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# Exemestane loaded polymeric nanoparticles for oral delivery

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**ABSTRACT**

The aim of the present study was to develop Exemestane loaded polymeric nanoparticles for improved oral bioavailability of Exemestane. Exemestane loaded nanoparticles were prepared by solvent displacement method with Eudragit RL 100 and Eudragit L 100 as polymers and Pluronic® F-68 as surfactant. The influence of various formulation factors (drug: polymer ratio and concentration of surfactant) on particle size, size distribution, zeta potential, encapsulation efficiency, in vitro drug release were investigated. The mean particle size of optimized formulations F5 and F13 were found to be 98.19nm and 48.16nm respectively. Zeta potential of optimized formulations F5 and F13 were found to be +22mV and -25mV respectively. Fourier Transform Infrared Spectroscopy (FT-IR) study indicated that, there was no interaction between drug and polymers. Scanning electron microscopy (SEM) study revealed spherical morphology of the developed NPs. The results of the present investigation indicate that the formulations F5 and F13 can be considered as best among various formulations with respect to particle size, entrapment efficiency and in-vitro drug release. In conclusion, this study indicates the capability of Eudragit nanoparticles in enhancing the oral bioavailability of exemestane.

**Keywords:** *Polymeric nanoparticles; Exemestane; Eudragit® RL 100; Eudragit L 100; Pluronic® F-68.*

## INTRODUCTION

The most important goal of cancer chemotherapy is to minimize the exposure of normal tissues to drugs while maintaining their therapeutic concentration in tumors [1-3]. Polymeric nanoparticles are one of the many carrier systems used for passive targeting and sustained release of the drug [4]. In addition to the potential for enhancing drug bioavailability via particle uptake mechanisms, particulate oral delivery systems can protect labile macromolecules from stomach acid and from the first-pass metabolism in the gastrointestinal tract.

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Nanoparticulate oral delivery systems also exhibit slower transit times than larger sized particles in various dosage forms increasing the local concentration gradient across absorptive cells, thereby enhancing local and systemic delivery of both free and bound drugs across the gut [5].

Exemestane (EXE) is an irreversible steroidal aromatase inactivator, with promising anti-tumor activity in postmenopausal women with hormonal sensitive (estrogen-dependent) breast cancer [6-7]. EXE is a neutral compound with steroidal structure characterized by high lipophilicity. This drug is orally active and a potent inhibitor of peripheral aromatase activity [8]. Chemical structure of EXE is given in Figure 1. Exemestane is a BCS class IV drug with poor aqueous solubility and low permeability [9-12]. Following oral administration of radio labeled EXE, 42% of radioactivity was reported to be absorbed from the gastrointestinal tract due to low solubility and first pass effect. Preclinical data obtained in rats and dogs, in which EXE was given intravenously, indicated that the absolute bioavailability of EXE was about 5% [13].

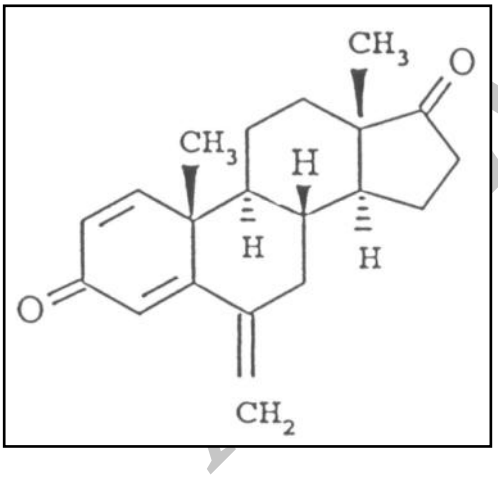


**Fig .1.** Chemical structure of Exemestane

In this study, EXE nanoparticles were prepared by nano-precipitation with non-biodegradable polymers (Eudragit® RL 100 and Eudragit® L 100). The nanoparticles were supposed to improve the oral bioavailability of exemestane by avoiding its first pass metabolism. Eudragit polymers (Eudragit® RL 100, Eudragit® L 100) are suitable inert carriers for oral drug delivery due to their capability to form nanodispersion with smaller particle size, good stability and biocompatibility. Exemestane nanoparticles were prepared by using solvent displacement method and evaluated for various physicochemical parameters.

