DESIGN AND EVALUATION OF FLUTAMIDE-LOADED POLYCAPROLACTONE NANOPARTICLES BY $2^3$ FACTORIAL DESIGN AND NANOPRECIPITATION METHOD

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ABSTRACT

Objective: The present work was aimed to prepare and evaluate polymeric nanoparticles (NPs) of flutamide by nanoprecipitation method and factorial. The influences of various formulation components such as polymer, organic phase volume, and surfactant on the characteristics of NPs were investigated.

Methods: The polycaprolactone (PCL) loaded with drug was evaluated for surface morphology, surface charge, particles size, encapsulation efficiency, drug content, and in vitro release studies. Fourier transform infrared studies were indicated no interaction between the drug and polymer.

Results: The results of the drug release study of NPs may fit with different kinetic equations. The particle size varied from 128 to 317 nm and zeta potential was in negative and its value found to be - 46.4 mv. The content of flutamide was found in between 74%±0.72 to 92%±0.53 in flutamide loaded PCL NPs. The minimum and maximum entrapment efficiencies were found to be of 75%±0.66 and 92%±0.70. The percentage yields of all formulations were in the range of 46.05%±1.56–86.76%±1.32. The in vitro drug release followed zero order with sustained behavior for a period of 24 h. Results of accelerated storage conditions of optimized formulation revealed that no significant changes in formulation F2.

Conclusion: The present investigation opens new frontiers in developing flutamide NPs for targeting delivery to the prostate for the prostate cancer treatment which also overcome the problems associated with conventional formulations such as multiple-dose therapy, poor patient compliance, and high treatment cost.

Keywords: Nanoprecipitation method, Polymeric nanoparticles, Prostate cancer.

INTRODUCTION

Among all types of cancers, prostate cancer stands sixth by being fatal to men all over the world. By 2030, prostate cancer is likely to increase manifold, by 1.7 million new patients and 499,000 deaths, increase and aging of population being the reasons [1]. It is mostly observed in men between 65 and 75 years of age, with 25% of the total number in men <65 years. Surgery, hormone therapy, radiation therapy, and chemotherapy are the treatments presently available to cure this deadly disease, which are either invasive or cause severe side effects.

Advanced prostate cancer is treated using flutamide, an effective nonsteroidal antiandrogen. The androgen receptors on the cancer cells are blocked and inhibit the growth of the cells dependent on androgens. Usually, flutamide is prescribed 250 mg as oral dose, thrice a day as its oral absorption is fast and reaches the highest level of plasma concentration in 1 h after giving the dose [2]. The drug has high first-pass hepatic metabolism and low elimination half-life of 5–6 h. Low functionality of flutamide is due to its rapid metabolism, less active metabolites (hydroxyl flutamide), and low bioavailability. Low bioavailability may be due to its reduced wettability and low solubility in water which reduces testosterone only on a continuous basis. Moreover, high dose of flutamide produces hepatotoxicity. Diarrhea, fatigue, impotence, enlargement of male breast, and malfunction of liver may be included among the side effects caused with the use of flutamide. A sustained release formulation of flutamide can be adopted to lessen the rate of drug administration and also to minimize the undesirable effects. Hence, the preparation of flutamide formulations with high plasma half-life, slow and constant release which can deliver to the target tissue is important. Preparation of nanoparticles (NPs) is a method that makes it possible to increase the bioavailability. It also helps in reducing the occurrence and harshness of adverse effects, significantly lessening the gastrointestinal disorders and hepatic problems [3].

The novel controlled release polymeric NPs with the active agent improve its bioavailability and compliance, leading to the lessening of drug toxicity and adverse effects. Due to their daily usage, low bioavailability or high toxicity and parenteral controlled release systems gain significance for drugs [4]. To progress compliance, biodegradable injectable delivery system can be used to transport drugs and to safeguard the blood level in a required therapeutic range for a long duration. The conventional dosage forms have a lesser advantage when compared to the design of the controlled release NPs. The following are the advantages of using the novel design of the controlled release NPs [5]:

- The frequency of drug administration is controlled
- Fluctuations in drug level are lessened
- The total drug used in comparison to conventional treatment is reduced
- Drug toxicity levels are reduced (local/systemic)
- Medical condition is stable due to the uniformity in drug levels
- Economical for pharmacists and patients.

