DEVELOPMENT OF FLUTAMIDE LOADED POLYMERIC NANOCARRIERS FOR CHEMOTHERAPY OF PROSTATE CANCER.

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ABSTRACT:

Flutamide is a potent nonsteroidal anti-androgen used to treat advanced prostate cancer. It blocks the androgen receptors on the cancer cells and inhibits the androgen dependent cell growth. This work was planned to compile the research work available in the scientific publications in the formulation and evaluation of Flutamide loaded polymeric nanoparticles for the treating the prostate cancer including the study of influence of various formulation components such as polymer concentration, organic phase volume, drug content, stabilizer concentrations and the ratio between aqueous to organic phase in the characteristics of nanoparticles. Literature study revealed that, methods like Ionic Gelation Technique, Solvent Evaporation method and Nanoprecipitation have been utilized and polymers like Chitosan, casein, Methacrylic acid, PHEA-IB-p(BMA) graft copolymer, mPEG – PLGA and PVA have been utilized. The drug loaded polymeric nanoparticles were evaluated for their physiocochemical properties along with characterization with FTIR, in vitro release and in vitro and in vivo anticancer potential. From the literature it was understood that, nanoprecipitation method was the most commonly used method in various research perspectives. Based on the findings of the present review, Flutamide loaded polymeric nanoparticles may be used as a treatment adjunctive for prostate cancer.

KEYWORDS: Flutamide, Nanoparticles, Prostate Cancer and Nanoprecipitation.

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, this orderly process breaks down and the cells become more and more abnormal, survive longer and new cells form when they are not needed. These extra cells mutate rapidly without stopping and form growths called tumours.

Prostate cancer is the sixth leading cause of cancer death among men worldwide and is expected to grow to 1.7 million new cases and 4,99,000 new deaths by 2030 simply due to the growth and aging of the global population (J. Ferlay, et al., 2012). This type of cancer is prevalent in men between the ages of 65-79 years old, with 25% of cases are occurring in men younger than 65 years old. Treatments that are currently available for prostate cancer include surgery, hormone therapy, radiation therapy and chemotherapy. These treatment methods are either very invasive or have harsh side effects. Though chemotherapy is successful to some extent, the main drawbacks of chemotherapy is the limited accessibility of drugs to the tumour tissues requiring in high doses, intolerable toxicity, development of multiple drug resistance and their nonspecific targeting.

Flutamide (FLT) is a potent nonsteroidal anti-androgen used in the treatment of advanced prostate cancer. It blocks the androgen receptors on the cancer cells and inhibits the androgen dependent cell growth. The usual oral dose of flutamide is 250 mg three times daily. Its oral absorption is rapid and it attains peak plasma concentration in 1 hr after a single dose (Goldspiel BR., 1990). It under goes high first pass hepatic metabolism into less active metabolites (hydroxyl flutamide), which was further worsened by the lower elimination half-life that ranges from 5-6 hrs. This poor therapeutic profile of flutamide is contributed by faster metabolism, and subsequently retarded bioavailability. Its low bioavailability may be due to its poor wettability and low aqueous solubility which reduce testosterone on a continuous basis. Treatment with flutamide may cause a variety of side effects including diarrhoea, tiredness, impotence, enlargement of male breast, liver malfunction and hepatotoxicity on high doses. In order to decrease the frequency of drug administration and also the incidence of adverse effects, a sustained release formulation of flutamide is desirable. Development of Nano drug carriers loaded with Flutamide that can yield enhanced plasma half-life, optimal release at the required site of action, improved bioavailability, reduced incidence and severity of adverse effects and the hepatic impairment can be achieved.

Nanoparticles (NPs), an evolution of nanotechnology, have the potential to successfully address these problems related to drug delivery and retention and are considered potential candidates to carry drugs to the desired site of therapeutic action. Polymer nanoparticles are playing an important role in the recent years for clinical administration of various anticancer drugs. Most anticancer drugs have limitations in clinical administration due to their poor solubility and other unfavourable properties. Thus, biodegradable polymeric controlled delivery systems bring the higher drug efficacy and better applications for the potent

anticancer drugs. The advantages of this carrier system include the controlled and targeted delivery, facilitated extravasation into the tumour, reduced systemic side effects and high capability to cross various physiological barriers (Brigger I, et al., 2002). Parenteral controlled release systems are of considerable importance for drugs as they require daily administration or have low bioavailability or high toxicity (Sandrap and Moes, 1993). Biodegradable injectable drug delivery system to transport and maintain the drug level in a desired therapeutic range for a long period of time improves compliance. The design of the controlled release nanoparticles has other advantages over conventional dosage forms.

