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Formulation, Evaluation and Comparison of Exemestane Nanoparticles Prepared by Nanoprecipitation Method and Spontaneous Emulsification Solvent Diffusion Method

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Abstract: The main objective of this study was to develop polymeric nanoparticles of Exemestane, for breast cancer. To achieve this goal Exemestane loaded poly (ε-caprolactone) (PCL) nanoparticles were prepared by Nanoprecipitation and Spontaneous emulsification solvent diffusion method. Nanoparticles prepared by both methods were characterized in terms of particle size, surface morphology, scanning electron microscope (SEM) and Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and Differential scanning calirometry (DSC). Exemestane loading was analyzed by high performance liquid chromatography. *In vitro* drug release studies were performed in phosphate buffer saline (PBS, pH 7.4 at 37° C). The cytotoxicity was evaluated using MCF7 breast cancer cell line and Vero cell line using MTT assays. FTIR and DSC studies demonstrated no interaction between drug and polymer. Among the above two method nanoparticles prepared by Nanoprecipitation method resulted more effective than prepared by Spontaneous emulsification solvent diffusion method to achieve small size (37.84 nm), uniform distribution, more recovery, high encapsulation efficiency(63.86%), higher percentage of drug release with time and more % Cell growth inhibition.
Keywords: Exemestane, Nanoparticles, Nanoprecipitation, Spontaneous Emulsification Solvent Diffusion.

Introduction

Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012. This represents about 12% of all new cancer cases and 25% of all cancers in women[1]. Exemestane is a drug used to treat breast cancer. It is a member of the class of drugs known as aromatase inhibitors. Some breast cancers require estrogen to grow. Those cancers have estrogen receptors (ERs), and are called ER-positive. They may also be called estrogen-responsive, or hormone-receptor-positive. Aromatase is an enzyme that synthesizes estrogen. Aromatase inhibitors block the synthesis of estrogen. This lowers the estrogen level, and slows the growth of cancers[2]. The existing anticancer agents do not greatly differentiate between the cancerous and normal cells, leading to systemic toxicity and adverse effects. This greatly limits the maximum permissible dose of the drug. Drug permeation into the cancer cells from the conventional formulation is very poor due to less distribution and quick elimination. The extensive distribution and rapid elimination from targeted organs result in a greater requirement of the drug by the tissue, which causes undesirable toxicity as well as being economically unsound[3]. Nanoparticles have advantages of targeting cancer by simply being accumulated and entrapped in tumours (passive targeting). The phenomenon is called as enhanced permeation and retention effect, caused by leaky angiogenetic vessels and poor lymphatic drainage[4].

Delivering the drugs through the nanoparticles makes it possible to achieve the desired concentration of drug in the specific site, thus minimizing the side effects and reducing the toxicity, dose dumping, longer circulating half–lives and improved pharmacokinetics etc.

In the present study, we have entrapped Exemestane in poly (Ɛ-caprolactone; PCL) nanoparticles. PCL is biodegradable, biocompatible and water insoluble polymer suitable for controlled drug delivery due to a high permeability to many drugs and at the same time being free from toxicity[5]. PCL is much more resistant to chemical hydrolysis and is achiral, a feature that limits the possibility of property modulation through the configurational structure of polymer chains. It is a highly hydrophobic crystalline polymer that degrades very slowly in vitro in the absence of enzymes and in vivo as well[6].

Several manufacturing techniques, including salting-out, emulsion evaporation, emulsification diffusion, and solvent displacement (Nanoprecipitation) are used to produce biodegradable nanoparticles from preformed, well-defined polymers[7]. We have prepared PCL NPs by two methods Nanoprecipitation and spontaneous emulsification solvent diffusion (SESD) method. The solvent displacement method is a convenient, reproducible, fast, and economic one-step manufacturing process for the preparation of monodisperse, polymeric nanoparticles in a size range of approximately 50–300nm[8]. This technique requires the use of amphiphilic organic solvents that are completely miscible with water, for example acetone. Progressive addition of polymer dissolved in acetone to an aqueous phase under stirring leads to the formation of colloidal particles. The molecular mechanism of instantaneous particle formation involves complex interfacial hydrodynamic phenomena and has been explained by interfacial turbulences between two liquid phases which are governed by the Marangoni effect[9,10,11].

Another method used is the spontaneous emulsification solvent diffusion (SESD) method in which nanoparticles can be effectively obtained by pouring the polymeric organic solution into an aqueous phase with moderate mechanical stirring. The technical characteristic of this method is the use of a binary mixture of a water-miscible organic solvent such as acetone and a water immiscible solvent such as dichloromethane (DCM) as the solvent of the polymeric solution and the particles are formed via an emulsification process and a subsequent solvent-evaporation process[12]. The prepared NPs were characterized for Drug Incorporation Efficiency, particle size, zeta potential, External morphological study, in vitro drug release Studies and % cell inhibition. Nanoparticles prepared by both the methods were compared and best method for EXE PCL nanoparticle was selected.

Materials and Methods

Materials

EXE was obtained as a gift sample from Sun Pharma Advanced Research Centre, Vadodara, India. PCL with a number-average molecular weight of 14 800 Da were purchased from Signet Chemicals, Mumbai. Poloxamer 188 nonionic surfactant composed of poly(ethylene oxide)/ poly(propylene oxide)/ poly(ethylene oxide) tri-block copolymer, was kindly supplied by the cadila pharmaceuticals, Ahemdabad. MTT Dye with molecular wt 414.3 Da was purchased from MP Biomedicals. Dulbecco's Modified Eagle's Medium (DMEM) was purchased from US Biological. MCF7 breast cancer cell line and Vero cell line were purchased from National centre for cell science (NCCS) Pune. All other chemicals and solvents were of analytical grade and obtained commercially. Deionized distilled water was exclusively used for the preparation of aqueous solutions.

