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COMPARSION OF THREE PLATFORM TECHNOLOGIES: MICROSPHERES, INSITU IMPLANTS & SOLID IMPLANTS FOR LEUPROLIDE ACETATE

Sumit Shah[1]*,
Arvind G[1], Noveen Konda[1], Prashanth P[1], Chandraswami[1]

[1]Celon Laboratories Limited, Hyderabad, Andhra Pradesh, India.

ABSTRACT
Leuprolide acetate is an LHRH agonist analog that is useful in the palliative treatment of hormonal related prostate cancer, mammary cancer, endometriosis and precocious puberty. Leuprolide acetate was prepared for controlled release of drug for a period of one month in the form of microspheres, in-situ implant and solid implant. Leuprolide acetate loaded microspheres were prepared by a double emulsion-solvent evaporation technique. In situ implant of Leuprolide acetate was prepared by polymer precipitation method. Solid implant of Leuprolide acetate was prepared by holt-melt excursion technology. Microspheres and solid implant having major drawback like cost and patient compliance as compare to in-situ implant. The characterization of all three platform technologies was carried out. In-Vitro release study of all three platform technologies was studied and it was observed that there was a significant difference in initial release of drug but more than 85% of drug was released after 28 days in all three platform technologies.

KEYWORDS : *Leuprolide acetate, Microspheres, Insitu Implant, Solid Implant*

Correspondence to Author

Sumit Shah

Plot no 123, Aleap Industrial Estate, Near Pragati nagar, Gajularamaram – 500072. A.P.

Email: shahsumit29@yahoo.com

INTRODUCTION

Leuprolide acetate is an LHRH agonist analog that is useful in the palliative treatment of hormonal related prostate cancer, mammary cancer, endometrosis and precocious puberty. With continued use, Leuprolide acetate causes pituitary desensitizing and down-regulation to affect the pituitary-gonodal axis, leading to suppressed circulating levels of luteinizing and sex hormones. In patients with advanced prostate cancer, achieving circulating testosterone levels of less than or equal to 0.5ng/ml (chemical castration level) is a desired pharmacological indicator of therapeutic action. Leuprolide acetate was launched in the United States as a daily subcutaneous injection of the analog solution. The inconvenience of chronic repetitive injections was later eliminated by the development of sustained release Formulations[1].

Microspheres have played a vital role in the development of controlled/sustained release drug delivery systems. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release[2]. The marketed preparation of Leuprolide acetate in the form of microspheres is available on the name of LUPRON DEPOT manufactured by ABOTT laboratories [3].

The injectable insitu-implant system comprised of a water insoluble bio degradable polymer, such as Poly (DL-Lactide), Poly (DL-Lactide-co-glycolide) and Poly (DL-Lactide-co-caprolactone) Dissolved in a water miscible, physiologically compatible solvent. Upon injection in to an aqueous environment, the solvent diffuses in to the surrounding aqueous environment while water diffuses into the polymer matrix. Since the polymer is water in soluble, it precipitates up on contact with the water and results in a solid polymeric implant [4]. The marketed preparation of Leuprolide acetate in the form of Insitu implant is available on the name of ELIGARD manufactured by TOLMAR, Inc [5].

There are two available commercial products for solid implant in market. A biodegradable implant containing Goserelin acetate, which is decapeptide analogue of lutinizing hormone releasing hormone (LHRH), is so-called Zoladex®. It uses PLGA or PLA as a carrier for the drug delivery system. The drug is dispersed in the polymer matrix using hot-melt extrusion method and the implant is distributed in the form of a prefilled syringe. The drug is continuously released over a period of 1 or 3 months [6]. Profact® Depot or Suprefact® Depot contains buserelin acetate, The trade name of this product depends on available locations. It is called Profact® Depot in Germany, PLGA in a 75:25 molar ratio (the ratio of lactide to glycolide) is used as a drug carrier, Profact® Depot implant has been designed for 2- and 3-month drug release. The duration of action is different due to the amount of drug and PLGA in the implants. 9.9 mg of buserelin acetate and 39.4 mg of PLGA are fabricated for each 3-month depot, whereas 6.6 mg of buserelin acetate and 26.4 mg of PLGA are produced for each 2-month implant [7].

