**Original Research Article**

# Preparation and Biopharmaceutical Evaluation of Novel Polymeric Nanoparticles Containing Etoposide for targeting the cancer cells


**Abstract:**

**Objectives:** Polymeric nanoparticles is a promising novel drug delivery system and have advantageous in cancer therapy. Etoposide is an anticancer agent that is used in the treatment of a variety of malignancies. The present study was aimed to prepare and evaluate the Novel polymeric nanoparticles containing etoposide. **Materials and Methods:** $3^2$ full factorial design was used to study the effect of Eudragit EPO and Pluronic F-68 on characterization of nanoparticles suspension. The polymeric nanoparticles was prepared by nano-precipitation technique. The prepared nanoparticles was evaluated by percentage yield, drug polymer compatibility using FTIR and DSC analysis, drug content, entrapment efficiency, zeta potential, particle size, SEM, XRD, *In-vitro* drug release studies, kinetic modeling, stability studies and *in-vivo* animal study. Response surface plots were studied which was generated using PCP dissolution software. **Results:** Scanning electron microscopic studies confirmed their porous structure with number of nanochannels. The FTIR spectra showed stable character of etoposide in mixture of polymers and revealed the absence of drug polymer interactions. DSC study revealed that drug was involved in complexation with nanoparticles. The average particle size of etoposide nanoparticles was found to be in the range of 114.4 nm to 136.7 nm. The results of zeta potential values were attained to ensure a good stability of nanosuspensions. *In-vitro* release of drug from nanoparticles follows peppas and showed controlled release behavior for a period of 24 h. The optimized nanoparticles were subjected to stability studies at 4ºC in refrigerator and found most suitable temperature for storage of Etoposide nanoparticles. The average targeting efficiency of drug loaded nanoparticles was found to be 41.88 ±0.030% of the injected dose in liver, 25.66±0.320% in spleen 13.82±0.090% in lungs, 4.52±0.300% in

kidney and 4.18±0.490% in brain. **Conclusions:** The study concluded that etoposide nanoparticles could be effective in sustained release and the drug loaded nanoparticles.
**Key Words:** Etoposide, Eudragit EPO, Pluronic F-68, $3^2$ full factorial design, Nanoparticles.

## INTRODUCTION

Cancer is a major public health problem in the world. There were 14.1 million new cancer cases and 8.2 million cancer deaths in 2012 worldwide. If these rates do not change, the global cancer burden is expected to nearly double to 21.4 million cases and 13.5 million deaths by 2030. Breast cancer is the most common cancer among women worldwide, with nearly 1.7 million new cases diagnosed in 2012 (the second most common cancer). This represents about 12% of all new cancer cases and 25% of all cancers in women. Cancer is the second leading cause of death worldwide, and was responsible for 8.8 million deaths in 2015. As per WHO, nearly 1 in 6 deaths is due to cancer.

A typical example for Topoisomerase inhibitors is etoposide and it is a first line of chemotherapeutic agents that are used in the treatment of many types of cancer. The mechanism of action of Etoposide by forming a ternary complex with topoisomerase II and DNA, causing DNA breaks and cell death (1). In addition to this, there are many side effects related to the drug (2–4), the administration of etoposide is rate limited by its low solubility in aqueous solutions (5, 6). Therefore, finding an effective approach to facilitate the transport of drugs and to improve the bioavailability of therapeutics is necessary.

The drug candidate etoposide has variable oral bioavailability and ranging from 24-74% and has terminal half-life of 1.5 hours by intravenous route and 0.44 hours by oral route. The conventional oral therapy has drawback of low bioavailability and parenteral therapy causes inconvenience and pain to the patients as it has to be given through a continuous IV infusion over 24-34 h.

Hence, the present study was aimed to prepare and evaluate the formulations of Eudragit EPO based nanoparticles. A nanoparticles suspension was prepared by nano-precipitation technique using Eudragit EPO. Eudragit EPO is a cationic non-biodegradable synthetic polymer which is used for the designing of controlled drug delivery system. $3^2$ factorial designs widely used to study the effect of Eudragit EPO and Pluronic F-68 on characterization of nanoparticles suspension. The optimized formulation was subjected to lyophilisation. The prepared nanoparticles was characterized with respect to particle size and its surface morphology, Surface charge-zeta potential, drug content, entrapment efficiency, In-vitro drug release studies, kinetic modeling, stability studies, animal study like biodistribution studies.

## MATERIALS AND METHODS

### Materials

Etoposide was a gift sample from Biocon Limited, Bangalore, India; Eudragit® EPO and HPMC K-15 were gifts from Cipla Pharmaceuticals, Mumbai, India. Pluronic® F-68 gifted from Alembic pharmaceuticals, Mumbai, India. Synthetic cellulose membrane (Mol.cut off value 12,000) was procured from Himedia Labs, Mumbai, India. All other reagents and chemicals used in this study were of Analytical Grade.

### Solubility study

Solubility profile of etoposide was carried out by different solvent system such as methanol and purified water as per the standard procedure.

### Preparation of Eudragit EPO based Nanoparticle Suspension:

Nanoparticles suspensions were prepared by nanoprecipitation method. Dissolved the 50 mg of the drug and specific amount of Eudragit®-EPO in 15 ml of methanol. The organic solution quickly injected to 40 ml aqueous solution containing Pluronic® F-68 under stirring at 2000 rpm. Stirring was continued for 2 hours at 40°C for the evaporation of methanol. The volume was adjusted upto 40 ml with aqueous solution of 200 mg of HPMC K-15 to obtain a nanoparticle suspension. The optimized nanoparticles suspension was lyophilized at – 42°C for 72 hours and which also redispersed in water to get aqueous nanoparticles suspension[7]. Blank nanoparticles

(without the drug) were prepared under the same conditions without the drug.

**Formulation Designing by $3^2$ Factorial Designing Techniques:**

A prior knowledge and understanding of the process and the variable under investigation led to preliminary experiments. Based on the preliminary data, the $3^2$ factorial design was used to optimize the amount of Eudragit®-EPO (X1) and Pluronic® F-68 (X2) identify the independent variable affecting the drug content and the percentage drug encapsulation efficiency (dependent variable). The response surfaces of the obtained result was plotted. The coded and the actual values of the experimental design are given in Table 1. The data analysis of values obtained from various batches for drug content and entrapment efficiency was subjected to multiple regression analysis using PCP dissolution software, the equation fitted is

$$\text{Response } Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad \ldots (1)$$

Where y is the measured response; X is the level of factors; β is the coefficient computed from the responses of the formulations(quadratic form).

Physical mixtures of the drug, Eudragit EPO, Pluronic F-68 and HPMC K-15 was prepared by dry blending using same ratios as that used for the preparation of optimized batch of nanoparticles suspension[7].

**Characterization of Nanoparticles**

**Practical yield:**

Percentage practical yield[8] is calculated to know about the efficiency of the method. Thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of nanoparticles recovered from each and every batch in relation to the sum of starting material. The percentage yield of prepared nanoparticles was calculated by practical yield/theoretical yield x 100.

**Compatibility Studies**

**a) Fourier Transform Infra-Red Spectroscopy (FTIR):**

The FTIR spectra of drug[9], lyophilized nanoparticles were determined by using Shimadzu FTIR-801 spectrophotometer. The pellets were prepared by gently mixing of 10 mg sample with 200 mg potassium bromide at high compaction pressure. A base line correction was made using dried potassium bromide and the spectra of dried mixture of drug and polymers was recorded . Thus the prepared pellets were scanned at a resolution from 4000 cm $^{-1}$ to 400 cm $^{-1}$.

