**TITLE:**

**Development of surface conjugated Block Co Polymeric Micelles as Targeted Therapeutics: Characterization and In-vitro cell Viability**

# INTRODUCTION

The most prevalent kind of cancer is lung cancer diagnosed globally.(1) It is one of the major cancer-related mortality factors, resulting in 1,38 million cases of cancer fatalities worldwide each year.(2) Amongst these types of lung cancer,  NSCLC (non-small cell lung cancer) accounts for about 80% of all lung cancers., with about half of patients having advanced illness when they are diagnosed.(3) In recent decades, traditional therapies (surgery, chemotherapy, and radiation) appear to have reached a threshold of unintended consequences in terms of improving NSCLC patient outcomes, which remain unsatisfactory, especially in advanced stages. It paves the door for the development of targeted therapies.(4) The epidermal growth factor receptor (EGFR) is mutated and over-expressed in many human malignancies, including head and neck, breast, ovarian, and in a non-small cell lung cancers..(5) In the treatment of patients with advanced NSCLC, epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are utilized as first-line and targeted treatments.(6,7)

EGFR Moiety is an orally accessible low molecular mass EGFR inhibitor that competitively attaches to the EGFR kinase domain at ATP binding site and inhibits EGFR tyrosine kinase autophosphorylation through inhibition of intracellular domain.(8) However in Asian country EGFR Moiety are authorized for the treatment of non-small cell lung cancer. EGFR Moiety, on the other hand, revealed a survival benefit with few side effect like rashes and diarrhea for all included participants, but gefitinib only exhibited a survival benefit for adenocarcinoma patients or never smokers with major side effects.(9)

Anticancer drug shows lower solubility and hence less bioavailability. Even salt of drug is also not improving much solubility. As a result, enhancing bioavailability with minimal toxicity remains a major concern. Compared to traditional dosage forms, colloidal drug delivery methods provide a number of benefits.(10) Injectable of EGFR Moiety formulations are not available on the market. such a formulation with reduced dose may be helpful in cases of patient survival and gastrointestinal disorders. In addition, treatment for cancer requires long-term therapy and targets both normal and tumor cells. Thus, Encapsulation in Novel nano carriers provides the benefits of far more beneficial pharmacokinetics, safety of medications, elimination of possible toxic side effects and enhancement of patient satisfaction by minimizing regular injections of the bolus.(11)

Polymeric micelles are a novel drug delivery platform technology that enables for the formulation of poorly soluble EGFR Moiety in an efficient and effective manner. They have qualities that make them particularly well-suited for biomedical applications. Polymeric Micelles using single polymer gives premature burst release of encapsulated hydrophobic drug. Several methods for achieving effective drug solubilization and sustained drug release from polymeric micelles have been attempted.(12) Several attempts has done on chemically crosslinked of Di block and Tri block copolymer which can give sustain release as well incorporation of hydrophobic segment in block can increase drug entrapment.(13) Block copolymer micelles have small size, distinctive nanoscopic architecture, stability, and flexibility to be customized for optimal drug compatibility which are desired features for a drug delivery method.(14) Small size of polymeric micelles can passively target tumor site because of enhanced retention and permeation.

In our research work, we have synthesized tri block polymer of Pluronic F68 and Polycaprolactone. Tri Block copolymer micelles are normally formed in an aqueous medium by the self-assembly of either an amphiphilic or oppositely charged copolymer.(16,17) Pluronic f 68 is tri block of Hydrophilic polyethylene oxide and Hydrophobic polypropylene oxide. Commercially available Pluronic F-68 is non cytotoxic and nonionic surfactant, which will act as backbone for Block copolymer.(18) However, Pluronic’s applicability is limited due to its high CMC value.(19) Therefore polycaprolactone (PCL) was introduced as segment in Pluronic F-68.(20) Free hydroxyl group of Pluronic will react with caprolactone by esterification reaction. The exterior of the micelles contains the PEO block as hydrophilic polymers of the block copolymers.(21) They bind with cell membranes and thereby provide micellar structures with improved steric resistance. The charge, lipophilicity, micelle size, and hydrophilic block surface density are all determined by the shell of the micelle’s backbone.(22) The shell also provides sufficient reactive groups that can be effectively used for chemical conjugation with biotin or folic acid thereby adjusting the micelles for targeted delivery.(21,23) Biotin and Folic acid receptors are over expressed in tumor site which can further used to target lung cancer.(24) Biocompatibility, biodistribution, pharmacokinetics, blood circulation duration, surface adsorption into biomacromolecules, bio-surface adsorption, and targeting are all controlled by the micellar carriers' fundamental biological features..(25,26)