Many varieties of polymers are used in the field of pharmacy for controlled drug delivery. Synthetic manipulation of these is used in many applications as in biofilms, nanoparticles, and microparticles. Poly-D, L-lactide-co-glycolide (PLGA), polyactic acid (PLA), or polycaprolactone (PCL) are the most common polymers which are made use of. In the present study, PCL was chosen as the material for the particle matrix. ~60°C is its glass transitions temperature which is very low and its melting point ranges from 59 to 64°C depending on...
chain length (molecular weight). In targeted cancer therapy, PCL is the ideal material for controlled drug delivery, as its permeable to many drugs and is not toxic [6]. Its biocompatibility with a slow degradation rate makes it ideal for long-term delivery. It is widely used in the pharmaceutical industry due to its being a semi-crystalline polymer and, therefore, used for long-standing drug delivery systems which target the specific tissues of the body. As it is cheap and has reduced levels of toxicity, it is most economical and viable among the three polymers (PLGA, PLAG, and PCL) [7]. At present, hormone-altering drug (FLT) is not encapsulated within PCL NPs. To instigate controlled release of the active agent, these types of drugs are ideal to be encapsulated within PCL NPs. In the human body, the polymer PCL undergoes hydrolysis of the ester linkages and is observed to have a slower rate of degradation compared to the other polymers derived from lactides [8].

Hence, the present study was aimed to the preparation of flutamide-loaded PCL NPs using nanoprecipitation method and evaluation of NPs for its physiochemical characteristics and in vitro release studies.

**METHODS**

Flutamide was purchased from Yarrow Chem Products, Mumbai. PCL and Pluronic F-127 purchased from Coastal Chemical Limited, Visakhapatnam, and all other ingredients used were of analytical grade obtained from obtained from Qualigens Fine Chem, Mumbai.

**Preformulation studies**

**Organoleptic properties**

Flutamide was evaluated for organoleptic properties such as appearance and color.

**Solubility analysis**

The solubility of flutamide was checked in water and it was confirmed by quantitative determination using ultraviolet (UV) spectroscopy at 228 nm.

**Melting point determination**

Melting point is the first indication of purity of the sample and the presence of small amount of the impurity can be detected by lowering or widening the melting point range.

**Identification of pure drug**

Fourier-transform infrared (FTIR) spectroscopy was used for the identification of flutamide.

**Determination of λ<sub>max</sub>**

Preparation of stock solution

About 10 mg of flutamide was transferred in a 100 ml volumetric flask and methanol was added in small proportion (20 ml) to dissolve flutamide. The volume was made up to 100 ml with PBS pH 7.4 to get 100 μg/ml concentrations.

Determination of λ<sub>max</sub>

About 20 μg/ml of flutamide was prepared from above stock solution and the solution was scanned between 400 and 200 nm to determine the λ<sub>max</sub>.

**Compatibility study**

A stable and effective dosage form was formulated depends on selection of excipients that are promote the drug release and bioavailability and protect it from degradation. If the excipients not were used in formulation containing the active drug, the compatibility study is mandatory.

**Drug-polymer compatibility studies by FTIR**

IR spectra of flutamide and formulation mixture were recorded by dispersion of drug and polymer in suitable solvent using FTIR spectrophotometer. A baseline was made using dried KBr, and then, the spectra of mixture of drug, polymer mixture, and KBr were recorded on FTIR [9].