**b) Differential scanning calorimetry**

DSC studies was performed using a Differential scanning calorimeter (Shimadzu W70 thermal analyzer) to know the thermal behavior of drug, lyophilized nanoparticles.The 5 mg samples were weighed into sealed aluminium pans and heated in a hermetically sealed aluminum pans in the temperature range of 100-300ºC at heating rate of 10ºC /min under nitrogen flow of 30ml/min[10].

**Estimation of Drug Content:**

Accurately weighed the equivalent to 10 mg of each batch of polymeric nanoparticles and dissolved with methanol. The solution was filtered by using 0.45µm Millipore filter as per the reported method. The drug content was estimated by using UV -Visible spectrophotometer (Shimadzu UV-1700) at 286 nm against blank solvent system containing the same concentration of drug in the formulation[11].

$$\text{\% drug content} = \frac{\text{Amount of drug found}}{\text{Label claim}} \times 100$$

**Estimation of Entrapment Efficiency:**

The entrapment efficiency of the prepared formulation was determined by measuring the concentration of free drug in the dispersion medium. The entrapped drug was determined by adding 1 ml of nanosuspension to 9 ml methanol in order to dissolve the entrapped drug. The nanoparticles suspension is need to centrifuge for 2 hours at 14000 rpm. The supernatant liquid was separated and filtered through 0.45µm Millipore filter. The filtrate was diluted with solvent

system and measured spectrophotometrically (Shimadzu UV-1700). Entrapment efficiency was calculated using the following equation[12].

$$\% \text{ drug entrapment efficiency} = \frac{\text{W initial drug} - \text{W free drug}}{\text{W initial drug}} \times 100$$

**Particle Size Analysis:**

Particle size analysis of nanoparticles was performed by photon correlation spectroscopy (PCS). This technique yields the mean particle diameter and particle size distribution[13]. Lyophilized nanoparticles was analyzed using Mastersizer 2000 (Malvern Instruments, Malvern, UK).

**Scanning Electron Microscopy (SEM) Analysis:**

Shape and Surface morphology of nanoparticles was studied by using scanning electron microscopy. SEM analysis used to determine particle shape, surface topography, texture and to examine the morphology of fractured structure. Small volume of nanoparticles suspension sufficient to mount on metal stubs using double-sided tape and coated with gold under vacuum. Stub is visualized under scanning electron microscope [14].

**Zeta Potential Measurement:**

The surface of particles in suspension develops a charge due to adsorption of ions or ionization of surface groups and the charge is correspondingly dependent on both the surface chemistry and environment of the particles. The zeta potential was determined by zeta potentiometer. Sample was filled into the cell; an electrode inserted was placed under the microscope and connect them to the zeta meter. Electrode energized and the colloids was watched to move across a grid in microscope eye piece. Track one by simply pressing track button and holding it down while the colloid traverses the grid. When the track button released, the zeta meter instantly calculates and displays the colloids zeta potential (Zetasizer, Malvern, UK )[13].

**X ray diffractometry Analysis:**

The X- ray Diffraction pattern of drug and lyophilized nanoparticles was recorded using Philips X ray diffractometer with copper target. The condition was: voltage 30kV;

current 30 mA; scanning speed -1º/min; temperature of acquisition: room temperature; detector: scintallisation counter detector; sample holder: non-rotating holder[7].

***In-Vitro* Drug Release Study:**

The in-vitro drug release of drug, physical mixture and lyophilized nanoparticles was carried out by using dialysis membrane method[15]. The formulation equivalents to 50 mg of drug was poured into dialysis bags (with a cutoff of 12,000 Da, Sigma). The dialysis bag suspended in a beaker containing 100 ml of phosphate buffer pH 7.4 on a magnetic stirrer at 100 rpm, with temperature adjusted to 37±0.5°C at selected time interval. 5 ml sample was removed and replaced with fresh medium. The sample filtered through 0.45µm Millipore (Millipore filter). The samples should analyze for drug release by measuring absorbance at 286 using UV- visible spectrophotometer (Shimadzu UV-1700). The rate of etoposide release was obtained using the standard curve.

**Kinetics of *In-Vitro* Drug Release:**

PCP dissolution software was used to study the mechanism and kinetics of drug release from etoposide nanoparticles. The data obtained from *invitro* release study was applied into PCP dissolution software to study the various kinetic equations like zero order (% cumulative drug release Vs. time), first order (log% cumulative drug remaining Vs. time) higuchi matrix (% cumulative drug release Vs. square root of time). In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by peppas equation. The value of n indicates a measure of the primary mechanism of drug release. $R^2$ values was calculated for the linear curves obtained by regression analysis.

**Statistical Analysis:**

A prior knowledge and understanding of the process and the variable under investigation led to preliminary experiments. Based on the preliminary data, the $3^2$ factorial design helpful adopted to optimize the amount of Eudragit®-EPO (x1) and Pluronic® F-68 (x2) identify the independent variable affecting the drug content and the percentage drug encapsulation efficiency (dependent variable). The response surfaces of the obtained result were plotted. The data analysis of values obtained from various batches for drug content and encapsulation efficiency

is subjected to multiple regression analysis using PCP dissolution software the equation fitted was

$$Y: \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X^2 + \beta_{12} X 1X2 \quad ....1$$

Where Y is the measured response; X is the level of factors; β is the coefficient computed from the responses of the formulations.

**Stability Studies:**

The stability studies of the optimized nanoparticles was evaluated [16] by storing formulation at 4ºC ±1ºC in refrigerator as per ICH guidelines. The nanoparticles was stored in screw capped amber-glass bottles. Physical instability like change in appearance, settling behavior was also observed. The sample is withdrawn and analyzed for its drug content, drug entrapment efficiency and *in-vitro* drug release profile.

***In-vivo* drug targeting Studies:**

An experimental protocol approved by the institutional Animal Ethical Committee (APCP/IAEC/409/01) prior to start the animal studies. Experiments were performed in accordance with the current guidelines of CPCSEA.

Healthy rats weighing 200-250 g selected; a constant day and night cycle maintained and they fasted for 12 hours. The animals are divided into 3 groups, each containing 6 rats.

Group I rats treated as control (received orally 0.5% CMC dispersion only)

Group II rats received 9 mg/kg of etoposide given orally after redispersing them in 0.5% CMC dispersion;

Group III rats received nanoparticles equivalent to 9 mg/kg of etoposide given orally after redispersing them in 0.5% CMC dispersion,optimized formulation (F6) was selected for the study.

After, 24 hour the rats were sacrificed and their liver lungs, spleen, kidney, heart and brain were isolated. Individual organs of each rat homogenized separately by using a tissue homogenizer. The tissue homogenate were made using methanol and 1.2 ml of tert-butyl methyl ether is mixed with a 0.1 ml aliquot of the tissue sample in a 2.0 ml polypropylene microtube. Then the homogenate centrifuged at 15,000 rpm for 30 min. Collected the supernatant liquid and filtered through 0.22 µm filters and samples were analyzed by HPLC System.

