Advances in Bioresearch
Adv. Biores., Vol 17 (5) May 2026: 296-305

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.17.5.296305

Advances in Bioresearch

ORIGINAL ARTICLE

Formulation Development *In- Vitro* Characterization of Paclitaxel Loaded Chitosan Nanoparticle for Brain Cancer

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ABSTRACT
*The present study aimed to develop, optimize, and characterize Paclitaxel-loaded chitosan nanoparticles (PTX-CS NPs) using the ionic gelation method **for** enhanced intranasal brain delivery. The primary objective was to improve the bioavailability of Paclitaxel (PTX) and enhance its uptake across the blood–brain barrier via the intranasal route. Nanoparticulate systems are particularly advantageous in protecting labile drugs from degradation in physiological environments, offering controlled release and targeted delivery.In this work, PTX-loaded chitosan nanoparticles were prepared using varying concentrations of sodium tripolyphosphate (Na-TPP) as a cross-linking agent. The formulations were optimized by evaluating critical parameters including particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), drug loading (DL), and in-vitro drug release. The optimized formulation demonstrated a mean particle size of 134.5 ± 2.96 nm, zeta potential of -32.4 ± 1.63 mV, and a maximum drug entrapment efficiency of 92.9%. In-vitro drug release studies, performed using the dialysis membrane method, showed a biphasic release profile characterized by an initial burst release followed by sustained release over 72 hours. Cumulative drug release in phosphate-buffered saline (PBS) at pH 5.5, 6.4, and 7.4 was 96.3%, 91.3%, and 86.7%, respectively. These findings confirm that the developed PTX-CS NPs offer a promising strategy for non-invasive, site-specific drug delivery to the brain, potentially improving therapeutic outcomes for brain cancer while minimizing systemic side effects.*
Keywords: *Nanoparticle, Drug delivery, Paclitaxel, Chitosan, Na-TPP, Ionic gelation method, brain delivery, Intra nasal delivery etc.*

Received 19.04.2026 Revised 04.05.2026 Accepted 24.05.2026

How to cite this article:
Khanderao R. J, Disha Shivaji P, Vidya B. P, Vrushali V. N. Formulation Development *In- Vitro* Characterization of Paclitaxel Loaded Chitosan Nanoparticle for Brain Cancer. Adv. Biores. Vol 17 [5] May 2026. 296-305

INTRODUCTION

Brain cancer, particularly glioblastoma multiforme (GBM), is one of the most aggressive and life-threatening forms of cancer, characterized by rapid cell proliferation, invasiveness, and resistance to conventional treatment approaches. Despite advancements in neurosurgery, radiotherapy, and chemotherapy, the prognosis for patients with brain tumors remains poor, largely due to the presence of the blood-brain barrier (BBB). This physiological barrier, composed of tightly joined endothelial cells, effectively restricts the entry of many chemotherapeutic agents into the brain, resulting in inadequate drug concentrations at the tumor site and subsequent therapeutic failure [1].

Paclitaxel is a widely used antineoplastic agent belonging to the taxane class, originally isolated from the bark of *Taxus brevifolia* (Pacific yew tree). Chemically, paclitaxel has the molecular formula $C_{47}H_{51}NO_{14}$ and a molecular weight of 853.91 g/mol. It exerts its cytotoxic effect by promoting tubulin polymerization and stabilizing microtubules, thereby preventing their depolymerization. This disrupts the mitotic spindle assembly, arrests the cell cycle at the G2/M phase, and ultimately leads to apoptotic cell death. Although paclitaxel is clinically effective against breast, ovarian, and lung cancers, its application in brain cancer

therapy is severely limited by its poor aqueous solubility (approximately 0.3 μg/mL), low oral bioavailability, and inability to cross the BBB[2]. Furthermore, paclitaxel is a known substrate of the P-glycoprotein (P-gp) efflux transporter, which actively pumps the drug out of the brain endothelial cells, further reducing its intracranial accumulation.

To overcome these challenges, nanoparticle-based drug delivery systems have gained prominence, offering controlled release, improved solubility, and enhanced site-specific targeting. Among various biodegradable polymers, chitosan has emerged as a promising carrier due to its favourable biological properties, including biodegradability, biocompatibility, mucoadhesive Ness, and the ability to transiently open tight junctions in the BBB. Chitosan's cationic nature allows strong electrostatic interaction with negatively charged cell membranes, promoting endocytosis and improved cellular uptake of the loaded drug [3].

The present study focuses on the formulation and in-vitro characterization of paclitaxel-loaded chitosan nanoparticles for targeted delivery in brain cancer therapy. This nanocarrier system is designed to enhance the solubility and bioavailability of paclitaxel, facilitate its transport across the BBB, and ensure sustained release at the tumor site. In-vitro studies will evaluate parameters such as particle size, zeta potential, drug entrapment efficiency, and release kinetics, aiming to establish an effective and safe platform for brain-targeted chemotherapy [4.5].