In this investigation, the PCL–F68–PCL (PFP) block co polymeric micelles system is utilized to deliver EGFR Moiety for lung cancer therapy. Nuclear magnetic resonance spectroscopy (1H NMR) and Fourier transform infrared spectroscopy (FTIR) were performed to confirm the crosslinking of PCL and Pluronic f 68. From 1:1 to 1:5, the mass ratio of PCL–F68–PCL (PFP) and drug was increased. Based on micellar size and entrapment efficiency, we chose the rations of 1:1 and 1:5. More over statical approach was applied to formulation. Quality by Design (QbD) is gaining traction to increase customer confidence in safe, effective medicine delivery while also promising to improve manufacturing quality. Micellar size and entrapment efficiency were chosen as dependent variables, with an optimized ratio of PCL–F68–PCL (PFP) with drug, solvent volume, and Ultrasonication duration as independent variables. 23 full factorial design was applied, and optimized batch was further characterized. The block co polymeric micelle system exerted favorable particle size of 115.1 ± 3.55 nm determined by Zeta sizer and encapsulation efficiency (EE%) of 94.34 ± 0.20%. The critical micelle concentration (CMC), in vitro drug release profile and stability was also assessed. Cell viability was performed by MTT cell assay. Conclusively the block co polymeric micelle system showed suitable biocompatibility and enhanced cellular uptake.

# Objectives

* Procurement of anticancer drugs and extensive literature review related to drugs.
* Preformulation study of drugs in terms of Melting point, FTIR study and DSC Study.
* Development of analytical method for estimation of drugs.
* Preparation of block co polymeric micelles by suitable method using DOE approach.
* Analysis and Interpretation of responses by contour plots.
* Characterization of block co polymeric micelles containing drug.
* Drug release study.
* Invitro cytotoxicity cell line study

# Materials and Methods

## Material

Pluronic F-68 (PEG-PPG-PEG, MW-8400) was purchased from Sigma Aldrich (India). Polycaprolactone (PCL, MW-14000) was purchased from Sigma Aldrich (India). Dimethyl aminopyridine (DMAP) and Dicyclohexylcarbodimide (DCC) were obtained from Sigma Aldrich (India). Dimethyl sulfoxide (DMSO), Acetone and Ethanol (pharmaceutical grade) was procured from Sigma-Aldrich, India. Distilled water was obtained using Millipore water purification system. Acetonitrile (HPLC grade), Methanol (HPLC grade), purified water (HPLC grade), orthophosphoric acid were purchased from Sigma-Aldrich (India). Mannitol as a (Cryoprotectant) was purchased from Qualikems Fine chem (Vadodara). saline was purchased from Otsuka pharmaceutical India Pvt Ltd. The dialysis membrane (Mw= 12,000, 31.3mm×21.5mm) was purchased from Hi-media Laboratories pvt.ltd. All chemicals were in analytical grade.

## Synthesis of PCL-F68-PCL Block Co-polymers

PCL-F68-PCL (PFP) is synthesized. The esterification of the hydroxyl group of F-68 with the carboxyl group of PCL is the mechanism involved in crosslinking. Pluronic F-68 (0.02 mM), Polycaprolectone (0.06 mM), Dimethyl aminopyridine (0.02 mM), and Dicyclohexylcarbodimide (0.06 mM) were added in DMSO (10ml) and agitated for 36 hours at 25°C. DCC will act as Initiator and DMAP will act as acyl transferase in formation of ester. Freeze the mixture for 24 hours and then the mixture is lyophilized for 24 hours. FTIR and H1 NMR were used to confirm the structure of PCL-F68-PCL.

## Characterization of PCL-F68-PCL Block Co-polymers

PCL-F68-PCL Block Co-polymers structure was determined using the 1HNMR apparatus. At a temperature of 25°C, NMR spectra were obtained with a Bruker 400 MHz instrument. The solvent was Dimethyl sulfoxide (DMSO) as analytical grade of this solvent dose not shows peak in NMR. The FT-IR spectrum was used to confirm the formation PCL-F68-PCL Block Co-polymers. The crosslinked PFP was combined with KBr and scanned with an FTIR spectrophotometer across a spectral range of 400–4000 cm-1.

## Biotin conjugation with PCl-F68-PCL:(27)

Biotinylation requires freeze drying process because it does not tolerate water in reaction mixture. Biotin, PCL-F68-PCL, Dimethyl aminopyridine (DMAP) and Dicyclohexylcarbodimide (DCC) were dissolve in 4 ml of NMP (N-methyl Pyrrolidone). Reaction mixture was followed by incubation with gentle stirring for overnight. Solvent exchange was performed by dialysis bag for 48 hours to remove unreacted biotin. NMP was replace by distilled water by solvent exchange. Solvent exchange was followed by freeze drying for 48 hours to obtain solid biotinylated polymer.

