ETOPOSIDE AMORPHOUS SOLID DISPERSION FOR IMPROVED ORAL BIOAVAILABILITY: FORMULATION, CHARACTERIZATION, PHARMACOKINETIC AND CYTOTOXICITY STUDIES

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ABSTRACT

Etoposide is a well-known anti-tumor agent used to treat a variety of cancers. Although it is a BCS class IV drug, applications are restricted due to poor solubility and bioavailability. Hence, the current research was designed to overcome these pitfalls. A total of 16 formulation batches were developed using the physical mixture and kneading method and optimized by Design-Expert® software. A selected batch was evaluated using solubility, differential scanning calorimetry, X-ray diffraction, motic microscopy, scanning electron microscopy, Fourier transform infrared (FTIR), gastrointestinal distribution, pharmacokinetic and cytotoxicity study. The results showed that the saturated solubility of formulation was 19.76 mg mL^{-1}. FTIR showed C-O=1646 cm^{-1}, and C-H=2956 cm^{-1}. The distribution study indicated 9.11, 5.39 and 4.23 µg mL^{-1} colon concentrations at 8h, 16h, and 24h, respectively. The Cmax and AUC were found at 741.17±12.29 ng mL^{-1} and 3089.23 ±34.69 ng mL^{-1} with less viability on HeLa cells. Therefore, the study investigates the developed solid dispersions enhanced solubility and bioavailability with an antiproliferative effect.

Keywords: Etoposide, amorphous solid dispersions, poor aqueous solubility, dissolution, oral bioavailability, cytotoxicity

INTRODUCTION

Worldwide, cancer is the primary reason for death and a common public health problem[1]. More than 19,300,000 new cancer cases were identified and reported, resulting in nearly 10 million deaths worldwide in 2020[2]. Skin, breast, lung, stomach, colon, rectum, and prostate are the most common types of cancer. It affects approximately 4,00,000 children annually[3]. By 2025, 420 million new cases of cancer will be diagnosed as predicted by global demographic characteristics[4]. This indicates an increase in the incidence of cancer over the upcoming years. Tobacco used, poor diet, obesity, inactivity, excessive alcohol consumption and other factors like ionizing radiation, infection and environmental pollutants are the leading causes of cancer[5]. Therefore, numerous novel technologies and treatments have been adopted to reduce the harm caused. Despite their high therapeutic activity, most newly discovered chemical entities are poorly absorbed in the gastrointestinal (GI) tract due to less water solubility and low bioavailability[6]. As a result, there is a lot of interest for increasing oral bioavailability with efficient, reliable, cost-effective by using and scalable strategies[7]. The common approaches to solve this problem are surfactants[8], cosolvents[9], lipid-based formulations[10,11], cocrystals[12], cyclodextrins[13], nanonization methods[14] and amorphous solid dispersions (ASDs)[15]. From all the above approaches, the most successful method for improving the dissolution profile is solid dispersion formulation[16]. Numerous anticancer drug play a crucial role in the formulation of ASDs. Among others, etoposide is a well-known anticancer medication used to treat a variety of cancers[17].

The semisynthetic podophyllotoxin, etoposide (ETO) has the molecular formula $C_{29}H_{32}O_{13}$[18]. The basic structure of ETO is depicted in Fig.1[19]. It is the drug used for first-line treatment of various cancer types including leukemia, colon, small cell lung cancer, lymphoma, and oropharyngeal cancer[17]. It can be used alone or in conjunction with other therapeutic drugs[20,21]. The ETO suppresses the enzyme topoisomerase II by controlling the DNA conformational arrangement and synthesis[22]. It is well absorbed orally and has no evidence to show the first-pass effect. It is cleared by renal and non-renal processes

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and does not cross the (BBB) Blood-Brain Barrier. But ETO is a BCS IV drug; its applications are restricted due to poor aqueous solubility[23], low bioavailability, high drug resistance and systemic toxicity[18]. Therefore, we developed etoposide solid dispersions (E-SD) to raise the solubility, dissolution, bioavailability, permeability, and, finally, bioactivity of the pure drug.

Solid dispersions (SDs) were prepared with different polymer concentrations (1:1 & 1:2) using a physical mixture and kneading method in this research study. The DOE was used to select the optimized batch. After that, various techniques were employed to characterize the optimized formulation. A shake flask method was utilized to perform solubility. The possibility of a dissolution rate limiting profile was verified. Apart from that, a gastrointestinal (GI) distribution study was conducted to determine cell viability using various GI tissues. The animal model (PK Solver 2.0 USA) was used for the pharmacokinetic study to examine oral bioavailability. Finally, the cytotoxicity study was investigated using an MTT assay on HeLa cells to determine the bioactivity. Overall, this research illustrates the formulation and characterization strategies for enhancing oral drug absorption.

MATERIALS AND METHODS

Materials

Etoposide was procured from Aurobindo Pharma Pvt. Ltd., Hyderabad, as a gift sample. The polymer Gelucire® 50/13 was purchased from Gattefosse Pvt. Ltd., India and Poloxamer 407 (Pluronic® F-127) was obtained from Merck, Ahmedabad. Sigma Aldrich provided the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (MTT reagent A). Additional excipients like methanol (methanol AR/ACS), dimethyl sulfoxide (DMSO), polyvinyl pyrrolidone (PVP) K30, PEG 4000 (polyethylene glycol 4000 for synthesis), and SCMC (sodium carboxymethyl cellulose) were availed from Loba Chemie Pvt. Ltd., Mumbai. All solvents and chemicals used were of analytical grade in the research study. Animal ethical committee CPCSEA, Ministry of Environment and Forests Government of India (CRY/2021/032) had approved the protocol for animal study.

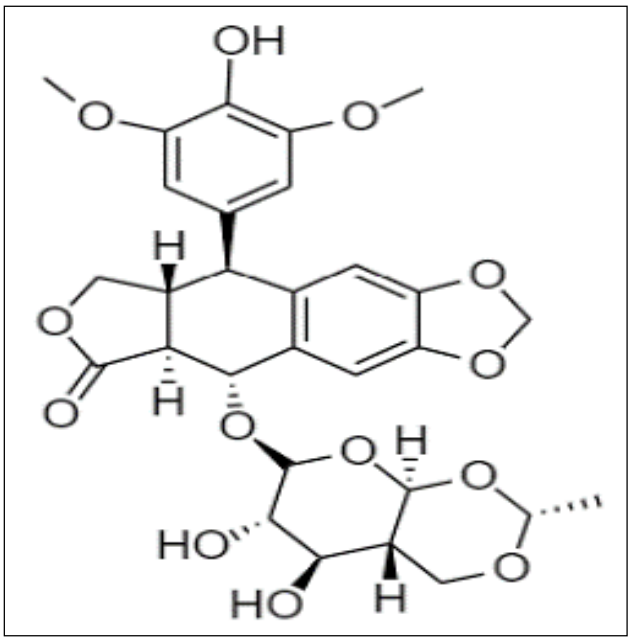


Fig. 1: Basic structure of etoposide

Methodology

Formulation of etoposide solid dispersions

Etoposide SDs were formulated by following two techniques;

Physical mixture method

Etoposide and selected polymers (polyethylene glycol 4000 for synthesis, Gelucire®50/13, PVP K30, and Pluronic® F-127) were assembled to form 8 batches (Table III). Drug and one polymer were taken in the ratio of 1:1 and 1:2 separately. This step was repeated for another polymer in the same composition. Basis of selection of this ratio was found to be suitable for achieving the desired drug release profile and stability of the solid dispersion formulation. The drug and the selected polymers were accurately weighed, pulverized and thoroughly mixed using a glass mortar for 5 minutes with light trituration until homogeneous. The final mixture was stored for further evaluation[24].

Kneading method

The 8 SDs batches were prepared in the same ratio of the drug with polymer (polyethylene glycol 4000 for synthesis, Gelucire®50/13, PVP K30, and Pluronic® F-127) as mentioned in the physical mixture method. Initially, the amount of ETO and excipient was weighed and ground for a few minutes using a mortar pestle. After that, alcohol: water (1:1V/V) 5 mL was added and triturated until the mixture evaporated. Overnight preserved in a desiccator the obtained dry dispersions were passed through the sieve of 100-mesh. The dry dispersion was kept in a moisture-free atmosphere until further utilization[25,26].