Microspheres and solid implant having major drawback like cost effective and patient compliance as compare to in-situ implant.

MATERIALS AND METHODS:

Materials

Leuprolide acetate was obtained from Hemmo pharma Ltd. India, poly (Lactide -co-glycolide) PLGA 50:50 (RG504) was obtained from Evonik, Germany. All solvents were HPLC grade and were obtained from Merck chemicals, Mumbai.

Preparation of Microspheres

Leuprolide acetate-loaded microspheres were prepared by a double emulsion-solvent evaporation technique. Briefly, 500 mg PLGA 5050 was dissolved in 5 mL dichloromethane (oil phase). An aqueous solution containing 50 mg of Leuprolide acetate in 1 ml of water was prepared separately (inner aqueous phase or W1). The first aqueous (W1) phase was emulsified into the oil phase (containing PLGA), using a high-speed homogenizer (T18 basic, IKA, Germany) at 2-8 °C, 10000 rpm for 2 mins to form water in oil primary emulsion. This primary emulsion was added in to 100 ml of external aqueous phase containing 1 % PVA solution to form secondary emulsion at 6000 rpm speed for 3 mins at 2-8°C temperature. The wet microspheres were then stirred at 1000 rpm for 2 hrs at 2-8°C to permit evaporation of DCM and solidification of microspheres. The wet microspheres obtained were collected by centrifugation followed by filtration and Lyophillization [8, 9].

Preparation of *in-situ* Implant

In situ implant of Leuprolide acetate was prepared by polymer precipitation method. PLGA is dissolved in hydrophilic solvent such as N-Methyl 2-pyrrolidone until the formation of a clear solution. The polymer concentration will be 35% w/w. Drug solution is prepared by dissolving 10 mg of drug in 0.5 ml of W.F.I and filled in to 2 ml clear USP type II glass vial for Lyophillization**.** Polymer solution was added in to 10 mg of lyophilized Leuprolide

acetate, mixed thoroughly with 1 ml tuberculin syringe until it formed clear solution [10, 11].

Preparation of Solid implants

Solid implant of Leuprolide acetate was prepared by holt-melt excursion technology. Calculated quantity of PLGA (3.2 gm) and Leuprolide acetate (900 mg) were dissolved in glacial acetic acid (25 ml) to form the Drug Polymer solution, The above drug polymer solution is frozen using liquid nitrogen (temp approx – 80° C). The resulting frozen materials were freeze dried for a minimum period of 24 hrs using a freeze dryer to remove glacial acetic acid from the samples. Samples were retained in vacuum desiccators prior to extrusion. Drug/polymer blends prepared in this manner were extruded using a three piece, one gram capacity, SS extrude consisting of a nozzle, base and die with a diameter of 1 mm to 3 mm. The temperature of the extruder was initially raised to a nominal value of 80 to 90°C over a period of 30 to 60 mins and then held at this temp for a further 90-120 mins. Extrusion of the drug/polymer melt was then performed by applying pressure of up to 1 ton to the extruder die. The resulting extrudes were cut into the required lengths for example 1 mm to 5 mm and desired length of solid implant was Filled and sterilized by Gamma Irradiation [12].

Characterization of microspheres

Particle size

The mean diameter of microspheres was determined by laser diffractometer (Mastersizer X, Malvern Instrument, UK). Microparticles were suspended in 0.3% aqueous solution of Tween 80 and sonicated for 15 s prior to particle size determination.

Scanning Electron Microscopy (SEM)

The morphology of microparticles was examined by scanning electron microscopy (MW2300, Cam Scan-England). Samples were mounted on metal stubs and sputter-coated with gold for 4 min prior to examination under.

