Ind. J. Pharm. Edu. Res., 2024; 58(4):1184-1188.
https://www.ijper.org

Original Article

Enhancement of Solubility and Dissolution Rate of Poorly Soluble Anticancer Drug Using Nanostructured Lipid Carrier Based Drug Delivery System

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ABSTRACT

Background: Prostate cancer is the leading cause of death in men over the age of 50, making it one of the most common cancers. Current treatments available such as chemotherapy, surgery are associated with many side-effects. Due to non-specificity and poor solubility of many anticancer drugs, less amount of drug is available at the absorption site. The current study aimed to develop Nanostructured Lipid Carriers (NLCs) of the poorly soluble anticancer drug Flutamide. **Materials and Methods:** Flutamide-loaded Nanostructured Lipid Carriers (NLCs) containing biocompatible lipids Precirol ATO 5 (Solid lipid), flaxseed oil (Liquid lipid) and surfactants were synthesized using the melt emulsification ultrasonication method. Koliphor RH40 and Tween 80 were used in combination in 1:1 ratio. **Results:** The interaction of drug and excipients was investigated using Fourier Transform Infrared (FTIR). Phase transition of Flutamide was confirmed using differential scanning calorimetry during the processing of NLCs. Solubility Flutamide increased significantly when it was prepared in the form of NLCs. Flutamide-loaded NLCs were found to be eighteen times more soluble in distilled water than the pure drug. Flutamide loaded NLCs were five times, three times, and four times more soluble in pH 1.2, 6.8, and 7.4 PBS than the pure drug. **Conclusion:** According to the findings, Flutamide can be formulated into nanostructured lipid carriers with improved solubility.

Keywords: Flutamide, Solubility, Nanostructured lipid carriers, Lipids.

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Received: 13-03-2023;
Revised: 23-03-2023;
Accepted: 22-05-2024.

INTRODUCTION

Prostate cancer is the leading cause of death in men over the age of 50, making it one of the most common cancers.[1] It is characterized by uncontrolled growth of cells in the prostate gland, which can spread to other parts of the body over time. Initially, cells grow slowly and remain localized, but they may spread when they begin to metastasize to other organs via lymphatic circulation and blood stream. Although organ-confined tumors can be treated with radical prostatectomy, many patients develop advanced metastatic disease. The high mortality rate is due primarily to the lack of a curative option for this stage of prostate cancer.[2]

Flutamide is a non-steroidal substance with anti-androgenic characteristics. Chemically it is 2-methyl-N-[4-nitro-3-(trifluoromethyl) phenyl] propanamide. It is used to treat prostatic carcinoma palliatively.[3] It works by preventing the receptor from recognizing and/or absorbing dihydrotestosterone.[4] Flutamide has been found to be both safer and more effective than steroidal anti-androgens due to its increased specificity for androgen receptors.[5] Its poor aqueous solubility, rapid hepatic metabolism, short half-life, and hepatotoxicity, however, frequently affect its clinical efficacy.[6] As a result, developing novel formulations that enhance solubility and dissolution rate of Flutamide will result in its higher bioavailability.

Lipid-based systems such as liposomes, Solid Lipid Nanoparticles (SLNs), and Nanostructured Lipid Carriers are promising carriers for a lipophilic molecule (NLCs). This could be due to their ability to improve the solubility of lipophilic drugs.[7,8] SLNs made from solid lipids had a number of drawbacks, including drug expulsion and less drug payload. As a result, NLCs have been developed to overcome drawbacks of SLNs. NLCs were fabricated by combining solid and liquid lipids, which resulted in the formation of lipid matrix imperfections.[9] The imperfect lipid matrix prevents leakage of the drug and ensures high drug loading.[10-12] The objective of the research work was to improve solubility of Flutamide. First, Flutamide NLCs were fabricated and tested for various physicochemical parameters and *in vitro* dissolution properties.

DOI: 10.5530/ijper.58.4.130

MATERIALS AND METHODS

Materials

Precirol ATO 5 was gifted by Gattefosse, Mumbai, India. Flutamide was procured from Cipla Limited, Bangalore, India. Flaxseed oil, Tween 80, and Koliphor RH 40 and all other chemicals were purchased from Loba Chemicals, Mumbai, India.