Optimization of prepared solid dispersion

Prepared batches were further processed for optimization. Based on the solubility and dissolution study, further selection was carried out.

Solubility study

A shake flask technique was utilized to solubilise pure drug and formulations. The pH 7.4 phosphate buffer (PB) was used as a solvent for SD, whereas water and buffer with varying pH were used for the pure drug. An excessive quantity of ETO and equivalent prepared SD were agitated with a fixed volume (25 mL) of a buffer. The study was conducted for 48 h at 37 °C. The samples were allowed to stand for 24 h. Then, the samples were collected and passed through Whatman filter paper 0.45 µm. The filtrate was diluted with the specific solvent and analysed at 285 nm by a UV-visible spectrophotometer[27,28].

In vitro dissolution study

Type II dissolution apparatus (USP) was employed to ascertain all batches' dissolution rates. The dissolution media was taken with 900 mL of water and pH of 7.4 PB with 50 rpm, and a temperature of 37 °C. ETO was detected at 285 nm after the withdrawal of 5 mL sample at various points of time (5, 10, 15, 20, 30, 45, and 60 minutes). The dissolution study was done in triplicate and the final results was discussed[29,30].

Based on Design-Expert® software

Based on solubility and dissolution results of 16 batches, drug: polymer (1:2) batches was selected for further optimization using Design-Expert® software version-7 (Stat Ease Inc., MN, USA). The particle size (X1) and polymer concentration (X2) were selected as independent variable, whereas % cumulative drug release (CDR) was chosen as dependent variable. ANOVA was used to examine how independent variables affected dependent variables. The F-value and p-value were also determined to find the best fit of the models[31].

Characterization of prepared solid dispersion

Utilizing the solubility and dissolution study, the optimized formulation was characterized. The procedure is described in detail below.

Saturation solubility study

The solvent used for the saturation solubility study was distilled water. In a conical flask, with a fixed volume of 25 mL of buffer, an excessive amount (50 mg) of the drug and equivalent solid dispersions were taken. The resulting mixture was shaken for 48 h with an incubator shaker at 200 rpm and 37 °C. After 48 h, the samples were removed, allowed to stand for 24 h, and filtered using Whatman 0.45 µm filter paper. Further, the filtrate was diluted and analysed at 285 nm by a UV-visible spectrophotometer[27].

Differential scanning calorimetry

Using a differential scanning calorimeter (DSC-TA-60, Japan), thermal evaluation of the drug, excipient, and E-SD was done. A sealed aluminium pan contained approximately 2 mg of ETO, Gelucire®50/13, and SD of the etoposide- Gelucire®50/13. A blank aluminium pan was considered a point of reference, and the samples were scanned at 10 °C min^{-1} with the temperature rising from 30 °C to 300 °C[32].

Powder X-ray diffraction

The X-ray diffraction (XRD) method was run to elucidate the structure and material properties. The solid dispersion XRD patterns were captured using a K filter X-ray spectrometer. Data were collected with a voltage of Cu/40 with kV/40 mA current. The sample was scanned with a rate of 10 min^{-1} and the diffraction angle was covered 2θ from 5.00° to 80.00°. The same procedure was repeated for pure ETO and the structure of pure drug and formulation observed[33].

Particle size analysis

Motic digital microscopy was used to examine the morphology and surface topography of prepared SD-6. The samples were mounted on a glass slide and observed under 10X object with the magnification power 2048 x 1536 at room temperature[34].

Scanning electron microscopy

The morphology of SD as well as ETO was visualized by using scanning electron microscopy (SEM). Using double-sided sticking tape, the ASDs were mounted to the SEM sample stub. Around 200 nm thick, gold film was coated. Reduced pressure (0.0001 mm Hg) was maintained throughout the analysis. The same procedure was repeated for pure drug and the particle size analysed[33].

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was chosen to determine the chemical interplay between excipients, ETO and optimized SD. Approximately 2 mg of the formulation was measured and combined with IR-grade potassium bromide pellets. Each analysis included 25 scans with a 1000-35000 cm^{-1} scanning range and 1 min resolution. Repeated the same procedure for the pure drug and the spectral analysis of the pure drug and the solid dispersion determined[35].

Gastrointestinal distribution study

The gastrointestinal (GI) distribution study was assessed using a healthy adult rat model by comparing

ETO suspended in 0.5% w/V sodium carboxy methyl cellulose (SCMC) as a control treatment. Animals were kept fasted for 12 h overnight before the study. All animals were provided proper care, water, and *ad libitum* throughout the experiments. Table I describes the study design for the GI distribution study. The administered, ETO dose was calculated using the colon's surface area of the rat (0.0023×500×7 = 8.05mg). The appropriate formulations of ETO were given to animals by the peroral route and for blood collection, retro-orbital route was used under mild anaesthesia. Blood was centrifuged, for 10 min at 3000 rpm and stored at -20^0 C for the estimation of ETO concentration by using high-performance liquid chromatography (HPLC). To suppress the stress effects, the animals were randomized before the blood collection at each time point.

Table I: Study design for GI distribution study

S. No.	Group	Treatment	Animals used
1.	I	Normal saline (NS)	6
2.	II	Etoposide fast-release Carboxymethyl Cellulose Sodium Salt formulations	6
3.	III	Etoposide solid dispersion	6
Time intervals of 0, 1, 2, 4, 6, 12, and 24h after administration			

Deep anesthesia was used to euthanize the animals, and the whole GI tract was taken out. Additionally, the mesenteric tissues and fatty layer were segregated. The NS (0.9% w/V NaCl) was used to wash the GI tract, which was divided and cut longitudinally into the stomach, colon, small intestine, and cecum. Using a tissue homogenizer (RQ127A, Remi Motors Ltd., Mumbai, India), small pieces of cut organs were homogenized at 4 °C at 3000 rpm and centrifuged for 10 minutes. The fatty layer was also discarded, and HPLC was used to estimate the concentration of ETO in the supernatant[36].

Pharmacokinetic study

All animals fasted overnight prior to the study. Food, water and care were provided *ad libitum* throughout the study. Extracts were dosed orally by making suspension in carboxy methyl cellulose to the targeted rats by intragastric tube by the oral route. A total of 24 rats divided into 2 groups were used for the pharmacokinetic study shown in Table II. Blood (250 µL) samples from the retro-orbital plexus route were collected into vials at different time points. Blood samples were centrifuged to obtain plasma and stored at-20 °C for further processes[37,38].

Table II: Study design for pharmacokinetic study

S. No.	Group	Treatment (mg kg^{-1})	Animals used
1.	I	Etoposide 20mg	6
2.	II	Etoposide solid dispersion 20 mg (equivalent to standard etoposide)	6
Time intervals: 1, 2, 4, 6, 8, 12, 24 & 48 h after administration			

Protein precipitation

The protein precipitation technique was used and analyzed by the HPLC method. It was used to extract, drugs from plasma and drug content. The gradient mobile phase composition ammonium acetate (20 mM, pH 4.5) 45%, and acetonitrile 55% was followed for analysis[39].

Cytotoxicity study

Preliminary evaluation of *in vitro* bioactivity

Cytotoxicity was carried out with a cervical cancer cell line (HeLa). The procedure is shown in Fig. 2. The MTT colorimetric assay was employed to evaluate the viability of the cell. Dimethyl sulfoxide was used to solubilize intracellular formazan crystals, and colour intensity was measured at 550 nm[40].

MTT assay

ETO dilutions were prepared with 100 mL solution and placed in the first well. It was diluted serially, into seven different concentrations. Various concentrations of ETO and E-SD were applied to HT-29 cell lines after 48 h of incubation. MTT dye reduction was used to count the remaining cells. In a nutshell, the plates were subjected to a 24 h for microscopic evaluation and incubated for three days at 37 °C in 5% CO_2. After 72 h, the drug solutions were removed, and 50 mL of MTT in PB was poured into each well. After being gently shaken, the plates were incubated for 3 h at 37°C and 5% CO_2. As soon as the supernatant was removed, 100 mL of propanol was added, and mild shaking was done to dissolve the formed formazan. At 540 nm, the absorbance was measured[41].

RESULTS AND DISCUSSION

Formulation of etoposide solid dispersions

Etoposide ASD was prepared, using different concentrations of drug with polymers using two methods: Physical mixtures and Solid dispersions. The batches were prepared by physical mixtures coded as PM (1-8) and for solid dispersions coded as SD (1-8) (Table III).