EUDRAGIT® RL 100 is a copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups. The molar ratios of ethyl acrylate, methyl methacrylate and trimethylammonioethyl methacrylate in this polymer, is approximately 1:2:0.2. Eudragit RL 100 is insoluble at physiological pH and capable of limited swelling and thus appears to be a good polymeric carrier for the dispersion of drugs. The presence of quaternary ammonium group renders positive charge to the polymer by which it can interact with anionic drugs and GIT mucus [14-17]. Eudragit L 100 is an anionic copolymer based on methacrylic acid and methyl methacrylate. The ratio of the free carboxyl groups to the ester groups is approx. 1:1. Eudragit L100 shows pH-dependent solubility and is therefore specifically soluble in the region of the digestive tract where juices are neutral to weakly alkaline.

## EXPERIMENTAL

### *Materials and methods*

Exemestane was a kind gift from Celon labs, Hyderabad, India. Eudragit® RL 100 was purchased from Cipla Pharmaceuticals, Mumbai, India. Pluronic® F-68 was purchased from Sigma Aldrich Limited, Bangalore, India. Dialysis membrane was purchased from HI media, Hyderabad. All other reagents and chemicals used in this study were of HPLC grade.

### *Formulation of nanoparticles*

Nanoparticles of Exemestane were prepared with Eudragit RL 100 and Eudragit L 100 by solvent displacement technique. Exemestane 10mg and specific amount of Eudragit RL 100 or Eudragit L 100 were dissolved by sonication in 10 ml of methanol. The organic solution was added drop wise to the 20ml of aqueous solution containing pluronic F 68 under moderate magnetic

stirring. Finally, the organic solvent was evaporated under reduced pressure at 40°C using rotary evaporator. The process variables involved in NPs preparation is presented in Tables 1 and 2.

**Table 1.** Formulation variables used in the preparation of Eudragit RL 100 nanoparticles

| Formulation code | Drug : Eudragit RL 100 | Pluronic F68 (% w/v) |
|---|---|---|
| F1 | 1:2 | 1 |
| F2 | 1:4 | 1 |
| F3 | 1:6 | 1 |
| F4 | 1:8 | 1 |
| F5 | 1:10 | 1 |
| F6 | 1:12 | 1 |
| F7 | 1:10 | 0.25 |
| F8 | 1:10 | 0.5 |
| F9 | 1:10 | 0.75 |

**Table 2.** Formulation variables used in the preparation of Eudragit L 100 nanoparticles

| Formulation code | Drug : Eudragit L100 | Pluronic F68 (%W/V) |
|---|---|---|
| F10 | 1:8 | 1 |
| F11 | 1:10 | 1 |
| F12 | 1:15 | 1 |
| F13 | 1:20 | 1 |
| F14 | 1:20 | 0.25 |
| F15 | 1:20 | 0.5 |
| F16 | 1:20 | 0.75 |

***Evaluation of Nanoparticles***

- **Particle size and zeta potential analysis**

Particle size analysis of nanoparticle formulations was performed by photon correlation spectroscopy (PCS). This technique yields the mean particle diameter and particle size distribution. Samples were analysed using Malvern nano-(zs) zetasizer Ver. 6.20.

- **Estimation of drug entrapment efficiency**

The entrapment efficiency of the formulation was determined by measuring the concentration of free drug in the dispersion medium using centrifugation technique. The amount of free drug was determined by taking one ml of formulation and dissolved in a minimum quantity of buffer. This solution was centrifuged at 14,000 rpm for 90 minutes. One ml of supernatant was taken and the volume was adjusted to 10 ml with pH 7.4 phosphate buffer. The solution was analyzed spectrophotometrically at 249nm. Percentage drug entrapment was determined by the following formula:

$$DEE = \frac{\text{Amount of drug found in nanoparticles}}{\text{Total amount of drug used}} \times 100$$

- **In-Vitro Drug Release Studies**

The in-vitro drug release of the nanoparticle suspension was studied by using dialysis method. The formulation equivalent to 5 mg of Exemestane was placed in a dialysis bag. The dialysis bag was suspended in a beaker containing 100 ml of pH 7.4 phosphate buffer solution on a magnetic stirrer. At selected time intervals, samples were withdrawn and replaced with fresh medium. The samples were analysed for drug release by measuring absorbance at 249nm using UV- Visible spectrophotometer.

- **FTIR Studies**

The Fourier transform infrared analysis was conducted to verify the possibility of chemical interactions between drug and polymer. Samples of pure EXE and optimized formulations (F5 and F13) were scanned in the IR range from 400–4000 $cm^{-1}$

with carbon black as reference. The detector was purged carefully by clean dry helium gas to increase the signal level and reduce moisture.