Table – List of Materials

S.NO	RAW MATERIALS	CATEGORY
1	Flutamide	API
2	Poly Caprolactone (PCL)	Polymer Matrix
3	Methoxy Poly (ethylene glycol) Poly Lactic Acid	Polymer Matrix
4	Methoxy Poly (ethylene glycol) Poly Caprolactone	Polymer Matrix
5	Pluronic F-127	Stabilizer
6	Hydrochloric acid	pH adjacent Agent
7	Sodium Hydroxide	Buffering Agent
8	Potassium Dihydrogen Phosphate	Buffering Agent
9	Methanol	Mobile phase
10	Acetonitrile	Mobile phase
11	PC – 3 Cell lines	Human cell carcinoma
12	Fetal Bovine Serum (FBS)	Culture Medium
13	Dulbecco's modified Eagles medium (DMEM)	Culture Medium
14	Dimethyl Sulfoxide (DMSO)	Culture Medium

PREFORMULATION STUDIES:

Oraganoleptic Properties:

a. Colour: Yellow colour.

b. Odour: Unpleasant smell.

c. Physical Form: Crystalline, very fine powder.

Solubility Studies: The solubility of FLT in water was found to be 0.0094 mg/ml. FLT was poorly soluble in water and highly soluble in organic solvents like acetone, diethyl ether, ethanol, methanol and dimethyl formamide.

Melting Point Determination:

After performing capillary method melting point of Flutamide found in range of 111 -113^{0}C.

Identification of Pure Drug:

The IR spectrum of Flutamide was recorded using brukers spectrophotometer with KBr pellet method. FT - IR spectroscopy was used to determine the functional group present in the pure drug sample. The FTIR spectrum of pure Flutamide has shown the characteristic peaks at 554.28, 3360.06, 1717.71, 1541.46 and 1344.97 cmí 1. The wave numbers observed at 1541.46 and 1717.71 may be assigned to the N = O and C = O bonds, respectively and the sharp peak occurred at 554.28 and 1344.97 indicates presence of C – F3 and C - N group attached to C = C. The absorption band between 3450 and 3050 cm-1 indicates the presence of –NH stretching. which are within the standard range of absorption frequencies of Flutamide.

Table- IR spectra of Flutamide

Functional Groups	**Drug Wave Number (cm^{-1})**	**Range of Absorption Frequencies**
N – H Stretching (Amide)	**3360.06**	**3450 – 3050**
C = O Stretching (Carboxylic Acid)	**1717.71**	**1760 – 1690**
N = O Stretching (Nitro Compound)	**1541.46**	**1550 – 1475**
C – N Stretching (Aromatic Amine)	**1344.97**	**1355 – 1250**
C – F3 Stretching (Alkyl halide)	**554.28**	**850 – 550**