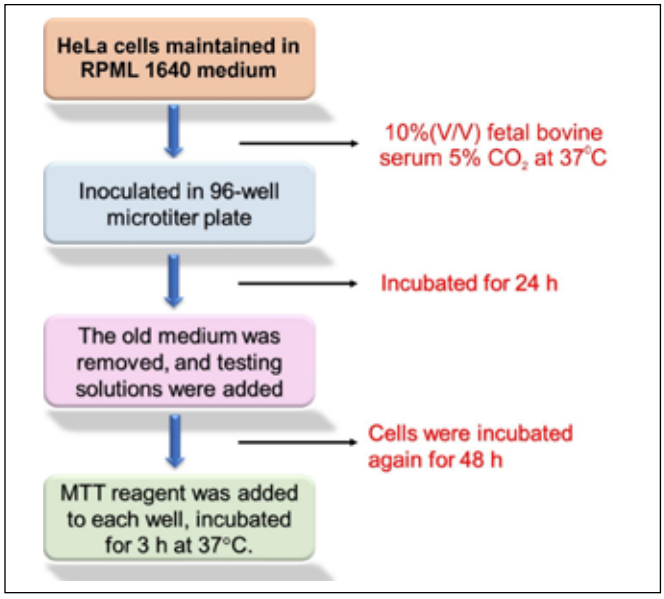


Fig. 2: Procedure for cytotoxicity study

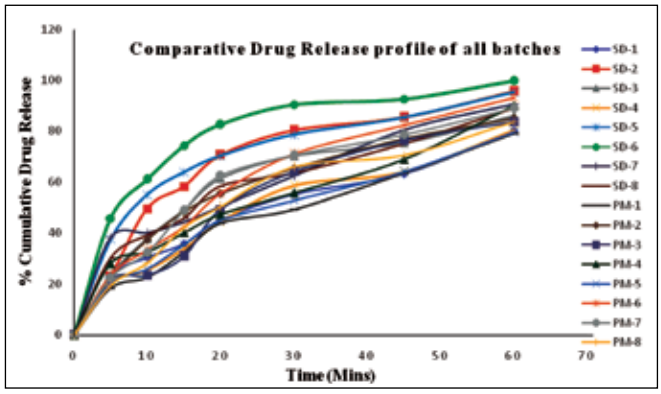


Fig. 3: Dissolution profiles of all batches in water

Optimization of prepared solid dispersion

After the formulation of all batches by using the above methods, the evaluation was done to select the optimized batch.

Solubility study

The first step of evaluation is to elucidate the drug solubility in different solvents, because it influences the bioavailability, drug release rate, and subsequently the therapeutic efficacy of the pharmaceutical products. The solubility was assessed in water and buffers at various pHs; the values are mentioned in Table IV and Table V. The solubility of ETO was much less in water than in other buffers at different pHs. As a result, we prepared SD to enhance the solubility. The SD-6 solubility is shown at 30.84 µg mL^{-1} which is highest compared to the pure drug and other formulation batches. Additionally, it indicates

Table III: Composition of solid dispersions and physical mixtures

Batch code	Composition
SD-1	Drug: Polyethylene glycol 4000 for synthesis (1:1) solid dispersion
SD-2	Drug: Gelucire®50/13 (1:1) solid dispersion
SD-3	Drug: PVPK30 (1:1) solid dispersion
SD-4	Drug: Pluronic® F-127 (1:1) solid dispersion
SD-5	Drug: Polyethylene Glycol 4000 For Synthesis (1:2) solid dispersion
SD-6	Drug: Gelucire®50/13 (1:2) solid dispersion
SD-7	Drug: PVPK30 (1:2) solid dispersion
SD-8	Drug: Pluronic® F-127 (1:2) solid dispersion
PM-1	Drug: Polyethylene Glycol 4000 For Synthesis (1:1) physical mixture
PM-2	Drug: Gelucire®50/13 (1:1) physical mixture
PM-3	Drug: PVPK30 (1:1) physical mixture
PM-4	Drug: Pluronic® F-127 (1:1) physical mixture
PM-5	Drug: Polyethylene glycol 4000 for synthesis (1:2) physical mixture
PM-6	Drug: Gelucire®50/13 (1:2) physical mixture
PM-7	Drug: PVPK30 (1:2) physical mixture
PM-8	Drug: Pluronic® F-127 (1:2) physical mixture

that the solid dispersions showed better solubility and more solubilized in solvents compared to physical mixture of formulation batches.

Table IV: Drug solubility study in different solvents

S. No.	Solvent	Solubility (µg mL^{-1})
1.	Water	2.06
2.	Acetate buffer pH 1.2	3.35
3.	Acetate buffer pH 4.5	4.23
4.	pH 6.8 PB	4.98
5.	pH 7.4 PB	5.89

In vitro dissolution study

The profile of dissolution evaluation of all formulation batches in different media is shown in Fig.3 and Fig.4. The SD-6 showed improvement in dissolution performance

Table V: Solubility study of solid dispersion in pH 7.4 PB

S. No.	Formulation code	Solubility (µg mL^{-1})
1.	SD-1	13.4
2.	SD-2	14.8
3.	SD-3	15.69
4.	SD-4	15.43
5.	SD-5	18.67
6.	SD-6	30.84
7.	SD-7	23.31
8.	SD-8	28.07
9.	PM-1	3.31
10.	PM-2	4.07
11.	PM-3	6.31
12.	PM-4	6.12
13.	PM-5	8.78
14.	PM-6	12.76
15.	PM-7	10.89
16.	PM-8	11.47
17.	Pure Drug	2.11

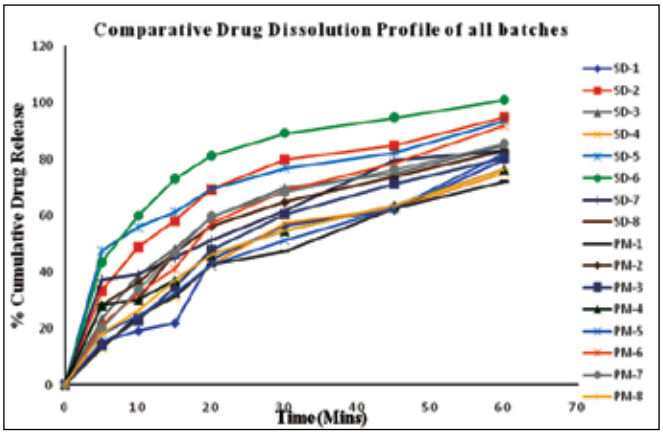


Fig. 4: Dissolution Profiles of all batches in pH 7.4 PB

in all media compared to ETO physical mixture. The dissolution rate of SD-6 was found to be 99.68±2.32% and 100.95± 3.5% in water and phosphate buffer 7.4, respectively, whereas the PM-1 batch showed a dissolution rate of 78.96 ±2.88 % and 72.15± 3.1% in water & phosphate buffer. The rationale behind the improvement in the dissolution of SD signifies that as the solubility increases, the dissolution also increases with an amorphous form compared to the physical mixing of drug and excipients. Based on the evaluation parameters