Preparation of nanoparticles

We have prepared PCL NPs by two methods Nanoprecipitation and spontaneous emulsification solvent diffusion (SESD) method.

a) Nanoprecipitation method (Method 1)

EXE (10% w/w) was dissolved in 100 ml of the acetone by mild heating containing 625 mg of PCL. The organic phase solution was slowly poured into 200 ml of aqueous solution containing poloxamer 188 (1:1 ratio with polymer) with moderate stirring. Stirring was continued for 3–4 h to allow complete evaporation of organic solvent. The suspension is passing through wattman filter paper. Sterilization is done by membrane filtration. Formulation was filtered through 0.45µm membrane filter (Millipore, India) in the aseptic area. The

resulting suspension of nanoparticles was centrifuged at 12 600 rpm for 1 h in Remi RM 12C micro centrifuge. The supernatant, consisting of acetone and water, was discarded. The pellet was washed twice with deionised distilled water. Nanoparticulate pellet was re-dispersed in water (10 ml) and lyophilized using mannitol (5%) as cryoprotectant. Blank NPs were also prepared by the method described above with the exception of adding EXE. Preliminary trial batches and optimize batch is shown in table 1.

Table 1 Preliminary formulations with different variables (Method 1)

Batch	B_1	B_2	B_3	B_4	B_5
Drug	Without drug	Without drug	Without drug	Without drug	62.5 mg
Polymer (PCL)	138 mg	100 mg	625 mg	625 mg	625 mg
Surfactant	Span 80-0.5ml Tween80-1 ml	Span 80-0.5ml Tween80-0.75 ml	Poloxamer-312.5 mg	Poloxamer-625 mg	Poloxamer-625 mg
Acetone	25 ml	25 ml	100 ml	100 ml	100 ml
Distilled water	50 ml	50 ml	200 ml	200 ml	200 ml
Final Product& Yield	Sticky Product	Sticky Product	More than 50% Particle in macro size	Near to 20% particle in macro size	Near to 20% particle in macro size

b) Spontaneous Emulsification Solvent diffusion Method (Method -2)

EXE (10% w/w) 62.5 mg and 625 mg of the polymer are dissolved in a mixture of acetone and dichloromethane in the ratio 1:1. This organic solution is then poured slowly into 200 ml of aqueous solution containing 625 mg of poloxamer 188 as the stabilizer under moderate stirring (1000 rpm) for 3 hours. After the addition of the organic phase, stirring was continued for 3 hours at the same speed. After 3 hours, the colloidal dispersion was subjected to heating under reduced pressure to remove acetone & dichloromethane and the solution was concentrated to 20 ml. Thus obtained dispersion was ultracentrifuged and the supernatant was analyzed for the amount of free drug. The pellet obtained after ultracentrifugation was freeze-dried in a lyophilizer after resuspending in a lyoprotective solution of mannitol (5%).

Table 2 Preliminary formulations with different variables (Method -2)

Batch	D_1	D_2	D_3	D_4	D_5
Drug	Without drug	Without drug	Without drug	Without drug	62.5 mg
Polymer (PCl)	625 mg	625 mg	625 mg	625 mg	625 mg
Surfactant	No surfactant	Poloxamer-312.5 mg	Poloxamer-625 mg	Poloxamer-625 mg	Poloxamer-625 mg
Acetone	40 ml	40 ml	60 ml	50 ml	50 ml
DCM	60 ml	60 ml	40 ml	50 ml	50 ml
Distilled water	100 ml	100 ml	200 ml	200 ml	200

Drug-Excipient Compatibility Study

The victorious formulation of a stable and effective Nanoparticulate targeted drug delivery system depends on the careful choice of the excipients, which are added to the formulation. Ideally these excipients mustn't act with the drug resulting in its degradation.

a) Fourier Transform Infrared (FTIR) Spectroscopic Analysis

Drug polymer interaction study is carried out using stability chamber. Drug and Polymer in magnitude relation of 1:1 taken and kept in stability chamber for one month at 40ºC 75% RS. At a measure of 1 week samples were taken FT-IR was applied. FTIR spectrums of moisture free powdered samples were obtained using a spectrometer (FTIR-8300, Shimadzu Co., Kyoto, Japan). Drug and potassium bromide (KBr) were mixed in equal proportion (app. 5 mg sample in 5 mg KBR) and scanned in vary of 400-4000 cm-1 and also the resolution was 1 cm^{-1}.

b) Differential scanning calorimetry (DSC) analysis

DSC scans of the fine-grained sample of EXE plane drug, Poly (ε-caprolactone), mixture of drug and polymer were recorded using DSC- Shimadzu 60 with TDA trend line software. The thermal traces were obtained by heating from 50°C to 300°C at heating rate of 10°C below inert N2 dynamic atmosphere (100 $ml.min^{-1}$) in open crucibles. Aluminium pans and lids were used for all samples. Pure water and indium were used to calibrate the DSC system of measurement and enthalpic response.

Evaluation of Nanoparticles

a) Drug Incorporation Efficiency

For determination of drug incorporation efficiency 1mg of Exemestane -NPs was dissolved in 20mL of acetonitrile and sonicated for 30min to extract drug completely. Exemestane in the solution was measured by HPLC[13]. Drug incorporation efficiency was expressed as drug encapsulation efficiency (%, w/w) and drug content (%, w/w), represented by Eqs. (1) and (2), respectively.

Drug encapsulation efficiency (%)

$$= \left[\frac{\text{Drug remained in the nanoparticles}}{\text{Feeding weight of the drug}} \times 100 \right] \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

$$\textbf{Drug content (\%)} = \left[\frac{\text{Drug weight in nanoparticles}}{\text{Total weight of nanoparticles}} \times 100 \right] \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(2)}$$

b) Particle Size & Zeta Potential

The particle size distribution studies of the nanoparticle formulations were carried out by laser diffraction method employing Malvern Master Sizer, UK .The freeze-dried nanoparticles were reconstituted and diluted with double distilled demineralized water (Millipore, India). Size analysis of Nanoparticle was carried out by dynamic light scattering with Zetasizer HSA 3000 (Malvern Instruments Ltd., Malvern, U.K.). Samples were placed in square glass cuvettes and droplet size analysis was carried out of optimized nanoparticle formulation (dil. Factor 100). Zeta potential for nanoparticulate suspension was determined using Zetasizer HSA 3000 (Malvern Instrument Ltd., U.K.). Zeta limits ranged from −200 to +200 mV. Average of 3 measurements of each sample was used to derive average zeta potential.

c) External morphological study

- **Scanning electron microscopy (SEM)**

Scanning electron photomicrographs of nanoparticles (batch B_5 and D_5) were taken. A little quantity of nanoparticles was unfolded on metal stub. Afterwards, the stub containing the sample was placed within the scanning electron microscope chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification.