- **Surface morphology**

The morphology of nanoparticles was examined using scanning electron microscopy (HITACHI-SU3500). A concentrated aqueous suspension was spread over a slab and dried. The sample was coated with gold for 2min. The coated sample was observed from the cryo chamber of microscope and viewed at different working distances. Photographs were taken by an image processing program.

- **Stability studies**

Following ICH guidelines, optimized nanoparticle formulations( F5 and F13) containing exemestane were subjected to stability studies at 25°C±2°C/60±5% RH and 40°C±2°C/ 75±5% RH for 3 months. The samples were withdrawn after three months and drug content was analyzed spectrophotometrically at 249nm.

## RESULTS AND DISCUSSION

In this study, we used solvent displacement method to prepare EXE loaded polymeric nanoparticles with different ratios of polymers and surfactant.

***Particle size analysis***

The mean diameter of EXE nanoparticles was determined by Particle size analyzer (Malvern nano-(zs) zetasizer Ver. 6.20) at temperature 25°C. The mean particle size of the optimized Eudragit RL 100 nanoparticle formulations (F1-F9) was found to be in the range of 98 to 120 nm. The mean particle size of the Eudragit L 100 nanoparticle formulations (F10-F16) was found to be in the range of 48.16 to 55.19 nm. Increase in Eudragit RL 100 concentration from 0.2 to 1%w/v resulted in gradual increase in particle size (98 to 120nm) was observed. Similarly increase in eudragit L 100 concentration from 0.8 to 2%w/v, increase in particle size (48.16 to 55.19nm) was observed. The lower polymer concentration support internalization of the polymer-solvent phase because of efficient distribution. Increased polymer concentration might have hindered the distribution and subsequent entrapment resulting in increased particle sizes. Particle size distribution of optimized formulations (F5 and F13) is shown in Figures 2 and 3.

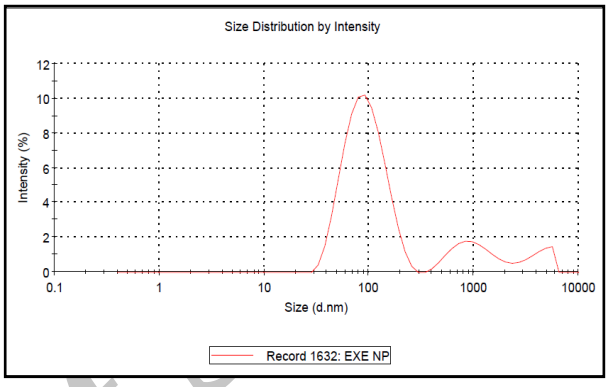


**Fig. 2.** Particle size of optimized formulation F5

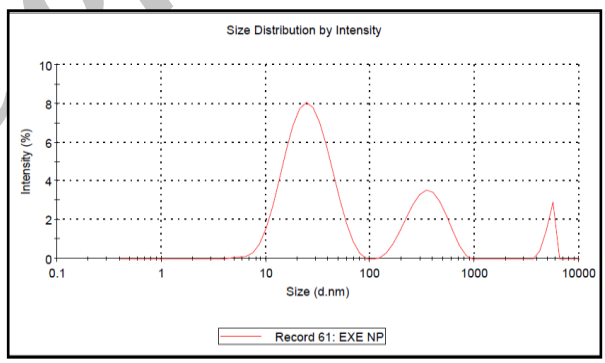


**Fig. 3.** Particle size of optimized formulation F13

All EXE loaded Eudragit RL 100 formulations showed a positive zeta potential value in the range of +22 to +34 mV. This positive charge is due to the presence of the quaternary ammonium groups on Eudragit RL 100. EXE loaded Eudragit L 100 formulations showed a negative zeta potential value in the range of -15 to -29 mV. Zetapotential of optimized formulations (F5 and F13) are shown in Figures 4 and 5.