***In-vitro* drug release**

The in-vitro drug release from the microspheres was carried out by using a regenerated cellulose membrane dialysis apparatus Float-A-lyzer. 2ml of microspheres suspension containing known amount of drug was placed in the Float-A-lyzer and this was placed in 250 ml of PBS (pH 7.4), maintained at 37°C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh PBS (pH 7.4) to maintain constant volume. The samples were analyzed for drug content by HPLC at 220nm. Upon completion of one week, the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium.

Characterization of Insitu Implant Viscosity

Viscosity of different formulations was determined using Brookfield viscometer 2000^{+} (Brookfield engineering laboratories, USA) with spindle no.6 at 50 rpm at temperature 25±1°C [13, 14].

Molecular weight determination after gamma irradiation

Gel permeation chromatography (GPC) is a type of size exclusion chromatography that separates analytes on the basis of size. Gel permeation chromatography is conducted almost exclusively in chromatography columns. GPC is often used to determine the relative molecular weight of polymer samples as well as the distribution of molecular weights. Samples are dissolved in an appropriate solvent, in the case of GPC these tend to be organic solvents and after filtering the solution it is injected on to a column [15].

***In-vitro* drug release**

In vitro drug release studies were performed by injecting the formulation in to 50 ml 7.4 pH phosphate buffers at 37°C. At 1, 3, 7, 14, 21, and 28th day time intervals, 5 ml sample were withdrawn and replaced with fresh medium and withdrawn samples analyzed for drug content by UV visible spectrophotometer at 220nm. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium [16].

Characterization of Solid Implant

Determination of Size and Diameter

The size and diameter of solid implant was determined by vernier caliper.

***In-vitro* drug release**

Several methods have been used to determine *in-vitro* drug releases from implant drug delivery systems. The compendial apparatus 4 device (Flow-Through Cell) is only equipment, which has been recommended on FIP/AAPS Guideline for drug release testing of implants. The apparatus consists of a flow-through cell, a pump and a water bath to maintain the release medium at 37 ± 0.5 °C. The standard flow rates as recommended in the USP are 4, 8 and 16 ml/min. The flow-through cell is made from transparent and inert materials and built to the vertical setting. During the test, the critical parameters including volume, temperature and flow rate of a medium have to be monitored and controlled [17].

RESULTS AND DISCUSSIONS:

Characterization of microspheres

Particle size

The mean diameter of microspheres was determined by laser diffractometer (Mastersizer X, Malvern Instrument, UK). Microparticles were suspended in 0.3% aqueous solution of Tween 80 and sonicated for 15 s prior to particle size determination. The mean particle size of Formulation was shown below in Fig 1.