➢ **Transmission electron microscopy (TEM)**

External morphology of nanoparticles was determined using transmission electron microscopy (TEM) Topcon® EM 002B, 200 kV. Usually the samples are prepared by placing one preparation drop on a collodion support on copper grids, followed by negative staining with an aqueous sodium phosphotungstate solution.

d) In vitro release study

The *in vitro* release of the entrapped drug was determined under sink conditions. 10 mg of the formulation containing known amount of drug was added to 40 ml of phosphate buffer saline (pH 7.4) in capped Erlenmeyer flask. The flasks were kept in a shaker bath incubator maintained at 100 rpm and 37˚C. Known volumes of release media were withdrawn and collected at definite intervals of time and replaced with equal volume of fresh buffer. The cumulative drug released was estimated by analyzing the drug concentration in the release media using HPLC. The assays were carried out in triplicate.

e) Kinetics of drug release

To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix and Krosemeyer and Peppas model using PSP-DISSO – v2 software[14]. Comparing the r2-values obtained, the best-fit model was selected for nanoparticles prepared by method 1 and method 2.

f) In vitro cytotoxicity studies by MCF7 breast cancer cell lines and Vero cell line.

The efficiency of the Exemestane-loaded PLGA nanoparticles on the viability of MCF-7 cells was assessed using standard MTT assays[15]. Human breast carcinoma MCF-7 and Vero (procured from National Cell Science Centre Pune, India) cells were grown in monolayer in Dulbecco's modified Eagle's medium (DMEM). The cells were cultured in media supplemented with 10% heat-inactivated foetal bovine serum (FBS) along with BSS at 37° C in a humidified atmosphere containing 5% carbon dioxide (CO2) in a CO2 incubator (RS Biotech, mini galaxy A, Scotland)[16]. Culture media were also tested for microbial contaminations. To prevent fungal contamination 2.5% Amphotericin B (25µg/ml) was supplemented in to media which act as working concentration. Bacterial contamination was prevented by addition of 1 % of Antibiotic, 100X (10000 U/ml Penicillin G, 10000µg/ml Streptomycin) in to culture medial. All sub culturing activities were done under class-II Bio safety cabinet. Cell viability, Density and Population Doubling Time were calculated for both cell lines. Pre incubate cells at a concentration of 1×10^6 cells/ml in culture medium for 3 h at 37°C and 6.5% CO2. Seed MCF7 cells and Vero cells at a concentration of 5×10^4 cells/well in 100 µl culture medium and various concentrations of Exemestane and Exemestane loaded in PCL nanoparticles (final concentration *e.g.* 100µM - 0.005µM) into microplates (tissue culture grade, 96 wells, flat bottom). Incubate cell cultures for 24 h at 37°C and 6.5% CO2. Add 10 µl MTT labelling mixture and incubate for 4 h at 37°C and 6.5% CO2. Add 100 µl of DMSO a solubilisation solution each well & incubate for overnight. Measure the spectrophotometrical absorbance of the samples using an ELISA microplate's reader (Thermo, USA). All the experiments were performed in triplicate. The wavelength to measure absorbance of the formazan product is between 450 and 600 nm according to the filters available for the ELISA reader, used. The reference wavelength should be more than 650 nm. Measure the spectrophotometrical absorbance of the nanoparticulate samples after 48, 72, and 96 hrs using a microplate (ELISA) reader and find out % cell inhibition. This assay depends on the cellular reductive capacity to metabolise the yellow tetra zolium salt, (3- [4, 5-dimethylthiazol -2 -yl] -3, 5-diphenyltetrazolium bromide dye (MTT), a highly coloured formazan product[17].

❖ Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:-

$$\%\ \text{Cell survival} = \frac{(At - Ab)}{(Ac - Ab)} \times 100 \quad \text{.....3)}$$

Where, At = Absorbance of Test,
Ab= Absorbance of Blank (Media),
Ac= Absorbance of control (cell line)
% Cell inhibition = 100 - % Cell survival .....4)

Results and Discussion

Fourier Transform Infrared Spectroscopy (FT-IR) Study

In the present work, sample of pure PCL, pure EXE and EXE/PCL physical mixture 1:1 were characterized by the FTIR. The obtained spectra were illustrated in figure 7 to 12. It showed that no significant differences on shape and position of the absorption peaks could be clearly observed for Drug, Polymer and drug polymer mixture after 4 week. Exemestane showed major peaks at 2943 cm^{-1} for –C-H stretching, 1732.08 cm^{-1} for ketone, 1658 cm^{-1} for alkene, and 690-900 $cm^{"1}$ for out-of-plane CH bending. PCL displays a characteristic absorption band at strong bands such as the carbonyl stretching mode around 1720 cm^{-1} (C=O), asymmetric stretching 2940 cm^{-1}(CH2) symmetric stretching 2865 cm^{-1}(CH2)[18].