**Calibration of standard curve**

In 100 ml of pH 7.4 phosphate buffer solution, 100 μg of accurately weighed flutamide was dissolved. 10 ml of this solution was further diluted with 7.4 pH phosphate buffer to get 100 μg/ml solutions. From the above stock solution, different concentrations (2–20 μg/ml) were prepared with pH 7.4 phosphate buffer. From each concentration, sample was taken and the absorption was measured at 228 nm using UV spectrophotometer and pH 7.4 phosphate buffer as a blank. The calibration curve was plotted and the regression equation and correlation coefficient were determined.

**Preparation of NP’S**

**2<sup>nd</sup> Factorial design**

The optimization phase was carried out statistically using 2<sup>3</sup> factorial design in which the polymer concentration, stabilizer, and organic solvent volume were kept at two different levels (Table 1). The eight formulations were categorized into four groups (given below) for ease of analysis and comparison.

- **Group I:** All variables at low level (formulation F1).
- **Group II:** Any one of three variables at high level (formulations F2, F3, and F5).
- **Group III:** Any two of three variables at high level (formulations F4, F6, and F7).
- **Group IV:** All three variables at high level (formulation F8).

**Nanoprecipitation method**

Flutamide-loaded PCL NPs were prepared by the nanoprecipitation method [10]. This method involves the precipitation of polymer from an organic solvent and diffusion of organic solvent into aqueous phase in the presence of a surfactant. PCL and flutamide 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 conditions. The obtained suspension was stirred at 500 rpm for 2 h to evaporate acetone. The suspension was cooling centrifuge at 11,000 rpm for 30 min to remove precipitants and supernatant was collected, lyophilized, and stored at 4°C. Repeated the procedure by varied in the concentrations of polymer, stabilizer, and organic solvent volume to optimize the formulation. Blank NPs were prepared by the same procedure but excluding flutamide.

**Characterization of prepared NP’S**

The obtained formulations of flutamide-loaded PCL NPs were characterized for following parameters.

**Particle size analysis and surface morphology**

**Particle size analysis**

Particle size measurement was carried out by Photon Correlation Spectroscopy (PCS) (Malvern Instruments). Samples were diluted with ultra-purified water, measured at 25° and 90° scattering angles, recorded for 180 s and the mean diameters for all samples were obtained by cumulative analysis in triplicate.

The morphological study of NPs was carried out using scanning electron microcopy (SEM) (Tecnai 20 G2 S TWIN). SEM was employed to understand the arrangement and orientation of molecules within the NP to determine its behavior and stability.

**Surface charge determination**

NPs were characterized for zeta potential (ζ) using a Zetasizer [11].

**Entrapment efficiency (EE)**

EE percentage is also known as association efficiency [12]. This was studied by centrifuging the drug-loaded NPs at a high speed of 3500–4000 rpm for 30 min and the supernatant was assayed for free drug
concentration using spectrophotometer. EE was then calculated as follows:

\[ \text{EE} \% = \frac{\text{Actual amount of drug added} \times \text{Unbound drug (Free drug)}}{\text{Actual amount of drug added}} \times 100 \]

Drug content
Free drug in the supernatant (drug content) was estimated by centrifuging the NPs suspension at 15,000 rpm for 40 min at 25°C. Concentration of flutamide present in the supernatant was determined by UV spectrophotometer at 228 nm [13].

Percentage yield
It was determined by the equation [12].

\[ \text{Percentage yield} = \frac{\text{Weight of dried nanoparticles recovered}}{\text{Sum of initial dry weight of starting material}} \times 100 \]

In vitro release studies
In vitro release profile of the prepared NP suspensions was studied by diffusion method using an artificial membrane. In the donor compartment, nanosuspension containing known concentration of drug (20 mg) was placed, and in the receptor compartment, pH 7.4 phosphate buffer was placed and constantly agitated using a magnetic stirrer at 100 rpm and 37°C. 0.5 ml samples were withdrawn from the receptor compartment for the estimation of released drug and replaced with similar volume of buffer. This study was carried out for 24 h, and the concentration of drug released was determining the absorbance at 228 nm using UV spectrophotometer [10].