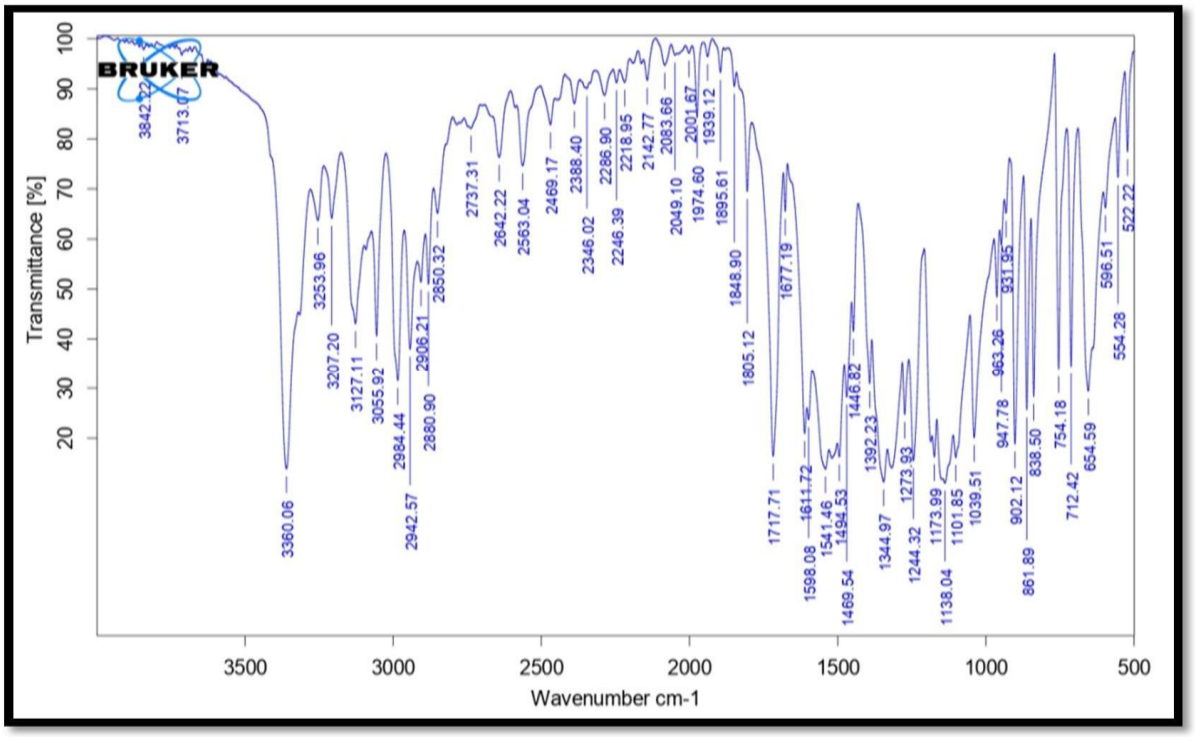


Figure –IR Spectra of Flutamide

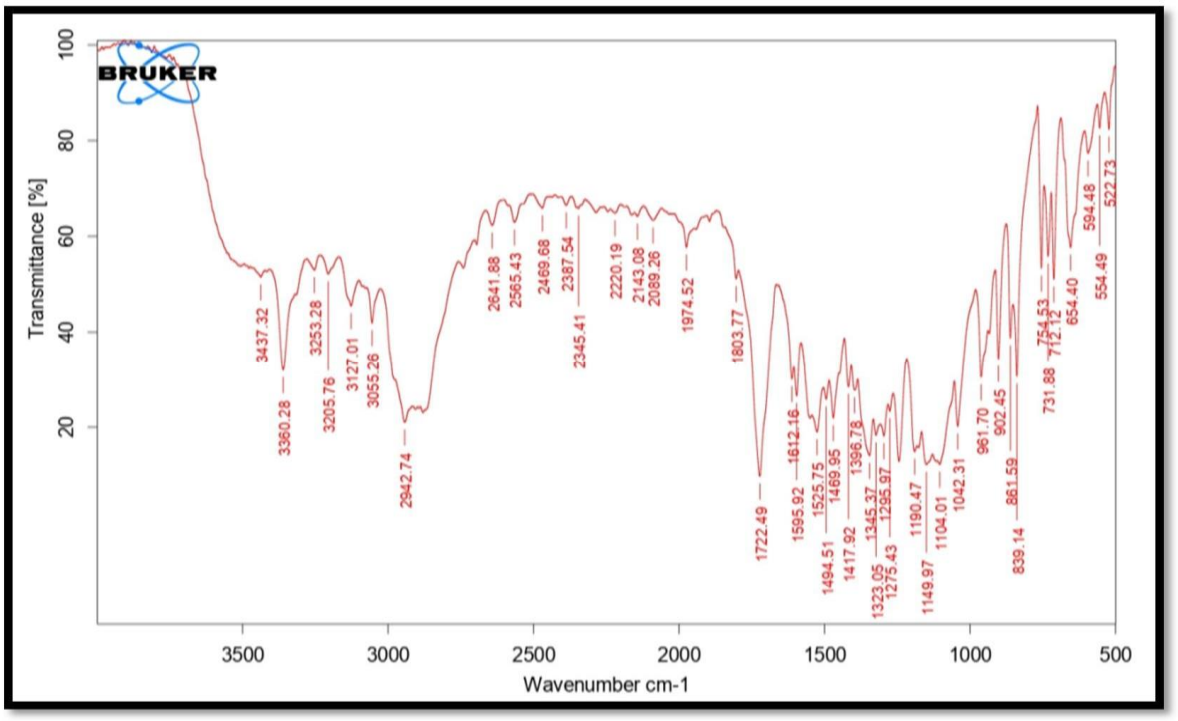