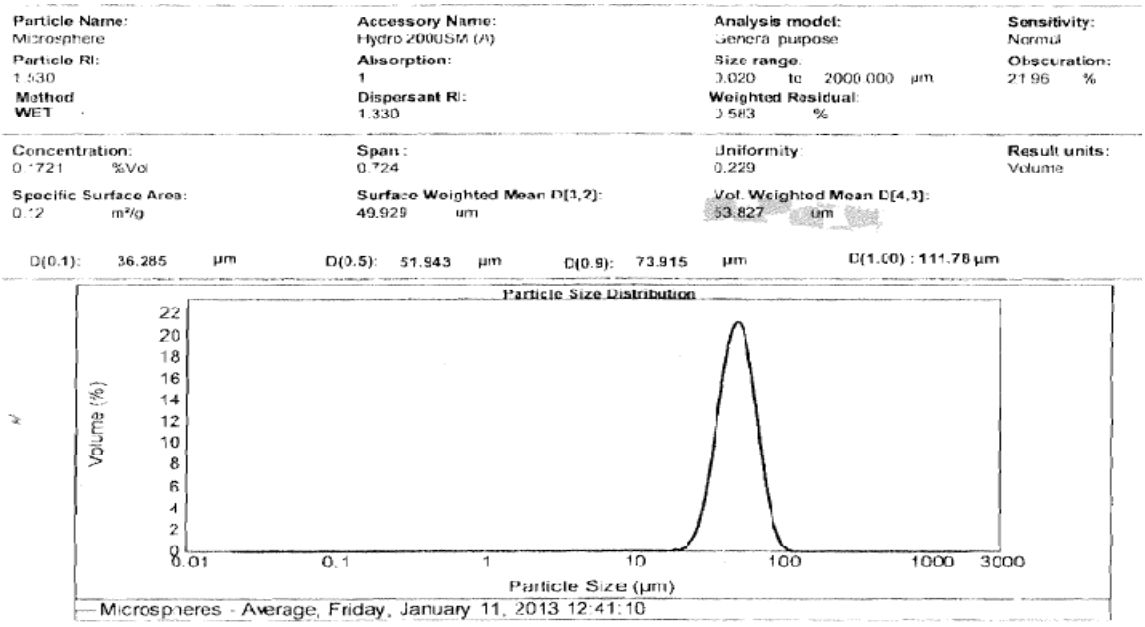

Fig 1: Particle size distribution of leuprolide acetate microspheres

Scanning Electron Microscopy SEM

The morphology of microparticles was examined by scanning electron microscopy. The SEM picture showed in Fig 2 that the shape of the microspheres was spherical and smooth surface with less porosity.

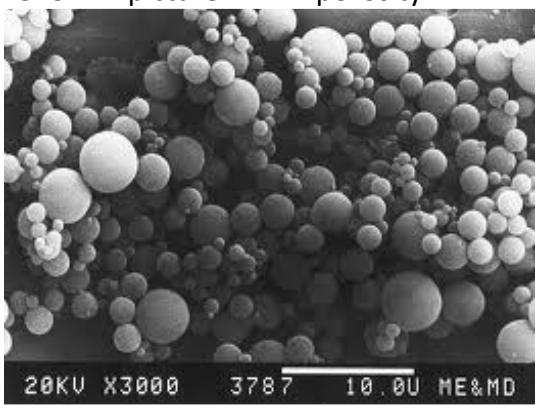

Fig 2: SEM image of leuprolide acetate microspheres

***In-vitro* drug release**

The cumulative percent release of leuprolide acetate microspheres at various time intervals was calculated. The cumulative percent drug release was plotted against time in Figure 3. It has been shown that the % initial burst release of drug was 15% and more than 85% drug was released after 28 days.