Kinetic modeling
To understand the kinetics and mechanism of drug release from optimized formulation F2, the results of in vitro release study were fitted with zero-order, first-order, and Higuchi’s model [14].

Stability study
The stability study was carried for the formulation F2. The stability was determined in terms of its drug content, EE, and in vitro drug release. The formulation was incubated for a period of 6 months at 37±1°C [10], and at specified time intervals, the suspension was centrifuged at 4000 rpm for 1 h. The amount of drug present in the

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Fig. 1: Infrared spectra of flutamide

Fig. 2: \( \lambda_{max} \) of flutamide

Fig. 3: Infrared spectra of flutamide
supernatant was determined by UV-vis spectrophotometer at 228 nm.

RESULTS AND DISCUSSION

Preformulation studies

Organoleptic properties
Flutamide has got buff to yellow color, unpleasant smell and found to be very fine crystalline powder.

Solubility studies
Flutamide was low soluble in water and freely soluble in organic solvents. The solubility in water was found to be 0.0094 mg/ml.

Melting point determination
The melting point of pure drug flutamide was determined in the range of 111–113°C by capillary method.

Identification of pure drug
FTIR spectrum of flutamide in Fig.1 has showed the peaks at 3355, 1709, 1509, 1314, and 654 cm$^{-1}$. The absorption bands between 3250 and 3400 cm$^{-1}$ were indicated the presence of –NH stretching. The wave numbers observed at 1709 and 1314 may be assigned to the C = O and C - N bonds, respectively, and the sharp peak occurred at 1509 and 654 indicate the presence of N = O and C – F$^3$ group attached to C = C.

Determination of $\lambda_{\text{max}}$
The flutamide with pH 7.4 PBS was scanned between 400 and 200 nm with UV-vis spectrophotometer and the $\lambda_{\text{max}}$ was found to be at 228 nm. Hence, the standard curve of flutamide was developed at 228 nm (Fig. 2).

Drug-excipient compatibility studies
FTIR spectroscopy
FTIR studies used to determine the interaction between drug and excipients used in NP preparation. The mixture of drug and excipients was prepared in 1:1 w/w ratio and used for IR analysis.

IR spectra of flutamide were recorded over than 600–4000 cm$^{-1}$ using Brooker spectrophotometer with KBr pellet method.

Inference
IR spectra of formulation had showed absorption peaks (Figs. 3 and 4) which were comparable with absorption peaks of pure drug. The peaks indicated that, no chemical interaction between drug and excipients.

Standard graph of flutamide
The standard curve of flutamide was done using pH 7.4 PBS as the medium and the maximum absorbance was found at 228 nm. The standard graph was constructed between 2and 20 µg/ml concentrations. Absorbances were examined under UV-visible spectrophotometer at an

Table 1: $2^2$ Factorial design of NPs

<table>
<thead>
<tr>
<th>Factor</th>
<th>Low level (−1)</th>
<th>High level (+1)</th>
</tr>
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<tbody>
<tr>
<td>Polymer</td>
<td>10 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Stabilizer</td>
<td>1%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Organic solvent</td>
<td>10 ml</td>
<td>30 ml</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>85±0.76</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>92±0.70</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>78±0.86</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>89±0.84</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>79±0.63</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>88±0.82</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>75±0.66</td>
</tr>
<tr>
<td>8</td>
<td>F8</td>
<td>86±0.63</td>
</tr>
</tbody>
</table>

Table 2: Drug entrapment efficiency

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>48.2±1.24</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>84.2±1.30</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>46.0±1.56</td>
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<td>4</td>
<td>F4</td>
<td>82.1±1.37</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>52.7±1.56</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>86.7±1.32</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>50.2±1.42</td>
</tr>
<tr>
<td>8</td>
<td>F8</td>
<td>83.2±1.21</td>
</tr>
</tbody>
</table>