Figure – IR Spectra of Flutamide + PCL + Pluronic F 127

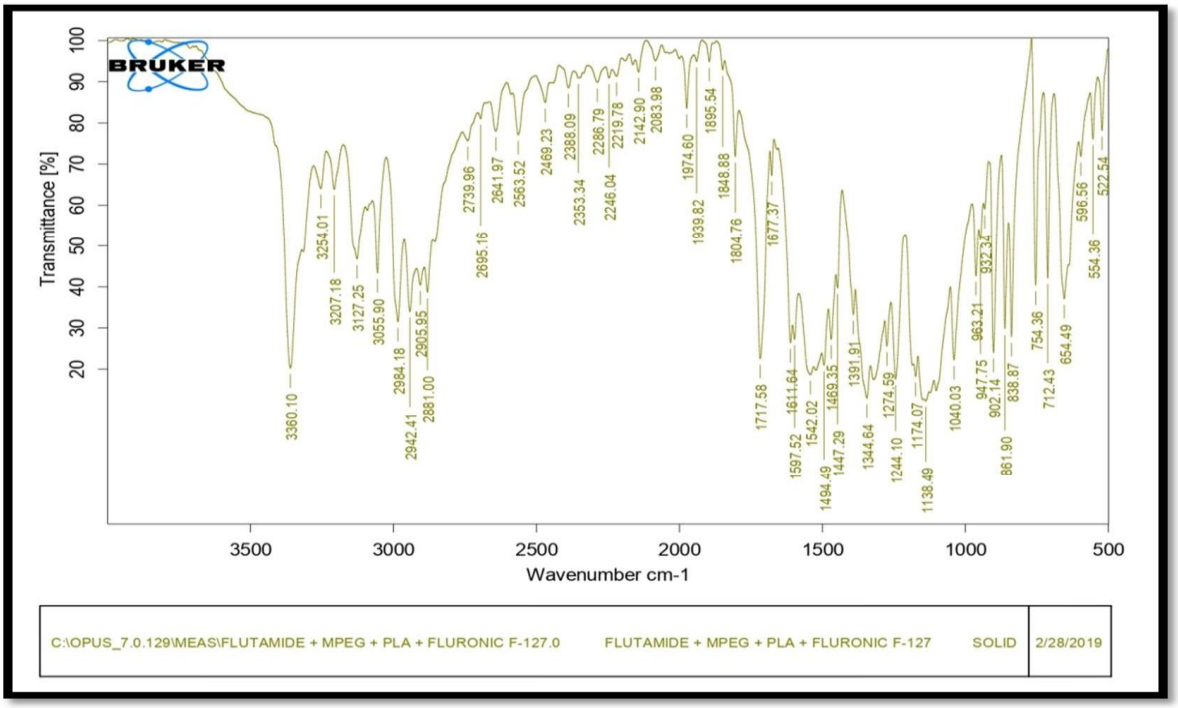
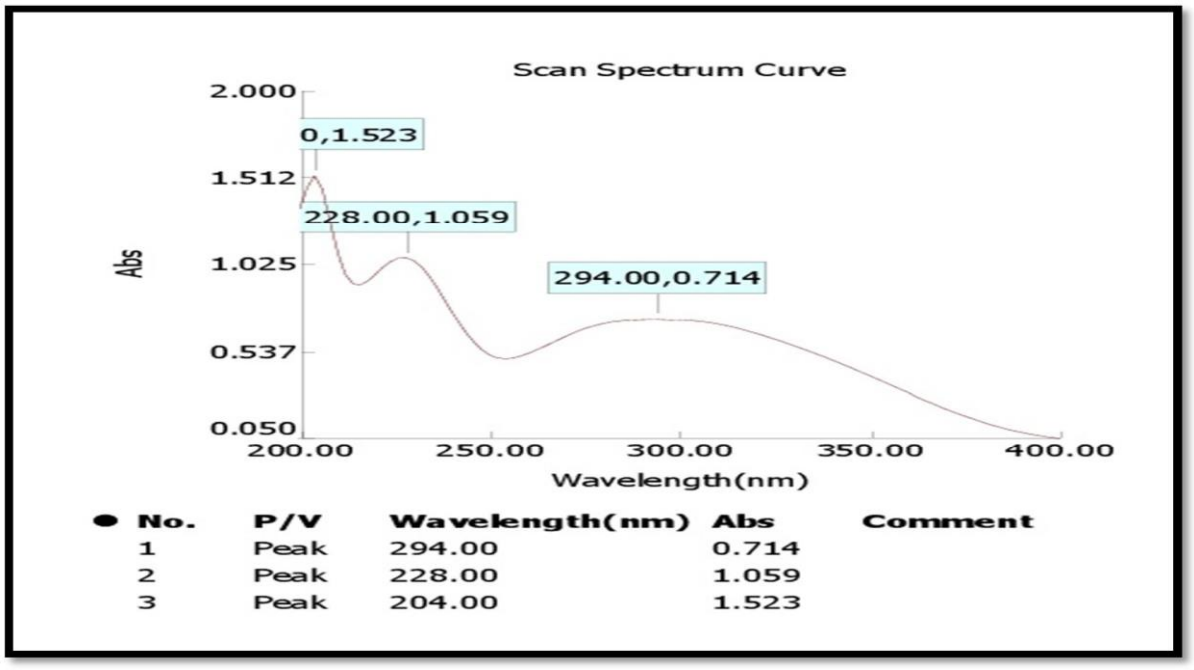