## Preparation of Block Co-polymeric micelles

Drug loaded block co-polymeric micelles were prepared by a solvent evaporation method.(28) Water does not dissolve the block copolymers easily so that an organic solvent is selected which is common to both the copolymer and the drug (such as Ethanol, dimethyl sulfoxide, N,N-dimethyl formamide, acetonitrile, THF, acetone or dimethyl acetamide). The mechanism by which micelle are stimulated depends on the process of solvent removal. With different mass ratios of PCL-F68-PCL, Drug was mixed. The combinations were ultrasonically dissolved in Ethanol (a water miscible organic solvent). After the copolymers had completely dissolved, the combined solution was dropped into clean water using a micropipette and agitated for 3 hours until the methanol had evaporated. To eliminate any big aggregates, the final solution was filtered with a 0.2µM filter.

## Initial risk assessment

For given formulation risk assessment was done based on quality by design. Quality target product profile (QTPP) elements were selected in first step with their targets and justification.

Table no 1shows QTPPs for block co polymeric micelles with their targets and justification.

|  |  |  |
| --- | --- | --- |
| **Elements** | **Targets** | **Justification** |
| Disorder | Lung cancer | Lung cancer is one of the major factor among all cancer which has high mortality rate (56%). |
| Route of administration | Parenteral | Drug will directly reach to target site without first pass metabolism which will improve bioavailability. |
| Target | Epidermal growth factor receptor (EGFR) | EGFR is mostly mutated in lung cancer. drug is targeted EGFR Inhibitor which is Important in treatment of lung cancer. |
| Nanocarriers | Polymeric Micelles | Polymeric micelles size should be in 80-200 nm. Because of nano size it will improve surface area. decreases particle size lead to high dissolution and better bioavailability. Furthermore, Block copolymeric micelles Gives effective entrapment over other formulation. |
| Dissolution profile | Sustain release profile | Dissolution and absorption are two critical parameters. Parenteral Block co polymeric micelles give sustain release which reduce patent comfort due to frequent dosing and toxicity with high oral dose. |

In second step critical quality attributes (CQA) and Critical process parameters (CPP) were identified.(29) Table no 2 shows CQA for block co polymeric micelles with specifications.

|  |  |
| --- | --- |
| **Critical quality attributes (CQA)** | **Acceptance criteria** |
| Micelle size | Not more than 200nm |
| PDI | Not more than 0.9 |
| Entrapment efficiency | Not less than 50% |
| Zeta potential | Should be in range -10mv-+10mv |
| Sterility | Yes |
| Physical stability | No change in Micelle size |
| Chemical stability | No change in Entrapment efficiency |

Minitab 20.1.0.0.x64 software was used to improve quality of formulation based on QBD approach.

### Optimization of Product Variables Using Full Factorial Design

Three factors were assessed at two levels each, and experimental trials were conducted in all conceivable combinations, according to the Full Factorial Design. The concentration of Polymer (X1), the Solvent volume (X2) and Ultrasonication time (X3) were selected as independent variables. Micelle size (Y1) and Percent Entrapment Efficiency (Y2) were used to optimize the influence of independent variables. All the batches were made according to the design in Table 3 and evaluated with the Minitab 20.1.0.0.x64 program. Analysis of variance was used to determine the model's dependability. The relationship between three variable can be explain by following polynomial equation.

Y1=β0+ β0X1+ β0X2+ β0X3+ β0X1X2+ β0X2X3 + β0X1X3 + β0X1X2X3

Where Y1 is dependent variable, β0 is constant, X1, X2, X3 are individual linear coefficient for independent variable and X1X2, X2X3, X1X3 are interaction effect of independent variable. On other hand we can write for Y2 for second dependent variable Statistical data analysis were performed to calculate different coefficients.

## Characterization of Block co-polymeric micelles.

### Size determination:

Using dynamic light scattering (DLS). the size as volume weighted hydrodynamic diameter and the size distribution of occurring particles as micelles were assessed.  DLS measurements were carried out in all cases utilizing a photon correlation spectrometer in Zetasizer NanoZS, Malvern Instruments Ltd., UK, which can detect particle sizes between 0.6 nm and 6 m at a constant scattering angle..(30) All measurements were made in triplicate at 25°C after 5 minutes of equilibration, and all results are the average of three separate samples. To prevent losing particles such as bigger vesicles, the generated samples were often examined without dilution or filtration to get information on all species that appeared during sample preparation. The electrophoretic mobility was translated into the zeta potential using a clear disposable zeta cell with a filed strength of 20 V/cm and aqueous medium as a dispersion media.(31) The polydispersity index was studied to determine the distribution of the molecular mass in the polymer.