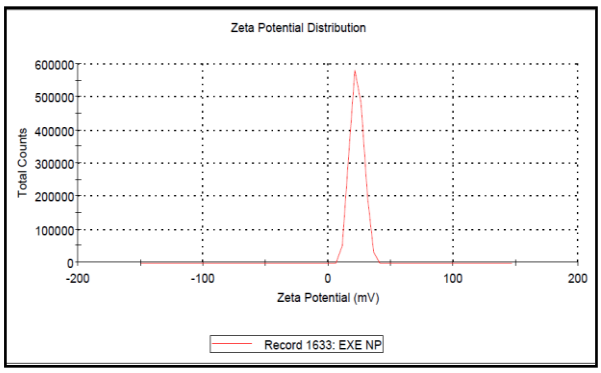


**Fig. 4.** Zeta potential of optimized formulation F5

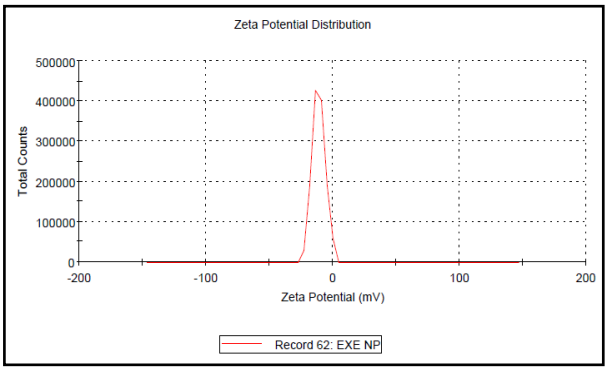


**Fig. 5.** Zeta potential of optimized formulation F13

### *Drug entrapment efficiency (DEE)*

The entrapment efficiency of the Exemestane loaded Eudragit RL 100 nanoparticles was found to be maximum in formulation F5 with 80.42%. The entrapment efficiency of the Exemestane loaded Eudragit L 100 nanoparticles was found to be maximum in formulation F13 with 75.63%.

- **Effect of Polymer Proportion on DEE**

The entrapment efficiency was affected by drug: polymer ratio. Increase in eudragit RL 100 concentration from 0.2 to 1%w/v (drug: polymer 1:2 to 1:10) led to increased DEE from 34.68% to 80.42%. The entrapment efficiency of eudragit L 100 nanoparticles increased from 67.78% to 75.63% as the polymer concentration increased from 0.8 to 2%w/v (drug: polymer 1:8 to 1:20). As the polymer concentration in organic phase increases, it results in significantly higher drug entrapment efficiency due to increase in organic phase viscosity, increased the diffusional resistance to drug molecules from organic phase to aqueous phase and higher drug entrapment. The results are shown in Figures 6 and 7.

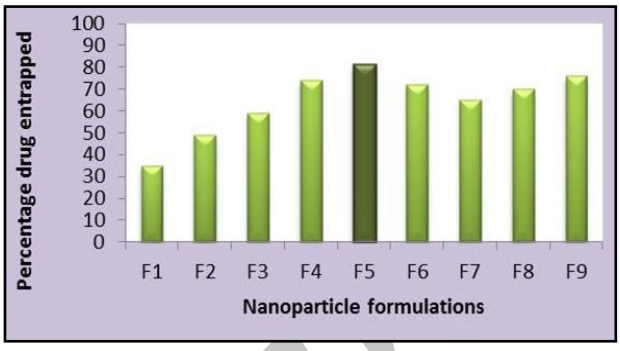


**Fig. 6.** Drug entrapment efficiency of Eudragit RL 100 nanoparticles

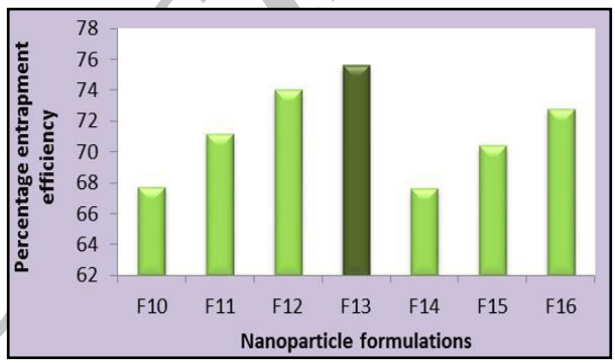


**Fig.7.** Drug entrapment efficiency of Eudragit L 100 nanoparticle

- **Effect of Stabilizer Proportion**

Upon increasing the proportion of pluronic® F-68 from 0.25% w/v to 1.0% w/v DEE increased from 65.14% to 80.42% for eudragit RL 100 nanoparticles and for eudragit L 100 nanoparticles EE increased from 67.78 to 72.81%. The increased EE can be attributed to the formation of stable emulsion and formation of uniform dispersion which increased drug encapsulation efficiency and prevented drug loss.

### *In-Vitro Drug Release Studies*

The formulations F5 and F13 were optimized based on the drug release studies. The formulation F5 showed 82.41% drug release and formulation F13 showed 88.02% drug release at the end of 24 hrs. The release curve suggests initial fast release. This may be due to the unentrapped drug being adsorbed on the surface of the nanoparticles.

