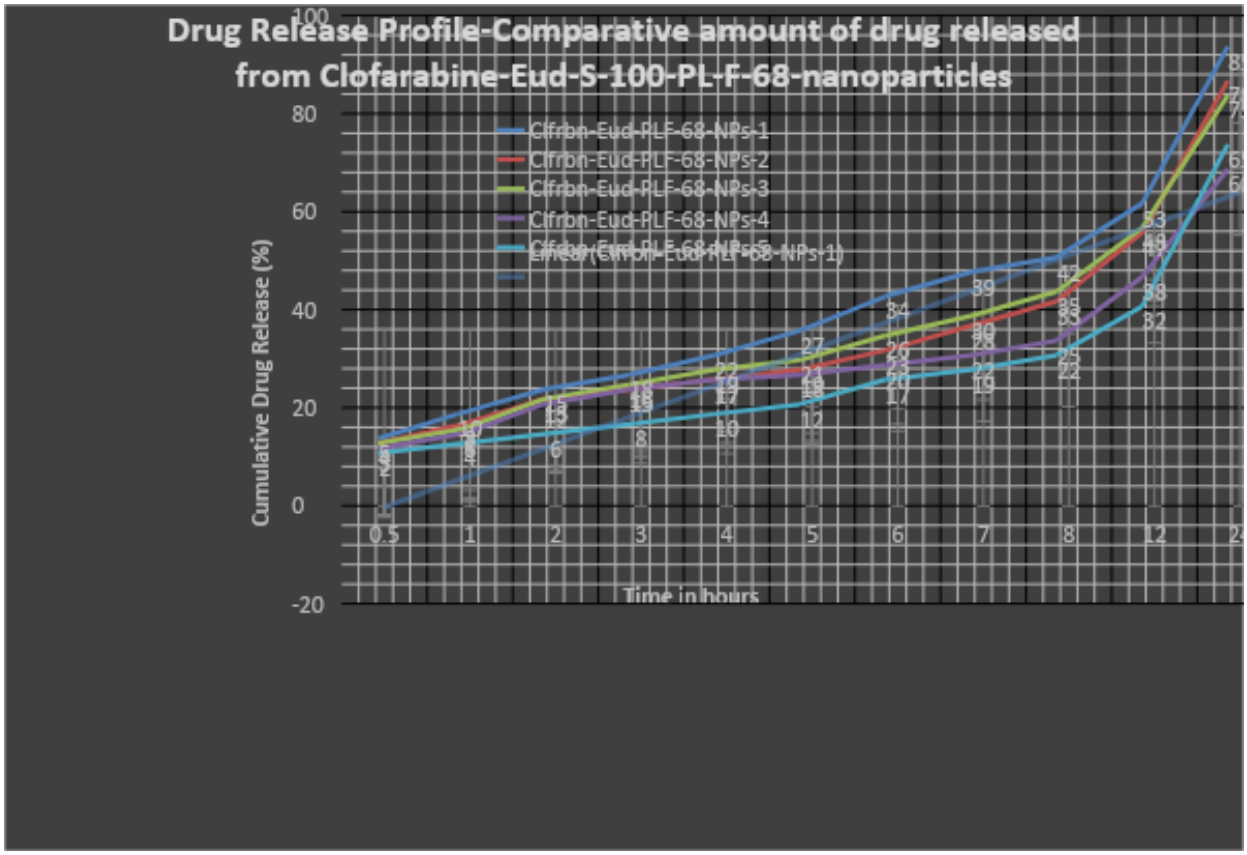

3.5. Drug Release Kinetics:

The manufactured Eudragit S 100 nanoparticles were subjected to the investigation of medication release kinetics and mode of drug release. The prepared formulations were evaluated by fitting the medication discharge time profile with the different pharmacokinetic models, for example, Zero-order rate of release, First-order rate of release, Higuchi, Korsmeyer-Peppas and Hixson-Crowell. All the five optimized nanoparticle dosage forms, CLFRBN1-to-CLFRBN5 were studied for the mode of drug release and the outcomes were introduced in the Table 3.

Table 3: Kinetic Models

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All the formulations from CLFRBN1-to-CLFRBN5 exhibited the best fit with Korsmeyer-Peppas model. The best formulation CLFRBN1 exhibited the R^2 estimation of 0.8826. The 'n' estimation of CLFRBN1 nanoparticle suspension was 0.807 demonstrated non-fickian patterns of dispersion. The 'n' is an exponent used to portray distinctive drug release systems. The 'n' estimate in the event if 'n'> 0.89 demonstrates super case transport and on the off chance that 0.45<n<0.89, at that point it is a non fickian-mode of drug release by diffusion.

In the other nanoparticle formulations, for example, CLFRBN2, CLFRBN3, CLFRBN4 and CLFRBN5 likewise showed the Korsmeyer-Peppas energy. The 'n' estimation of details showed to be around 0.994, 0.889, 0.873 and 0.955 separately for CLFRBN2, CLFRBN3, CLFRBN4 and CLFRBN5. Among these Nano-suspensions, CLFRBN2 and CLFRBN5 exhibited the super simplicity transport with the exception of CLFRBN1, CLFRBN3 and CLFRBN4 which followed non fickian atypical mode of drug release pattern.