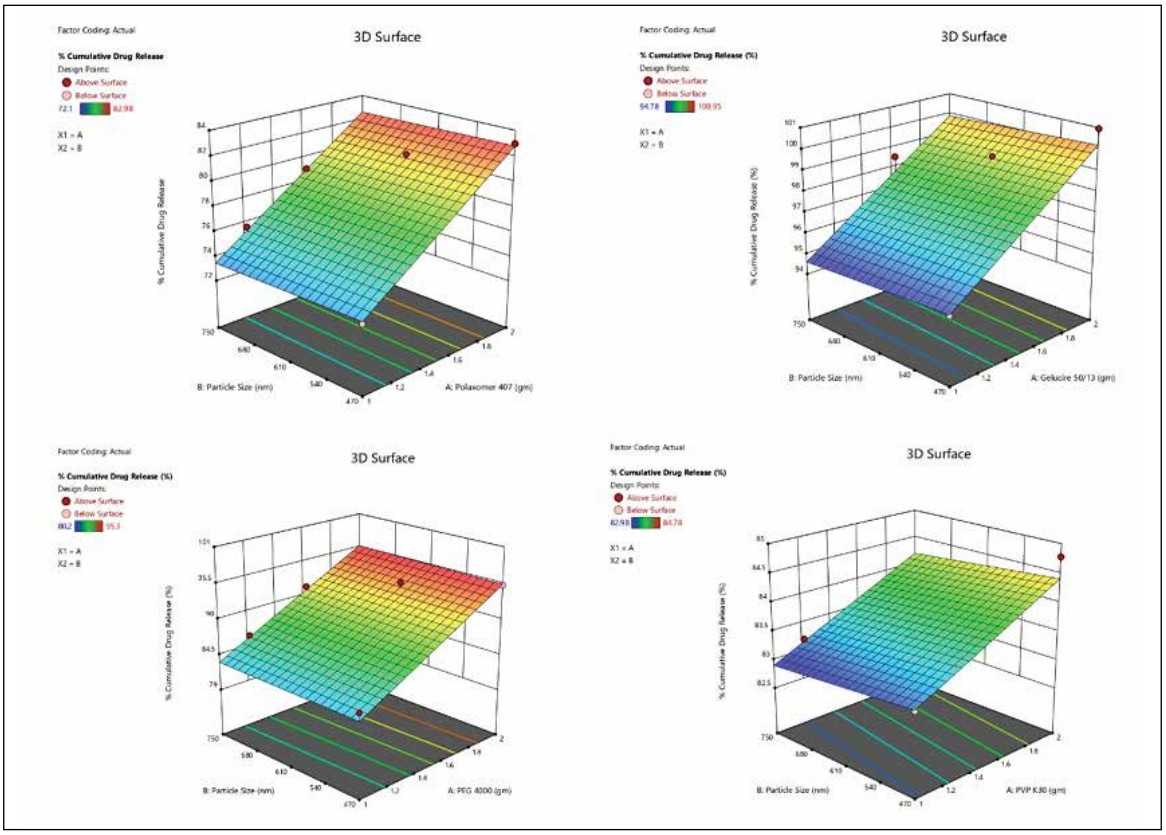


Fig. 5: 3D response surface plot showing the relationship between various levels of Pluronic® F-127, Gelucire®50/13, polyethylene glycol 4000 for synthesis, and PVP K30 concentration and particle size on cumulative drug release

of all the batches, the SD-6 batch (Drug: Gelucire®50/13, 1:2 ratio) was selected as an optimized batch, and further characterization was done.

Based on Design-Expert® software

Quadratic models were used to fit the generated data. The influence of each response's independent components was assessed us ing an analysis of variance (ANOVA). It was utilized to determine the model, its variables, and the statistically significant value. At 5% level of significance, the model and its terms were deemed necessary if the probability value (p-values) was less than 0.05. The associations between the independent and dependent variables were displayed using 2D counterplots and 3D response surface plots (Figs.5 - 8). Results of percentage cumulative drug release (%CDR) obtained from optimization batches are depicted in Table VI.

Table VI: Results of %CDR obtained from optimization batches

S. No.	Formulation (1:2)	% CDR
1.	Drug: Pluronic® F-127 SD	82.98
2.	Drug: Gelucire®50/13 SD	100.95
3.	Drug: Polyethylene glycol 4000 for synthesis SD	95.30
4.	Drug: PVP K30 SD	84.78
5.	Drug: Pluronic® F-127PM	72.98
6.	Drug: Gelucire®50/13PM	91.98
7.	Drug: Polyethylene glycol 4000 for synthesis PM	82.10
8.	Drug: PVP K30 PM	85.51

Optimization data for physical mixtures

The results of % CDR is shown in Table VI.

Following the ANOVA test's analysis and evaluation of all the data collected from each response, the desirability approach was used to create an optimized batch. The batch that contains Gelucire®50/13 was considered the optimized batch, as it demonstrates the excellent % CDR among all batches, whereas the batch containing Drug: Pluronic® F-127 PM shows the lowest, 72.98%, drug release. The predicted R^2 of 0.9728 signifies a good fit with the adjusted R^2 of 0.9854. Regarding ANOVA, the

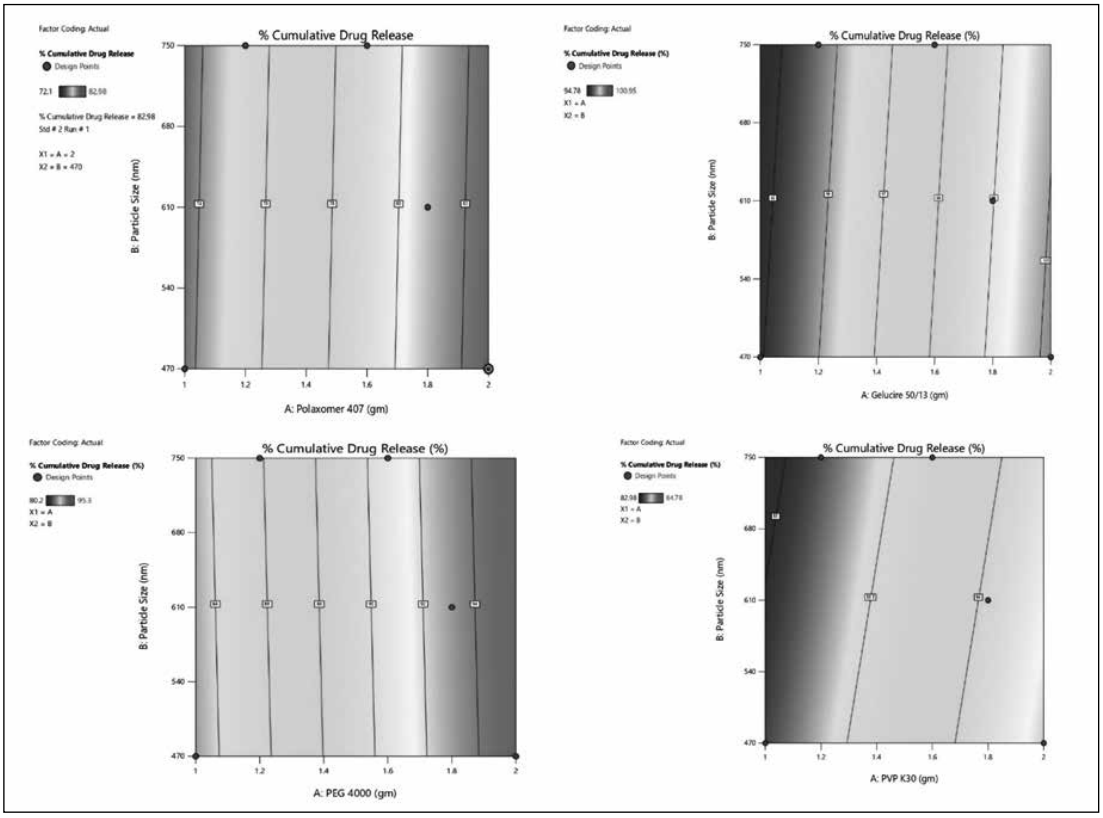


Fig. 6: Contour plot showing the influence of two factors on % CDR of solid dispersion using Polyethylene glycol 4000 for synthesis, Gelucire®50/13, PVPK-30, Pluronic® F-127