The release rate was related to polymer and surfactant concentration. It was observed that the drug release was increased with an increasing amount of polymer as shown in Figures 8 and 9.

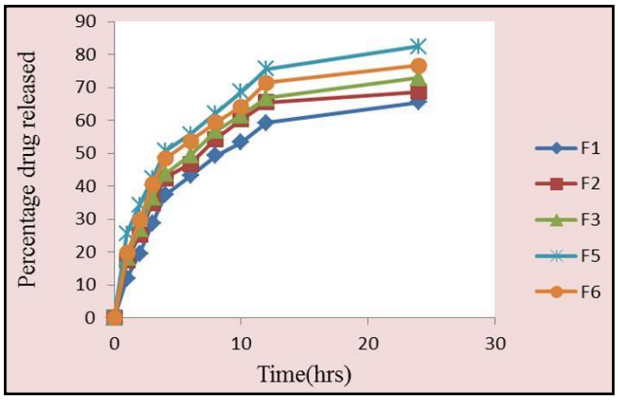


**Fig. 8.** Percentage drug release Vs time graphs EXE NP (F1-F6)

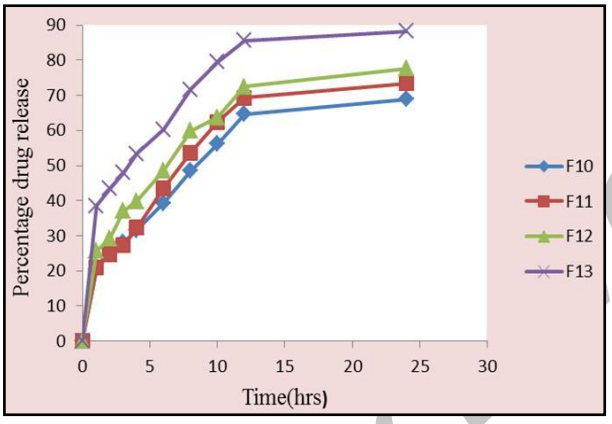


**Fig. 9.** Percentage drug release Vs time graphs EXE NP (F10-F13)

The concentration of surfactant also affects drug release from NPs. It is evident that the formulations with higher concentration of Pluronic F 68 (1%w/v) resulted in faster drug release than the formulations with lower concentration of Pluronic F 68 (0.25%w/v) as shown in Figures 10 and 11. This could be due to the fact that increased Pluronic F 68 resulted in decreased average particle size, which increased the effective surface area exposed to the drug release media, resulting in increased drug release.

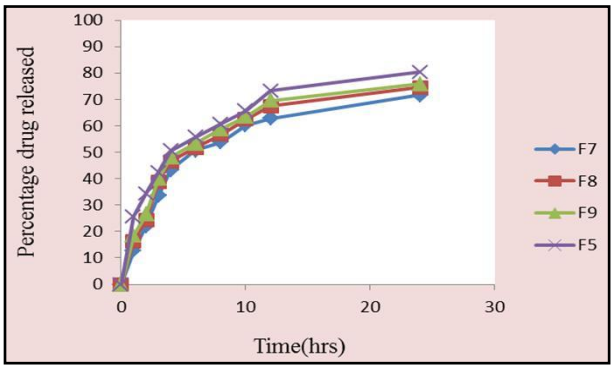


**Fig. 10.** Percentage drug release Vs time graphs EXE NP (F7-F9)

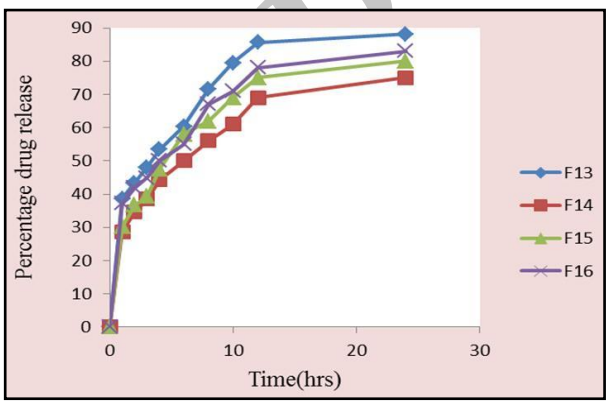


**Fig. 11.** Percentage drug release Vs time graphs EXE NP (F13-F16)

### *FTIR Studies*

Pure Exemestane has characteristic IR peaks at 1732 $cm^{-1}$ (CO stretch), 3076$cm^{-1}$ (=CH2), 2943$cm^{-1}$ (CH), 1654 $cm^{-1}$ (C=C). The characteristic peaks of the optimized formulations followed the same trajectory as that of the drug alone with minor differences as shown in Figures 12, 13 & 14.