4. Stability Studies:

The Optimized formulation of the overall formulation design was injected into 10ml ampoules and sealed for storage at 2-8°C for short term stability study. The parameters such as clarity, appearance, and drug release pattern and entrapment efficiency were determined. The best formulation CLFRBN1 was subjected to stability studies in terms of short term and it was found to be stable during 30-day study period. The parameters such as physical appearance, % Entrapment efficiency and drug release profiles were evaluated after 24hrs, 7-days and 30-days. The results were fulfilling the acceptance criterion and there was a negligible deviation in these results indicated that the optimized formulation is anticipated to be stable for long term stability studies. However, the long term stability studies are recommended for data analysis in further formulation development process.

5. In vitro Cytotoxicity Study:

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The cytotoxic and anticancer property of free and encapsulated Clofarabine was determined in MCF-7 cell lines using MTT dye assay[8-10]. Control experiments were carried out using the complete growth culture medium only (serving as non-toxic control).

5.1. Anti-breast Cancer /Cytotoxic activity Procedure

Cell line Culture Cell lines were obtained from National center for cell sciences Pune (NCCS). The medium and Trypsin-Phosphate-Versene-Glucose (TPVG) was brought to room temperature by thawing. The tissue culture bottles were observed for growth, cell degeneration, pH and turbidity in inverted microscope. After the cells became 80% confluent and free-flowing then sub culturing was done. The growth medium was discarded 4-5 ml of Minimum-Essential-Media (MEM) was added without Fetal-Calf-Serum (FCS) and rinsed gently by tilting. The non-viable cells and excess FCS were washed out, and the medium was discarded. TPVG was added over the cells, incubated at 37º C for 5 minutes to develop the cell as individual and discrete entities. The cells become individual and were present as suspension. 5ml of 10% MEM was added to FCS by using serological pipette. Passaging was formed with serological pipette. After passaging the cells were split into 1:2 and 1:3 ratios for cytotoxicity studies by plating method. The breast cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), (100U) 20µg/ml penicillin, and 100 µg/ml streptomycin. Incubation was carried out at 37 °C with an atmosphere of 5% CO2. Normal breast (MCF-7) cells were cultured in 1:1 mixture of DMEM and Ham's F12 medium with 20 mg/ml of epidermal growth factor (EGF), 100 µg/ml cholera toxins, 0.01 mg/ml insulin and 500 µg/ml Cortisol, and 5% chelex treated horse serum. Purified berberine and tamoxifen were dissolved in dimethyl sulfoxide (DMSO) and used for the bioassays.

$$\text{Cell viability (\%)} = \frac{\text{Mean Optical Density}}{\text{Control Opticla Density}} \times 100\%$$

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5.2. MTT-(3-(4,5-dimethyl thiazol-2-yl) -2,5-diphenyl tetrazolium bromide, a tetrazole) Assay: **Principle:** Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT(3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The assay relies on the reduction of MTT, a yellow water-soluble tetrazolium dye, primarily by the mitochondrial dehydrogenases, to purple colored formazan crystals. The formazan product is analyzed spectrophotometrically (550-570 nm) after dissolution in DMSO, the spectra of nanoparticle-treated and untreated cells giving an estimate of the extent of cytotoxicity. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The MTT Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.

MTT Assay The anticancer activity of samples on breast cancer cells were determined by the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay was used to assess the cytotoxicity Horiuchi et al (1988). Cells (1×10^5 /well) were plated in 0.2 ml of medium/well in 96-well plates. For MTT assay the medium from the wells was removed carefully after incubation. Each well was washed with MEM (w/o) FCS for 2-3 times and 200µl of MTT (5mg/ml) was added. The plates were incubated for 6-7 hrs in 5% CO2 incubator for cytotoxicity. After incubation, 1ml of DMSO (solubilizing reagent) was added to each well and mixed well by micropipette and left for 45sec. Presence of viable cells was visualized by the development of purple color due to formation of formazan crystals. The suspension was

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transferred to the cuvette of a spectrophotometer and the OD (optical density) values were read at 595nm by using DMSO as a blank. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically Standard Graph was plotted by taking concentration of the drug in X axis and relative cell viability in Y axis