Formulation of Flutamide-loaded NLCs

The melt emulsification ultrasonication technique was used to prepare Flutamide-loaded NLCs. Precirol ATO-5 (solid lipid) and flaxseed oil (liquid lipid) was melted together at temperatures higher than their melting points. Then, Flutamide was added to the molten mixture and stirred. Tween 80 and Koliphor RH 40 in a ratio of 1:1 were added in the distilled water to get the aqueous phase. The aqueous phase was heated to the same temperature as the lipid phase. The aqueous phase was then added to the hot lipid phase while being magnetically stirred at 70°C at 500 rpm to obtain pre-emulsion. The final dispersion was sonicated by using a probe sonicator (Branson ultrasonics, India) at 80% amplitude.

Characterization of Flutamide-loaded NLCs

Determination of Lambda max and standard curve of Flutamide

Dissolving 10 mg of pure Flutamide in 100 mL of methanol, pH 1.2 buffer and pH 7.4 PBS produced a stock solution of 100 ug/mL was prepared. The working solutions ranging 5-30 ug/mL were prepared by using stock solution and analysed at wavelengths ranging from 200 to 400 nm. The stock solution was appropriately diluted with methanol, pH 1.2 buffer, and pH 7.4 PBS in order to measure the absorbance.

Drug content

Flutamide-NLC 10 mg equivalent Flutamide was sonicated for 2 min after being dispersed in methanol and centrifuged at 10000 rpm for 10 min in a lab centrifuge (Remi Equipments, India). The supernatant was removed and its drug content was determined using a UV-spectrophotometer.

Particle size

The Dynamic Light Scattering (DLS) method was used to determine particle size of Flutamide-NLC.[13] Malvern Nano ZS (Malvern, UK) and Zetasizer were used to measure particle size, which was then analyzed by Zetasizer software using an average of 10 measurements. Before measuring particle size, the sample was diluted 500 times. The sample was equilibrated for 120 sec before being measured at 25°C.

Zeta potential

It is one of the most important factors influencing the stability of any colloidal formulation.[14] Zeta potential was measured using the zeta-sizer. Flutamide-NLCs were dispersed in distilled water to make 0.02% w/v prior to the measurement, which was then followed by 5 min of ultrasonication.

FTIR study

FTIR analysis was performed to identify any potential interactions between the individual components of formulation. FTIR Spectrophotometer (Shimadzu, Japan) was used to record the FTIR spectra of Flutamide, Precirol ATO 5, and Flutamide-NLCs. The spectra were analyzed in the 4000 cm^{-1} to 400 cm^{-1} scanning range, with a resolution of 4 cm^{-1} for each sample. Background scanning and correction were performed prior to each measurement.[15]

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was used for thermal analysis (DSC; Mettler-Toledo, Zurich, Switzerland). The calibration of the instrument was carried out using indium. Flutamide and Flutamide-NLC DSC thermograms were obtained. The samples (2 mg) were heated in a sealed aluminium pan at a heating rate of 10°C/min from 30 to 300°C. Nitrogen was continuously purged over the sample cell at a rate of 40 mL/min throughout the measurement.[16,17]

Determination of Solubility

Flutamide (100 mg) and Flutamide-NLCs (corresponding to 100 mg Flutamide) were added in 100 mL of distilled water, pH 1.2 buffer, and pH 7.4 PBS. For 12 hr, the flasks were shaken in a water bath at 25°C. Following appropriate dilutions, ten milliliters of the sample was taken and the dissolved drug was measured using UV spectrophotometer at 306 nm.

In vitro dissolution study

Cellophane membrane dialysis tubing (molecular weight cut off 12,000) was used for the *in vitro* drug release study.[18] The NLC formulation (3 mL) containing 5 mg of the drug was sealed in the dialysis bag with the drug release medium. The dialysis bag was then immersed in a beaker containing 200 mL of pH 7.4 PBS as the drug release medium and stirred at 50 rpm with a magnetic stirrer. A 10 mL aliquot was collected at predetermined intervals, and the sink condition was maintained by adding same volume of dissolution medium. The drug content of the withdrawn samples was determined using a "UV spectrophotometer" set to λ_{max} 295 nm.

RESULTS AND DISCUSSION

Determination of λ_{max} and standard calibration curve

The Flutamide with methanol, pH 1.2 buffer, and pH 7.4 PBS was scanned with a UV-vis spectrophotometer between 400 and 200 nm, and the max was found to be at 295 nm, 299 nm, and 301nm. Flutamide standard curves were developed using methanol, pH

1.2 buffer, and pH 7.4 PBS. The standard graph was built between concentrations of 5 and 30 µg/mL. Absorbance was measured using a UV-visible spectrophotometer at a wavelength of 295 nm, 299 nm, and 301 nm. The standard graph was developed by plotting absorbance on the Y-axis and concentrations on the X-axis. The relationship between drug concentration and absorbance was linear and the correlation coefficient (R^2) is 0.9993, 0.9999, and 0.9996.