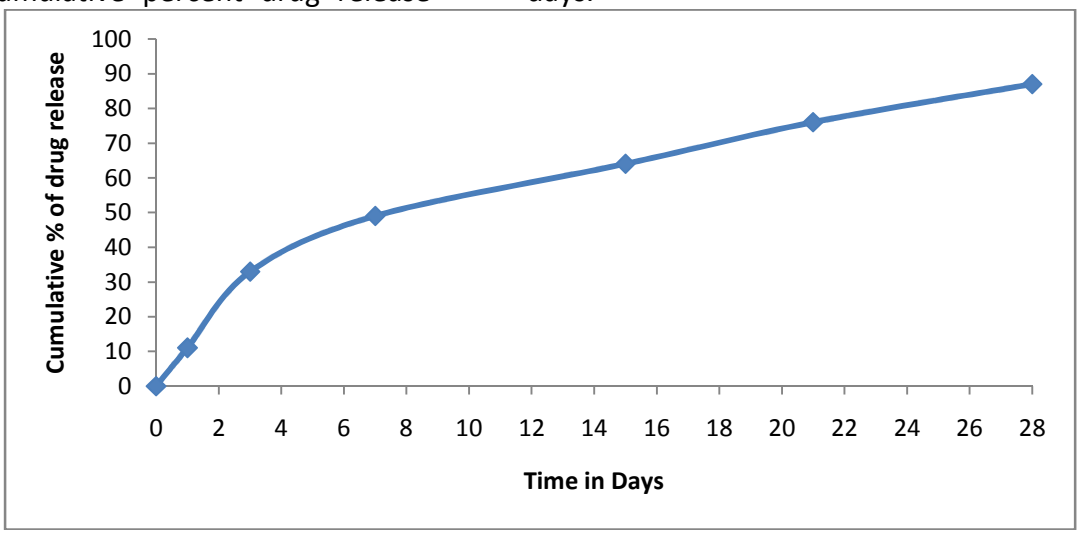

Fig 3: *In- vitro* release studies for optimized formulation of Microspheres

Characterization of Insitu Implant

Viscosity

Viscosity of polymer solution before and after gamma radiation was determined using Brookfield viscometer (50 rpm at temperature 25±1°C). Viscosity of polymer solution before gamma irradiation was 2000 cps and after gamma irradiation was 1700 cps.

***In-vitro* drug release**

The *in vitro* dissolution profile (Figure 4) after γ irradiations was performed. The dissolution was carried out for a period of 28 days in 7.4 pH saline phosphate buffer. The initial burst release of drug was shown 20% and more than 90% of drug was released within 28 days.

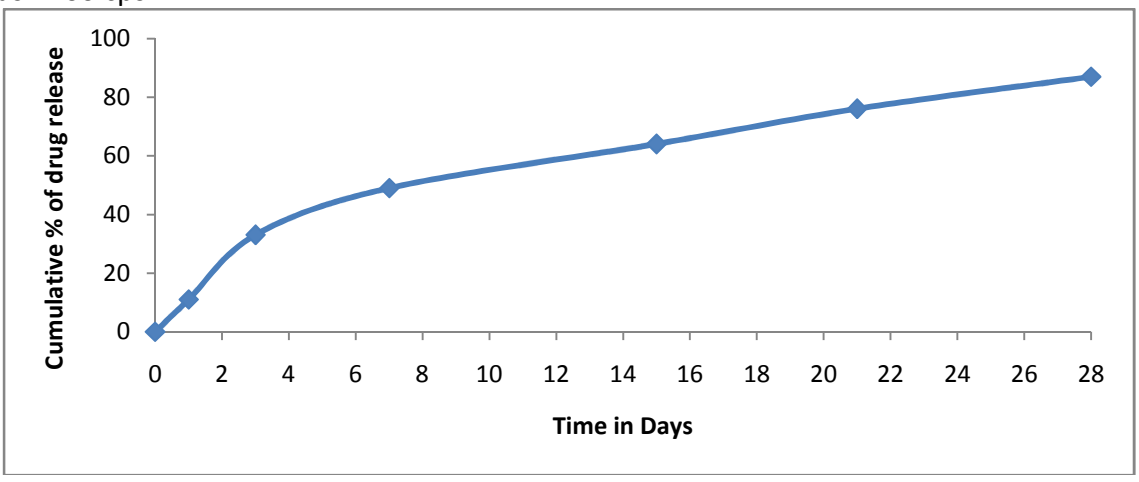

Fig 4: *In- vitro* release studies for optimized formulation of *Insitu*- Implant.

Molecular weight determination after gamma irradiation

Gel permeation chromatography (GPC) is a type of size exclusion chromatography that separates analytes on the basis of size. Initial molecular weight of polymer solution was 58,549 Daltons and it was significantly reduced after gamma irradiation i.e. 33,251 Daltons (Figure 5).