## RESULTS AND DISCUSSION

Spectral data of etoposide sample and standard etoposide confirmed the identity of the compound as etoposide. The solubility of etoposide in 10 mg/10 mL of solvent was carried out and it reveals that it is freely soluble in methanol, poorly soluble in water (less than mg/ml) at 37° C. Nanoprecipitation technology was selected for the production of submicron particle complying with the low aqueous solubility of etoposide. On the basis of drug solubility and miscibility in aqueous phase, methanol was selected as a choice of solvent. The rapid diffusion of methanol from dispersed droplets into aqueous phase with subsequent evaporation leads to fast precipitation of dissolved drug and polymer in the form of nanoparticles[17].

Drug content and encapsulation efficiency of nanoparticle suspensions were in the range of 61 to 89% and 48 to 94% respectively (Table I and 1a), which were mainly influenced by polymer concentration. The curvilinear relation observed between the drug content, encapsulation efficiency with Eudragit® EPO concentration. It can be explained on the basis of lipophilic–lipophilic interaction between etoposide and Eudragit® EPO. Consequently with increase in the Eudragit® EPO amount, etoposide gets preferentially dispersed in the internal organic phase [18]. Pluronic® F-68, also displayed similar trend and increase in encapsulation efficiency which can be due to the formation of interpenetrated network chain between the hydrophobic portions of Pluronic® F-68 with Eudragit® EPO during precipitation(synergistic effect evidenced from positive co efficient value for X1X2 interaction term)[19]. It is confirmed by the positive regression values of X1X2 term as shown in Table 1a. The particle size also shown similar effects. It influence of polymer–polymer interaction as compare to

polymer–pluronic interaction signifies the stabilizing effect of the latter by minimizing dispersion and distribution of drug outside the matrix. In this research, two responses were evaluated, and each response was plotted in relation to the modified factor. Both the experimental design and the linearity and response surface plots for drug content and encapsulation efficiency are shown in Figure 1a,1b.

As shown in Table 2, particle size of the nanoparticle suspension was in range of 114 to 136 nm, which was almost smaller than the etoposide (1120 nm). The increase in particle size of nanoparticle suspension with decrease in polydispersity index was observed with increase in polymer content. The smaller particle size obtained at low polymer content may be due to high distribution efficiency of the internal polymer-solvent phase into the external phase [20–22]. Increase in the viscosity of internal phase with increased amount of polymer also provides resistance for mass transfer in turn diffusion of polymer solvent phase into the external phase leading to particle enlargement. The zeta potential values of the nanoparticle suspension are presented in Table 2. All formulations exhibited strongly positive zeta potential values due to polycationic Eudragit® EPO comprising of various ammonium groups. The increased zeta potential values in initial batches may be attributed to Eudragit® EPO available at the surface of the particles due to high viscosity of external aqueous phase. The subsequent decline in values of zeta potential is an inverse function of particle size[23]. As solid state pharmaceutics have many advantages over liquid formulation mainly improved physicochemical stability and less susceptibility to microbial contamination, attempts were made to obtain dry powder nanoparticle suspension by lyophilization technique. Based on the results of the factorial design batch F6 having drug content of 88.36±0.075 %, encapsulation efficiency of 94.28±0.198%, zeta potential of 26.2±0.208

mV, was further processed to obtain dry powder. When it was compared with blank batch no significant variations in particle size and zeta potential were observed (Table 2). The lyophilized nanoparticles (F6) have the average particle size of 131.4±0.057 nm. Almost twice increase in size of particles could be due to changes in the internal structure of the particles, originated during the freeze drying process caused by the formation of ice crystal in the water phase or, more likely, to particle aggregation during freeze-drying resulting in poor redispersion[24]. Figure 3 and 4 shows the DSC thermogram of etoposide and lyophilized nanoparticle. Etoposide exhibit a sharp melting endotherm at 266.9 °C (78.39 J/g), whereas the thermogram of lyophilized nanoparticle suspension displayed a sharp endotherm at 262.7°C (60.38 J/g). It explains monotectic behavior of the system, where drug gets completely dissolved below its melting temperature in molten mass of the excipients. The similar behavior was also reported for the nifedipine with Pluronic® F-68, Gelucire and paracetamol with PEG [25,26]. The PXRD diffraction patterns as shown in Figure 4, reveal characteristic peaks at 4.2, 9.46, 10.22, 13.18, 16.15, 17.08, 17.67, 19.26, 19.89, 22.14, 23.03, 23.67, 24.17, and 26.78 which can be inferred to traits of a high crystalline structure. The complete disappearance of peaks in lyophilized powder may be due to formation of an amorphous complex while undergoing the nanoprecipitation with intermolecular interaction occurring within the matrix. Peaks of reduced intensity were observed in physical mixture. The intermolecular interaction in nanoparticle suspension was established by FT-IR shown in Figure 5 and 6. Etoposide exhibits the characteristics intensities of C=O stretching absorption band at 1764 cm−1 and the O-H stretch at 3452 cm−1. However, FTIR spectra of the lyophilized powder showed C=O stretching

absorption band of etoposide and O-H stretching. These result suggested no interaction between drug and polymer. The surface topography of the nanoparticle suspension was studied using SEM, which displayed uniform sized spherical shaped nanoparticles with size range correlating with particle size studies. SEM photograph of nanoparticles was shown in figure 7. *Invitro* drug release profile of etoposide, and lyophilized nanoparticles in Phosphate pH 7.4 buffer is shown in Figure 8, 9 and 10. The formulated nanoparticles showed a most favorable release within 24 hours. Only 14.09±0.19% drug release obtained from raw material of etoposide. In the 24th hour, the drug release 79.09±0.58%, 81.34±0.37%, 82.81±0.63%, 94.22±0.56 %, 96.02±0.31%, 99.22±0.50%, 86.02±0.18%, 89.90±0.33% and 91.93±0.28% for F1, F2, F3, F4, F5, F6, F7, F8 and F9 respectively. This followed by a steady state drug release pattern.

From these above data, it showed formulation F6 released drug mostly at the end of 24 hours. The release rate of Etoposide decreased with increasing concentration of Eudragit. But increase in the rate of release found with increasing amount of pluronic.

As compared with pure drug, lyophilized nanoparticle showed significant increase in dissolution rate with maximum and complete drug release with F6 formulation. However, lyophilization retarded the drug release, the retardation of drug release of lyophilized formulation is probability due to the aggregation of the particles in lyophilization, but still particles exhibited size below 1 micrometer.The kinetics of in-vitro drug release was determined by applying the drug release data to various kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas. The result obtained was shown in Table 3.

The optimized nanoparticle suspension subjected to stability study at 4 ± 1°C. During stability study at 4 ± 1°C, no significant difference in drug content (87.69 ± 0.043%), encapsulation efficiency (93.32 ± 0.015%) and in-vitro drug release (97.92 ± 0.037%) was observed over the period of 1 year. The stability results are shown in Table 4. There is no significant difference in Physical instability like change in appearance, settling behavior was also observed. The average targeting efficiency of drug loaded nanoparticles found to be 41.88 ±0.030% of the injected as dose in liver, 25.66 ±0.320% in spleen, 13.82±0.090% in lungs, 4.52±0.300% in kidney, 4.18 ±0.490% in brain as compared to the concentration of pure drug 28.47 ±0.041% in liver, 16.40 ±0.080% in spleen, 13.79 ±0.195% in lungs, 11.83 ±0.065% in kidney, 3.63±0.180% in brain. The results are shown in figure 11. The drug loaded nanoparticles showed preferential drug targeting to liver followed by spleen, lungs, kidney and brain.