Drug content

The total drug content of Flutamide-NLC was found to be 100.60±2.17%. Flutamide was encapsulated in the NLC system, as evidenced by this result.

Particle size

Particle size of Flutamide-NLC was significantly smaller, with a mean size of 29.54±3.12 nm (Figure 1) and a lower polydispersive index, indicating a narrow distribution of nanoparticles within the system.

Zeta potential

The Flutamide-NLCs showed a zeta potential of +12.7±2.42 mV 22 (Figure 1). The zeta potential denotes the particle stability in a continuous medium. A higher zeta potential value results in high particle repulsion and reduces the likelihood of particle aggregation.[19] A positive zeta potential value indicated that the Flutamide-NLCs had improved in terms of stability and dispersion.

DSC study

The thermal transition of components used in formulation is frequently determined using DSC. In addition, the crystallinity of drug and polymers in formulations can be evaluated using DSC. The thermograms of flutamide, and flutamide-NLC DSC were recorded (Figure 2). A sharp, single melting peak on the thermogram indicated the melting point of the crystalline Flutamide at approximately 115.4°C, whereas Flutamide-NLCs show melting points of 67.7°C.[20,21] This was attributed to the conversion of Flutamide the crystalline form to the amorphous form in the formulation.

FTIR study

Figure 3 shows the recorded FTIR spectra for Flutamide, solid lipid, and Flutamide NLCs. The FTIR spectrum of Flutamide showed the absorption bands corresponding to the principle functional groups. The absorption bands were recorded at 3360, 1716.55, 1390.68, and 1595.13 cm−1 for N-H stretching, carbonyl group, aromatic C=C stretching, and nitro group respectively. The FTIR spectra of Precirol ATO 5 showed absorption bands at 3500.80, 1732.08, and 1105.21 cm^{-1} corresponding to free OH, C=O, and C-O-C stretching vibrations. In the case of the Flutamide-NLCs showed no significant change in the IR spectra of Flutamide and excipients, demonstrating the compatibility of drug and polymer. The entrapment of the drug in the lipid matrix was confirmed by shifting of drug specific peaks by the lipids in the Flutamide-NLCs IR spectrum.

Solubility study

Flutamide loaded NLCs were found to be eighteen times more soluble in water (0.0493 mg/mL) than pure drug (0.00261 mg/mL), indicating the presence of a high amount of amorphous Flutamide in NLCs. Flutamide loaded NLCs were five times, three times, and four times more soluble in pH 1.2, 6.8, and 7.4 buffers (0.0746 mg/mL, 0.0653 mg/mL, and 0.0577 mg/mL) than the pure Flutamide (0.0127 mg/mL, 0.0130 mg/mL, and 0.01225 mg/mL). This may be because of the solubilizing effect showed by the components used in the formulation of NLCs.[22,23] This enhancement in the solubility of Flutamide could be due to the increased surface area, wettability, and solubilizing effect of components used in the formulations.

In vitro drug release study

As shown in Figure 4, Flutamide-NLCs showed higher drug release than that from the pure drug suspension in the pH 7.4 PBS dissolution medium. The percentage cumulative drug release of the Flutamide-NLCs and pure drug suspensions in pH 7.4 PBS was 69.09±5.8% and 43.67±2.36, respectively. Flutamide incorporation into NLCs drastically enhanced solubility and offered more space to entrap the drug in the lipid matrix, which resulted in a higher drug release from NLCs.

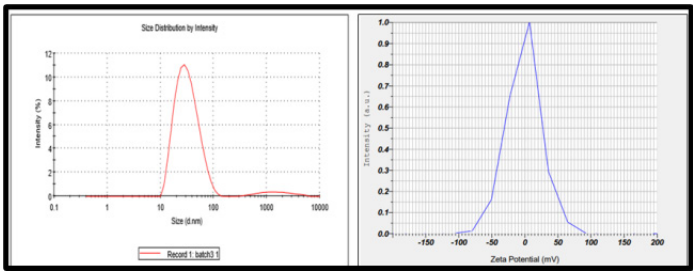

Figure 1: Particle size distribution and zeta potential of Flutamide-NLCs.