### *Surface morphology*

SEM photographs of Exemestane nanoparticles containing F5 and F13 formulation showed the smooth surfaced nanoparticles with spherical shape as shown in Figures 15 and 16.

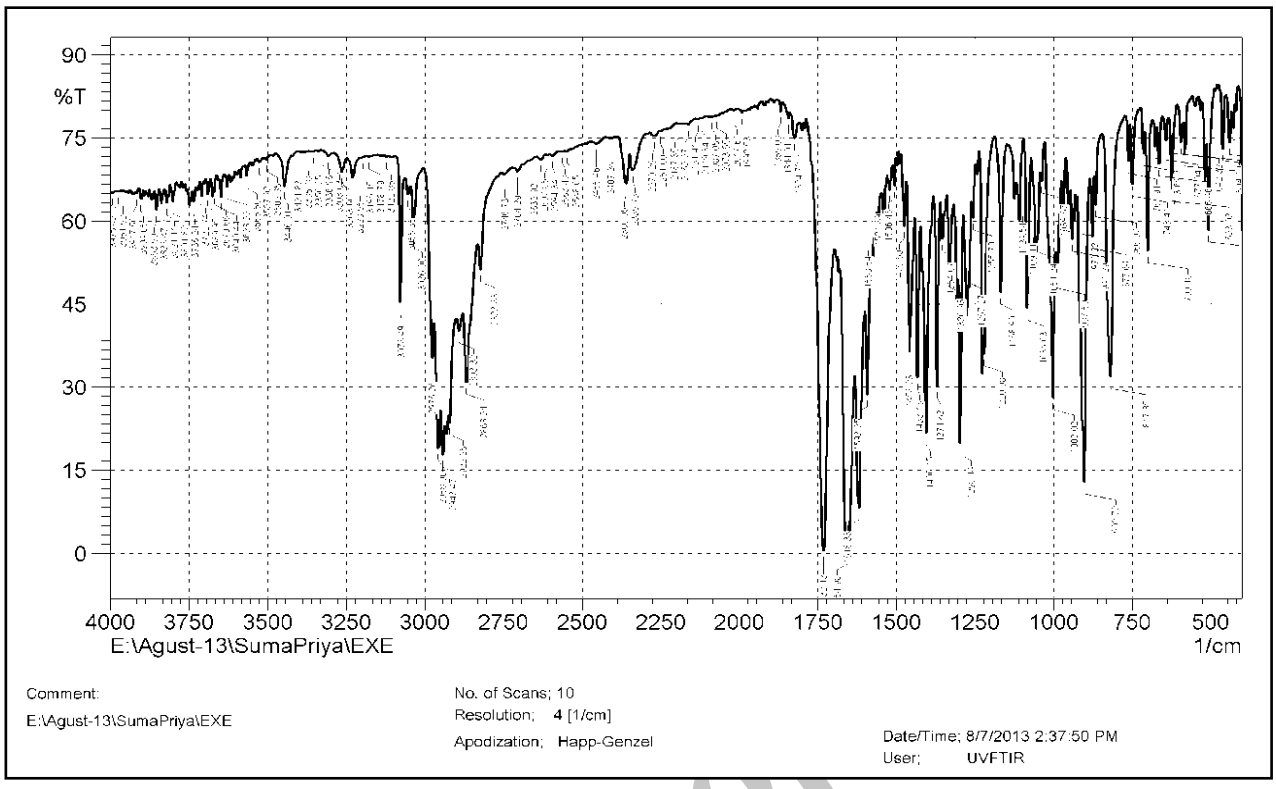


**Fig. 12.** FTIR spectra of Exemestane

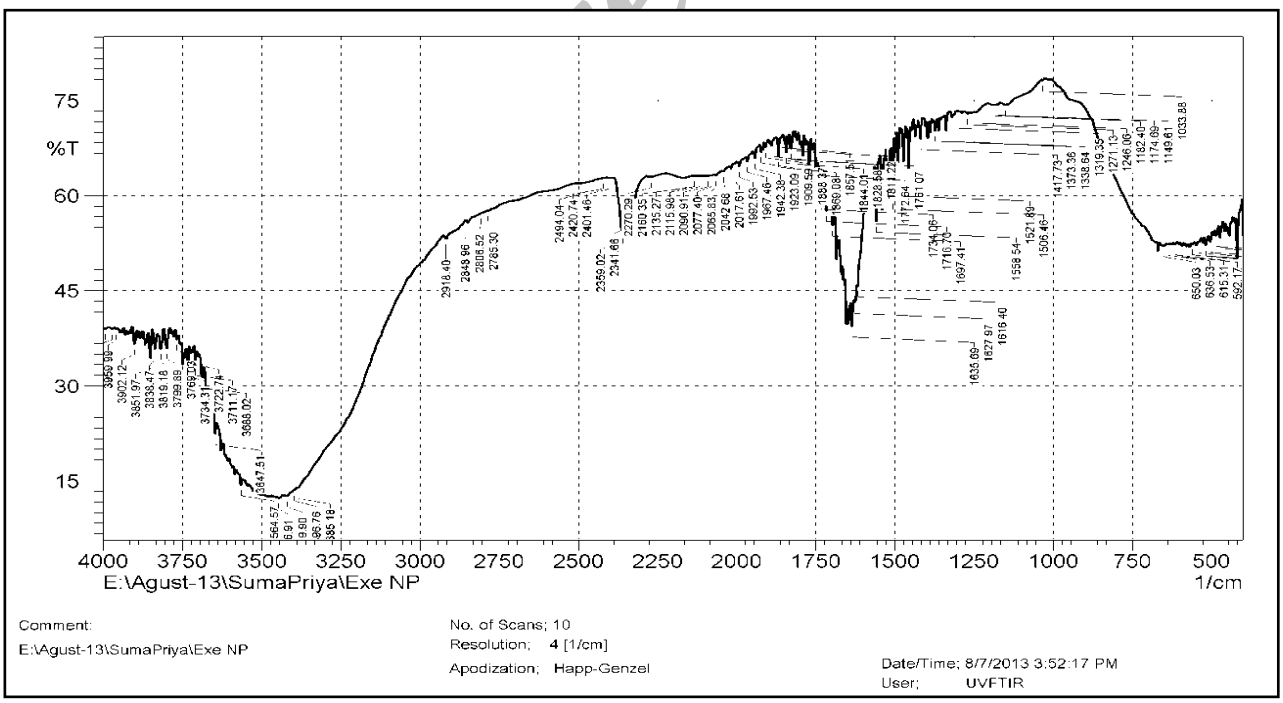


**Fig. 13.** FTIR spectra of formulation F5

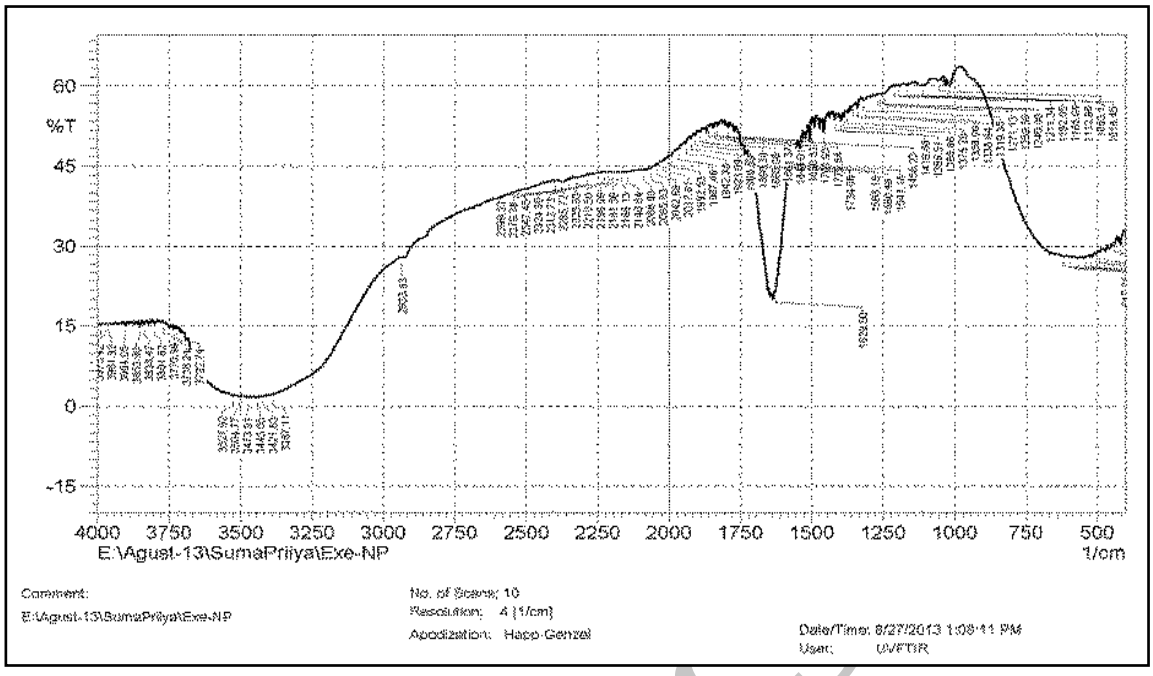


**Fig. 14.** FTIR spectra of formulation F13

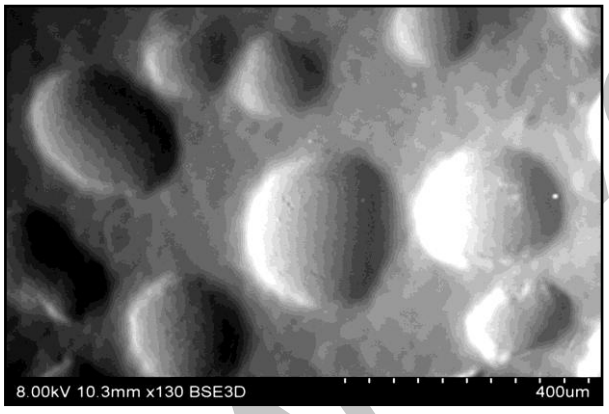


**Fig. 15.** SEM micrographs of Eudragit RL 100 Nanoparticle

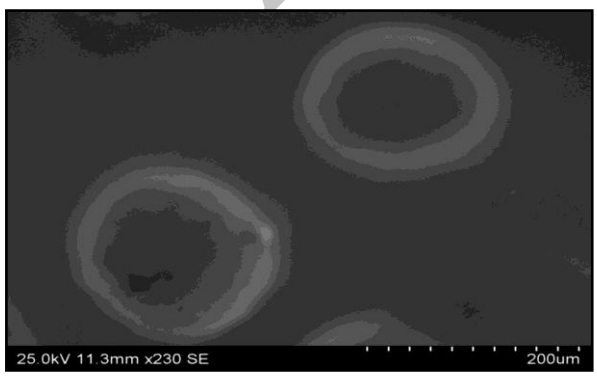


**Fig. 16.** SEM micrographs of Eudragit L 100 Nanopartice

***Stability studies***

The stability studies of EXE NPs were performed at 25°C±2°C/60±5% RH and 40°C±2°C/ 75±5% RH for 3months. The formulations were examined visually for