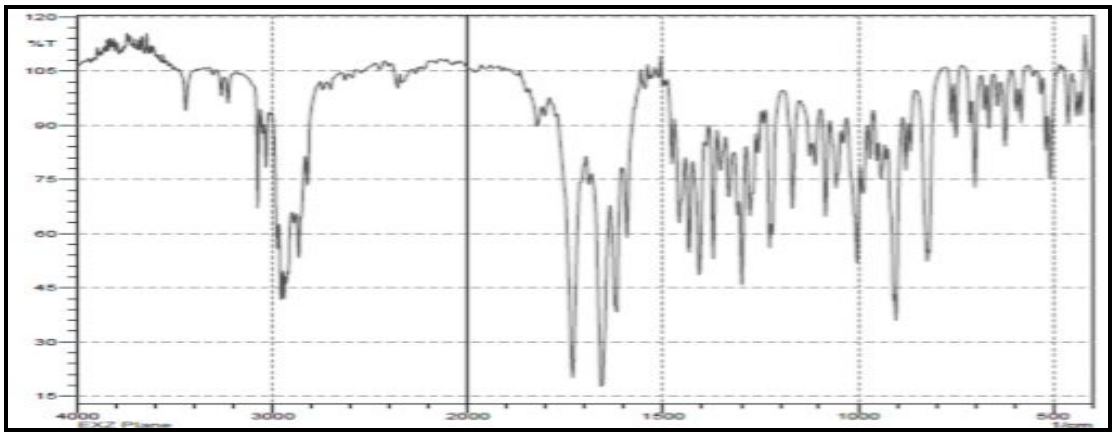

Figure 7 FTIR of Exemestane

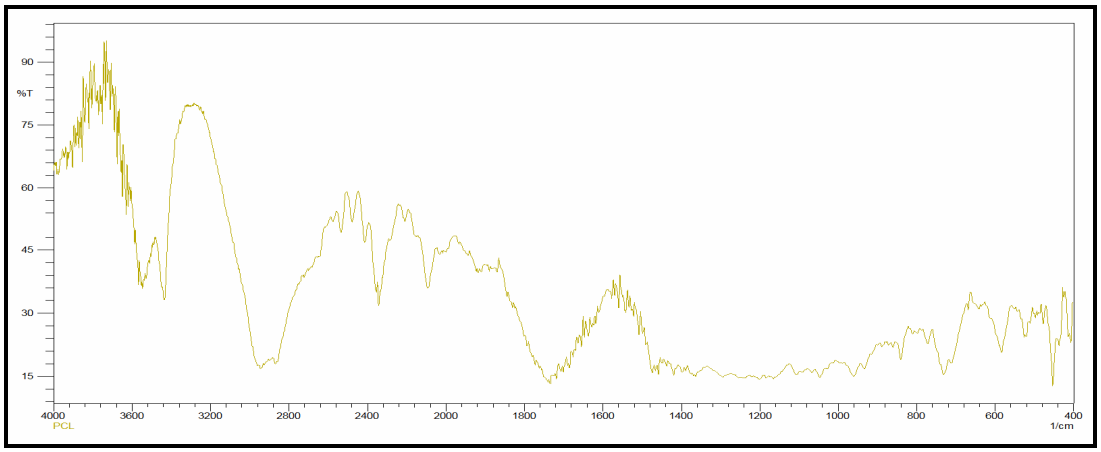

Figure 8 FTIR of PCL

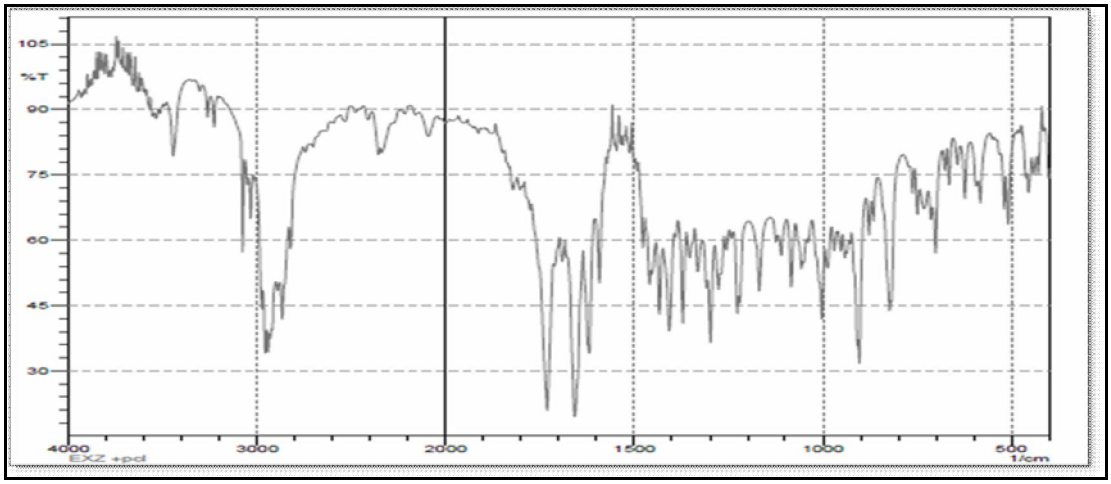

Figure 9 FTIR of Exemestane + PCL (after 1 week)

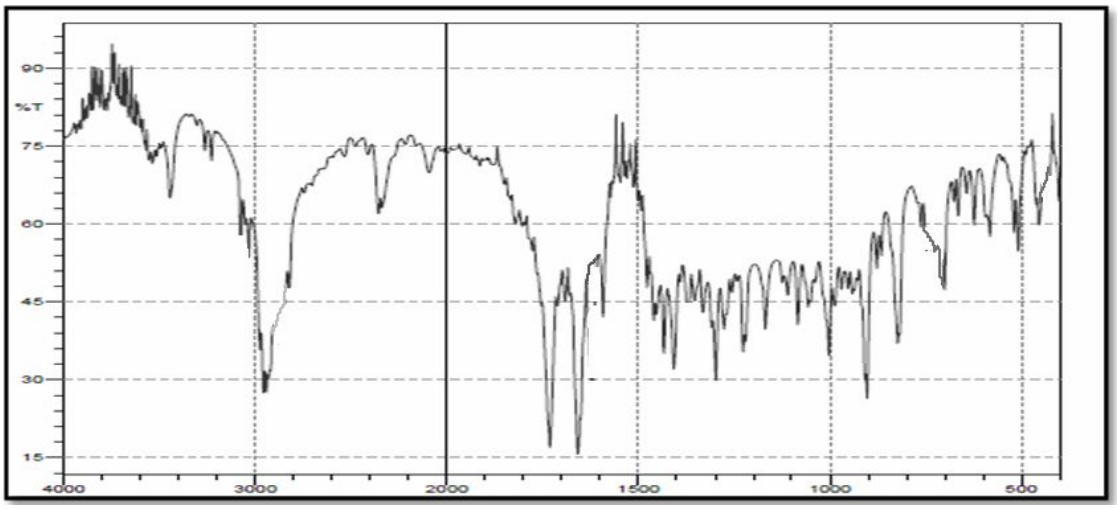

Figure 10 FTIR of Exemestane + PCL (after 2 week)