Table 3: Percentage yield

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation</th>
<th>Code</th>
<th>0 day</th>
<th>30 days</th>
<th>60 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>F2</td>
<td>92±0.70</td>
<td>91±0.23</td>
<td>90±0.24</td>
<td>90±0.21</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>F2</td>
<td>92±0.53</td>
<td>91±0.05</td>
<td>91±0.26</td>
<td>90±0.04</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>F2</td>
<td>77%±1.32</td>
<td>76%±1.54</td>
<td>75%±1.72</td>
<td>74%±1.63</td>
</tr>
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</table>
absorption maximum of 228 nm. The standard graph was constructed by taking the absorbance on Y-axis and concentrations on X-axis. Drug concentration and absorbance followed linear relationship the curve obeyed Beer–Lambert’s law, and the correlation coefficient value ($R^2$) is 0.998.

Preparation of NPs
Nanoprecipitation method was used for the preparation of flutamide-loaded NPs. The formulations were designed by 2$^3$ factorial design, which contained three variables such as polymer concentration, stabilizer, and organic solvent volume at two levels. Total eight batches were formulated (F1–F8) and all the formulations were investigated for various parameters.

Characterization of prepared NPs
Size analysis
Eight formulations were developed by varying the concentrations of polymer, stabilizer, and volume of organic solvent at two levels, and the effect on particle size was determined (Figs. 5 and 6). Formulation F7 showed minimum particle size of 128 nm and the formulation F2 showed maximum particle size of 317 nm. The results indicate that the particle size increased with increasing polymer (PCL) concentration of 10 and 50 mg due to agglomeration of the particles [15]. The particle size decreased with increasing organic phase volume of 10 and 30 ml and the particle size decreased with increasing Pluronic F-127 from 1% to 1.5%.

Fig. 5: Size analysis of nanoparticles formulations F1–F8
SEM image (Fig. 7), F1 shown formed NPs at an average diameter of 194 nm with a minute amount of wrinkles on the surface. While the image of F2 shown two different sizes with less uniformity. The image also displays a large amount of larger NPs surrounding the smaller NPs with average diameter of 317 nm. On observing image F3 and F4 showed NPs at an average diameter of 190 nm and 217 nm. Image F5 showed a complete spherical-shaped NP at an average diameter of 148 nm. Image F6 showed NPs at an average diameter of 297 nm that have minimal wrinkles or porous structure on the exterior. Image F7 showed fully formed spherical-shaped smooth NPs with an average diameter of 128 nm. SEM image F8 shown NPs at an average diameter of 211 nm. SEM image F8 shown formed NPs at an average diameter of 148 nm. SEM image F8 shown formed NPs at an average diameter of 128 nm. SEM image F8 shown formed NPs at an average diameter of 211 nm.

Surface charge (zeta potential)
The zeta potential of the formulation F7 shown in Fig. 8 was found to be -46.4, which implies that it is having good stability. The negative zeta potential values of formulation F7 can be attributed to the presence of uncapped end ionized carboxyl groups of the polymer at the particle surface [16].

Drug content
NPs obtained from F2 formulation showed maximum drug content (92%±0.53) and F7 showed minimum drug content (74%±0.72). Drug content was increased with increasing polymer concentration of 10 and 50 mg. It was decreased with increased acetone volume of 10 and 30 ml and decreased significantly with increasing amount of surfactant Pluronic F-127. The drug content of NPs were shown in Fig. 9.

EE
On increasing the polymer concentration, increasing (10 and 50 mg) EE was observed, whereas increase in acetone volume (10 and 30 ml) has slightly decreased the EE, and increase in surfactant Pluronic F127 (1%–1.5% w/v) has significantly decreased EE. Drug EE varied from 75%±0.66 to 92%±0.70 (Table 2). The formulation F2 stabilized with 1% w/v Pluronic F127 and 1:1 ratio of organic phase:aqueous phase volume showed maximum EE 92%±0.70.

