

DESIGNING AND CHARACTERIZATION OF DOCETAXEL LOADED NANOPARTICLES WITH PIPERINE

Abstracts

Objective: The main objective of this research was to develop a novel formulation with an herbal bioenhancer to boost the bioavailability of an anticancer drug that was poorly soluble in water and overcome its limitations of short half-life.

Methods: Eudragit RLPO was used as a polymer in an emulsification solvent evaporation process to generate Docetaxel loaded bioenhancer (Piperine) nanoparticles and to evaluate its parameters.

Results: The study demonstrated that encapsulating Docetaxel with herbal bioenhancers (Piperine) in a nanoparticle system effectively enhanced its in vitro release profile. The particle size range (142 to 189 nm) is optimal range for enhanced cellular uptake and improved bioavailability for nanoparticle drug delivery, and the drug release from all batches was significant, with a release percentage ranging from 83.69% to 96.44% over 24 hours. This indicates a controlled release profile, which is desirable for maintaining therapeutic drug levels over an extended period.

The release data adhered to Fick's law of diffusion, suggesting that the drug release mechanism is diffusion-controlled. Higuchi's model best described the release kinetics, indicating that the release rate is proportional to the square root of time, which is typical for systems where the drug release is controlled by diffusion through a polymer matrix.

Conclusion: The study demonstrated that encapsulating Docetaxel with herbal bioenhancers (Piperine) in a nanoparticle system effectively enhanced its in vitro release profile. The particle size range (142 to 189 nm) is optimal for nanoparticle drug delivery, and the release kinetics following Higuchi's model confirm the controlled release mechanism. The significant finding that higher amounts of Piperine enhance drug release rates underscores the potential of using bioenhancers to improve the bioavailability of poorly water-soluble drugs like Docetaxel.

These promising in vitro results pave the way for further in vivo pharmacokinetic and cytotoxicity studies to fully assess the therapeutic potential and bioavailability improvements provided by this novel formulation.

Keyword: Anti-cancer, Bioenhancer, Docetaxel, Nanoparticle, Piperine,

Introduction

Recently, nanomedicines have generated a lot of interest in the creation of innovative drug delivery vehicles. The tiny compounds known as nanoparticles (NPs) have certain characteristics within the body (1). Moreover, NPs have the advantage of gradually delivering chemotherapy and other drugs to affected cells in a targeted or site-specific manner, improving efficacy and reducing harmful side effects (2).

Docetaxel (DOX) is a type of chemotherapy drug primarily used to treat various cancers (3) and it can be made by extracting semi-synthesis of taxol, from the European yew tree.

Docetaxel has demonstrated efficacy in treating breast, ovaries, lung, and head and neck cancers (4). Docetaxel antitumor effects are thought to be mediated through two mechanisms: Inhibition of microtubule depolymerisation (5) and attenuation of the effects of bcl-2 and bcl-xL gene expression (6). Docetaxel is nearly twice as potent as popular cancer medicine like paclitaxel. The Drug Docetaxel (DTX) is classified as a class IV drug in the bio-pharmaceutics categorization system (BCS). Drugs classified as IV in the Bio-pharmaceutics Classification System (BCS) have low bioavailability due to their poor water solubility. One of the drawbacks of utilizing Docetaxel in treatment is its low solubility. This drug's high toxicity and limited hydro solubility lead to decreased bioavailability and decreased efficacy (7, 8 and 9).

Docetaxel has low selective distribution, fast in vivo elimination, poor solubility, and other issues restrict its therapeutic usefulness (10). In order to enhance the pharmacokinetic and pharmacological properties of these drugs, nanocarriers have been developed recently. The unique surface characteristics and size ranges that nanocarriers provide help to improve the permeability, solubility, and bioavailability of drug. Nanocarriers are therefore frequently employed in the identification and management of cancer (11, 12, 29, 30). Hence, a nanoparticle-based delivery method was selected for this study.

A bioenhancer is a substance that is able to boost the bio-efficacy and bioavailability of a drug which it is paired with, without having any additional pharmacological effects of its own (13). When used with medications, bioenhancers boost and extend their availability without indicating the impact of drug interactions (14). The addition of a bioenhancer reduces the drug dosage, costs, resistance, and the chance of any adverse reactions or side effects.

Piperine is an alkaloid mostly derived from plants in the Piperaceae family, including long and black pepper (15). PPN, commonly referred to as the "king of spices," possesses exceptional therapeutic qualities against a wide range of illnesses (16). PPN has a strong ability to block the metabolizing enzyme CYP3A4 and the efflux transporter P-glycoprotein. P-gp functions essentially as an efflux transporter, facilitating the removal of many medicines from cells following intestinal absorption (24). Bioenhancers have been shown to increase the bioavailability of companion medications through transepithelial electrical resistance factor (TER)-controlled intestinal mucosal permeability (25) or inhibition of the drug-metabolizing enzyme cytochrome P450 (26).