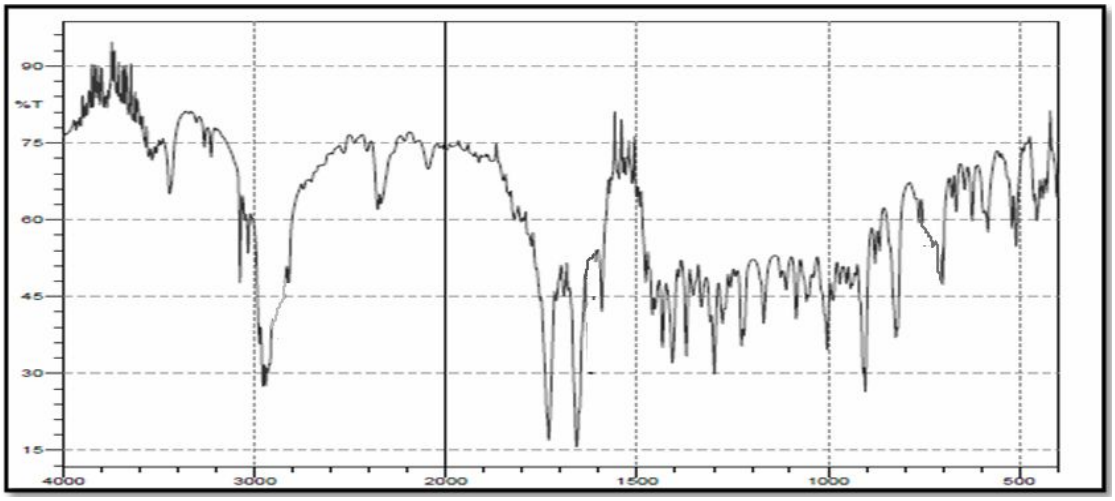

Figure 11 FTIR of Exemestane + PCL (after 3 week)

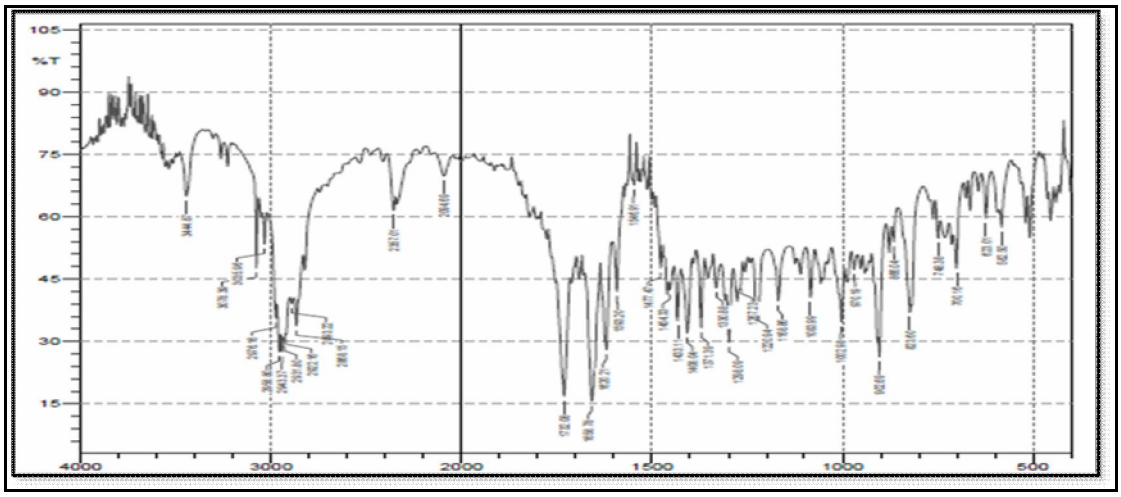

Figure 12 FTIR of Exemestane + PCL (after 4 week)

Differential scanning calorimetry (DSC)

The physical state of EXE inside the NPs was characterized by analysis of the DSC curves. The pure drug shows a characteristic endothermic peak that corresponds to melting at 186.05°C, indicating a crystalline nature (figure 13). The DSC scans revealed that EXE melting peak totally disappeared in the calorimetric curve of loaded NPs, thus evidencing the absence of crystalline drug in the samples. These results suggested that the nanoencapsulation inhibited the crystallization of EXE during NPs formation. Thus it can be assume that EXE was present in the NPs either in an amorphous or disordered crystalline phase or in the solid solution state.

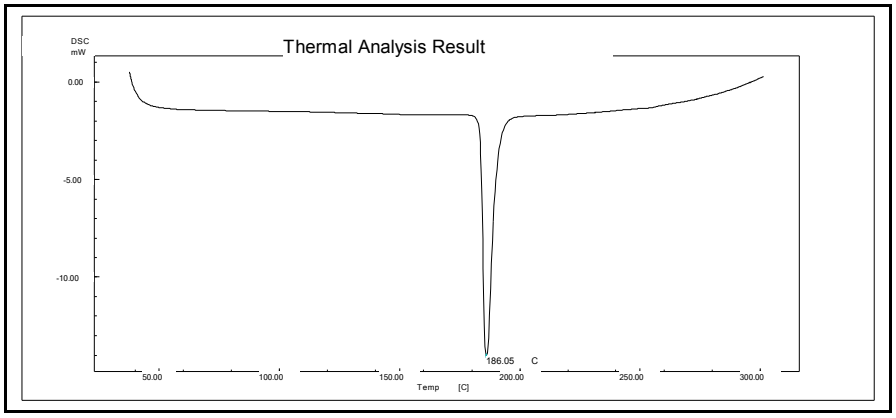

Figure 13 DSC thermogram of Exemestane

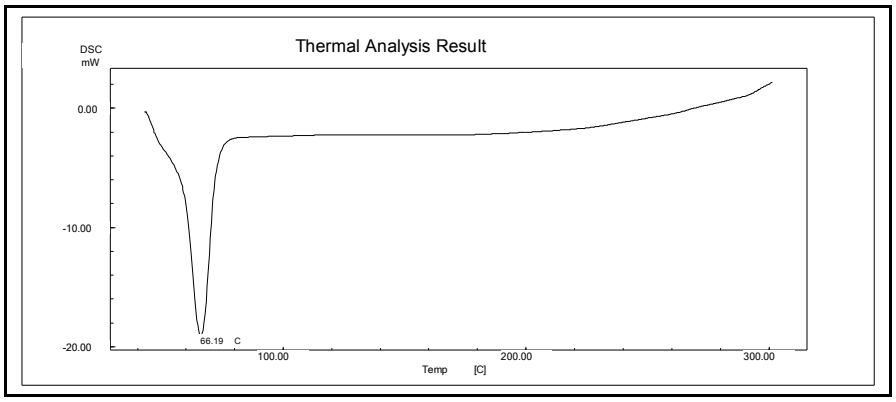

Figure 14 DSC thermogram of Poly (ε-caprolactone)