### Encapsulation Efficiency and drug loading:(32,33)

The drug concentration of PCL-F68-PCLmicelles were measured by HPLC Analysis at maximum absorption wavelength. Drug loaded micelles were passed from a 0.22 micron syringe filter to remove the unentrapped drug. To dissolve the core-shell structure, methanol was transferred to PFP micelles, which culminated in the release of drug. 1 ml of filtered micellar solution was pipetted out and made up to 10 ml with Methanol and allowed to sonicate for 15 minutes to further break the micelles.(34) The following equations were used to determine EE percent and drug loading (DL percent).

EE% = (1)

DL% = (2)

### Critical micelles concentration:

The dynamic light scatter technique was used to estimate the critical micelle concentration of micelles using Zetasizer. Dilutions of samples ranging from 0.01 mM to 0.05 mM were made in deionized water and ethanol, and changes in light intensity were measured at a scattering angle of 90° for each sample. The temperature was kept at 25 degrees Celsius. The light intensity and the sample's molar concentration were plotted on a graph. The CMC was calculated from the point where the slope of the intensity increased dramatically, indicating micelle production..(35)

### Surface Morphology:

### Surface morphology of polymeric micelles of drugs, dispersion of drug containing polymer and pure drug powder will be captured using a Transmission electron microscope (Philips, Philips XL 30 ESEM).(36) Samples will be fixed on an aluminum stub with conductive double-sided adhesive tape and coated with gold in an argon atmosphere (50 Pa) at 50mA for 50 sec.

### In vitro release:

To explore the in-vitro drug release behavior the validated HPLC method described above was used. The optimized batch of drug loaded block co polymeric micelles was evaluated for in vitro drug release using Franz diffusion cell via dialysis bag methodology,(37) in Phosphate buffer (pH 7.4 as well as pH 5.5). Lyophilized block co polymeric micelles were weighed accurately such that equivalent to 10 mg of the drug and further reconstituted in 10 ml of phosphate buffer (pH 7.4 and 5.5). The diffusion cell was put in a shaker incubator at 37.0±0.5°C with moderate shaking at a speed of 100 rpm. To keep the sink conditions, 1ml of the sample was removed and the incubation medium was replaced with freshly produced release medium at specified time intervals.

### Cell viability study:

The MTT assay was performed to assess cell viability potential of Block co polymeric micelles. Further assays were performed to check and compare cytotoxicity of different formulations with API solution of respective drug, in lung cancer cell line A549(38) Both formulations and API were diluted with serum free DMEM to prepare various concentrations of formulation mentioned in result table no 4. As a negative control, cells were given 200 µl of fresh incomplete medium (DMEM) (100 percent viability will be assumed from the absorbance of wells containing these cells).

### Stability studies:

The stability study of the optimized batch was carried out according to the ICH (International Conference on Harmonization) guidelines.(39) The stability chamber was placed at two different temperature conditions, i.e. 40±2°C/ 75±5% RH (Refrigerator, RF) for a period on 30 days. Samples were evaluated, 15- and 30-days interval. The physical characteristics were evaluated by Micellar size and Entrapment efficiency.

# Result and Discussion

## Characterizations of block co-polymer PCL-F68-PCL

FTIR spectrum and 1HNMR spectrum of Crosslinked PCL-F68-PCL is shown in figure 2.

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Description automatically generated FTIR spectroscopy was used to verify the synthesis of the PCL-F68-PCL. The F-68 hydroxyl group interacted through esterification with the PCL carboxyl group. In F-68, the stretching vibration peaks were referred to the-OH and-CH3 groups at 3395 and 2983 cm-1 simultaneously. The –OH and –COOH groups from PCL were linked to a broad stretching peak at 3288 cm-1, whereas the –CH2 group in PCL had a stretching peak at 2910 cm-1. The C=O stretching mode of ester in PCL was ascribed to the peak at 1726 cm-1. The –OH and –COOH, –CH3, –CH2, C=O groups from PCL–F68–PCL, r were identified as the stretching peaks at 3395 cm1, 2983 cm-1, 2910 cm-1, and 1726 cm-1 respectively. Additionally, 1H NMR spectroscopy were further used to validate PCL-F68-PCL block co polymer. Intense Peak at 3.28 ppm indicates CH2 group of PEO and 1.17, 3.428, 3.48 ppm peaks for CH2 of PPO for Pluronic F-68. 2.422, 1.5-2.0, 4.11, 1.229 peaks indicate CH2 groupin PCL. Combinedly both FTIR and 1HNMR successfully conform crosslinking of block co polymer.

## Characterizations of Biotin conjugated block co-polymer PCL-F68-PCL:

FTIR spectrum conform conjugation of biotin with Crosslinked PCL-F68-PCL is shown in figure 2.