precipitation. The drug content was also determined at the end of every month for 3 months. It was observed that there was no change in the physical appearance of the formulation. The drug content was analysed and there was marginal difference between the formulations kept at different temperatures as shown in Tables 3 and 4. Nanoparticle formulations retain good stability throughout the study.

**Table 3.** Stability studies of formulation F5

| Formulation stability temperature | Physical appearance | Assay | | | |
|---|---|---|---|---|---|
| | | Initial | 1 month | 2 months | 3 months |
| **25°C±2°C/ 60±5% RH** | Clear solution | 98.23% | 98.12% | 97.82% | 97.51% |
| **40°C±2°C/ 75±5% RH** | Clear solution | 98.23% | 97.25% | 96.5% | 95.04% |

**Table 4.** Stability studies of formulation F13

| Formulation stability temperature | Physical appearance | Assay | | | |
|---|---|---|---|---|---|
| | | Initial | 1 month | 2 months | 3 months |
| **25°C±2°C/ 60±5% RH** | Clear solution | 97.42% | 97.25% | 97.08% | 96.84% |
| **40°C±2°C/ 75±5% RH** | Clear solution | 97.42% | 96.5% | 96.18% | 95.63% |

## CONCLUSIONS

Exemestane loaded nanoparticles were successfully prepared by the nano precipitation technique. The results of the present investigation conclude that the formulation F5 and F13 were considered as best among various formulations with respect to particle size, entrapment efficiency and in-vitro drug release. Exemestane loaded nanoparticles can be a viable approach if scaled up for the treatment of breast cancer. However, further studies need to be conducted for establishing the same.

## ACKNOWLEDGMENTS

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