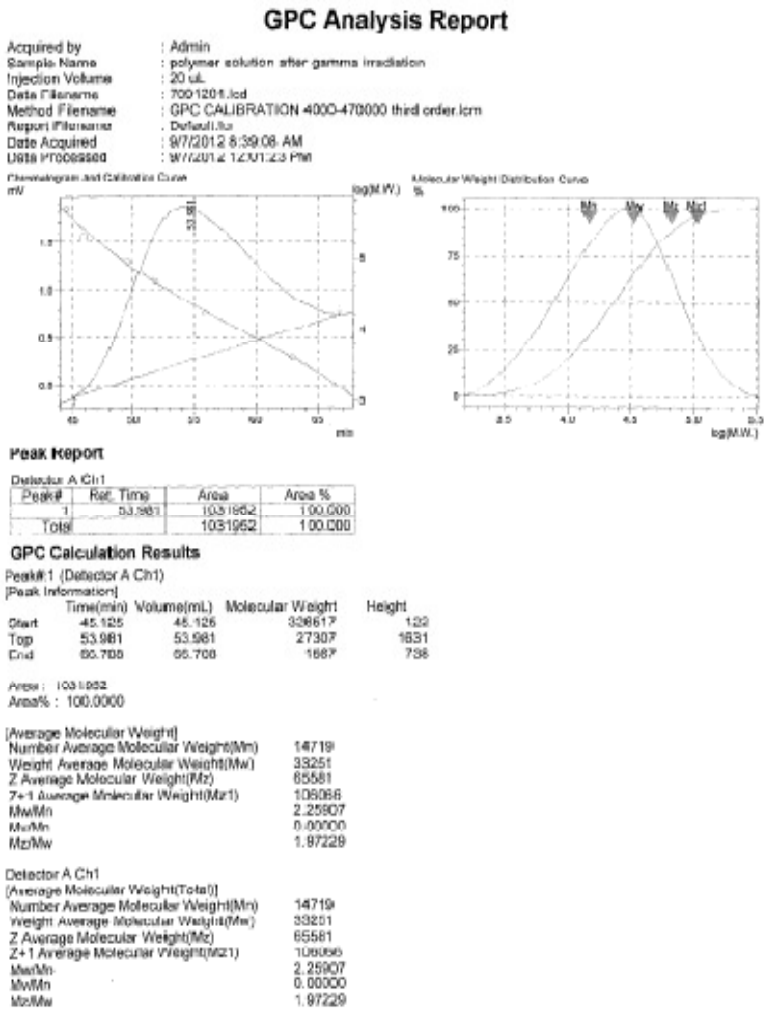

Figure 5: GPC after gamma irradiation

Characterization of Solid Implant

Size, Shape and Diameter

Size and diameter of solid implant was measured by vernier caliper. The measured length of solid implant was approximate 3 cm and diameter of solid implant was approximate 5 mm. The shape of solid implant was cylindrical.

***In-vitro* drug release**

The *in vitro* dissolution profile (Figure 6) after γ irradiations was performed. The dissolution was carried out for a period of 28 days in 7.4 pH saline phosphate buffer. The initial burst release of drug was shown 11% and more than 85% of drug was released within 28 days.

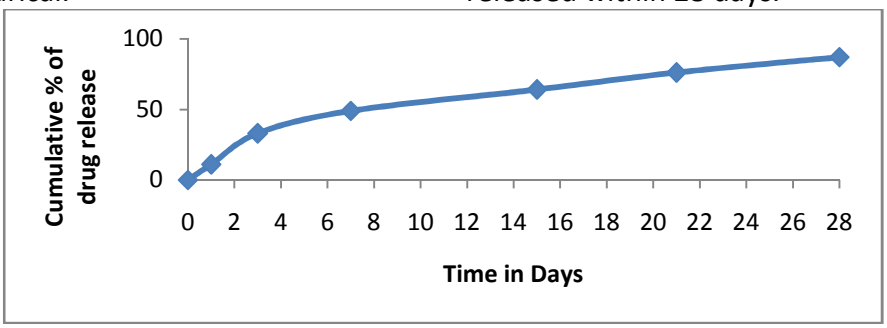

Fig 6: *In- vitro* release studies for optimized formulation of Solid- Implant

Comparison of In-vitro drug release for Microspheres, Insitu-Implant, Solid Implant

It has been observed from in-vitro dissolution study that initial burst release (Release of drug within 24

hrs) of three different technologies are different (Solid implant > Microspheres > *In-situ* implant) but more than 85% of drug was release after 28 days.