Figure – IR Spectra of Flutamide + mPEG-PLA + Pluronic F 127

Determination of ʒmax:

The Flutamide with pH 7.4 PBS was scanned in UV - Vis spectrophotometer from 400 – 200 nm to determine the ʒmax. The ʒmax was found to be at 228 nm, so the standard curve of FLT was developed at 228 nm.



No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	294.00	0.714	
2	Peak	228.00	1.059	
3	Peak	204.00	1.523	

Figure –Identification of ʒ max of Flutamide

Calibration of Standard Graph of Flutamide:

The calibration curve of FLT was done by using pH 7.4 PBS (Dipsingh SN. et al., 2018) and the ʒ max was found at 228 nm. The calibration curve was constructed by making the concentrations of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml, 14 µg/ml, 16 µg/ml, 18 µg/ml and 20 µg/ml solutions. The absorbance of solutions was examined under UV - visible spectrophotometer at an ʒmax of 228 nm. The calibration curve was constructed by taking the concentrations on X - axis and absorbance on Y - axis. The standard calibration curve of Flutamide in pH 7.4 PBS was shown in fig. The relationship between drug concentration and absorbance obeyed Beer - Lambert's law and the R^2 value is 0.998.

Table – Standard Calibration Curve of Flutamide

S. No	Concentration ʒg/ml)	Absorbance in pH 7.4 PBS
1.	0	0
2.	2	0.128
3.	4	0.234
4.	6	0.302
5.	8	0.420
6.	10	0.534
7	12	0.619
8	14	0.706
9	16	0.810
10	18	0.925
11	20	1.037

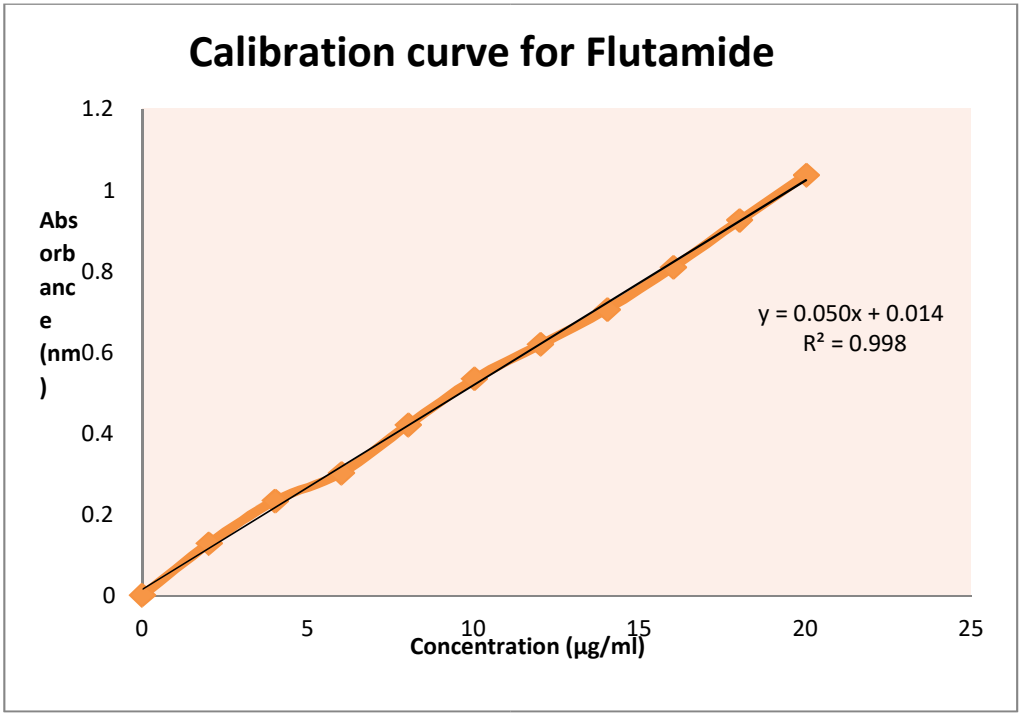


Figure –The Standard Calibration Curve of Flutamide in PBS pH 7.4

Nanoprecipitation method:

Flutamide loaded polymeric nanoparticles were prepared by the nanoprecipitation method (Joshi SA, et al., 2010). Drug and polymer were dissolved in Acetone and this solution was added to pluronic F-127 in PBS pH 7.4 at 1 ml/min speed using syringe under magnetic stirring. The obtained suspension was stirred at 500 rpm for 2 hr to evaporate acetone and centrifuged at 11,000 rpm for 30 minutes to remove precipitants, supernatant was collected, lyophilized and stored at 4°C. A blank nanoparticles was prepared by the same procedure, but excluding Flutamide. Total 8 formulations were prepared for ease of analysis and comparison (Yadav N. B. V., et al., 2016).

CHARACTERISATION OF PREPARED NPs:

Size Analysis:

Table –Mean particle size of FLT loaded PCL NPs (F1 – F8)

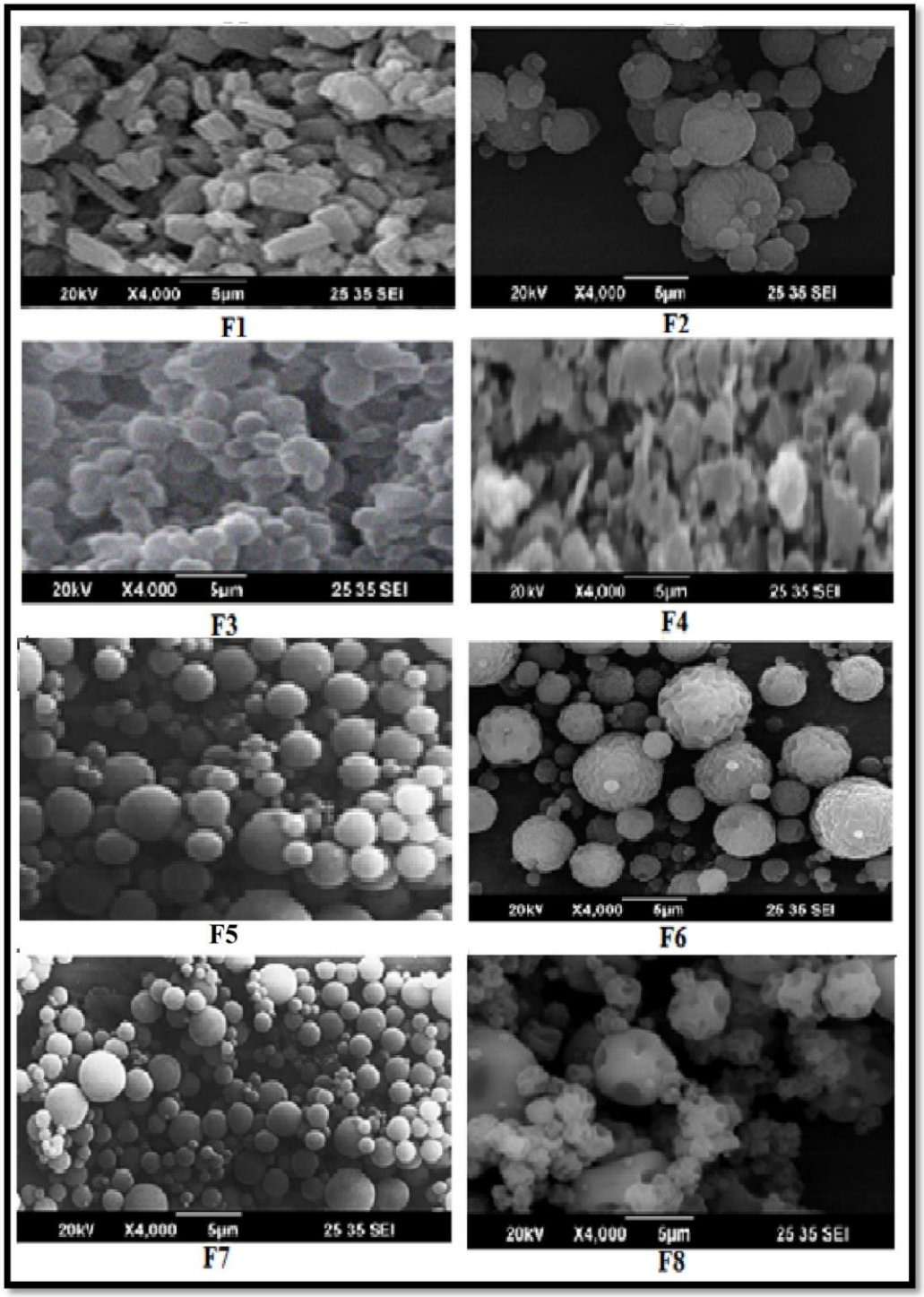
S. No	Formulation Code	Mean Particle Size* (nm)	Polydispersity*
1	F1	194 ± 1.32	0.51 ± 0.14
2	F2	317 ± 5.24	0.43 ± 0.12
3	F3	190 ± 1.36	0.50 ± 0.08