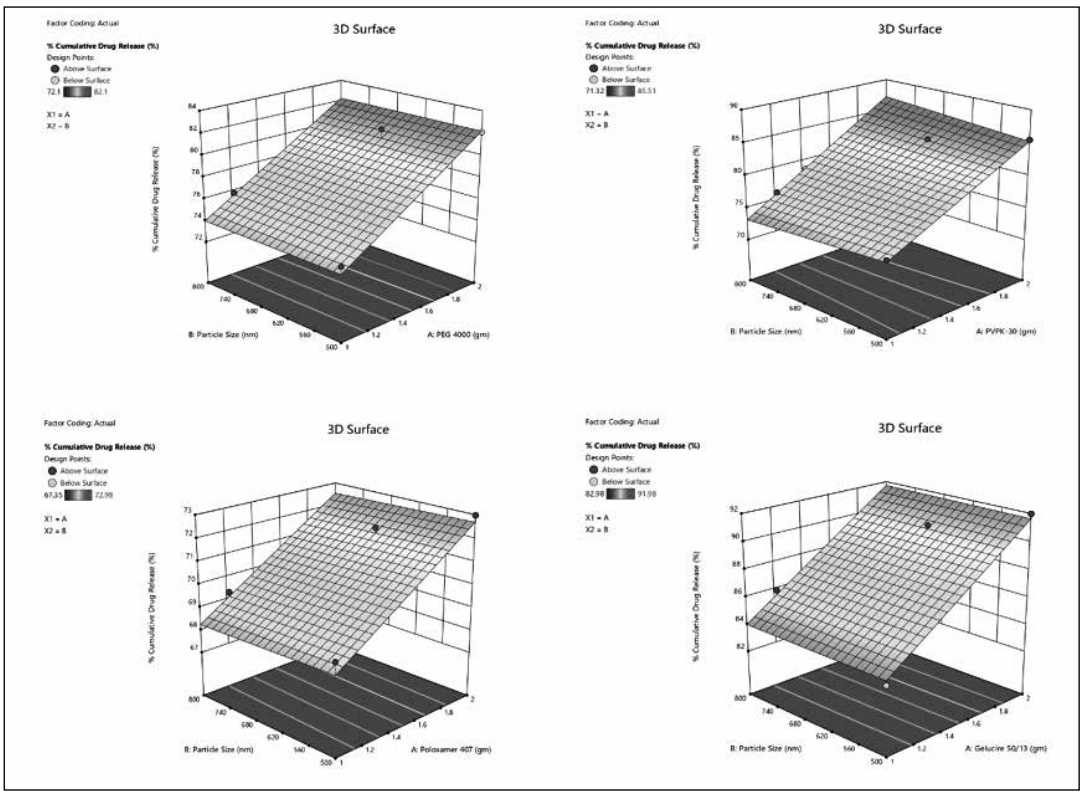


Fig. 7: 3D response surface plot showing the relationship between various levels of Pluronic® F-127, Gelucire®50/13, polyethylene glycol 4000 for synthesis, and PVP K30 for physical mixtures concentration and particle size on CDR

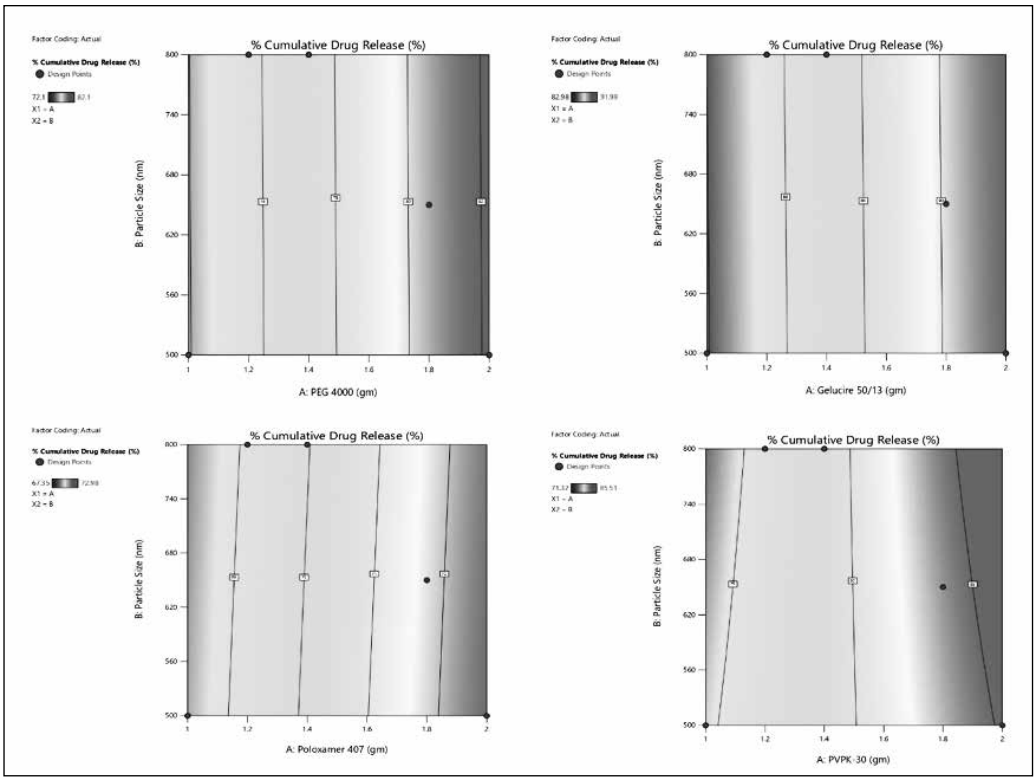


Fig. 8: Contour plot showing the influence of two factors on % CDR of physical mixtures using polyethylene glycol 4000 for synthesis, Gelucire®50/13, PVPK-30, Pluronic® F-127

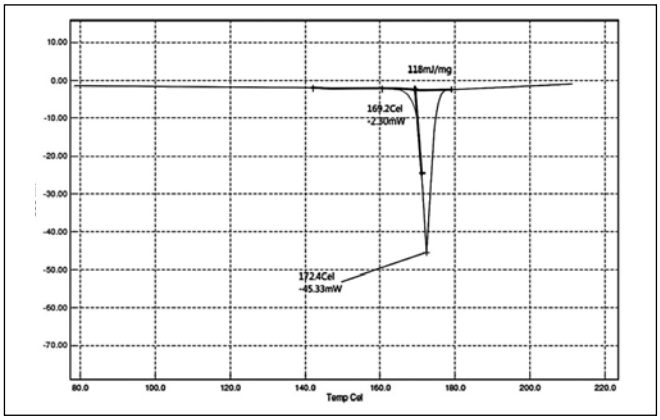


Fig. 9A

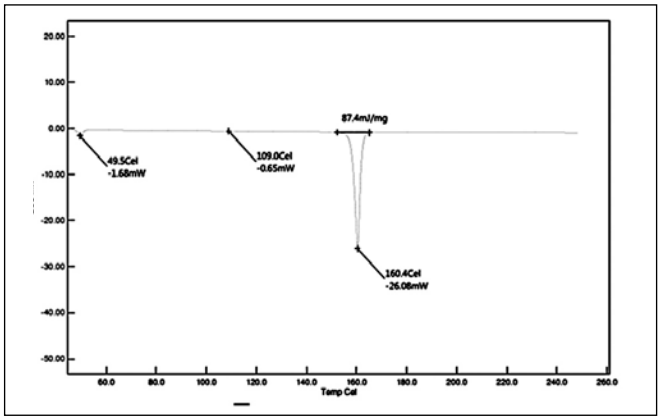


Fig. 9B

Fig.9: DSC thermogram of ETO (Fig. 9A) and ETO-Gelucire®50/13 (Fig. 9B)

Model F-value of 16.44 implies that terms with p-values < 0.05 are considered significant.

Characterization of prepared solid dispersion

Saturation solubility study

The solubility of SD-6 and ETO was found to be 19.76 and 2.28 mg mL^{-1}, respectively. The SD-6 showed better solubility as compared to ETO. It indicates enhancement as solid dispersions absorb more solvent due to amorphous form; these results increase in the surface area and maximizes, the solubility.