Mechanism of Action of Piperine as a Bioenhancer:

Various theories have been proposed to explain how piperine improves biological function. An increase in gastrointestinal absorption is caused by:

- Bile acid increases solubility by aiding in the formation of micelles, which are required for the absorption of lipids and lipid-soluble medications. Inhibiting bile acid metabolism while simultaneously increasing bile acid secretion leads to an increase in production. This enhances solubility and absorption.
- Improved blood flow: According to a study by Annamali et al (28). Trikatu is said to enhance gastrointestinal blood flow, which raises the absorption of drugs from the digestive system.
- Through its interaction with intestinal epithelial cells, piperine triggers the activity of gamma-glutamyltranspeptidase, increasing the uptake of amino acids by the epithelial cells and leading to enhanced. (27)

The objective of this study was to encapsulate paclitaxel and an herbal bioenhancer in a nanoparticle system and evaluate it in vitro for several properties, including its pharmacokinetic profile and impact on a lung cancer cell line. The drug works extremely effectively for treating breast cancer, ovarian cancer, non-small cell lung cancer, and other tumors (17).

Published research indicates that in healthy people, piperine enhances the bioavailability of several drugs, such as rifampin, theophylline, propranolol, and phenytoin. P-gp protein transporters and CYP3A4 enzyme activity suppression are piperine's main modes of action (18-19). In light of these encouraging results, we postulated that paclitaxel's anticancer effects and adverse effects might be reduced by mixing it with piperine, a bioenhancer (20-21).

Material and Method

Docetaxel (DOX) was received as a gift sample from Panacea Biotech, India. Polymer Eudragit RLPO (ERLPO), Piperine (PPN) was utilized as a bio enhancer, polyvinyl alcohol (PVA) was employed as an emulsifying agent, DMSO and acetone was employed as a solvent which was acquired from Merck in Mumbai, India.

Studies of Pre-formulation

Pre-formulation studies on the drug molecule are essential before developing a novel delivery system. In order to understand the qualities of the drugs, it is necessary to investigate its physicochemical properties. In this investigation, we applied the reported method to measure UV absorption and drug excipient interaction.

Preparation of NPs

In the current research, Docetaxel-loaded polymeric NPs were prepared by Emulsification solvent evaporation method using sonicator.

Emulsification solvent evaporation

Preparation of Organic Phase:

- Dissolve Docetaxel and Piperine, in Dimethyl sulfoxide (DMSO).
- Add Eudragit RLPO to the solution and stir until a uniform mixture is obtained.

Preparation of Aqueous Phase:

- Prepare an aqueous solution of PVA ((0.5%) by dissolving the required amount in distilled water.
- Heat the solution if necessary to ensure complete dissolution of PVA (0.5%).

Emulsification:

- Add the organic phase to the aqueous phase drop wise under continuous stirring at 300 rpm for three hours.
- Maintain the emulsification process for a specific period to form a stable oil-in-water emulsion.

Solvent Evaporation:

- Transfer the emulsion to a vacuum evaporator to remove the organic solvent under reduced pressure.
- Continue evaporation until all the DMSO is removed, resulting in the formation of nanoparticles.

Collection and Washing:

- Centrifuge the nanoparticle suspension to collect the nanoparticles.
- Wash the nanoparticles with distilled water to remove any unencapsulated drugs and residual stabilizers.
- Repeat the washing process several times.

Drying:

- Lyophilize (freeze-dry) the washed nanoparticles to obtain a dry powder form for further characterization and evaluation.

Six different formulations were prepared by using different components. Table 1 displays the formulation variables.

Table 1: Different formulation of Docetaxel loaded nanoparticles with Piperine

Formulation Code	Drug	Bioenhancer	Eudragit RLPO (mg)	PVA (%)
F1	20	20	100	0.5
F2	20	20	150	0.5
F3	20	30	100	0.5
F4	20	30	150	0.5
F5	20	40	100	0.5
F6	20	40	150	0.5

Evaluation Parameters

Particle Size, Zeta Potential and Polydispersity Index

The characterization and evaluation of Docetaxel-PIP (Piperine) loaded nanoparticles (NPs) involve several key parameters and techniques: Particle size, zeta potential and polydispersity index, in vitro drug release studies, scanning electron microscopy (SEM).

Particle Size: This parameter indicates the average diameter of the nanoparticles. Smaller particles usually have better cellular uptake and drug delivery efficacy. Dynamic Light Scattering (DLS) is commonly used to measure particle size.

Zeta potential measures the surface charge of the nanoparticles, which can affect stability, aggregation, and interaction with biological membranes. A higher absolute value of zeta potential (either positive or negative) typically indicates more stable nanoparticles.