Diagram

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The F-68 hydroxyl group interacted with the biotin In F-68, the stretching vibration peaks were referred to the-OH and-CH3 groups at 3390 and 3287 cm-1 simultaneously. The –OH and –COOH groups from PCL were linked to a broad stretching peak at 2971 cm-1, whereas the –CH2 group in PCL had a stretching peak at 2953 cm-1. The C=O stretching mode of ester to the peak at 1724 cm-1 conform conjugation of biotin with Pluronic F68.

## Optimization of Block co polymeric micelles using 23 full factorial Design:

The **Table:3** showing the design matrix 23 full factorial Design of in which the concentration of Co block polymer (X1), Solvent volume (X2) and Ultrasonication time (X3) were selected as independent variables and were further taken for the evaluation of the dependent variables such as Micellar size and % entrapment efficiency with total of 8 experimental runs by using the Minitab 20.1.0.0.x64 software.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Batches | (A) Conc. Of Co block Polymer (X1) | (B) Solvent volume (X2) | (C) Ultrasonication time (X3) | Entrapment efficiency(Y1)(%) | Micelle size(Y2)(nm) |
| F1 | 1 | 10 | 5 | 70.02 ± 0.23 | 83.31 ± 2.33 |
| F2 | 5 | 10 | 15 | 94.34 ± 0.20 | 115.1 ± 3.55 |
| F3 | 5 | 10 | 5 | 89.01 ± 0.41 | 154.3 ± 2.21 |
| F4 | 1 | 5 | 15 | 55.72 ± 0.28 | 87.7 ± 3.34 |
| F5 | 5 | 5 | 15 | 85.21 ± 0.31 | 173.1 ± 1.34 |
| F6 | 1 | 5 | 5 | 50.65 ± 0.55 | 100.2 ± 2.59 |
| F7 | 5 | 5 | 5 | 79.13 ± 0.36 | 265.3 ±4.33 |
| F8 | 1 | 10 | 15 | 81.7 ± 0.35 | 76.41 ± 3.78 |

Block co polymeric micelles from F1 to F8 batches showing the range of 75-265nm size shown in Table 3. which is suitable for the targeting at the cancerous site; hence this pH 7, solvent type and its volume, polymer concentration, Sonication time were selected respectively to obtain this range of particle size with uniform distribution and high entrapment of drug. EE% from F1-F8 ranges from 50% - 94%. Suitable micellar size with highest entrapment efficiency was shown by batch F2 that consisted highest polymer concentration, solvent volume, Sonication time. This exhibited to be the main reason behind the most suitable result showing the significant role of each factor on the Micellar size and %EE.

Regression statistics was applied and the fitting quadratic model of Micellar size, % EE are shown in the following equation 1 and 2.

Polynomial equation for Micelle size was found to be,

**Micelle size =** 45.45 + 80.69 Concentration of Polymer - 1.952 Solvent Volume + 1.367 Ultrasonication time - 5.890 Concentration of Polymer\*Solvent Volume - 3.177 Concentration of Polymer\*Ultrasonication time - 0.1250 Solent Volume\*Ultrasonication time + 0.2370 Concentration of Polymer\*Solvent Volume\*Ultronication time**….[Eq. 1]**

Polynomial equation for Entrapment Efficiency was found to be,

**Entrapment efficiency =** 23.60 + 8.446 Concentration of Polymer + 3.503 Solvent Volume - 0.3633 Ultrasonication time + 0.2905 Concentration of Polymer\*Solvent Volume + 0.2093 Concentration of Polymer\*Ultrasonication time + 0.1690 Solvent Volume\*Ultrasonication time - 0.03680 Concentration of Polymer\*Solvent Volume\*Ultrasonication time…(**Eq. 2]**

The contour Plots are showing the overall relation between the independent variables and dependent variables which is shown in **figure 3 and 4** to exhibit the combine and individual effect of independent variables on the response.

The result of all the experimental runs is shown in **Table 3**

Interpretation of polynomial equation and contour plot:

Particle size: The equation1 showing the interactive effects and individual effects of polymer concentration, solvent volume and Sonication time on the Micellar size indicating the positive response shown by concentration of polymer is most influencing the particle size distribution whereas individual effect of solvent volume and sonication time found to have negligible impact on the micellar size. The solvent type and the volume play the major role in decreasing the particles size. Both Ethanol and methanol having the high dielectric constant hence shown the prominent results when taken in a ratio. When all these factors interact that is their combined effect shows the positive response because all these factors are interrelated to each other.

The **fig 3** illustrating the overall effect of polymer content, solvent volume, and sonication time as a contour plot.