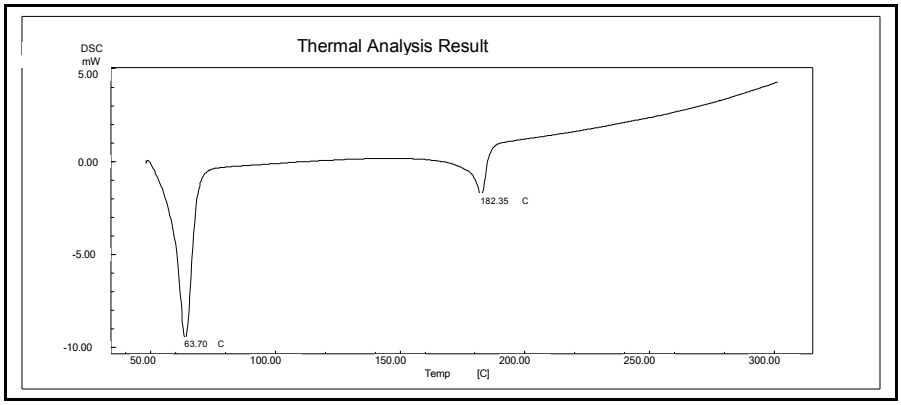

Figure 15 DSC thermogram of Drug, Polymer (PCL) physical mixture (1:1)

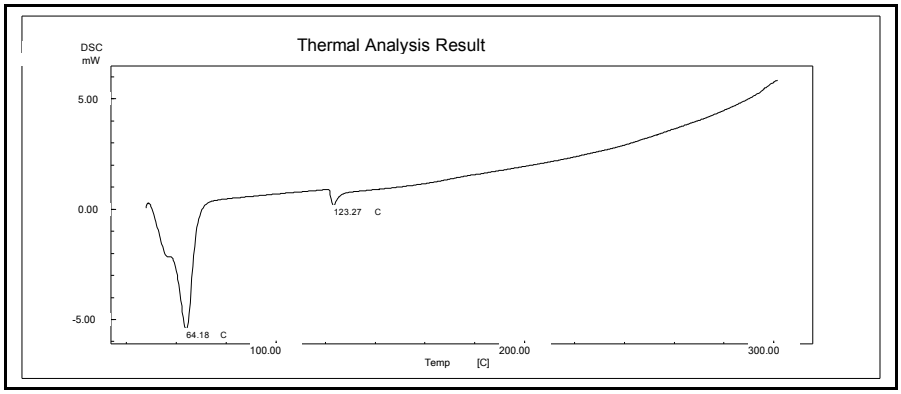

Figure 16 DSC thermogram of EXE -loaded PCL NPs

On comparison of the individual thermograms with that of the physical mixtures, it was observed that the peaks were at the same temperatures as that of the individual thermograms as shown in the figures 16 above. Thus, it was proved that there are no major degenerative drug-excipient incompatibilities and hence the excipients could be safely used to formulate nanoparticles.

In this study, EXE loaded polymeric nanoparticles were prepared by Nanoprecipitation and SESD method.

Drug Incorporation Efficiency

The drug incorporation with nanoparticles can either be in the form of entrapment in the matrix and/or adsorption onto the surface. Drug incorporation efficiency can be expressed both as drug content (% w/w), also referred to as drug loading and drug entrapment (%).Nanoparticle recovery, Drug content(% w/w) and Encapsulation efficiency (%) of batch B_{51} and D_{51} are shown in table 3. The recovery and Encapsulation efficiency of nanoparticles prepared by solvent displacement method is more compare to SESD method. This shows that method adopted for preparation contribute to a large extent in the preparation of nanoparticles. The large surface area of the nanoparticle geometry prepared by SESD method may have also contributed to loss of drug into the aqueous phase during preparation[19]. So, Encapsulation efficiency of batch D_{51} is less compared to batch B_{51}.

Table 3 Nanoparticle recovery, Drug content (% w/w), Encapsulation efficiency (%), Particle size (nm) and Zeta potential of batch B_{51} (prepared by Nanoprecipitation method) and D_{51} (prepared by SESD method).

Batch	Method of preparation	Nanoparticle recovery (%)	Drug content (% w/w)	Encapsulation efficiency (%)	Particle size (nm)	Zeta potential (mv)
B_{51}	Nanoprecipitation	55.01	8.656	63.86	37.84	− 35.8
D_{51}	SESD	52.28	9.10	59.54	85.2	− 33.21

Particle Size & Zeta Potential

The two most important parameters, which affect the recognition of injected nanoparticles in vivo by the serum components of the blood, are particle size and particle surface charge. Particle size and zeta potential of NPs are shown in table 3. It is observed that particle size of batch B_{51} NPs are smaller than batch D_{51} due to a considerable amount of residual dichloromethane, used as organic solvent in preparation of NPs by SESD method, particles are likely to aggregate during the solvent-evaporation process[20]. Normally a zeta potential value of around –40 mV to –50 mV is said to be sufficient to provide good stability and a shelf life of at least 2 years for colloidal pharmaceutical formulations21 [Washington C; Report # MRK036-03]. From the table 3 we observe that there is decrease in zeta potential value of batch $D_{51.}$ Zeta potential value is directly proportional to electrophoretic mobility (ratio of velocity of migration over potential gradient), as described by Helmho EXE-Smoluchowski equation[22]. Therefore, higher the average NP size, slower the velocity of migration of charged particles in a known applied electric potential and thus resulted in decreased zeta potential value compared to smaller mean size NPs, which has higher velocity of migration and higher zeta potential value[23].

Surface Morphology

Figure 5,6 showed the Scanning Electron microscopy (SEM), Transmission Electron Microscopy (TEM) images of EXE-PCL-NPs of batch B_{51} and D_{51}respectively. Studies unconcealed that the nanoparticles obtained had a swish surface and were spherical in all the formulations. Conjointly the particles were discreet (non-aggregated) which is a general requirement for particles meant for intra blood vessel administration.