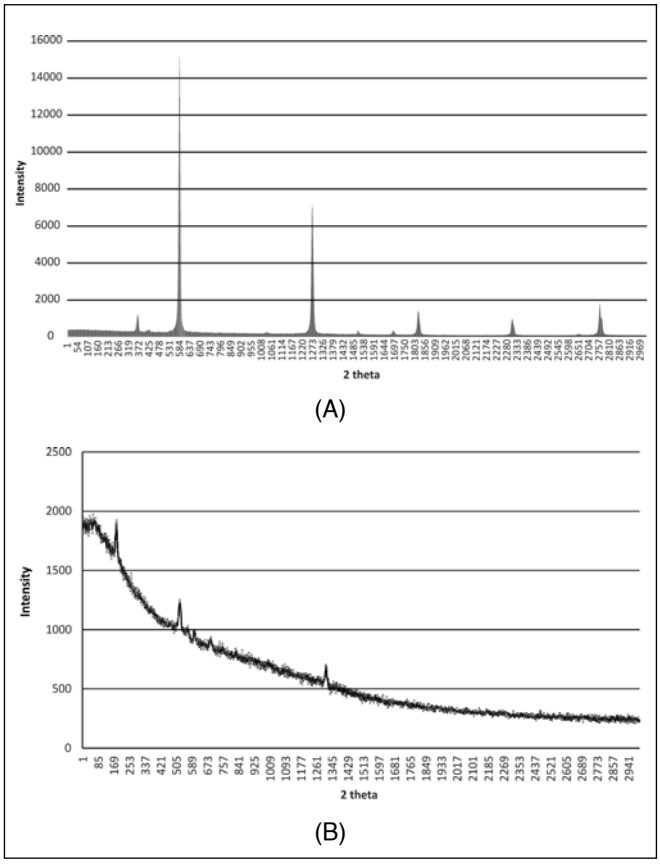
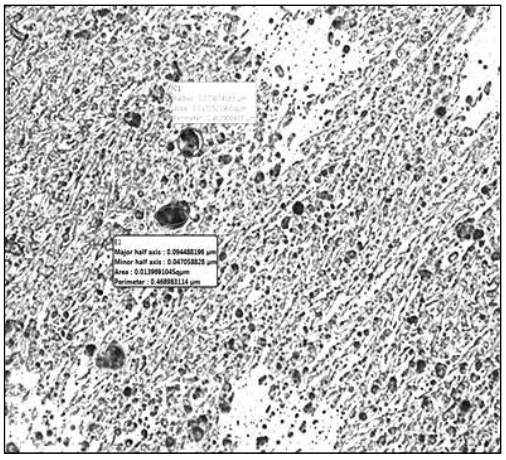
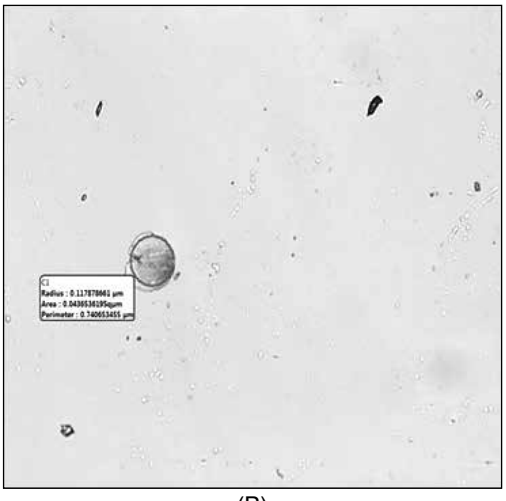


Fig.10: XRD analysis of ETO (Fig. 10A) and ETO and Gelucire®50/13 (Fig. 10B)



(A)



(B)

Fig. 11 (A,B): Particle size of SD-6

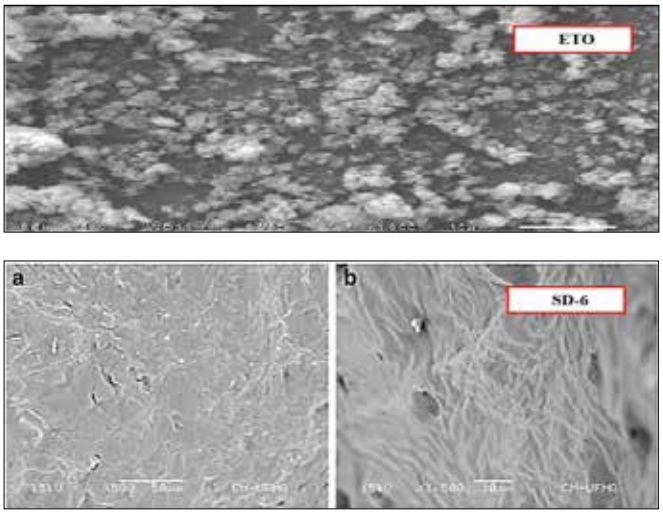


Fig. 12: SEM images of ETO and optimized solid dispersion (SD-6)

Differential scanning microscopy

The thermographic behaviour of etoposide was observed at a significant peak at 172.4 °C, which indicates the drug's crystalline nature, despite the SD-6 showing the peak at 160.4 °C. The changes in peak indicate that the crystalline nature of the drug is converted into an amorphous form due to SD formulation (Fig. 9).

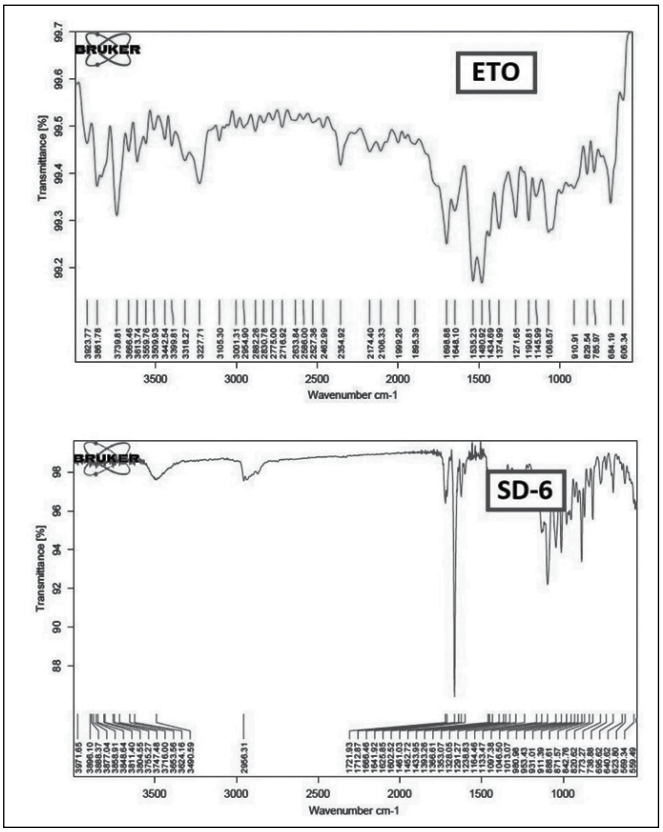
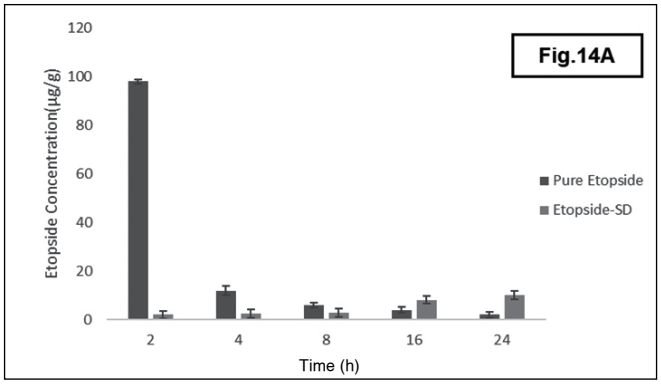
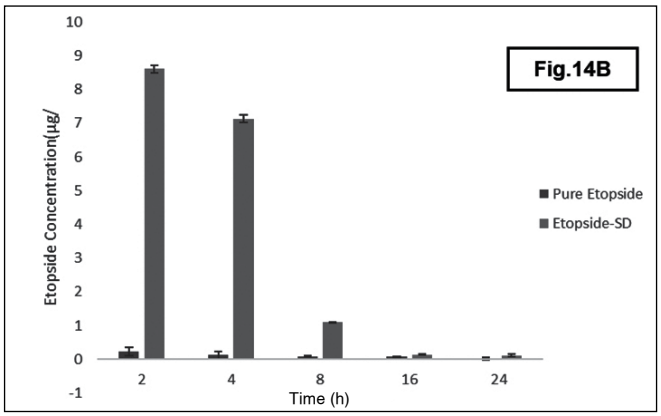


Fig. 13: FTIR spectra of ETO and SD-6





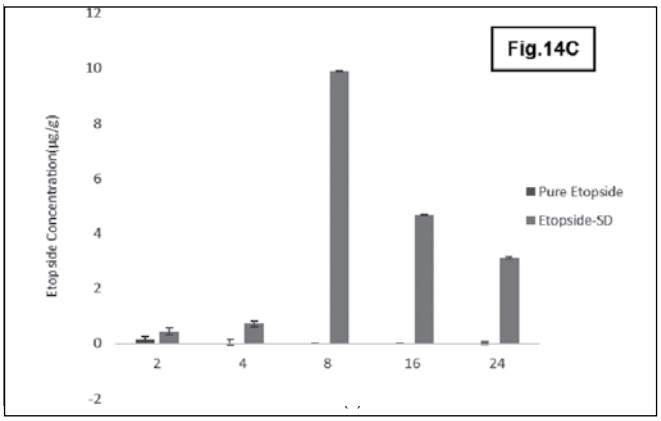


Fig.14: Concentration of ETO-SD in homogenates of (Fig. 14A) stomach tissue, (Fig. 14B) intestinal tissue and (Fig. 14C) cecum content