**CONCLUSION:**

Generally, oral etoposide administration compared to intraveneous administration may result in an improvement of patient's quality of life and reduced costs. Several studies confirmed comparable safety and efficacy of oral and intravenous etoposide. However, a greater use of oral etoposide is limited by its incomplete and variable bioavailability. The study utilizes the particle engineering to improve primary properties of the etoposide. The novel polymeric nanoparticles containing etoposide were prepared by nanoprecipitation technique. The polycationic polymer Eudragit® EPO and Pluronic® F-68 as stabilizer can be used to obtain stable nanoparticle suspension. In addition, ionic interactions between cationic polymers with GI mucosa may improve bioavailability. Drug: polymer ratio and concentration of stabilizer were found to influence the drug content and entrapment efficacy of etoposide nanoparticles but the concentration of stabilizer had great influence of both dependent variables. *In-vitro* drug release study of selected factorial formulations F6 showed, 99.22% drug release in 24 hours. The drug release was found to peppas release kinetics with fickian diffusion

mechanism for all batches. So, it concluded that etoposide nanoparticles could be effective in sustained release.

These results shows that, the etoposide loaded polymeric nanoparticles showed preferential targeting the drug to liver followed by spleen, lungs, kidney and brain. It also revealed that, as compared to pure drug, higher concentration of drug targeted to the organs like liver and lungs after administering the dose in the form of nanoparticles. This may lead to attributed to high macrophage load in these organs and large size of liver as compared to spleen and lungs.

The relatively high concentration of drug etoposide present in the liver which suggests their usefulness in the targeting of Liver cancer. The etoposide nanoparticles of relatively smaller particle size coupled with prolonged blood circulating property could be a beneficial delivery system for tumor targeting. Further investigations is required on the anticancer activity, Pharmacokinetics of selected factorial formulations F6 etoposide nanoparticles. Those studies are under progress in our laboratory.

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**Table 1: Coded levels and actual values of the variables along with the measured responses of $3^2$ factorial design.**

| Batches | Coded levels | | Concentration of Eudragit® EPO (% w/v) | Concentration of Pluronic® F-68(% w/v) | Drug Content# (%) | Entrapment Efficiency# (%) |
|---|---|---|---|---|---|---|
| Etoposide | X 1 | X 2 | - | - | - | - |
| F1 | -1 | -1 | 0.3 | 0.4 | 61.86±0.130 | 48.96±0.135 |
| F2 | -1 | 0 | 0.3 | 0.5 | 67.43±0.075 | 54.40±0.150 |
| F3 | -1 | +1 | 0.3 | 0.6 | 72.87±0.015 | 59.97±0.198 |

| | | | | | | |
|---|---|---|---|---|---|---|
| F4 | 0 | -1 | 0.45 | 0.4 | 82.21±0.075 | 81.82±0.274 |
| F5 | 0 | 0 | 0.45 | 0.5 | 86.25±0.075 | 88.23±0.276 |
| F6 | 0 | +1 | 0.45 | 0.6 | 88.36±0.075 | 94.28±0.198 |
| F7 | +1 | -1 | 0.6 | 0.4 | 89.90±0.080 | 89.95±0.202 |
| F8 | +1 | 0 | 0.6 | 0.5 | 87.66±0.130 | 94.42±0.430 |
| F9 | +1 | +1 | 0.6 | 0.6 | 89.5±0.130 | 94.10±0.135 |

#all the determinations were performed in triplicate and values were expressed as mean±S.D, *n* = 3; X1: Polymer Eudragit® EPO; X2: Stabilizer Pluronic® F-68.

**Table 1a: Prediction of Regression Value**

| Variable | CONSTANT | X1 | X2 | X1X2 | X1X1 | F | $R^2$ |
|---|---|---|---|---|---|---|---|
| Drug Content (R1) | 85.6067 | 11.2727 | 2.7933 | 2.7364 | 6.9473 | 721.12 | 0.9986 |
| Entrapment Efficiency (R2) | 88.1100 | 19.1767 | 4.6033 | - | 14.4633 | 194.77 | 0.9915 |

**Table 2. Zeta potential, Particle size and poly dispersity of polymeric nanoparticles**

| Batches | Zeta potential * (mV) | Mean particle size* (nm) | Poly dispersity* |
|---|---|---|---|
| Etoposide | - | 1120±0.200 | 1.547±0.005 |
| F1 | 18.30±0.135 | 114.4±0.305 | 0.734±0.002 |

| | | | |
|---|---|---|---|
| F2 | 19.46±0.305 | 125.5±0.862 | 0.715±0.001 |
| F3 | 20.2±0.115 | 134.6±0.200 | 0.707±0.002 |
| F4 | 24.6±0.200 | 128.5±0.100 | 0.564±0..005 |
| F5 | 25.6±0.200 | 129.3±0.100 | 0.548±0.001 |
| F6 | 26.2±0.208 | 131.4±0.057 | 0.522±0.001 |
| F7 | 22.3±0.152 | 134.4±0.115 | 0.693±0.001 |
| F8 | 23.6±0.200 | 135.3±0.152 | 0.684±0.002 |
| F9 | 24.60±0.152 | 136.7±0.100 | 0.353±0.005 |
| Blank (F6) | 26.50±0.208 | 136.3±0.208 | 0.526±0.002 |

*All the determinations were performed in triplicate and values were expressed as mean±S.D, *n* = 3