PDI indicates the size distribution of the nanoparticles. A lower PDI (closer to 0) suggests a more uniform size distribution, while a higher PDI (closer to 1) indicates a broader size

distribution. PDI values less than 0.3 are generally considered acceptable for monodisperse nanoparticles.

The dynamic light scattering method was employed to investigate these features with the Zetasizer Nano ZS (Malvern Instruments Ltd, UK) device. ZP refers to the total charge that a particle receives. The particle dispersion was contained in a cell with two electrodes at either end that received a voltage applied across them. The oppositely charged electrode attracted charged particles, and the particles' velocity was recorded and expressed as electrophoretic mobility in units of field strength (22).

SEM provides detailed images of the surface morphology and structure of the nanoparticles. It helps in visualizing the shape, surface texture, and aggregation state of the nanoparticles. The surface characteristics of the NPs were investigated using SEM (JSM-T20, Kyoto, Japan). After mounting the nanoparticle sample to metal (aluminium) stumps using double-sided adhesive carbon tape, the sample was divided using a razor blade (23). The samples were sputter-coated with gold/palladium for 120 s at 14 mA in an argon atmosphere and examined for morphology at a 15 kV acceleration voltage for secondary electron emissive SEM.

These characterization techniques collectively provide a comprehensive understanding of the physical and chemical properties of Docetaxel-PIP loaded nanoparticles, which is crucial for evaluating their potential efficacy and stability as a drug delivery system.

Fourier Transform Infrared (FTIR) Analysis

FTIR was used to determine the compatibility of all components; thus, we intended to discover any drug-polymer interaction by collecting the data between 400 and 4,000 cm^{-1} .

Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was utilized to analyse the thermal properties of the samples. This study used a differential scanning calorimeter (TA Instruments, Wilmington, USA) to examine five milligrams of samples sealed in an aluminum pan at a temperature heating rate of 10 $^{\circ}\text{C}/\text{min}$ between 35 and 350 $^{\circ}\text{C}$. Nitrogen was added at a flow rate of 100 mL/min and pressure of 40 bar. An empty pan served as the reference during the indium calibration process for the DSC.

Determination of Entrapment Efficiency and Drug Content

Drug Content: Initially, the free drug in each formulation was identified in the supernatant by selecting a solvent that dissolved the free drug and left the remaining ingredients behind. 50 mg of the drug equivalent to the formulation was precisely weighed and put into a 100 mL beaker with 50 mL of DMSO in order to ascertain the drug content.

The mixture was stirred for three hours at 700 rpm using a magnetic stirrer. After the proper dilution, the resultant solution was filtered, and the amount of drug in the filtrate was measured using a UV spectrophotometer set to 229 nm.

Percentage entrapment efficiency (% EE)

To calculate the entrapment efficiency (EE %) of Docetaxel-PIP loaded nanoparticles using the described method, we need to follow these steps and use the appropriate formula:

- 1. Preparation and Equilibration:**
 - Combine 10 mg of nanoparticles (NPs) with 10 mL of distilled water.
 - Allow the mixture to reach equilibrium solubility.
- 2. Centrifugation:**
 - Perform high-speed cooling centrifugation to separate the supernatant from the nanoparticles.
 - Collect and refine the supernatant.
- 3. Sample Preparation for UV-Vis Analysis:**
 - Mix 4 mL of methanolic HCl with 1 mL of the filtrate from the supernatant.
- 4. UV-Vis Spectrophotometry:**
 - Measure the absorbance of the final sample at 229 nm using a UV-visible spectrophotometer.

Calculation of entrapment Efficiency (EE %):

The percentage entrapment efficiency can be computed using formula 1 as follows:

$$\% \text{ Entrapment efficacy} = \frac{\text{Amount of drug encapsulated in the formulation}}{\text{The total amount of drug in the formulation}} \times 100$$

In-vitro Drug Release Study

The dialysis bag diffusion method was used to conduct an in vitro drug release investigation. Within the dialysis bag, pre-weighed nanoparticles (equal to 20 mg Docetaxel) were added. Next, this bag was submerged in the USP type-II dissolving apparatus's phosphate buffer, which has a pH of 7.4. The temperature was kept at $37 \pm 1^\circ\text{C}$ at a speed of 100 rpm. To keep the sink condition throughout the experiment, five millilitres of aliquots were taken at regular intervals and replenished with new buffer. Using a UV 1800 Shimadzu UV-vis spectrophotometer, the filtered aliquots were calculated by computing the absorbance at the 229 nm λ max

Stability Studies

In order to ascertain the physical and chemical alterations in the produced nanoparticles, short-term stability experiments lasting six months were conducted on them. In order to do this, the microcrystals were maintained in stability chambers at four distinct temperatures and relative humidity levels: 5°C , $25^\circ\text{C}/60\% \text{ RH}$, $30^\circ\text{C}/65\% \text{ RH}$, and $40^\circ\text{C}/75 \text{ RH}$. They underwent FTIR analysis six months later to determine any changes in functional groups brought on by chemical instability. They also looked at permeability, drug release, and crystal size measurement.