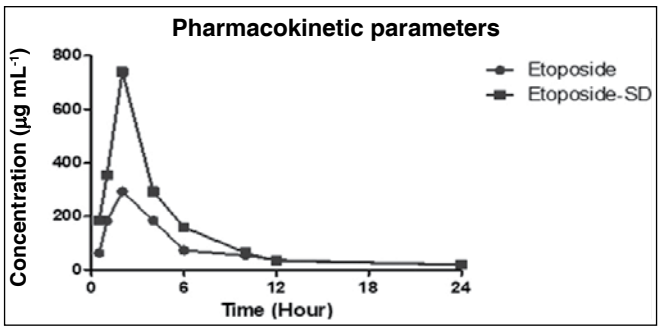


Fig. 15: Maximal observed concentration-time curve of ETO and E-SD

Table VII: Pharmacokinetic parameters for ETO and ETO solid dispersion in rats upon oral administration (dose=20 mg kg^{-1})

PK parameter	Unit	ETO	ETO solid dispersion
Cmax	ng mL^{-1}	293.25±1.80	741.17±12.29
Tmax	H	2±0.00	2±0.00
$t/_{1/2}$	h	9.28±0.28	4.77±0.19
Kel	h	0.07±0.002	0.14±0.005
AUC (0-24)	h.ng mL^{-1}	1728.88 ±129.43	3089.23 ±34.69
AUC (0-∞)	h.ng mL^{-1}	1979.32±159.79	3225.50 ±41.53
AUMC (0-∞)	h.ng mL^{-1}	20597.60±2605.80	19660.10±463.50
MRT (0-∞)	h.ng mL^{-1}	10.22±0.52	6.09±0.068
Cl/F	(mg kg^{-1})/(ngmL^{-1})h^{-1}	0.01±0.0008	0.006±0.0008
Vdapp	(mg kg^{-1})/(ng mL^{-1})	0.13±0.01	0.042±0.00142

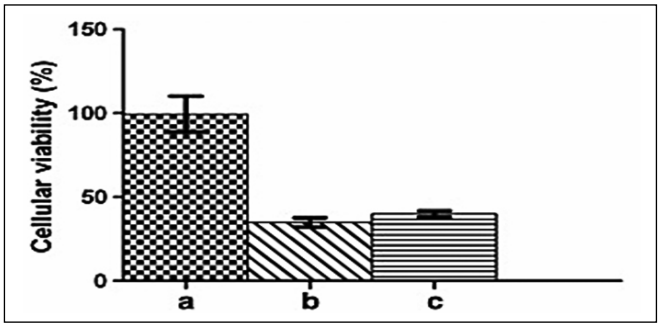


Fig. 16: HeLa cells viability treated with different samples: (a) culture medium, (b)solid drug dispersion (SD6), (c) the drug solution of 50 µg mL^{-1}

Powder X-ray diffraction

The XRD designs of ETO and SD-6 are depicted in Fig. 10. The ETO exhibited a significant peak. In contrast, the SD-6 showed a flat curve, and no major peak was found, which indicates the formation of an amorphous form of solid dispersion.

Particle size analysis

Particle size is important for quality and performance. It also evaluates the rate of substance dissolution. The particle size of SD-6 formulation was determined, using motic microscope and was found to be in the range of 470-800 nm (Fig. 11).

Scanning electron microscopy

The SEM was done to determine the external surface and drug structure. Etoposide's SEM image has a crystalline structure, whereas SD-6 does not show crystalline nature like ETO. It exhibits irregularly shaped particles with a smother surface (Fig. 12), and its solid state has changed to an amorphous form.

Fourier transform infrared spectroscopy

The FTIR spectra were studied to explain the API-excipients compatibility. FTIR spectra of ETO and SD-6 are presented in Fig. 13. ETO alone showed C-H stretching at 2956 cm^{-1}, secondary N-H at 3483 cm^{-1} and C-O absorption band at 1646 cm^{-1}, which remained the same in the case of SD-6. These bands showed diagnostic value to elucidate no drug-excipient interactions.

Gastrointestinal distribution studies

Etoposide tissues or organ concentration indicates specificity, effectiveness, and pharmacological activity. The GI distribution study indicated a high concentration of ETO in the colon for SD-6 in comparison to the etoposide in sodium carboxymethyl cellulose (Fig.14). The concentration was obtained at 8h, 16h and 24h after administration of the SD was 9.11, 5.39 and 4.23 µg mL^{-1} respectively. This concentrations was more than CTC 50 of etoposide needed for resistance of HT-29 cell lines, showing that the minimum ETO was discharged to the non-target site and restricted tumor progression. Etoposide usually crosses the intestinal membrane by passive transcellular absorption without the help of uptake transporters, but once inside the cell, they can serve as substrates for apical efflux transporters. Efflux proteins located at the apical membrane, such as P-glycoprotein (P-gp, MDR1), MRP2 and BCRP, can pump drugs from inside the enterocyte back into the intestinal milieu, hence limiting their absorption.

But in case of etoposide SD formulation, made up by using non-ionic surfactants, the efflux transporter (p-gp) activity is reversed and reaches maximum amount of concentration to intestinal site.

Pharmacokinetic study

The PK Solver 2.0 USA was used to determine pharmacokinetic parameters. The maximal Observed Concentration (Cmax), maximum Observed Time (Tmax), Half-life ($t^{1/2}$), Area Under the Curve (AUC), Area Under Moment Curve (AUMC), Rate of Elimination (Kel), Mean Residence Time (MRT), Mean Apparent Volume of Distribution (Vdapp), and Mean Apparent Clearance (CL/F), are depicted in Table VII. The results indicate a significant enhancement in Cmax and AUC of SD-6 at 2 h compared to ETO (Fig. 15). Based on the above results, it was concluded that the oral bioavailability of SD-6 was increased as compared to pure drug.

Cytotoxicity study

The antiproliferative effect was determined, as a cell growth percentage with the control of the different treatments. The results showed less cell viability and minimum cell growth in the case of SD-6 Fig.16(b) compared to culture media and drug solution Fig.16(a and c), which directly indicates the antiproliferative effect of SD-6.

CONCLUSION

In this research study, etoposide solid dispersions were successfully prepared using a physical mixture and kneading technique and then optimized using DOE software version-7 for the selection of a suitable batch. The physicochemical characteristics of the optimized SD were evaluated by using various parameters. The solubility, as well as dissolution rate of solid dispersion, were improved contrasted with the ETO as well as physical mixture. The amorphous nature of the solid dispersion was confirmed by XRD and DSC. Motic microscopy and SEM study showed the morphology of SD. The GI distribution study also described that colon-targeted delivery could be accomplished by E-SD. The pharmacokinetic study depicted that the E-SD efficiently increased drug absorption through oral administration. The concentration of etoposide in E-SD was achieved to arrest the tumor growth with an antiproliferative effect analyzed by cytotoxicity study.

Current advances in SD can be divided into four major fields a) creating new preparation and evaluation techniques, b) explaining the mechanism of thermodynamics for formulation, dissolution, and storage, c) adding new additives like pH modifiers, super disintegrants, as well as surfactants, and d) using new carriers.

All the findings showed that amorphous solid dispersion are a prominent approach as they can improve solubility, dissolution, particle porosity and bioavailability of ETO.