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The contour figure depicts the combined influence of polymer concentration and solvent volume on particle size, demonstrating that when protein concentration rises, particle size rises as well. Because more the concentration leads to increases hydrophobic chain length in the natural building blocks of micelles which produces bigger size and higher the volume leads to increase in solubility of polymer which results into the small particle size.

%Entrapment efficiency: The interaction and individual effects of polymer concentration, solvent volume, and sonication on the percent EE are shown in equation 2. Individual impacts of polymer concentration on entrapment efficiency have demonstrated a strong positive response. Higher the concentration of polymer more the capacity to entrap the drug. However, when this factor interact with solvent volume leads to the positive outcome of the response (%EE); because solvent volume play a role of supporting by producing more uniform distribution to meet the characteristics for the IV formulation, so this increases the tendency of the polymer to entrap more amount of drug. On the other hand quadratic terms are also showing the positive effect on %EE; whereas individual effect of sonication time and solvent volume is found to have a negligible response on entrapment efficiency.

**Fig. 4** showing contour plot representing the combined effect of polymer concentration and solvent volume on entrapment efficiency which shows that as the polymer concentration and solvent volume increases the entrapment efficiency increases.

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## Characterization of block co-polymeric micelles.

### Micellar size and zeta potential

Accumulation of micelles in tumor site is highly dependent on its size. If micellar size is more than 200 nm, It will be easily eliminated by spleen. While size distribution in the range below 200 nm accumulate in tumor cell by EPR effect.(40) In addition to that small particle were suitable for IV administration. Micellar size of block co-polymeric micelles was found to be in range of 70-270 nm, PDI was 0.9 and Zeta potential of block co-polymeric micelles was found to be -0.84mV, which indicates stability of formulation. As we have chosen three independent variable each of them having have their individual effect on micellar size. 1% concentration of block co polymer forms micellar size in the range of 76.41 nm to 100.2 nm. As concentration increase to 5%, Micellar size is also getting increase with 115 nm to 265 nm. Ultrasonication time and solvent volume was provided to dissolve block co polymer. Increases in solvent volume and ultrasonication time decrease micellar size. From all baches f2 batch gives best entrapment and its micellar size is 115.1 nm.

### Encapsulation Efficiency and Drug loading.

A major problem of polymeric micelles is low drug entrapment into the micellar core. Drug to Polymer ratio is one of the important parameter for size, entrapment and PDI. Optimum ratio should be selected above to this ratio drug gets precipitated and Entrapment get decreases. As we have selected three independent variable, concertation of polymer is highly effecting entrapment of hydrophobic drug. In this research work drug to polymer ration selected was 1:5 and 1:1. The result of entrapment efficiency for all batches is shown in table no 4. As concentration of polymer increases the entrapment of drug is also increases. Other factors like solvent volume and sonication time are also influencing drug entrapment. Solvent such as Ethanol and ultrasonication time used as process condition to get dissolve polymer and drug aids to get clear solution. Furthermore, to overcome this issue, 0.9% NaCl and 37ºC temperature was used to aid entrapment in this research work and resulted in excellent entrapment efficiency. From all baches f2 batch gives best percentage entrapment efficiency 94.34 ± 0.20%.

### Critical micelles concentration

Dilutions of samples from 0.01 mM to 0.05 mM were prepared. Each sample were tested for light intensity. The point at which Intensity increase was selected as CMC value for Block co polymer. 3.3× 10-5 M Concentration was found to be critical micellar concentration which is equivalent to 1.08 mg Copolymer as shown in Figure 5.

Diagram

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CMC is a common metric for determining micelle self-assemble stability. Blank PCL-F68-PCL micelles exhibited a CMC of 1.08 mg/ mL, suggesting that they were thermodynamically stable enough to sustain micellization at therapeutic amounts. Dilution of polymeric micelles causes micelle dissociation, lowering drug loading capacity. so that lower CMC value is required for efficient drug loading under possible dilution.(41) As concentration of polymer increases CMC value of polymer decreases therefor a greater number of polymeric micelles forms which can incorporate more amount of drug.(42) Crosslinked block co polymer PCL-PluronicF68-PCL have more hydrophobic chain length. So as hydrophobic portion get increases it will increase agglomeration number which provide more core volume and entrapment of drug will get improve. However as hydrophobic length of polymer get increase CMC value of polymer will get decreases. Because of lower CMC levels of Pluronic F68 and Polycaprolactone, non-ionic surfactants are typically stronger solubilizing agents than ionic surfactants for hydrophobic drugs.

### Surface Morphology:

Morphology of optimized batch was examined by TEM. The TEM photographs of optimized batch are shown in the fig. 6.

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Description automatically generated Micelles having a consistent spherical shape were seen in TEM micrographs. It appeared to be evenly distributed, non-aggregated, and smooth.