Result and Discussion

Pre-formulation Studies

UV 1800 Shimadzu used UV spectroscopy to find the maximum and create the standard curve. At a wavelength of 229 nm, the drug's absorbance in phosphate buffered pH 7.4 was determined. Figure 1, the standard curve is shown.

Calibration Curve:

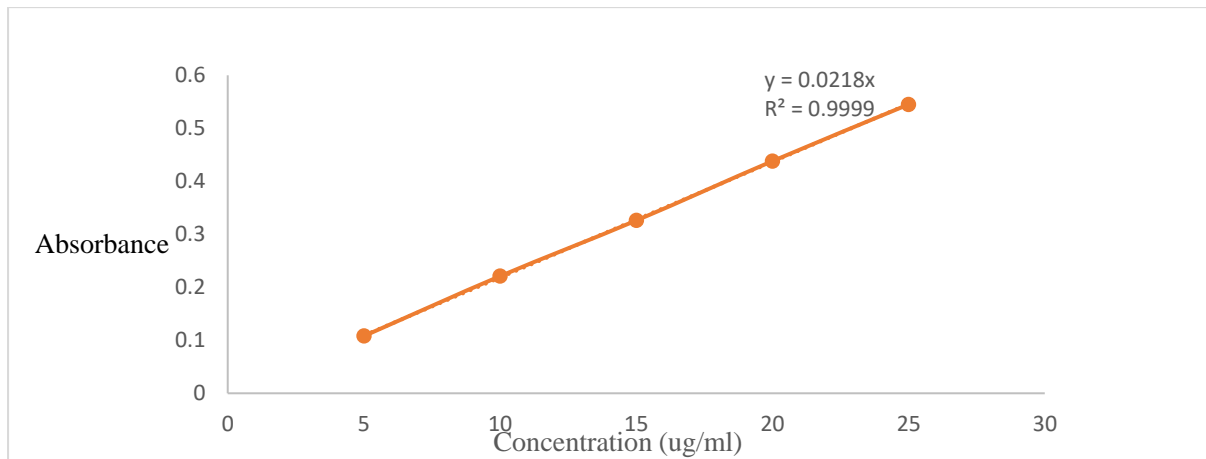


Figure 1 Calibration curve of Docetaxel.

The principal peaks of Docetaxel as indicated by FTIR analysis are displayed in Figure 2. These findings supported the drug's purity because these peaks are fairly similar to those of usual Docetaxel peaks.