Figure 10: Cytotoxic Studies on MCF-7-Cell Lines

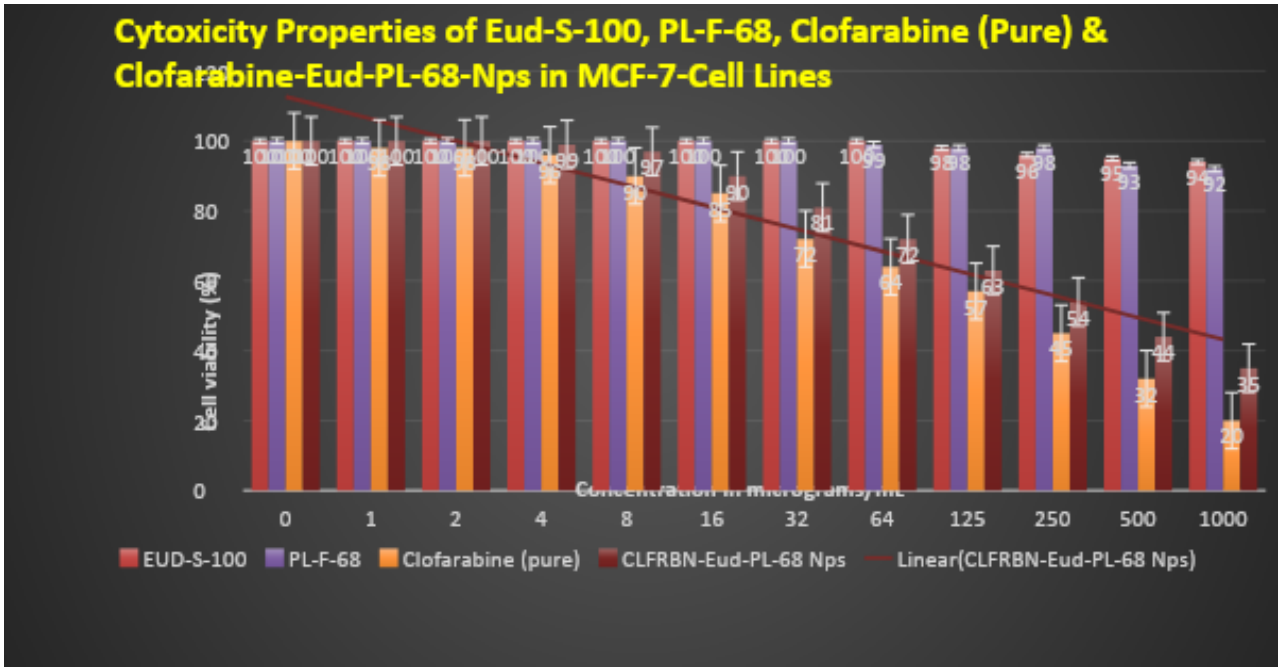

Figure 11: Uptake of nanoparticles by MCF-7 AND HUVEC Cell lines.

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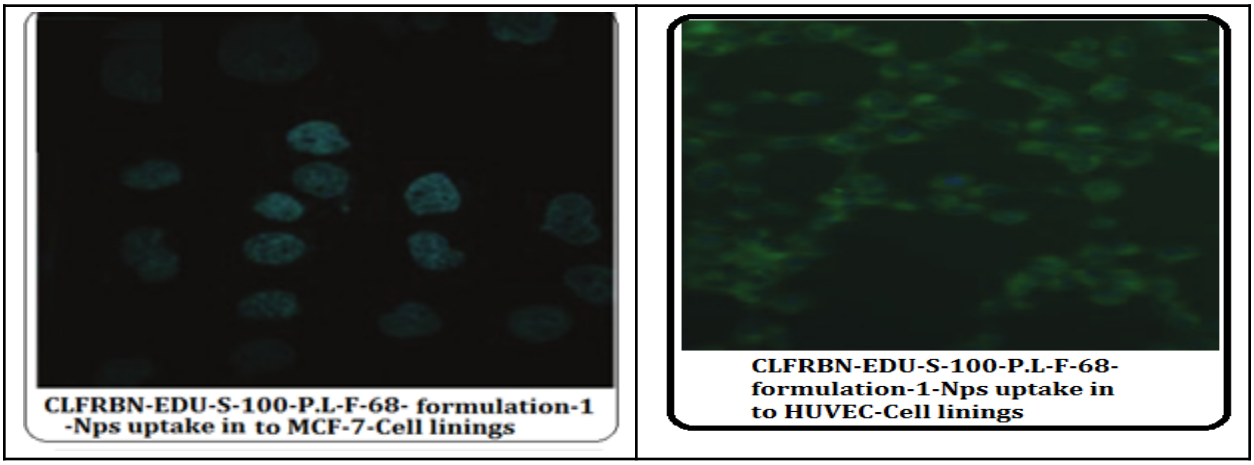

6. Conclusion

Present study is focused on formulation and Evaluation of biocompatible Eudragit-S-100 Nanoparticles. Preformulation studies are done and once we are sure that my drug is compatible with polymer we started with formulation of Nano suspension of Eudragit –S-100 loaded nanoparticles of clofarabine. We have formulated Five Nano -suspension using Nano-precipitation technique. The parameters such as drug to polymer ratio, agitation time and concentration of containing Pluronic F-68 were varied for various formulations based on the formulation design. Then have studied characterisation, In-vitro drug release, Drug release kinetics, Entrapment Efficiency of drug and have performed anti breast cancer study on different cell lines. Among 5 formulation Clofarabine formulation 1 was found to be best formulation.

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