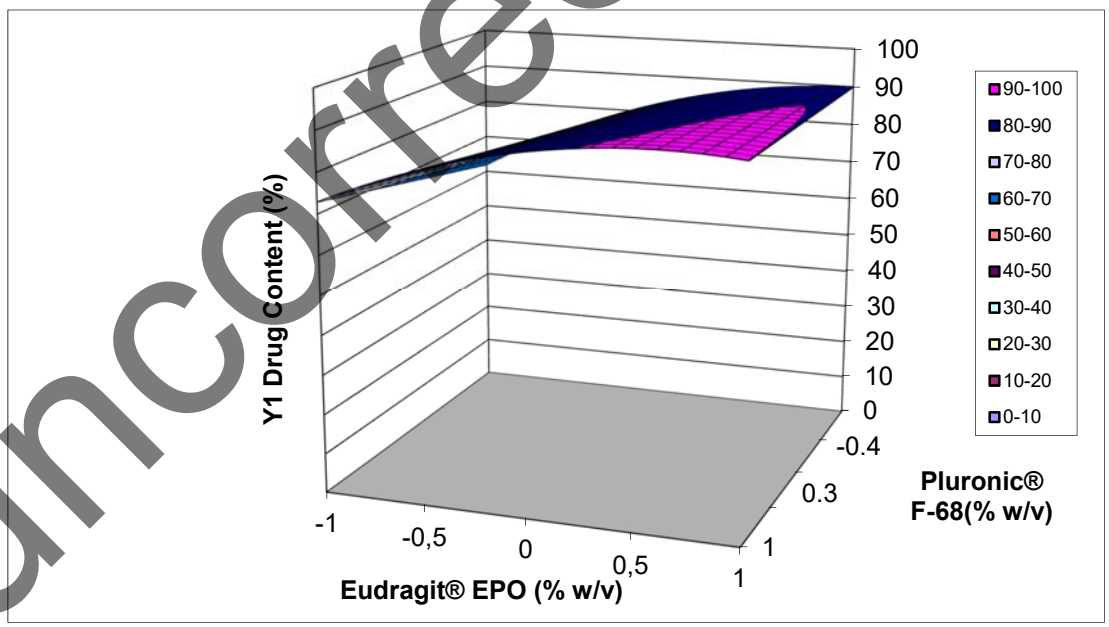

**Fig. 1**(a) Response surface plot showing the influence of polymer surfactant ratio on drug content.

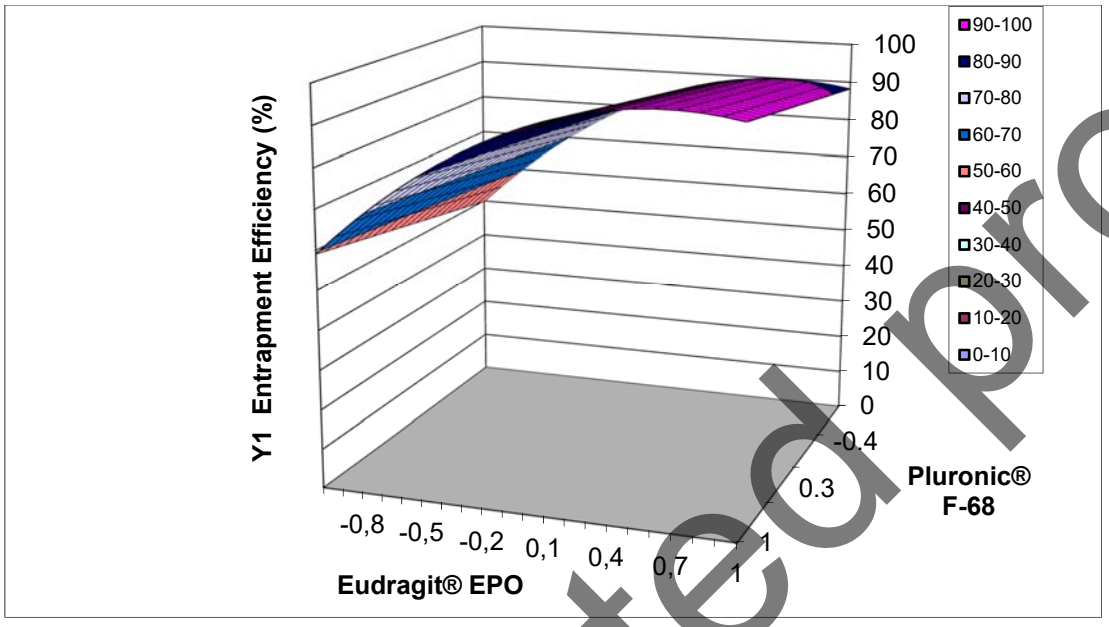


**Fig. 1**(b) Response surface plot showing the influence of polymer surfactant ratio on encapsulation efficiency.

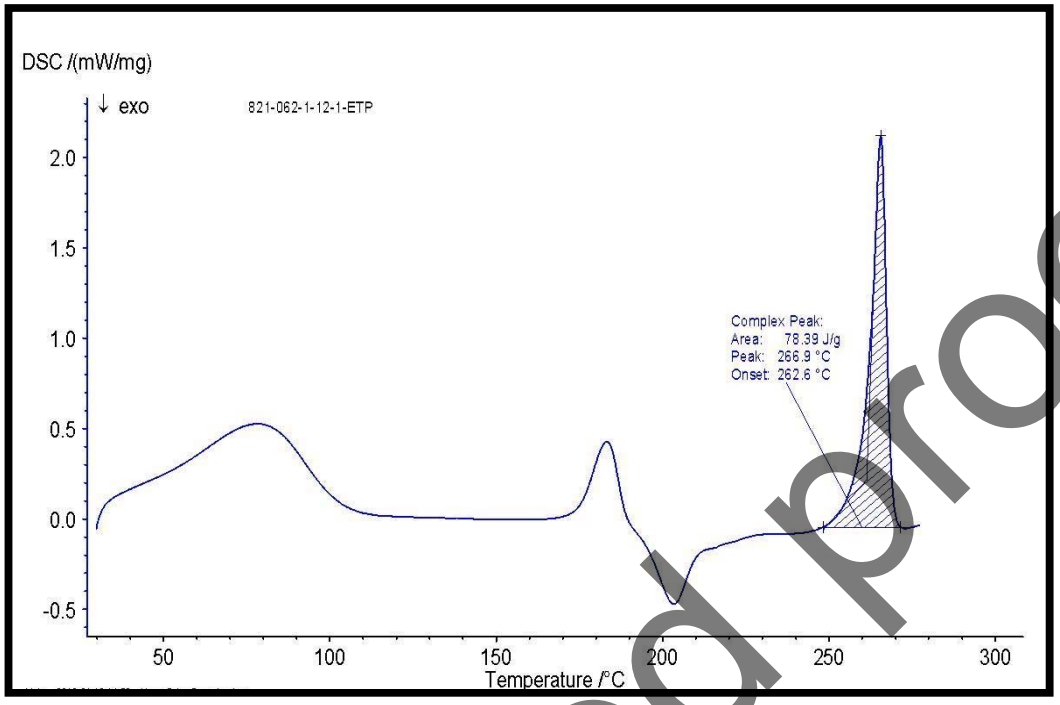