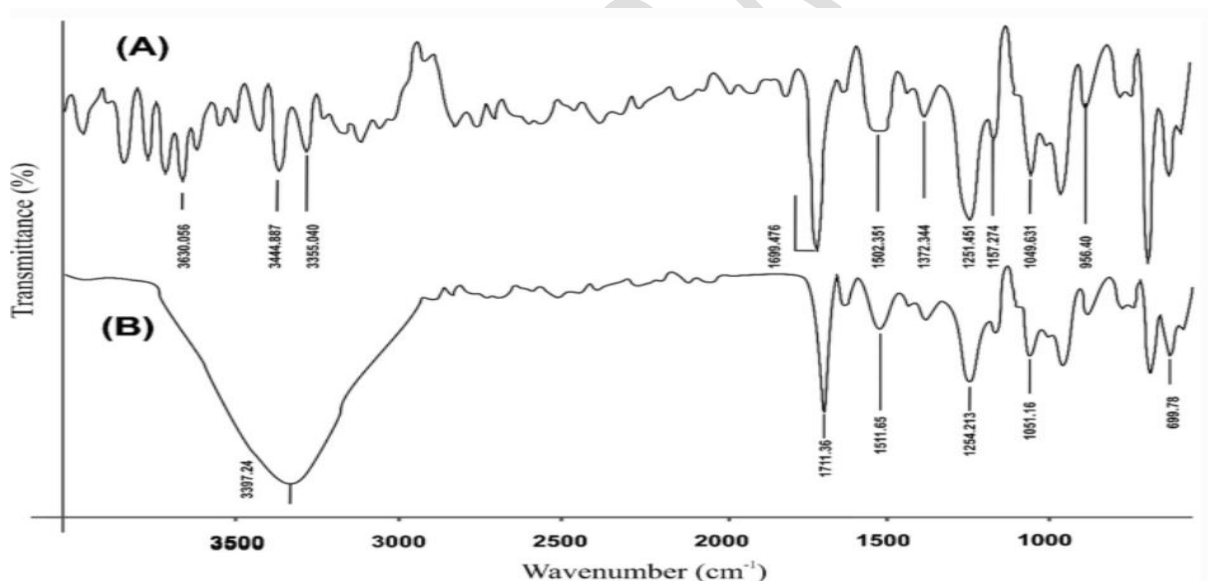


Figure 2: A) FTIR spectra of Docetaxel and B) Docetaxel polymer interaction

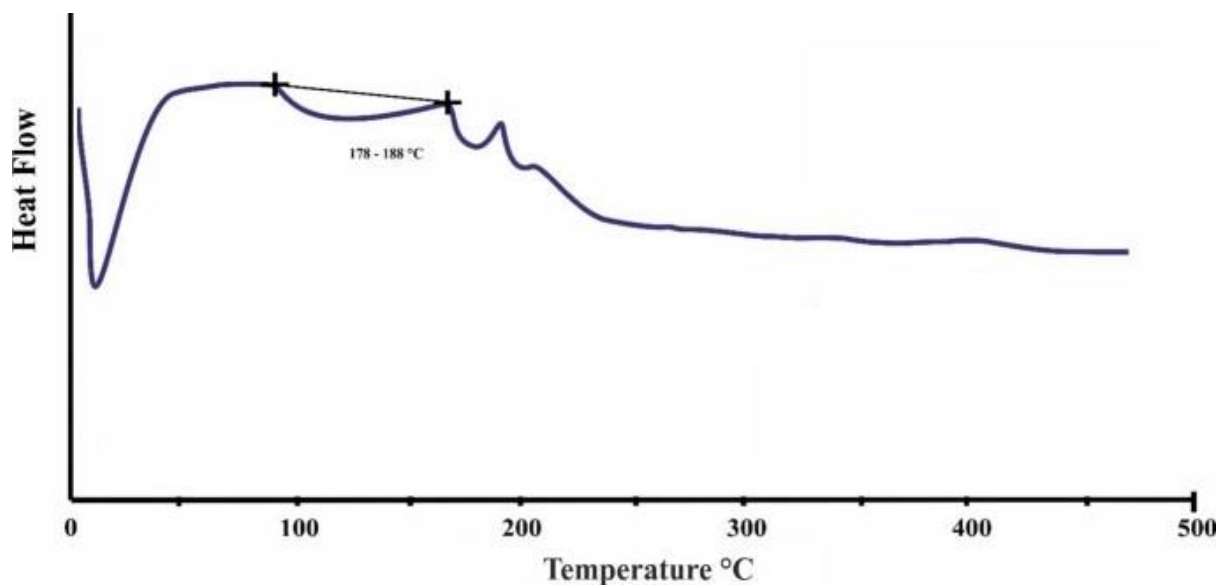


Figure 3: DSC thermograms of Docetaxel

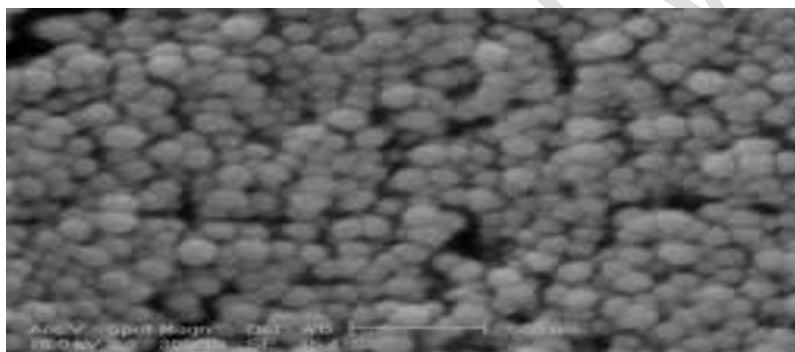


Figure 4: SEM Docetaxel-loaded nanoparticles

Determination of Drug Content and % EE

The drug content across all formulations of Docetaxel-loaded ERLPO nanoparticles was ranging from 61.58% to 90.16%. All formulations exhibited good drug content, demonstrating effective drug loading into the nanoparticles. The highest drug content was achieved with the formulation F6, which exhibited a drug content of 90.16% and indicating that this formulation has the most effective drug loading capacity. The consistent increase in drug content from F1 to F6 suggests that the optimization of formulation parameters, likely including the concentration of polymer and other formulation components, significantly impacts drug loading efficiency. These findings confirm that the formulations were successful in incorporating a substantial amount of Docetaxel into the nanoparticles, with F6 being the most efficient. This high drug content is promising for the efficacy of the drug delivery system

Percentage entrapment efficiency

The encapsulation efficiency for each formulation ranged from 58.54% to 88.29%. The entrapment efficiency increased as the amount of polymer increased from F1 to F6. The highest entrapment efficiency was observed in the F6 formulation, which exhibited 88.29% as indicated in Table 3, indicating that optimizing polymer concentration is crucial for maximizing drug entrapment. Increasing the polymer concentration, higher entrapment

efficiencies. The bioenhancer concentration (piperine) did not significantly affect the entrapment efficiency, suggesting that its primary role might be related to enhancing drug bioavailability rather than influencing the encapsulation process.