4	F4	217 ± 2.36	0.52 ± 0.17
5	F5	148 ± 1.21	0.44 ± 0.11
6	F6	297 ± 2.16	0.51 ± 0.12
7	F7	128 ± 3.45	0.50 ± 0.04
8	F8	211 ± 2.53	0.43 ± 0.12

***Mean ± S.D. of three trials**

The FLT loaded PCL nanoparticles (F1 – F8) showed the particle size in the range of 128 ± 3.45 to 317 ± 5.24 nm.

Scanning Electron Microscopy (SEM):



Surface morphology of Flutamide loaded PCL nanoparticles shown, minute amount of

wrinkles and quite smooth surface, slight deformities are seen attached to the surface. The figure also shows a large amount of big NPs surrounding the smaller NPs. The NPs have clumps of polymer formed due to increased organic solvent and attached to the outer surface during the formulation process.

Entrapment Efficiency:

Table –Drug Entrapment Efficiency of FLT loaded PCL NPs (F1 – F8)

S. No	Formulation Code	Entrapment Efficiency* (%)
1	**F1**	**85 ± 0.76**
2	**F2**	**92 ± 0.70**
3	**F3**	**78 ± 0.86**
4	**F4**	**89 ± 0.84**
5	**F5**	**79 ± 0.63**
6	**F6**	**88 ± 0.82**
7	**F7**	**75 ± 0.66**
8	**F8**	**86 ± 0.63**

* Mean □ S.D. of three determinations

Nanoparticles prepared with interfacial deposition / nanoprecipitation method could achieve higher encapsulation efficiency than with the simple emulsion technique. Drug entrapment efficiency of PCL nanoparticles (F1 – F8) varied from 75% ± 0.66 to 92% ± 0.70.

Drug Content:

Table – % Drug Content of FLT loaded PCL NPs (F1 – F8)

S. No	Formulation Code	Drug content* (%)
1	F1	87 ± 0.75
2	F2	92 ± 0.53
3	F3	79 ± 0.62
4	F4	88 ± 1.05

5	F5	79 ± 0.86
6	F6	81 ± 0.55
7	F7	74 ± 0.72
8	F8	76 ± 0.63

*(Mean □ S.D. of three determinations)

The increase polymer concentration, decrease organic solvent volume and increased surfactant may inhibit drug diffusion during organic solvent evaporation and increase drug polymer interaction results increasing drug content.

Percentage Yield:

Table –Percentage yield of FLT loaded PCL NPs (F1 – F8)

S. No	Formulation Code	Percentage Yield*
1	F1	48.24 ± 1.24
2	F2	84.29 ± 1.30
3	F3	46.05 ± 1.56
4	F4	82.13 ± 1.37
5	F5	52.78 ± 1.56
6	F6	86.78 ± 1.32
7	F7	50.25 ± 1.42
8	F8	83.28 ± 1.21

*Mean □ S.D. of three determinations

The % yield of NPs was recorded and it was determined by collected the NPs and weighed. The weight NPs was divided by the weight of starting materials, which were used in the NPs preparation. The percentage yields of PCL nanoparticles (F1 – F8) showed in the range of 46.05 % ± 1.56 to 86.78% ± 1.32.