**Fig. 2:** DSC thermogram of etoposide

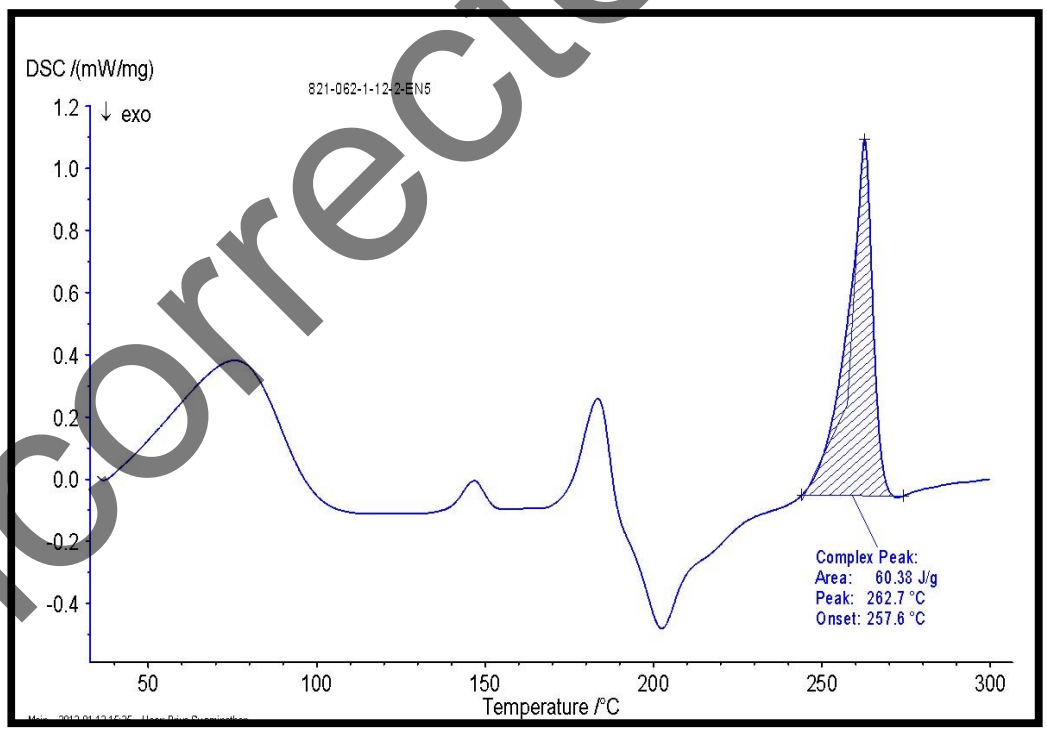


**Fig. 3:** DSC thermogram of etoposide nanoparticle

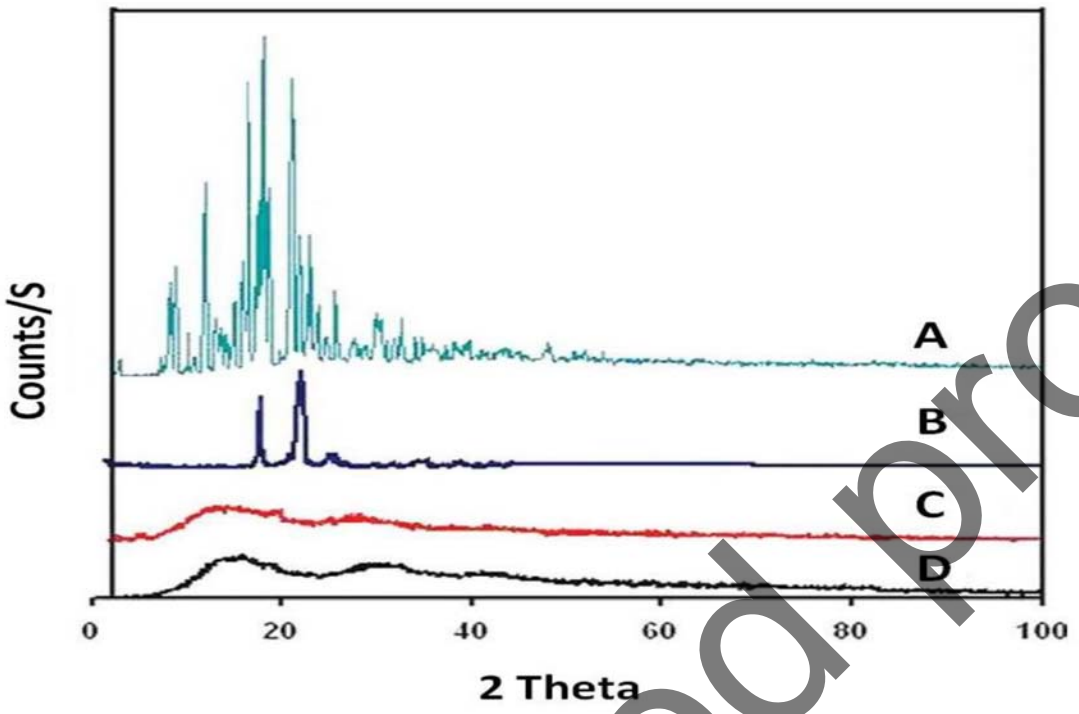


**Fig.4:** XRD spectra of (A) Etoposide, (B) Physical Mixture of Etoposide and polymers (C) polymeric nanoparticles (before lyophilisation) and (D) Etoposide loaded polymeric nanoparticles (after lyophilisation).