MATERIAL AND METHODS

Selection of the method of preparation

Various formulation techniques have been explored for the development of paclitaxel-loaded polymeric nanoparticles (PTX-PNP), including nanoprecipitation, ionic gelation, solvent evaporation, microemulsion, co-precipitation, polyelectrolyte complexation, and spray drying methods. Among these, the ionic gelation method was selected for the current study due to its simplicity, mild processing conditions, and suitability for encapsulating hydrophobic drugs like paclitaxel. This method allows for the formation of nanoparticles through electrostatic interaction between the cationic chitosan polymer and a negatively charged cross-linking agent, typically sodium tripolyphosphate (TPP) [5]. The cross-linking ionic gelation technique produced nanoparticles with optimal particle size, favorable zeta potential, and high entrapment efficiency, making it the preferred approach for the formulation of PTX-PNP aimed at brain cancer therapy.

Table 1: List of Materials with Specifications

Material	Supplier	Location	Grade
Paclitaxel (PTX)	Naprod Life Sciences Pvt. Ltd.	Boisar, Palghar, India	Pharmaceutical grade
Chitosan	Fine-Chem Industries	Mumbai, India	Low molecular weight, ≥75% deacetylated
Sodium Tripolyphosphate (TPP)	Purochem Pvt. Ltd.	Taloja, Maharashtra, India	Analytical reagent grade
Glacial Acetic Acid	IOL Chemical Ltd.	Mumbai, India	Analytical reagent grade
Methanol	S.D. Chemicals	Mumbai, India	HPLC grade
Acetonitrile	S.D. Chemicals	Mumbai, India	HPLC grade
Other chemicals and reagents	—	—	Analytical reagent grade

Preparation of chitosan NPs by using Ionic gelation method

Chitosan nanoparticles (CS-NPs) were prepared using the ionic gelation method, involving the electrostatic interaction between positively charged chitosan and negatively charged sodium tripolyphosphate (Na-TPP) as a cross-linking agent. Chitosan of medium molecular weight (approximately 750,000 Da) with a degree of deacetylation of ~70% was used. It was dissolved in 0.25% v/v glacial acetic acid to obtain various concentrations of chitosan solutions (0.05%, 0.075%, 0.1%, 0.15%, 0.175%, and 0.2% w/v). Separately, Na-TPP was dissolved in distilled water at different concentrations (0.1%, 0.15%, 0.2%, 0.25%, 0.3%, and 0.4% w/v)[6].

For nanoparticle formation, 4 mL of Na-TPP solution was added dropwise to 10 mL of chitosan solution under continuous stirring at room temperature. Nanoparticle formation was observed only at specific combinations of chitosan and Na-TPP concentrations, indicating critical ionic interaction ratios for stable nanoparticle synthesis. The stirring was continued for 1 hour to ensure complete cross-linking [7].

For drug loading, paclitaxel was added to the chitosan solution in varying drug-to-polymer ratios (1:1, 1:2, and 1:3 w/w) and allowed to equilibrate for 24 hours before the addition of Na-TPP. After

nanoparticle formation, the suspension was subjected to ultracentrifugation at 2000 rpm for 45 minutes at 10 °C to separate the nanoparticles from the aqueous medium. The supernatant was collected and analyzed to determine the unencapsulated drug content, which was used to calculate entrapment efficiency and drug loading.

The resultant nanoparticles were washed with distilled water or mannitol and subsequently freeze-dried for further characterization [8,9].

Particle size and zeta potential

The particle size, size distribution, polydispersity index (PDI), and zeta potential of the paclitaxel-loaded chitosan nanoparticles (PNPs) were determined using the Horiba Scientific SZ-100 Nanoparticle Analyzer**.** Measurements were performed at 25 ± 1 °C using a 4 mW standard laser **at a** fixed scattering angle of 90°**.** For analysis, 100 µL of each nanoparticle formulation was diluted with 6 mL of Milli-Q water and sonicated briefly to ensure uniform dispersion. The measurements were conducted in triplicate to ensure accuracy and reproducibility [10,11]

The analyzer provided values for mean particle size**,** PDI (to assess uniformity), and zeta potential (to determine surface charge and colloidal stability). These parameters are crucial for evaluating the stability, dispersion behavior, and potential biological performance of the nanoparticles.