Particle Size and Zeta Potential

According to the Zetasizer analysis, the particle sizes of all the batches varied from 142 to 189 nm. Larger particle sizes were observed in batches F2, F4, and F6 (146.51, 174.34, and 189.84 nm, respectively). This increase in size is likely due to a higher polymer concentration in these formulations. Every formulation had a good polydispersity index of less than 0.5, indicating that the particles were evenly distributed, suggesting a narrow size distribution and homogeneity in the nanoparticle formulations.

ZP has a major impact on nanoparticle colloidal stability. For colloidal nanoparticles, a high ZP value (>20 mV) is thought to be the optimal surface charge because it repels other particles, reduces the chance of aggregation, and exhibits great colloidal stability. There was no particle agglomeration in the ZP of batches with optimized nanoformulation. The findings suggest that the nanoparticle formulations are stable, with good size distribution and minimal aggregation. The polymer concentration appears to influence the particle size, but not the zeta potential. The F6 formulation showed optimal characteristics, with the best average zeta potential, indicating it might be the most stable formulation among the tested formulations.

Table 2: Drug content, percentage entrapment efficiency, zeta potential, and particle size of different formulation of Docetaxel nanoparticle

Formulation	Drug content (%)	Percentage EE	Zeta potential (mV)	Particle Size (nm)	PDI
F1	61.58	58.54	20.59	142.00	0.38
F2	68.42	65.15	22.08	146.51	0.46
F3	75.32	72.54	22.87	154.27	0.43
F4	82.20	79.20	24.02	174.34	0.44
F5	87.74	84.57	26.14	179.62	0.45
F6	90.16	88.29	28.88	189.84	0.43

In-vitro drug release study

These prepared Docetaxel loaded NPs in-vitro drug release was evaluated utilizing the dialysis bag diffusion method at pH 7.4 in phosphate buffer. Over the course of a day, it was discovered that the cumulative percentage of drug release from these NPs was sustained (Figure 5). All formulations show an initial burst release within the first few hours, likely due to the surface-bound drug. After the initial burst, the drug release continues at a slower, sustained rate. The range of percentages of drug released in a 24-hour period was 83.69% to 96.44% across all formulations. F6 demonstrates the highest cumulative release 96.44% at 24 hours, making it suitable for sustained drug delivery applications. Data were incorporated into the Higuchi kinetic, zero order, and first order analysis kinetic models. In Higuchi's kinetic, the regression coefficient value, or R², was highest. Therefore, it may be said that Fick's distribution law was being followed by the kinetic output.

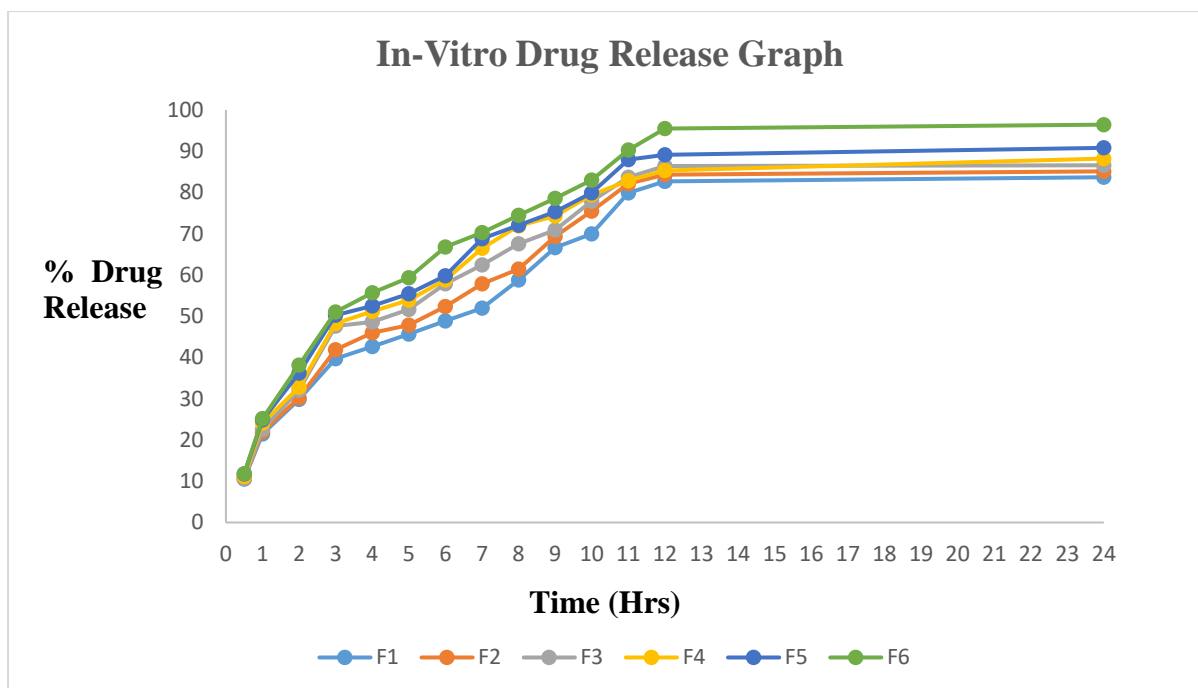


Figure 5: In-vitro drug release graph of Docetaxel

Kinetic Models Analysis

Higuchi Kinetic Model: The Higuchi kinetic model had the highest regression coefficient value (R^2), suggesting that Fickian diffusion preceded drug release.