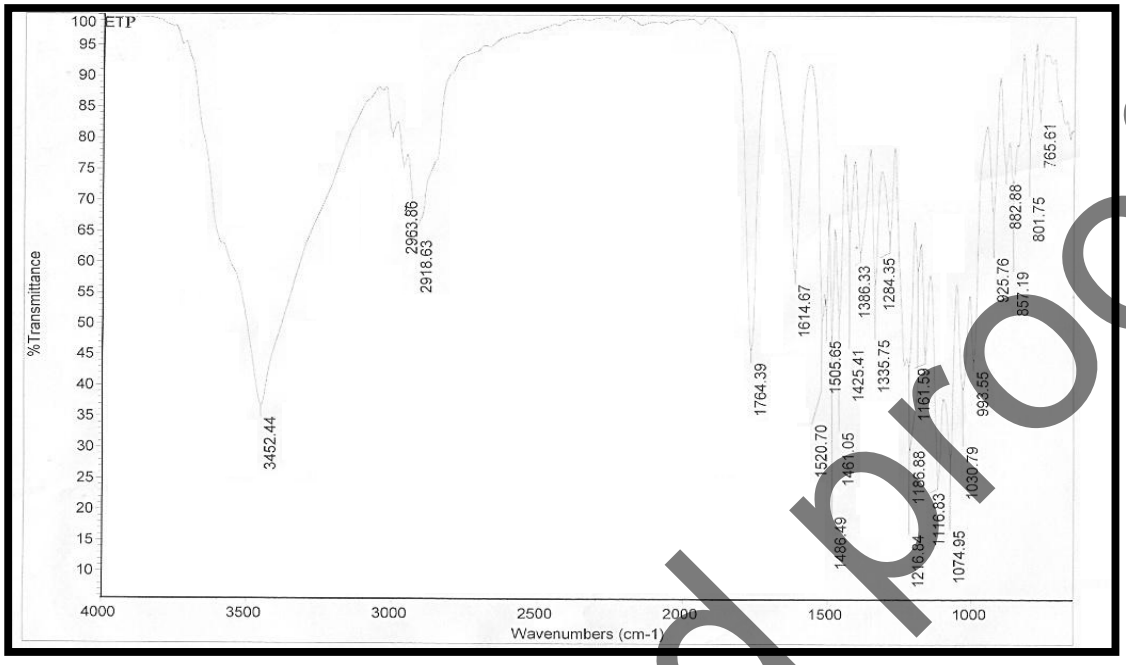


**Fig.5:** FTIR spectrum of Etoposide

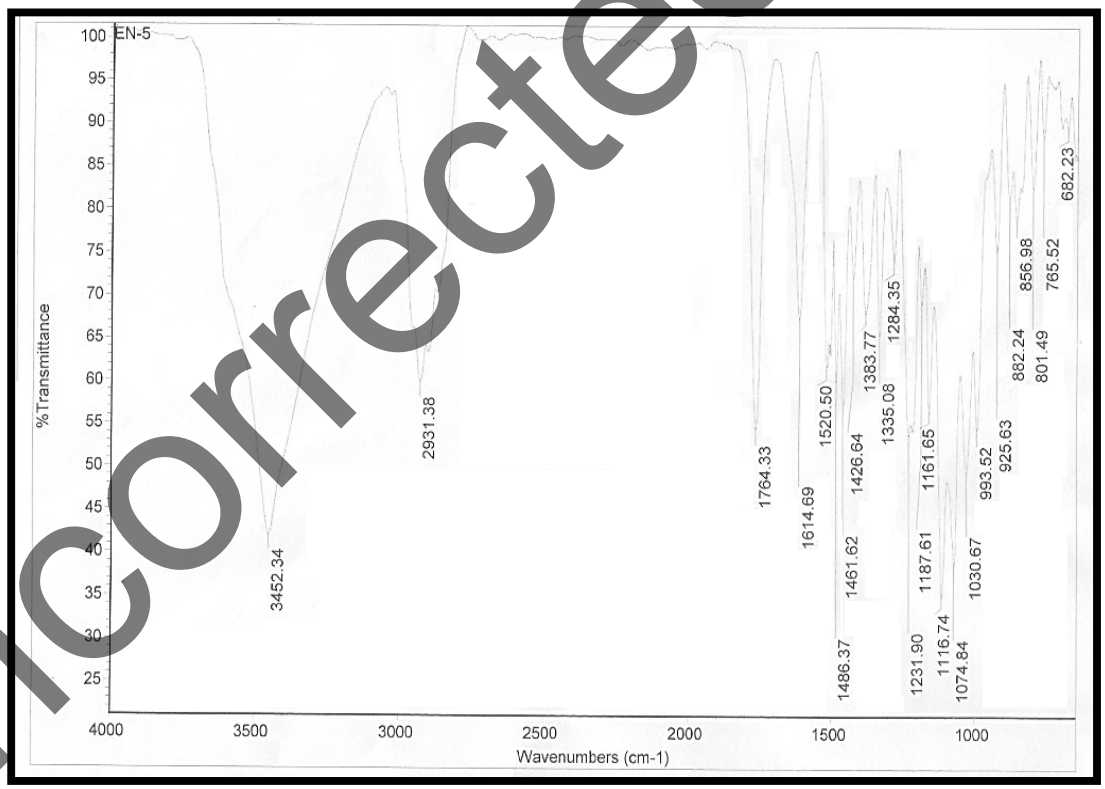


**Fig.6:** FTIR spectrum of Etoposide nanoparticles

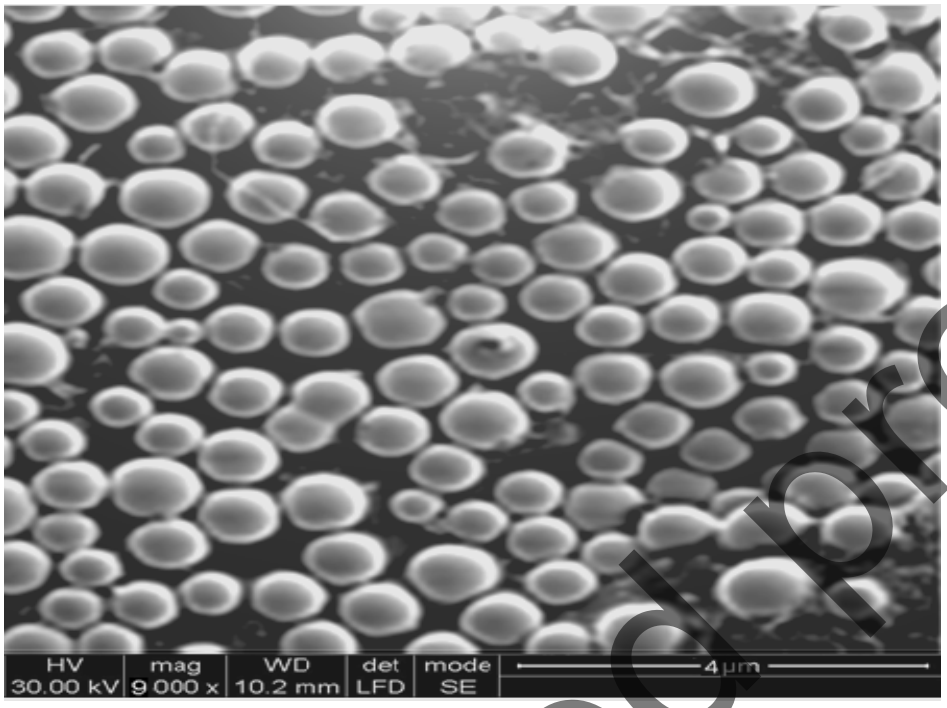


**Fig.7:** SEM photograph of the etoposide nanoparticle suspension

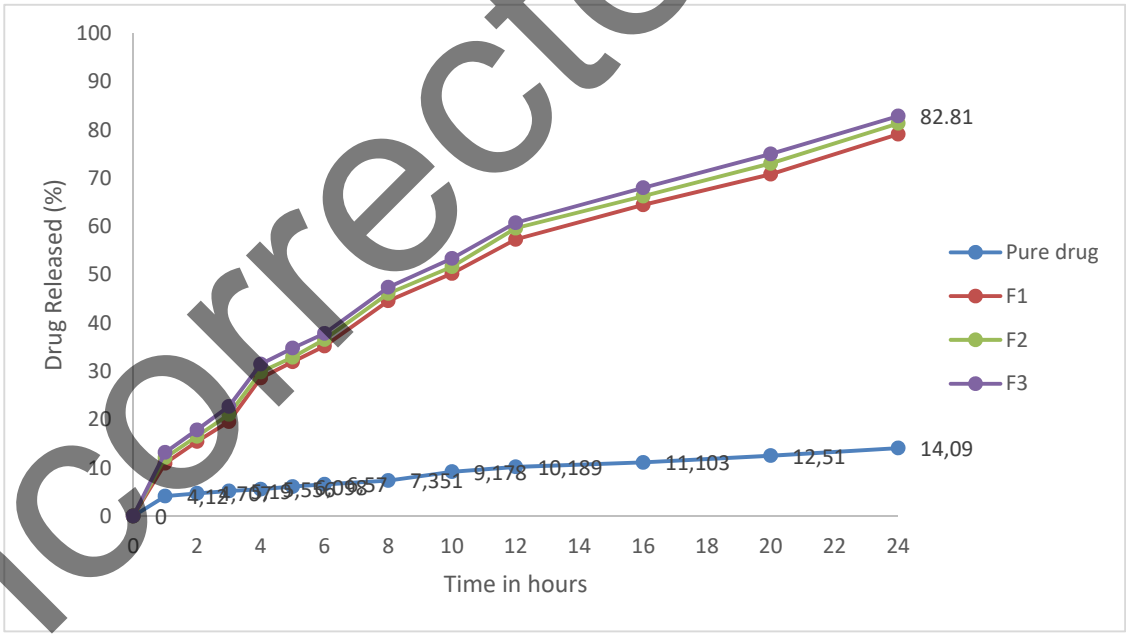


**Fig.8:** Comparative *in-vitro* drug release of the etoposide nanoparticles (F1, F2, F3), All the values expressed as mean ± S.D., n=3

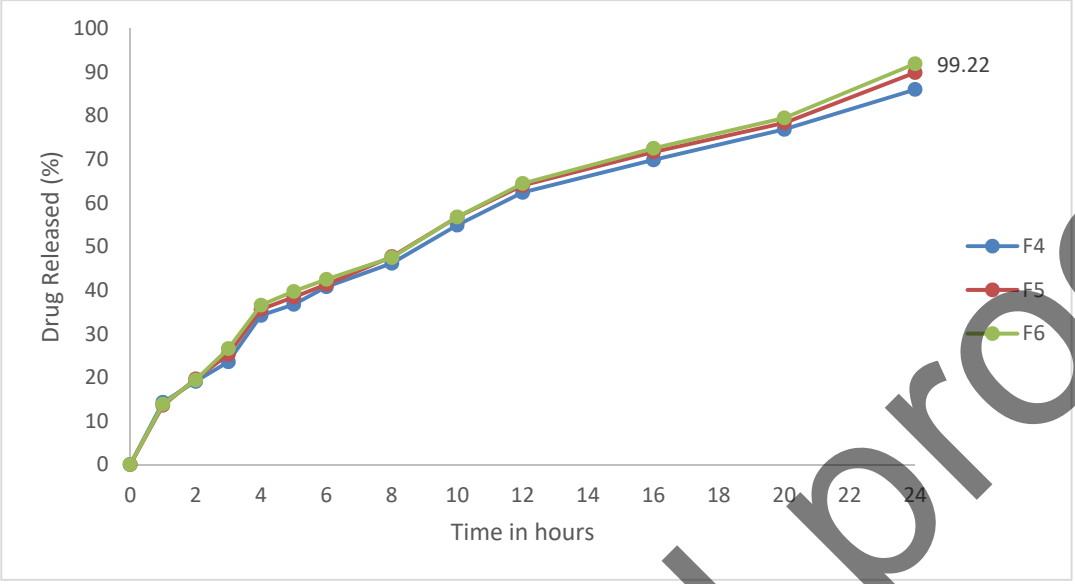


**Fig.9:** Comparative *in-vitro* drug release of the etoposide nanoparticles (F4, F5, F6), All the values expressed as mean ± S.D., n=3