REFERENCES

1. Siegel R.L., Miller K.D., Fuchs H.E. and Jemal A.: Cancer statistics, **Cancer J. Clinicians**, 2022, 72(1), 7-33.
2. Chhikara B.S., and Parang K., Global Cancer Statistics 2022: the trends projection analysis, Available at https://pubs.thesciencein.org/journal/index.php/cbl/article/view/451. Access Date- 31 October 2022.
3. Ferlay J., Ervik M., Lam F., Colombet M. and Mery L.: Global cancer observatory: cancer today, **IARC**., 2018, 3(20), 17.
4. Saini A., Kumar M., Bhatt S., Saini V. and Malik A.: Cancer causes and treatments, **Int. J. Pharm. Sci.**, 2020, 11, 3109.
5. Blackadar C.B.: Historical review of the causes of cancer, **World J. Clin. Oncol.**, 2016, 7(1), 54.
6. Lipinski C.A., Lombardo F., Dominy B.W. and Feeney P.J.: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, **Adv. Drug Deliv. Rev**., 1997, 23(1-3), 3-25.
7. Dannenfelser R.M., He H., Joshi Y., Bateman S. and Serajuddin A.T.M.: Development of clinical dosage forms for a poorly water-soluble drug I: Application of polyethylene glycol–polysorbate 80 solid dispersion carrier system, **J. Pharm. Sci**., 2004, 93(5), 1165-1175.
8. Williams H. D., Trevaskis N.L. and Charman S.A.: Strategies to Address Low Drug Solubility in Discovery and Development, **Pharmacol Rev.,** 2013, 65(1) 315-499.
9. Jain P. and Yalkowsky S.H.: Solubilization of poorly soluble compounds using 2-pyrrolidone, **Int. J. Pharm.**, 2007, 342(1-2), 1-5.
10. Cherniakov I., Domb A.J. and Hoffman A.: Self-nano-emulsifying drug delivery systems: an update of the biopharmaceutical aspects, **Expert Opin. Drug Deliv.**, 2015, 12(7), 1121-1133.
11. Miller J. M., Dahan A., Gupta D., Varghese S. and Amidon G.L.: Quasi-equilibrium analysis of the ion-pair mediated membrane transport of low-permeability drugs, **J. Control. Release,** 2009, 137(1), 31-37.
12. Thakuria R., Delori A., Jones W., Lipert M.P., Roy L. and Rodríguez-Hornedo N.: Pharmaceutical cocrystals and poorly soluble drugs, **Int. J. Pharm.,** 2013, 453(1), 101-125.
13. Brewster M.E. and Loftsson T.: Cyclodextrins as pharmaceutical solubilizers, **Adv. Drug Deliv. Rev**., 2007, 59(7), 645-666.
14. Chen H., Khemtong C., Yang X., Chang X. and Gao J.: Nanonization strategies for poorly water-soluble drugs, **Drug Discov.**, 2011, 16(7-8), 354-360.
15. Newman A., Knipp G. and Zografi G.: Assessing the performance of amorphous solid dispersions. **J. Pharm. Sci**., 2012, 101(4), 1355-1377.
16. Vo C.L.N., Park C. and Lee B. J.: Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs, **Eur. J. Pharm. Biopharm**., 2013, 85(3), 799-813.

17. Kim Y.R., Lee B., Byun M.R., Lee J.K. and Choi J.W.: Evaluation of pemetrexed and etoposide as therapeutic regimens for human papillomavirus-positive oral and oropharyngeal cancer, Teh MT, ed. **PLoS One**. 2018, 13(7).
18. Wang Y., Wang S. and Xu Y.: Etoposide amorphous nanopowder for improved oral bioavailability: Formulation development, optimization, *in vitro* and *in vivo* evaluation, **Int. J. Nanomedicine**, 2020, 15, 7601-7613.
19. Witterland A.H.I., Koks C.H.W. and Beijnen J. H:. Etoposide phosphate, the water-soluble prodrug of etoposide. **Pharm. World Sci**., 1996, 18(5), 163-170.
20. Girek M., Kłosinski K. and Grobelski B.: Novel tetrahydroacridine derivatives with iodobenzoic moieties induce G0/G1 cell cycle arrest and apoptosis in A549 non-small lung cancer and HT-29 colorectal cancer cells, **Mol. Cell Biochem**., 2019, 460(1-2), 123-150.
21. Lavoie J.M. and Kollmannsberger C.K.: Current Management of Disseminated Germ Cell Tumors. **Urol. Clin. North Am**., 2019, 46(3), 377-388.
22. Martin B., Seguin J. and Annereau M.: Preparation of parenteral nanocrystal suspensions of etoposide from the excipient free dry state of the drug to enhance *in vivo* antitumoral properties. **Sci. Rep**., 2020, 10(1), 18059.
23. Beig A., Miller J.M. and Lindley D.: Head-to-head comparison of different solubility-enabling formulations of etoposide and their consequent solubility–permeability interplay. **J. Pharm. Sci**., 2015, 104(9), 2941-2947.
24. Sinha S., Ali M., Baboota S., Ahuja A., Kumar A. and Ali J.: Solid dispersion as an approach for bioavailability enhancement of poorly water-soluble drug ritonavir, **AAPS Pharm. Sci. Tech**., 2010, 11(2), 518-527.
25. Nikghalb L.A., Singh G., Singh G. and Kahkeshan K.F.: Solid dispersion: Methods and polymers to increase the solubility of poorly soluble drugs, **J. Appl. Biomed**., 2012, 2(10), 170-175.
26. Tran P., Pyo Y.C., Kim D.H., Lee S.E., Kim J.K. and Park J.S.: Overview of the manufacturing methods of solid dispersion technology for improving the solubility of poorly water-soluble drugs and application to anticancer drugs, **Pharmaceutics**., 2019, 11(3), 132.
27. Shah J.C., Chen J.R. and Chow D.: Preformulation study of etoposide: Identification of physicochemical characteristics responsible for the low and erratic oral bioavailability of etoposide. **Pharm. Res**., 1989, 6(5), 408-412.
28. Patel J.S., Setty S.K., Chakraborty M. and Kamath J.V.: Antihyperlipidemic activity of Medohar Vati intriton x-100 induced hyperlipidemic rats, **Int. J. Pharm. Res. Devel**., 2011, 4, 125-130.
29. Goodman L.S.: Goodman and Gilman's the pharmacological basis of therapeutics, McGraw-Hill, New York 1996, pp.1361-1373.
30. Zhang X., Sun N., Wu B., Lu Y., Guan T. and Wu W.: Physical characterization of lansoprazole/PVP solid dispersion prepared by fluid-bed coating technique, **Powder Technol**., 2008, 182(3), 480-485.
31. Elsayed M. M. A., Okda T. M., Atwa G. M. K., Omran G. A., Abd Elbaky A. E. and Ramadan A.E.H.: Design and optimization of orally administered luteolin nanoethosomes to enhance its anti-tumor activity against hepatocellular carcinoma. **Pharmaceutics**, 2021, 13(5), 648.
32. Raju Y.P., Garbhapu A., Prasanna S.A., Rao B.S. and Murthy K.R.: Studies on enhancement of dissolution rate of etoposide, **Indian J. Pharm. Sci**., 2007, 69(2), 269.
33. Ascenso A., Raposo S. and Batista C.: Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes, **Int. J. Nanomed.**, 2015, 10, 5837-5851.
34. Yadav V., Jadhav P., Dombe S., Bodhe A. and Salunkhe P.: Formulation and evaluation of microsponge gel for topical delivery of antifungal drug. **Int. J. App. Pharm.,** 2017, 9(4), 30. doi:10.22159/ijap.2017v9i4.17760
35. Saifulislam M.D., Mehjabeen K. and Faribakhan I.: Improvement of dissolution of ibuprofen and etoposide by polaxamer based solid dispersion, **Pharm. Sci**., 2010, 13(1), 54-59.
36. Permezel N.C. and Webling D.D.: The length and mucosal surface area of the small and large gut in young rats, **J. Anat**., 1971, 108, 295-296.
37. Chen H., Shi S., Zhao M., Zhang L., He H. and Tang X.: A lyophilized etoposide submicron emulsion with a high drug loading for intravenous injection: preparation, evaluation, and pharmacokinetics in rats, **Drug Dev. Ind. Pharm**., 2010, 36(12), 1444-1453.
38. Bairagi S., Ghule P., Jithan A., Aher A. and Gilhotra R.: Amorphous solid dispersion: a promising technique for improving oral bioavailability of poorly water-soluble drugs. **SA Pharm. J**., 2018, 85(1), 50-56.
39. Mohammed S.A., Eissa M.S. and Ahmed H.M.: Simple protein precipitation extraction technique followed by validated chromatographic method for linezolid analysis in real human plasma samples to study its pharmacokinetics, **J. Chromatogr. B**., 2017, 1043, 235-240.
40. Vahedi F., Fathi Najafi M. and Bozari K.: Evaluation of inhibitory effect and apoptosis induction of Zyzyphus Jujube on tumor cell lines, an *in vitro* preliminary study, **Cytotechnology**, 2008, 56(2), 105-111.
41. Ciapetti G., Cenni E., Pratelli L., and Pizzoferrato A.: *In vitro* evaluation of cell/biomaterial interaction by MTT assay, **Biomaterials,** 1993, 14(5), 359-364.