Determination of Entrapment Efficiency (EE%) and Drug Loading (DL%)

To determine the entrapment efficiency (EE%**)** and drug loading (DL%) of paclitaxel-loaded chitosan nanoparticles, the nanoparticle suspensions were subjected to ultracentrifugation at 2000 rpm for 45 minutes at 10 °C using an Allegra™ Ultracentrifuge (Beckman Coulter**,** USA)**.** After centrifugation, the resulting supernatant was carefully collected and filtered through a 0.45 µm membrane filter to remove any residual particles [12].

The concentration of free (unencapsulated) paclitaxel in the supernatant was quantified using a UV-visible spectrophotometer at the drug's characteristic absorbance wavelength. All measurements were performed in triplicate, and the average value was used for calculations [13].

The entrapment efficiency (EE%) and drug loading (DL%) were calculated using the following equations:

EE = ($Amout\ of entrapped\ drug$ – free drug / $Total\ amount\ of\ drug$) x 100

DL= (Total drug in nanoparticles – free drug / Total weight of nanoparticles) x 100

Morphology and Shape

Transmission Electron Microscopy (TEM)

The morphology and surface characteristics of the optimized paclitaxel-loaded chitosan nanoparticles were examined using Transmission Electron Microscopy (TEM)**.** The analysis was carried out on **a** JEOL JEM-2100F TEM, equipped with a super twin lens system, operated at an accelerating voltage suitable for nanoparticle imaging [14].

For sample preparation, a small droplet of nanoparticle dispersion was carefully placed onto a carbon-coated copper grid. The sample was negatively stained with 1% (w/v) sodium phosphotungstate solution to enhance contrast. After complete air drying under ambient conditions, the grid was inserted into the TEM instrument, and the nanoparticles were visualized to assess their shape, surface morphology, and dispersion [15-16]

Differential Scanning Calorimetry (DSC) Study

Differential Scanning Calorimetry (DSC) analysis was carried out to evaluate the thermal behavior and possible changes in the physical state of paclitaxel (PTX) upon encapsulation in chitosan nanoparticles. The study was performed using a thermal analysis system (DSC7020, HITACHI, Japan)**.**

DSC thermograms were recorded for pure PTX**,** placebo nanoparticles (PNP without drug)**,** and the optimized PTX-loaded nanoparticle formulation (PTX-PNP)**.** Approximately 1 mg of each sample was accurately weighed and placed in a hermetically sealed aluminum pan. The samples were scanned over **a** temperature range of 30 °C to 300 °C **at a** heating rate of 10 °C/min**,** under a continuous flow of nitrogen gas to provide an inert atmosphere and prevent oxidative degradation.

This analysis was used to detect any shifts in melting point, endothermic or exothermic transitions, and possible drug–polymer interactions, indicating changes in crystallinity or molecular dispersion of PTX within the nanoparticle matrix [17].

In-vitro Drug Release Study

The in-vitro release profile of paclitaxel (PTX) from the synthesized PTX-loaded chitosan nanoparticles (PTX-CS PNPs) was evaluated using the dialysis membrane diffusion method under three different pH conditions**:** 7.4 (physiological), 6.4 (tumour extracellular), and 5.5 (endosomal/lysosomal

A pre-treated dialysis membrane (molecular weight cut-off ~12–14 kDa) was used. A 5 mL suspension of PTX-loaded nanoparticles containing 2 mg of paclitaxel was placed into the dialysis bag and tightly sealed. The bag was immersed in 100 mL phosphate buffer saline (PBS) at the respective pH values (7.4, 6.4, and 5.5) in separate beakers. The release study was conducted under continuous magnetic stirring at 150 rpm and maintained at **37 ± 0.5 °C** to simulate physiological conditions [18].

At predetermined time intervals**,** 1 mL of release medium was withdrawn and replaced immediately with an equal volume of fresh PBS to maintain sink conditions. The collected samples were analyzed for drug content using a UV-visible spectrophotometer at 230 nm**,** and the amount of drug released was quantified using a previously constructed calibration curve.

Each experiment was conducted in triplicate**,** and the results were expressed as the mean cumulative percentage drug release over time [19].

RESULT AND DISCUSSION

Formulation and Characterization of Chitosan Nanoparticles

Chitosan nanoparticles were formulated using varying concentrations of chitosan and sodium tripolyphosphate (Na-TPP) to optimize particle formation. Among the different formulations, those exhibiting opalescent appearance an indicator of stable nanoparticle formation—were selected for further evaluation. The results indicated that the optimum chitosan-to-TPP ratio yielded nanoparticles with a mean particle size in the range of approximately 200 nm**,** which is ideal for enhanced cellular uptake and brain-targeted delivery.

The optimized formulations demonstrated good colloidal stability, with a narrow size distribution and acceptable polydispersity index (PDI), indicating uniformity in particle size. These findings suggest that proper adjustment of the polymer and cross-linker concentrations is critical in achieving nanoscale particles with suitable physicochemical properties for drug delivery applications [20].