### In vitro release of Drug from Block co-polymeric micelles

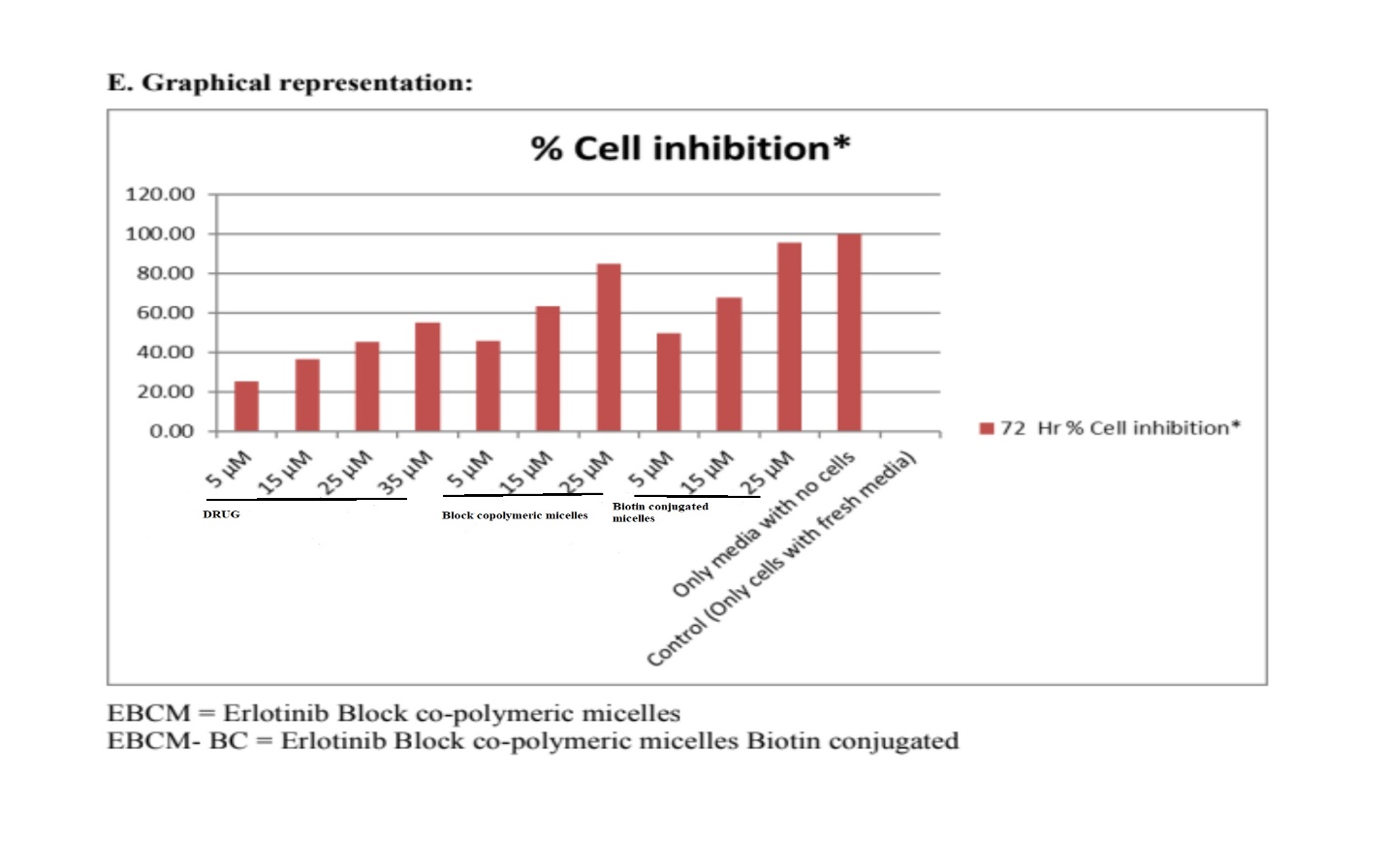
In vitro drug release study was performed using Franz diffusion cell. Receptor compartment was filled with 40 ml of 7.4 phosphate buffer. Ideal conditions, temperature 37±0.5°C and speed of 100 rpm were maintained in orbital shaker. Result of *in-vitro* drug release shows 84.45% up to 96 hrs. which is sustained release of drug from block co-polymeric micelles. Drug release kinetics are affected by many factors like Polarity of hydrophobic molecule, Size of block co polymer, compatibility of drug with polymer. As micellar size and hydrophobicity increases it gives more sustain release. The formulation provides the sustained release up to 96 hrs. so there is a decrease in frequency of administration resulting in better patient acceptance. Further drug release was also performed at 5.5 pH to mimic condition at tumor site. At 5.5 pH drug release was found to be 89.45%. The rate of drug release from block co-polymeric micelles, on the other hand, is influenced by the level of drug diffusion from micelles as well as micelle stability. Because the drug molecules are trapped in the vesicular system, the rate of release is slowed when a hydrophobic polymer is included in the formulation, the rate of biodegradation of the copolymer is dramatically reduced.(29)(43) Result of invitro drug release of shown in Figure no 7.