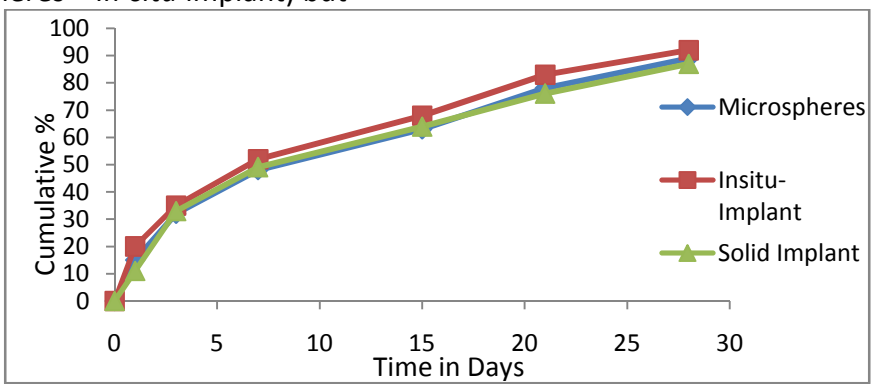

Fig 7: Comparison of *in- vitro* release studies for three different platform technologies

CONCLUSION:

From the in-vitro release study, we could conclude that all three platform technologies were worked as a controlled release of leuprolide acetate for a period of one month - palliative treatment of hormonal related prostate cancer, mammary cancer, endometriosis and precocious puberty. There was significant difference in initial burst release of all three different technologies. As in-situ implant was easy to manufacture as well patient compliance than microspheres and solid implant, it can be used as better platform technology for treatment of cancer therapy.

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REFERENCES

1. Richard L. Dunn, John Steven Garrett, Harish Ravivarapuram. US Patent, US 6773714 B2, 2004.
2. Raju T, Santhosh Kumar J, Ravindra babu D.S. Int J Pharm Pharm Sci, 2013; 1 [5]: 87-93.
3. http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=00c486b0-bd7b-4898-834d-8c656e5e73cb#nlm34089-3
4. A. Hatefia, B. Amsdena. Journal of Controlled Release, 2002; 80: 9 –28.
5. http://products.sanofi.us/eligard/eligard.html
6. http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=4ff62a5a-77ba-4bf5-0497-95ccca842315
7. http://products.sanofi.ca/en/suprefact.pdf
8. Yi-Yan Y, Tai-Shung C, Xin-Lia B. Effect of preparation condition on morphology and release of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method. Chemical Engineering Sci 2000; [55]: 2223-26.
9. Rajesh P, Jolly RP, Iieena Soni, Kishor NK. Poly (D,L- Lactide-co-Glycolide) microspheres containing 5-Fluorouracil:Optimization of process parameters. AAPS 2003;[4] :1-8.
10. Richard L. Dunn, Fort Collins, John Steven Garrett. US Patent, US 6773714, 2004.
11. http://www.medsafe.govt.nz/profs/datasheet/e/Eligardinj.pdf .
12. Francis G, Hutchinson, Lymm. US Patent, US 5004602, 1991.
13. Sanjay Dey, Bhaskar Mazumder, J.R. Patel. Int J Pharm Sci and Drug Res, 2009; 1 [1]: 13-18.
14. Reyhaneh Astaneh, Mohammad Erfan. J Pharm Sci, 2009; 98 [1]: 135-45.
15. http://en.wikipedia.org/wiki/Gel_permeation_chromatography.
16. Dewan and Islam, IJPSR, 2011; 2 (11): 3039-45
17. http://www.fip.org/files/fip/BPS/Dissolution/FIP_AAPS_Guidelines%20for%20Dissolution.pdf.

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