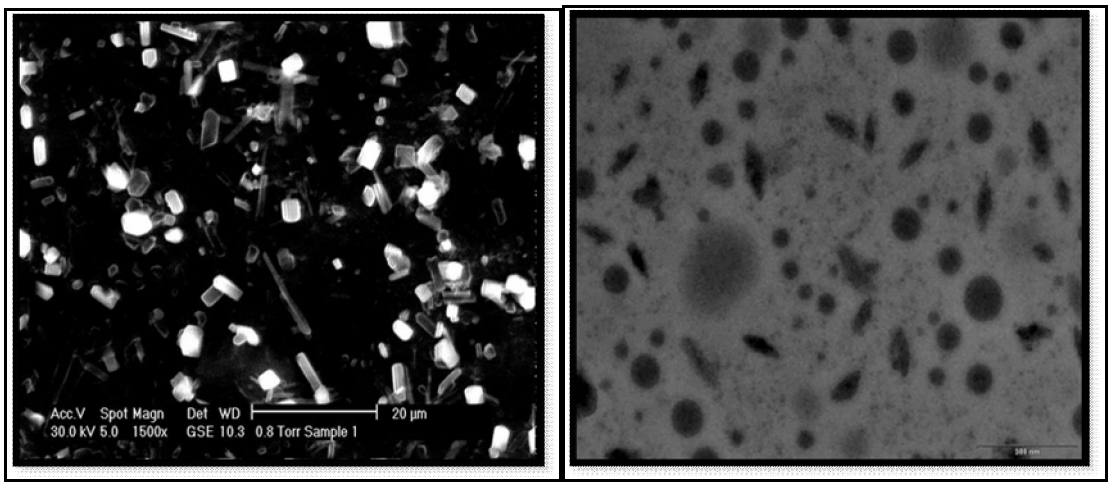

Figure 14 SEM and TEM photomicrograph of Exemestane PCL nanoparticles prepared by Nanoprecipitation method (batch B_{51})

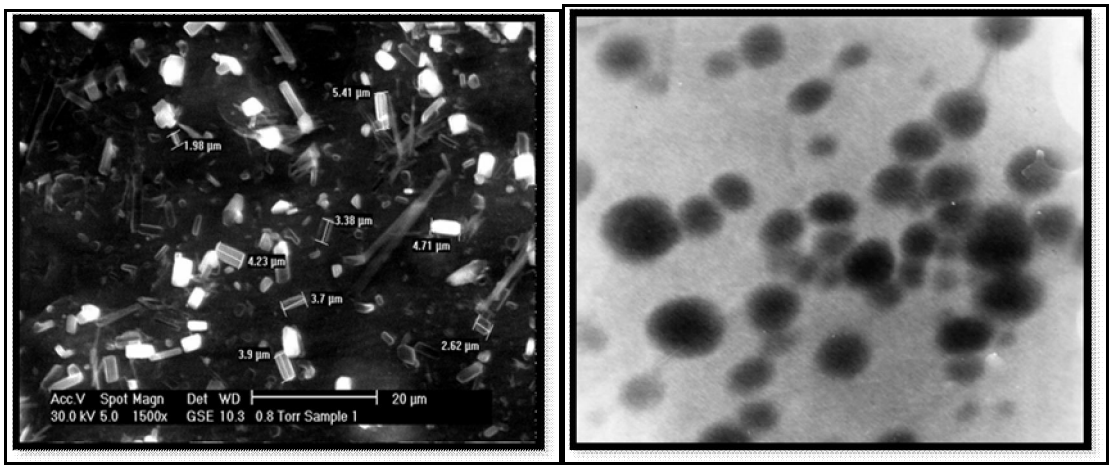

Figure 15 SEM and TEM photomicrograph of Exemestane PCL nanoparticles prepared by SESD method (batch D_{51})

In Vitro Release Study

In Vitro release profile of Batch B_{51} and Batch D_{51}were carried out in phosphate buffer saline at pH 7.4 in a shaker bath maintained at 37ºC and the results of cumulative percentage released over 100 h were shown in Graph 1. The results showed that there was a pronounced time prolongation of drug release from Exemestane - PCL-NPs. Only 68.63% and 66.05% of EXE were released only after 100 hrs. Batch B_{51} prepared by nanoprecipitation method shows higher % drug release then Batch D_{51} prepared by SESD method. Release profiles can be correlated with entrapment efficiency. The increase in drug entrapment increases the amount of drug close to the surface as well as the drug in the core of NPs and is responsible for an increased release of drug from NPs. Lower amount of drug in polymer matrix is more homogenously distributed than higher amount of drug distributed in same amount of polymer.

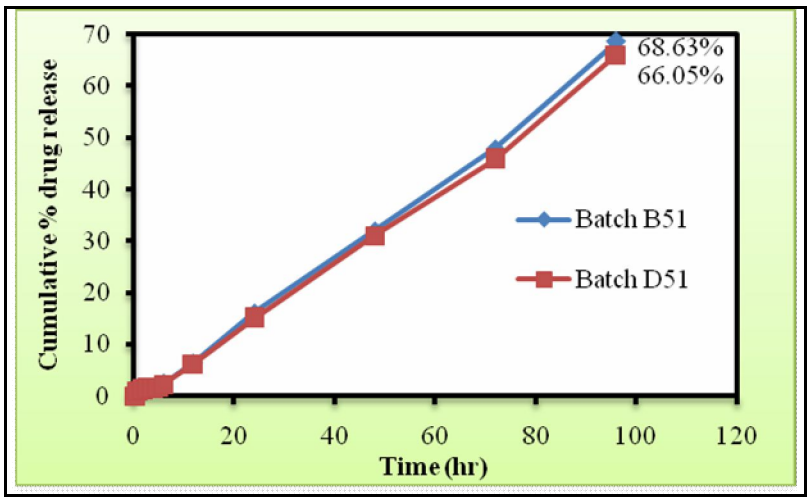

Figure 16 In-vitro Drug Release profile of EXE-PCL NPs (Batch B_{51} and Batch D_{51}) carried out in phosphate buffer saline at pH 7.4 in a shaker bath maintained at 37ºC.