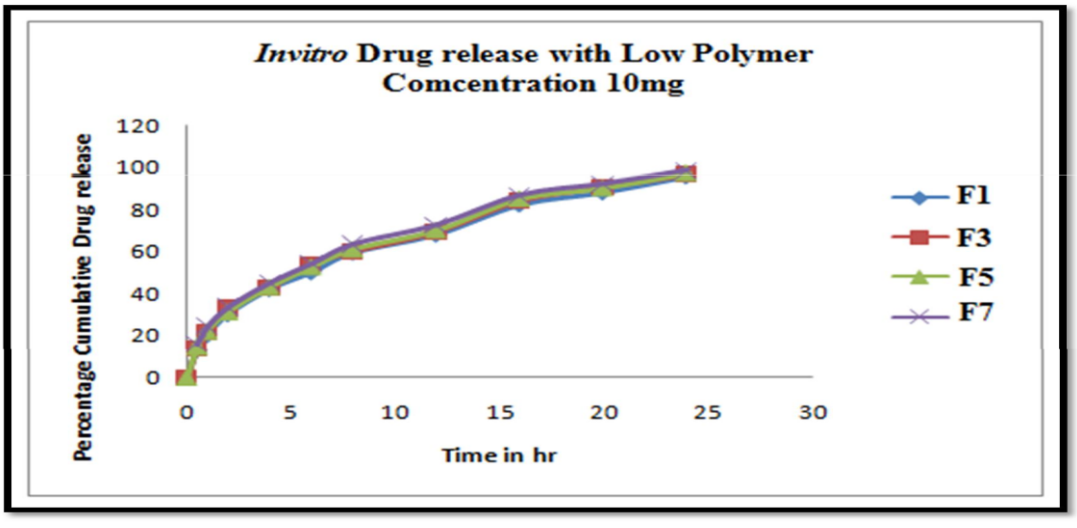


Figure –*Invitro* Drug Release of FLT loaded PCL NPs (F1 – F8) with Low Polymer 10 mg

The drug release showed two phases, a fast release followed by a slow release rate. The in vitro release exhibited initial fast release of first 4 hr followed by a slower and constant sustained release over 24 hr.

In vitro release studies:

The in vitro release profile of the prepared polymeric nanoparticular drug delivery system was studied by diffusion across the artificial membrane. Nanosuspension containing known concentration of drug (20 mg) was placed in the donor compartment and pH 7.4 PBS was placed in the receptor compartment and constantly agitated using a magnetic stirrer at 100 rpm and 37 °C. Samples of 0.5 ml were withdrawn from the receptor compartment for estimation of released drug and replaced with similar volume of buffer. This study was carried out for 24 hr, and the concentration of drug release was estimated by determining the absorbance at 228 nm using UV spectrophotometer. The experiment was carried out in triplicate, and the values are reported as mean value±standard deviation (Yadav, N. B. V, et al., 2016).
The conditions used for studying the drug release from the nanoparticles are: Apparatus : Franz Diffusion cell

Agitation speed (rpm) : 100

Medium : 20 ml of pH 7.4 PBS

Temperature : 37.0 ± 0.5 ºC

Time : 0 to 24 hr

Wavelength : 228 nm

In-Vitro Cytotoxicity Study:

Preparation of test solutions: The FLT and FLT loaded NPs were separately dissolved in distilled Dimethyl sulfoxide (DMSO) and prepared a stock solution of 1 mg/ml concentration, the volume was then made up with DMEM supplemented with 2% inactivated FBS. For cytotoxic studies, two fold serial dilutions were made.

Determination of cell inhibition by MTT assay: Cell proliferation was determined using the 3-[4, 5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide] (MTT) assay

(Fattahi1, A., 2018). The cell count was adjusted to 1.0×105 cells/ml using DMEM containing 10% FBS. A 96 well microtitre plate was used. To each well, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added and let it as such for 24 hr. Upon the formation of the partial monolayer, the supernatant was flicked off and increasing concentrations of the FLT and FLT loaded NPs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO2 atmosphere, and microscopic examination was carried out and noted. After 72 hr, the drug solutions in the wells were replaced with 50: l of MTT in PBS. The plates were gently shaken and incubated for 3 hr at 37°C in 5% CO2 atmosphere. The supernatant was replaced with 100: l of propanol and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a ELISA micro plate reader at a wavelength of 570 nm and the percentage growth inhibition was calculated. The concentration of test drug needed to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line (Jia, L., 2010).

Cell inhibitory rate % = 1-Abs of test cells/Abs of control cells X 100

Conclusion:

New approaches are being studied and investigated for the drug delivery to prostate cancer cells, since there are several limitations and side effects associated with the conventional formulations. Polymeric nanoparticles have been selected as promising candidates for drug delivery to the prostate cancer, presenting numerous advantages over conventional chemotherapy such as enhancing the drug solubility, protecting it from the macrophage uptake, thus increasing drug residence time in the blood, and providing controlled drug delivery to the target prostate cancer.

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