Chart

Description automatically generated Additionally, the release data was fitted to kinetics models such as Zero order, first order, Higuchi, and Korsmeyer Peppas to examine the drug release mechanism. The Higuchi model had the highest R2 0.9845, which was close to 1, indicating that it was the best-fitting model.

### Cell viability study:

The cell lining studies were performed for MTT assay on A549 cells for evaluating and comparing the cell viability studies of Drug alone, drug loaded polymeric micelles. Based on IC50 value three concentration was prepared (5µM, 15µM, 25µM) and incubated at 72 hrs. EGFR Moity itself is EGFR targeted moiety. At highest concentration 25 µM, 45.60 % inhibition was found for Drug. For drug loaded block co polymeric micelles 85.06% inhibition was found at 25 µM concentration. Biotin was further provided as targeting probe. Biotinylated co block polymeric micelles shows 94% inhibition was found at 25 µM concentration. Result shows drug loaded block co polymeric micelles more specifically target tumor cell than drug only. Result of the cell viability studies are shown in **figure 8**



### Stability study:

According to ICH standards, the block co polymeric micelles were tested for stability at 40°C ±2°/75±5% RH (Refrigerator, RF) for one month. The formulation shown good stability with no remarkable change in its physical appearance up to 1 month. Result of the stability data are shown in **Table 4**

|  |  |  |  |
| --- | --- | --- | --- |
| **Temperature and %RH** | **Interval** | **Micellar size (nm)** | **%EE** |
| 40±2°C/  75± RH% | Initial | 117.1 ± 3.55 nm | 94.26±0.15 |
| 15 days | 114.1 ± 2.44 nm | 93.98±0.18 |
| 30 days | 121.1 ± 3.39 nm | 94.14±0.16 |

# Impact of the research in the advancement of knowledge or benefit to mankind

# The main goal of this study is to develop EGFR-loaded Co block polymeric micelles for intravenous drug administration with low dosage and long-term drug delivery. Dose-related adverse pharmacological effects in anticancer medicines might impact patient survival. Nanocarriers of anticancer drug helps to reduce dose related toxic effect.

Furthermore, the EGFR moiety is targeted, which can affect both normal and malignant cells. Because biotin receptors are overexpressed at cancer sites, we conjugated biotin on the surface of micelles to precisely target malignant cells in our work. In terms of penetration, anti-cancer medicines are ineffective as a single targeted agent. Anti-cancer medication efficacy can thus be improved by making them as smaller as feasible and modifying their surface.

Anticancer drugs have a reduced solubility and, as a result, have a poorer bioavailability. Even the drug's salt doesn't seem to help much with solubility. We can increase solubility and thereby bioavailability and pharmacokinetics by using EGFR-loaded Co block polymeric micelles to promote solubility.

# In a summary, we developed Targeted EGFR-loaded Co block polymeric micelles for the treatment of Non Small cell lung cancer that have cost-effective formulations and lower doses, resulting in improved patient survival.

# Conclusion

The work illustrates the effective use of Pluronic F68 and Polycaprolactone in block co-polymeric micelles to improve EGFR Moiety solubility. When a drug enters the micellar core, the hydrophobic nature of the drug causes the micellar core to shrink, which supports the study's potential scope in the field of innovative drug delivery.

The study was undertaken for development and characterization of EGFR Moiety loaded lyophilized block co-polymeric micelles for treatment of cancer therapy. The study demonstrates that for Micellar size, shape, zeta potential and other characteristics of Lyophilized block co-polymeric micelles are appropriate and synthesis of cross linking of polymer by lyophilized parameter.ie temperature and percentage yield, entrapment efficiency, loading capacity and particle size characteristics of block co-polymeric micelles are in optimum range.

Block co-polymeric micelles Minimizes toxicity and side effect due to their encapsulation by polymerand ability to sustained drug release. Block co-polymeric micelles provide better solubility and high drug loading capacity and Block co-polymeric micelles may enhance the bioavailability. The notion of using block co-polymeric micelles in the solubility improvement of hydrophobic medicines has a significant potential to serve as a viable platform for intravenous drug administration, according to this research. Although the results are promising, the EGFR Moiety-loaded block co-polymeric micelles must be scaled up.

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