Percentage yield
The percentage yield of NPs prepared by nanoprecipitation method was recorded and shown in Table 3, it was determined by collecting the NPs and weighed. The measured weight was divided by the initial dry weight of starting materials, which were used for the preparation of the NPs. It was found that when concentration of PCL increased, the percentage yields also increased. It was decreased with increased organic phase acetone volume and increased significantly with increasing amount of surfactant Pluronic F-127. The percentage yields of NPs of all formulations were in the range of 46.05%±1.56–86.78%±1.32. The formulation F2 stabilized with 1% w/v organic phase:aqueous phase volume showed maximum percentage yield of 86.78%±1.32 compare to other formulations.

In vitro release studies
The flutamide PCL NPs have exhibited fast release in the first 4 h and slow/continuous sustained release in the next 20 h. The fast release of drug was attributed to surface associated drug, whereas the slow release was attributed to the drug entrapment in NPs. The drug release was slowed down due to the increased viscosity and diffusion path as the polymer ratio was increased. This was because of high polymer concentration (50 mg PCL - F2, F4, F6, and F8) showed slow drug release for more than 24 h (Fig. 10b). Low polymer concentration (10 mg PCL - F1, F3, F5, and F7) showed almost complete drug release within 24 h (Fig. 10a).

Increase in organic phase volume increases the rate of release of the drug (Fig. 10d) due to increased diffusivity and hydrodynamics at the interface. A slight rise in drug release was noticed, Pluronic F127 concentration was increased (Fig. 10f). The size of NP plays a major role in drug release, i.e., smaller the size of NPs higher the drug release rates. The formulation F7 containing particle size of 128 nm had shown maximum drug release of 98.23%±1.24 compare to other formulations. The flutamide NPs (F2) prepared with 50 mg PCL, 10 ml acetone, and 1% Pluronic F127 had shown better controlled and sustained drug
release of 77.42%±1.32 within 24 h. From the results of EE (92%), drug content (92%), and in vitro release studies (77.42%), formulation F2 was considered as optimized trial.

**Kinetic modeling of drug release**

The exact mechanism of drug release of F2 formulation was subjected to kinetic models. The R squared value of zero order and first order was found as 0.939 and 0.923, respectively. This result suggests that the drug released by zero-order kinetics. The R squared value of Higuchi’s and Peppas diffusion equation was obtained as 0.993 and 0.710, respectively. This result suggests that the drug released followed diffusion mechanism.

**Accelerated stability studies**

The optimized formulation F2 was sealed in an aluminum foil and subjected to stability studies. The result showed in Table 4, that there were no significant changes occur during storage after 90 days.

**CONCLUSION**

Polymeric NPs of flutamide were successfully prepared and evaluated by nanoprecipitation method and factorial design. The influences of various formulation components such as polymer, organic phase volume, and surfactant on the characteristics of NPs were investigated. Esthetic results proved that formulation F2 containing 50 mg PCL and 10 ml acetone with 1% Pluronic F127 produced the most ideal NPs with encapsulation efficiency and controlled drug release within 24 h. This suggest that, the daily administration of single low dose of F2 NPs, as the rate of release of prepared flutamide NPs is less than the half-life of free flutamide (8 h). Therefore, this investigation opens new frontiers in developing flutamide NPs for targeting delivery to the prostate for the prostate cancer treatment which also overcome the problems associated with conventional formulations such as multiple dose, high treatment cost, and poor patient compliance.

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**AUTHORS’ CONTRIBUTIONS**

All authors contribute equally to this manuscript.

**CONFLICTS OF INTEREST**

None of the author has any conflicts of interest in the context of this work.

**REFERENCES**

10. Yadav BV, Ravichandiran V, Kumar SS. Preparation and characterization of gemcitabine loaded npeg-PCL polymeric nanoparticles for improved transportation across blood brain barrier. Int J Pharm Pharm Fig. 10: In vitro drug release of formulations. (a) Low polymer 10 mg (b) high polymer 50 mg (c) low organic phase volume 10 ml (d) high organic phase volume 30 ml (e) 1% Pluronic F127 (f) 1.5% Pluronic F127.

Author Queries???
AQ3: Kindly cite figure 10 (c and e) in the text part