Zero Order and First Order Kinetic Models: The data were also incorporated into these models, but the Higuchi model provided the best fit.

Interpretation of Results

- Fickian Diffusion:** The high R^2 value in the Higuchi kinetic model suggests that the drug release mechanism is primarily diffusion-controlled, following Fick's law of diffusion.

Table 3: An overview of drug release Data

Formulation	Cumulative Release in 24 Hours (%)	Best Fit Kinetic Model	R^2 Value (Higuchi)
F1	83.69	Higuchi	0.98
F2	85.12	Higuchi	0.97
F3	86.54	Higuchi	0.99
F4	88.22	Higuchi	0.98
F5	90.87	Higuchi	0.97
F6	96.44	Higuchi	0.99

Stability Studies

For three months, each of the formulations in this study was exposed to a different temperature of 4°C, 25°C, or 40°C. After that, the formulations were once more examined for factors like particle size, FTIR, drug entrapment. Particle size results did not significantly change, however the polydispersity index did. It was discovered that this impact might be the result of the

nanoparticles aggregating during storage. The kinetics of Higuchi. Therefore, it may be said that Fick's distribution law was being followed by the kinetic output.

Nanoparticle drug entrapment was marginally lower than before, however the difference was not statistically significant. There were only 0.75 to 1.2% variations in the percentage of drug entrapment for the optimized batch F6. The synthesized formulation's FTIR spectra showed no alterations to its main peak, indicating no chemical modifications.

Conclusion

The study successfully encapsulated Docetaxel and an herbal bioenhancer (piperine) within nanoparticles using an emulsion solvent evaporation technique. The produced nanoparticles showed a notable reduction in particle size and good in vitro characteristics across all formulations. Formulation F6 was identified as the optimal formulation due to its:

- Highest entrapment efficiency (88.29%),
- Highest drug content (90.16%),
- Superior in vitro drug release profile.

The results indicate that combining a bioenhancer with an anticancer drug in a nanoparticle formulation can significantly improve the drug's bioavailability. This finding supports the potential for enhanced therapeutic efficacy through improved delivery and absorption of the drug, highlighting the benefits of this nanoparticle technology in cancer treatment.

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References:

1. Atal N, Bedi KL. Bioenhancers: Revolutionary concept to market. *J Ayurveda Integr Med* 2010; 1:96-9.
2. Nath B, Barbhuiya TF. Studies on the density and surface area of nanoparticles from *Camellia sinensis*, a natural source. *J Chem Pharm Res* 2014; 6:608-10.
3. Hanahan D., Weingberg R.A. the Hallmarks of Cancer. *Cell*. 2000; 100:57–70.
4. Ganesh T. Improved biochemical strategies for targeted delivery of taxoids. *Bioorg. Med. Chem.* 2007; 15:3597–3623
5. Greenlee R.T., Hill-Harmon M.B., Murray Thun M. Cancer statistics. *CA Cancer J. Clin.* 2001; 51:15–36.
6. Oh W.K., Kantoff P.W. Management of hormone refractory prostate cancer: Current standards and future prospects. *J. Urol.* 1998; 160:1220–1229.
7. Wang, L.; Liu, Z.; Liu, D.; Liu, C.; Juan, Z.; Zhang, N. Docetaxel loaded-lipid-based-nanosuspensions (DTX-LNS): Preparation, pharmacokinetics, tissue distribution and antitumor activity. *Int. J. Pharm.* 2011, 413, 194–201.
8. Yin, Y.M.; Cui, F.D.; Mu, C.F.; Choi, M.K.; Kim, J.S.; Chung, S.J.; Shim, C.K.; Kim, D.D. Docetaxel micro emulsion for enhanced oral bioavailability: Preparation and in vitro and in vivo evaluation. *J. Control. Release* 2009, 140, 86–94.
9. Zhao, P.; Astruc, D. Docetaxel Nanotechnology in Anticancer Therapy. *ChemMedChem* 2012, 7, 952–972.
10. Sanna V., Roggio A.M., Posadino A.M., Cossu A., Marceddu, Mariani A. Novel docetaxel-loaded nanoparticles based on poly(lactide-co-caprolactone) and poly(lactide-co-glycolide-co-caprolactone) for prostate cancer treatment: formulation, characterization, and cytotoxicity studies. *Nanoscale Res. Lett.* 2011;6(1):260–268
11. Allen TM, Cullis PR. Drug delivery systems: Entering the mainstream. *Science* 2004; 303:1818-22.
12. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: An emerging treatment modality for cancer. *Nat Rev Drug Discov* 2008; 7:771-82.
13. DB M, S S, KR M. Role of Piperine as an Effective Bioenhancer in Drug Absorption. *Pharm Anal Acta* 2018; 09(07):7–10.
14. Gottesman MM, Pastan IH. The Role of Multidrug Resistance Efflux Pumps in Cancer: Revisiting a JNCI Publication Exploring Expression of the MDR1 (P-glycoprotein) Gene [Internet]. Vol. 107, *Journal of the National Cancer Institute*. Oxford University Press; 2015.
15. Meghwal M., Goswami T.K. Piper nigrum and piperine: An update. *Phytother. Res.* 2013; 27:1121–1130. doi: 10.1002/ptr.4972.
16. Imam S.S., Alshehri S., Ghoneim M.M., Zafar A., Alsaidan O.A., Alruwaili N.K., Gilani S.J., Rizwanullah M. Recent advancement in chitosan-based nanoparticles for improved oral bioavailability and bioactivity of phytochemicals: Challenges and perspectives. *Polymers.* 2021; 13:4036. doi: 10.3390/polym13224036.
17. Yang W, Xia Y, Fang Y, Meng F, Zhang J, Cheng R, et al. Selective Cell Penetrating Peptide Functionalized Polymersomes Mediate Efficient and Targeted Delivery of Methotrexate Disodium to Human Lung Cancer In Vivo. *Adv Healthc Mater.* 2018; 7(7):1–8.