In Vitro Anti Tumor Activity Studies

The percent survival of the human breast cancer MCF7 cells following treatment with free Exemestane solution and Exemestane loaded nano-particles was determined using MTT cytotoxicity assay. The cytotoxic effect of nanoparticles increased with an increase in Exemestane concentration from 0.005 µM - 100 µM. The toxicity of Exemestane solution and Exemestane loaded nano-particles was also checked on Vero cell line (African green monkey's kidney cell line). Figure shows effect of plane EXE and EXE loaded NPs prepared by nanoprecipitation method on MCF7 and Vero cell line after incubating for 24, 48, 72, and 96 hrs. As the incubation time increased from 24 hr to 96 hr % cell inhibition also increased in MCF7 and Vero cell line. In this cytotoxicity test, EXE NPs caused more death of viable cells than EXE alone (free drug). EXE plane drug shows effect for 24 hr only while EXE loaded NPs having effect for more than 96 hrs so, it can be concluded that a single dose of EXE NPs will provide a much longer drug action (sustained) as compared to a single dose of free drug and may provide passive targeting due to the enhanced permeability and retention effect[24]. Exe NPs having more % cell inhibition on MCF7 cell line (86.93%) than on Vero cell line (55.11%) after 96 hrs so, we can say that toxicity of NPs is less on other non cancerous body cells. This increased toxicity may be due to the preferential uptake of nanoparticles than that of the plane EXE. Similar effect were observed with plane EXE and EXE loaded NPs prepared by SESD method on MCF7 and Vero cell line after incubating for 24, 48, 72, and 96 hrs as shown in figure. NPs prepared by method 1 shows more % Cell growth inhibition then NP prepared by SESD method. The cellular uptake of nanoparticles by MCF-7 breast cancer cells is influenced by nanoparticle shape, size, surface properties, and concentration of nanoparticles in the medium, incubation time, and temperature, etc[25].

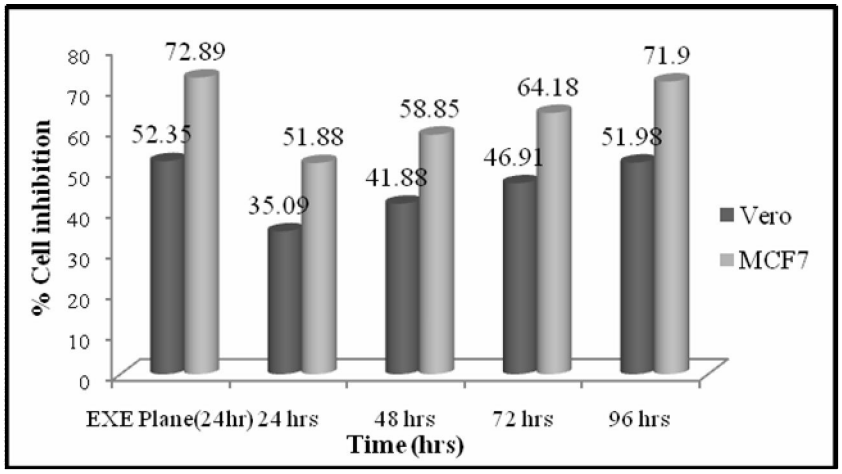

Figure 29 % Cell inhibition Vs time of EXE plane drug and EXE NPs of Batch B_{51}

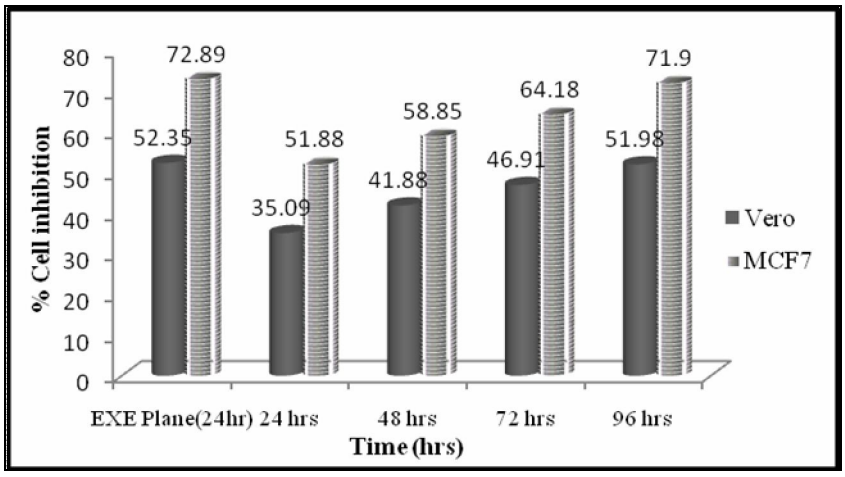

Figure % Cell inhibition Vs time of EXE plane drug and EXE NPs of Batch D_{51}

According to literature data, the higher Cytotoxicity of the drug formulated into NPs can be attributed to the combination of different but not exclusive mechanisms. In fact, the NPs were adsorbed on the cell surface leading to an increase in drug conc. near the cell membrane, thus generating a conc. gradient that promotes the drug influx in to the cell[26]. Besides, while part of free EXE molecules, transported in to the cytoplasm by a passive diffusion, were transported out by P- glycoprotein (P-gp) pumps, NPs are taken up by cells through an endocytosis pathway, thus resulting in a higher cellular uptake of the entrapped drug[27], thereby enabling them to escape from the effect of P-gp pumps. Also NPs prepared by SESD method have higher particle size so, low cellular uptake and less % Cell growth inhibition then Np prepared by Nanporecipitation method. Moreover, intracellular delivery of EXE NPs allows a drug accumulation near the site of action[28].

Conclusion

In this work Exemestane NPs were successfully formulated using Poly (Ɛ- Caprolactone) polymer by using Nanoprecipitation method and SESD method. Among the above two method NPs prepared by Nanoprecipitation method resulted more effective than NPs prepared by SESD method to achieve small size, uniform distribution, more recovery and high encapsulation efficiency. Nanoparticle prepared with Nanoprecipitation method shows higher percentage of drug release with time compare to Nanoparticle prepared with SESD method because of higher drug encapsulation efficiency, also particle of nanosize range lead to a shorter average diffusion path for matrix entrapped drug molecule, thereby causing faster diffusion. Ex- Vivo study on MCF7 breast cancer cell line also shows lower cell viability achieved by NPs prepared by Nanoprecipitation than NPs prepared by SESD method. In conclusion Nanoprecipitation method described here appeared to be a more suitable technique to formulate Exemestane loaded PCL NP.

Disclosure

The authors report no conflicts of interest in this work.

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