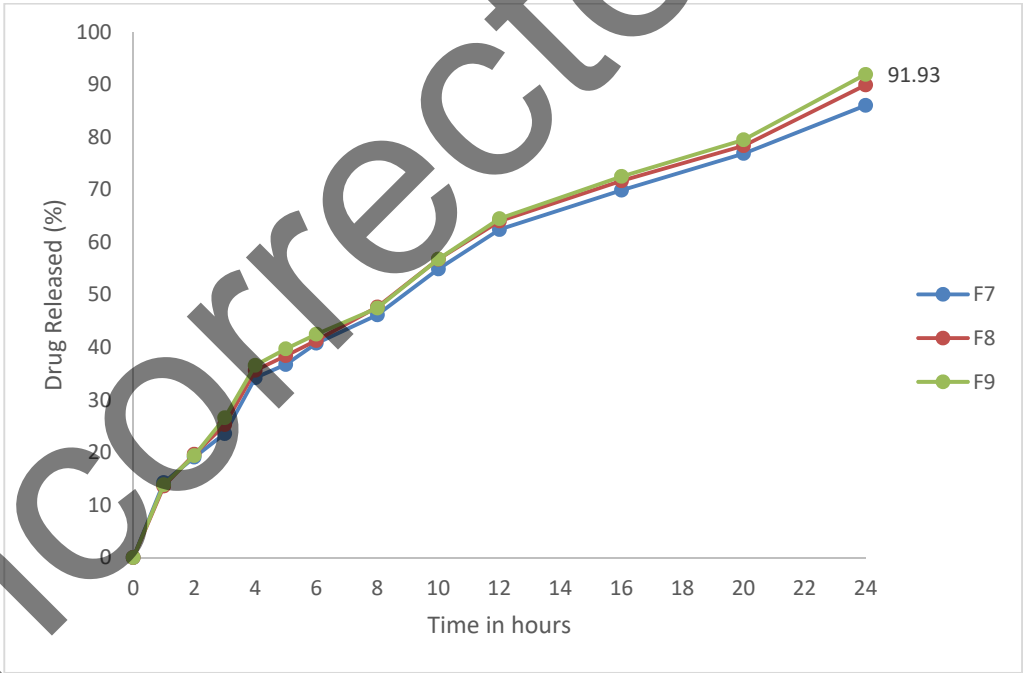


**Fig.10:** Comparative *in-vitro* drug release of the etoposide nanoparticles (F7, F8, F9) , All the values expressed as mean ± S.D., n=3

**Table 3:** Kinetics of *in-vitro* drug release profile for all etoposide nanoparticles

| Formulation code | Zero order $R^2$ value | First order $R^2$ value | Higuchi's $R^2$ | Korsmeyer | | Best fit models |
|---|---|---|---|---|---|---|
| | | | | $R^2$ value | n value | |
| F1 | 0.9043 | 0.9940 | 0.9880 | 0.9943 | 0.6513 | Peppas |
| F2 | 0.8976 | 0.9946 | 0.9898 | 0.9950 | 0.6303 | Peppas |
| F3 | 0.8846 | 0.9946 | 0.9926 | 0.9955 | 0.6061 | Peppas |
| F4 | 0.9058 | 0.9630 | 0.9914 | 0.9963 | 0.5978 | Peppas |
| F5 | 0.9013 | 0.9493 | 0.9923 | 0.9958 | 0.5848 | Peppas |
| F6 | 0.8895 | 0.8677 | 0.9940 | 0.9964 | 0.5617 | Peppas |
| F7 | 0.8769 | 0.9935 | 0.9938 | 0.9946 | 0.5873 | Peppas |
| F8 | 0.8789 | 0.9875 | 0.9938 | 0.9956 | 0.5982 | Peppas |
| F9 | 0.8787 | 0.9803 | 0.9938 | 0.9946 | 0.5959 | Peppas |

∗Results are expressed as mean ± standard deviation ($n$ = 3).

**Table 4:** Stability testing parameters of optimized nanoparticles (F6)

| Evaluation Parameters | Fresh Formulation | Storage condition at 4 ± 1°C (End of 1 year) |
|---|---|---|
| % Drug content | 88.36±0.075 | 87.69±0.043 |
| %Entrapment Efficiency | 94.28±0.190 | 93.03±0.020 |
| Percentage Drug Release | 99.22±0.50 | 97.92±0.037 |

∗Results are expressed as mean ± standard deviation ($n$ = 3).

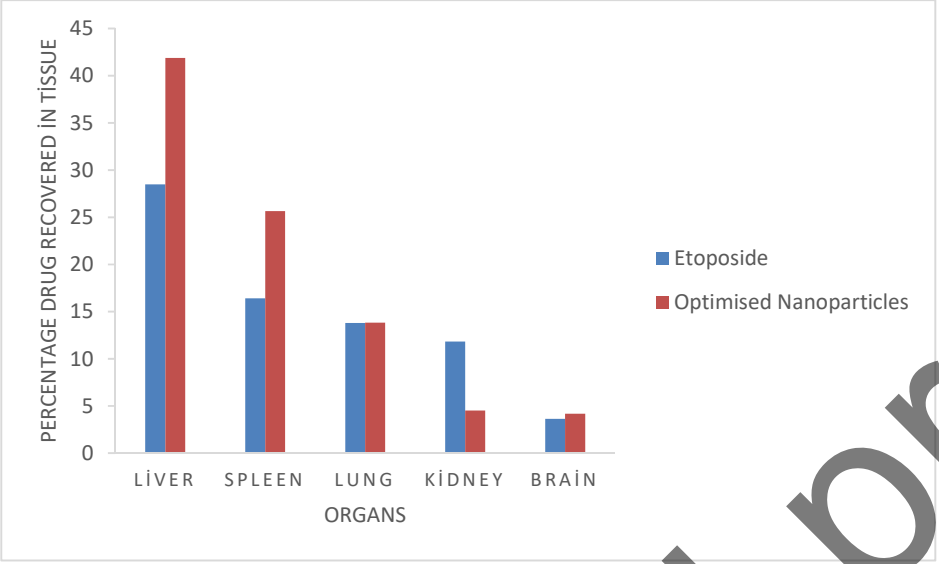


**Fig.11:** Comparative *in-vivo* drug targeting.

Each data point is the mean ± SD of 3 experiments.