18. Pradeep CR, Kuttan G. Piperine is a potent inhibitor of nuclear factor- κ B (NF- κ B), c-Fos, and CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells. *Int Immunopharmacol*. 2004; 4(14 SPEC.ISS.): 1795–803.
19. Greenshields AL, Doucette CD, Sutton KM, Madera L, Annan H, Yaffe PB, et al. Piperine inhibits the growth and motility of triple-negative breast cancer cells. *Cancer Lett [Internet]*. 2015; 357(1):129–40.
20. Motiwala MN, Rangari VD. ScienceDirect Combined effect of paclitaxel and piperine on a MCF-7 breast cancer cell line in vitro: Evidence of a synergistic interaction. *Synergy [Internet]*. 2015; 2(1):1–6.
21. Pandit a D, Ahuja A, Velpandian T, Lather V, Dutta T, Khar RK. Characterization and in vitro assessment of paclitaxel loaded lipid nanoparticles formulated using modified solvent injection technique. *Pharmazie [Internet]*. 2009 [cited 2021 Jun 29]; 64(5):301–10
22. Musmade KP, Deshpande PB, Musmade PB, Maliyakkal MN, Kumar AR, Reddy MS, et al. Methotrexate-loaded biodegradable nanoparticles: Preparation, characterization and evaluation of its cytotoxic potential against U-343 MG human neuronal glioblastoma cells. *Bull Mater Sci* 2014; 37: 945-51.
23. Hoa LT, Chi NT, Nguyen LH, Chien DM. Preparation and characterisation of nanoparticles containing ketoprofen and acrylic polymers prepared by emulsion solvent evaporation method. *J Exp Nanosci* 2012; 7:189-97.
24. Amar Singh, Amar Deep, Piperine: A Bioenhancer. *International Journal of Pharmacy Research and Technology* 2011, Volume 1, Issue 1, 01-05.
25. SB Bhise, and VY Pore, “Influence of CoAdministration of Piperine on Pharmacokinetic profile of Ciprofloxacin,” *Indian Drugs* 39, 166-168 (2002).
26. RK Reen, and J Singh, “In vitro and in vivo inhibition of pulmonary cytochrome P450 activities by piperine- a major ingredient of Piper species,” *Indian Journal of Experimental Biology* 29, 568-573 (1991)
27. Sanjiv Kumar Chaudhri, Sourabh Jain, A Systematic Review of Piperine as a Bioavailability Enhancer. *Journal of Drug Delivery and Therapeutics* 2023; 13(4):133-136
28. Annamalai AR, Manavlan R. Trikatu- A bioavailability enhancer. *Indian Drugs* 1989; 27: 595-604.
29. Engels, F. K., & Verweij, J. (2005). Docetaxel administration schedule: from fever to tears? A review of randomised studies. *European journal of cancer*, 41(8), 1117-1126.
30. Lyseng-Williamson, K. A., & Fenton, C. (2005). Docetaxel: a review of its use in metastatic breast cancer. *Drugs*, 65, 2513-2531.