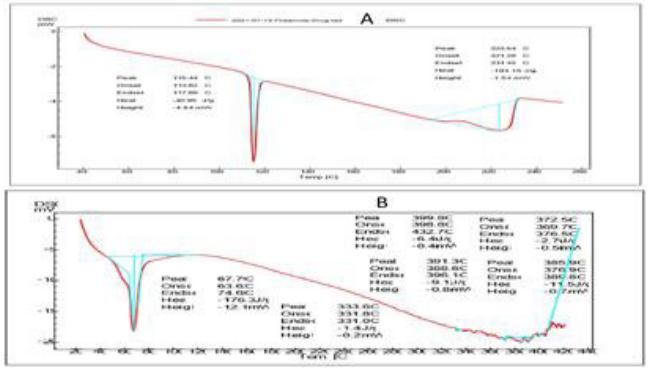

Figure 2: DSC thermograms of A) Flutamide B) Flutamide-NLCs.

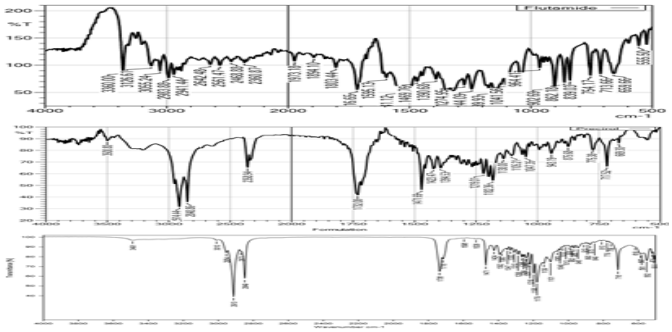

Figure 3: FTIR spectra of A) Flutamide B) Precirol ATO 5C) Flutamide-NLCs.

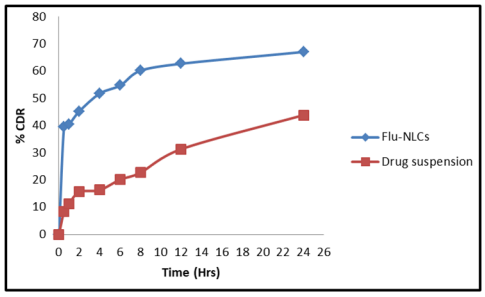

Figure 4: Comparative dissolution study of Flutamide suspension and Flutamide-NLCs.

DISCUSSION

Flutamide, a prostatic carcinoma anticancer drug, has poor aqueous solubility and oral bioavailability. NLCs are being developed to improve Flutamide solubility and thus bioavailability. The absence of crystallinity and the presence of the drug in an amorphous state are indicated by the shifting of the melting endotherm in the DSC thermogram of Flutamide NLCs. This could be due to transition of Flutamide from crystalline to amorphous phase in the NLCs.[24] Solubility of Flutamide could well have enhanced significantly due to the solubilizing effect of the components used in the preparation of NLCs. Flutamide may be more soluble in NLCs due to the increased surface area, wettability, and solubilizing effect of polymers used in the formulations.[22,23] When compared to pure Flutamide, the presence of hydrophilic surfactants that regulates drug release enhanced the initial dissolution rate of Flutamide in NLCs substantially. As a result, the developed lipid nanocarriers may have a better chance of improving Flutamide solubility.

CONCLUSION

The study found that Flutamide formulation containing biodegradable and biocompatible lipids is feasible for increasing Flutamide solubility and dissolution rate. These results showed transition of Flutamide from crystalline state to the amorphous state in the NLCs and nano range particle size could be the reasons for the enhancement of solubility. From the results, it can be concluded that NLCs are suitable for enhancing solubility and dissolution of Flutamide. This lipid-based nanocarrier considered to be a potential drug delivery system for the oral administration of Flutamide.

ACKNOWLEDGEMENT

The authors thank the Management and Principal for their support of our work on NLCs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NLCs: Nanostructured Lipid Carriers; **SLNs:** Solid Lipid Nanoparticles; **DSC:** Differential Scanning Calorimetry; **FTIR:** Fourier Transform Infrared; **DLS:** Dynamic Light Scattering; **PBS:** Phosphate Buffer Saline.

SUMMARY

Flutamide-loaded NLCs were prepared by melt-emulsification ultrasonication method with an aim to enhance solubility and dissolution rate of poorly water-soluble drug Flutamide. The drug was entrapped in the lipid matrix of Precirol ATO 5 and linseed oil. The particle size revealed nanometer range of Flutamide-loaded NLCs. DSC study showed reduction in crystallinity of Flutamide when formulated as NLCs. Solubility and drug release studies revealed enhancement in the solubility of Flutamide.

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Cite this article: Sabale V, Sabale P, Jiwankar M. Enhancement of Solubility and Dissolution Rate of Poorly Soluble Anticancer Drug Using Nanostructured Lipid Carrier Based Drug Delivery System. Indian J of Pharmaceutical Education and Research. 2024;58(4):1184-8.