The detailed results of particle size, PDI, and zeta potential are summarized in Table 1.

Formulation Code	Conc. of CS (Mg/ml)	Conc. of NA-TPP (mg/ml)	Mean Particle Size	Mean PDI
F1A	0.5	1	188 ±2.21	0.391 ± 0.028
F1B	**1**	**1**	**160.4 ± 1.09**	**0.268 ± 0.009**
F2A	1.5	1.5	324.6 ± 1.7	0.347 ± 0.001
F2B	1	1.5	281.0 ± 4.93	0.352 ± 0.004
F3C	1	2	440.1 ± 1.8	0.431 ± 0.005
F3D	2	3	250.7 ± 1.07	0.383 ± 0.011
F4D	2	1	270 ± 1.3	0.291 ± 0.006
F4A	1.5	3	487.1 ± 1.41	0.226 ± 0.011
F5A	1	3	502.7 ± 2.11	0.521 ± 0.021
F5C	2	0.5	380.6 ± 4.51	0.491 ± 0.006

Table 2: Optimization of placebo nanoparticle (CS-TPP) on the basis of ratio

The formulation F1B was selected for the preparation of paclitaxel-loaded chitosan nanoparticles (PTX-CS PNPs) due to its optimal particle size of 160.4 ± 1.09 nm and polydispersity index (PDI) of 0.268 ± 0.009. These values fall within the desired range for effective brain targeting via the intranasal route, where a particle size of less than 200 nm and a PDI below **0.5** are generally considered ideal to ensure efficient mucosal penetration, enhanced uptake, and uniform distribution [21]. Other formulations were excluded due to their significantly larger particle sizes and higher PDI values, which make them unsuitable for brain delivery via the nasal pathway. Among all the tested formulations, Formulation F2**,** prepared using 1 mg/mL of chitosan and **1** mg/mL of sodium tripolyphosphate (Na-TPP)**,** was identified as the optimized batch for loading paclitaxel. The particle size and PDI of the drug-loaded nanoparticles were determined using the Horiba Zetasizer**,** confirming consistent results with minimal variation (160.4 nm, PDI 0.268 ± 0.003) [22]. These results support the suitability of the selected formulation for further in-vitro characterization and nasal delivery targeting the brain.

Table 3: Optimization of paclitaxel (PTX)-loaded CS NPs.

Formulation Code	Polymer: Stabilizr Drug Ratio	Particle size (nm)	Zeta potential (mv)	Mean PDI	E.E. %	L.D. %
F-21D1	1:1:0.5	226.6	- 20. 0	0.424	87.2%	20.3%
F-22D2	**1:1:1**	**134.5**	**- 32.4**	**0.442**	**89.5%**	**35.3%**
F-23D3	1:1:1.5	214.5	- 21.8	0.413	92.9%	45.2%

Optimization of Drug-Polymer-Stabilizer Ratio

The drug-to-stabilizer ratio was optimized based on key formulation parameters, including particle size, zeta potential (ζ-potential), polydispersity index (PDI), and entrapment efficiency (EE%)**.** It was observed that increasing the drug content in the formulation led to a corresponding increase in both entrapment efficiency and drug loading**.** This enhancement can be attributed to the stabilizer's ability to improve the solubility and stability of the poorly water-soluble drug, paclitaxel [23].

Among the various batches studied, formulation F-22D2 (Table 2) was identified as the most optimized, exhibiting a mean particle size of 134.5 nm and a PDI of 0.442 ± 0.01, which are within the acceptable range for brain targeting via the intranasal route (i.e., particle size <200 nm and PDI <0.5). In contrast, other formulations were excluded due to significantly larger particle sizes and broader size distributions, making them unsuitable for nasal delivery.

Further optimization revealed that formulation F-21D2, containing 1 mg/mL of chitosan and 1 mg/mL of Na-TPP**,** was ideal for in-vitro and in-vivo studies [24-25] This formulation exhibited favorable physicochemical characteristics, including a mean particle size of 134.5 nm**,** ζ-potential of -32.4 mV**,** PDI of 0.442**,** entrapment efficiency of 89.5%, and drug loading of 35.3% (Figure 2).

Moreover, as the drug polymer stabilizer ratio increased from 1:1:0.5 **to** 1:1:2:1.5**,** the entrapment efficiency improved from 87.2% to 92.9%**,** and the drug loading increased from **20.3% to 45.2%**. These results clearly indicate that both polymer and stabilizer concentrations play a critical role in maximizing the encapsulation and payload of paclitaxel in chitosan nanoparticles.

The detailed comparative data are shown in Table 2.

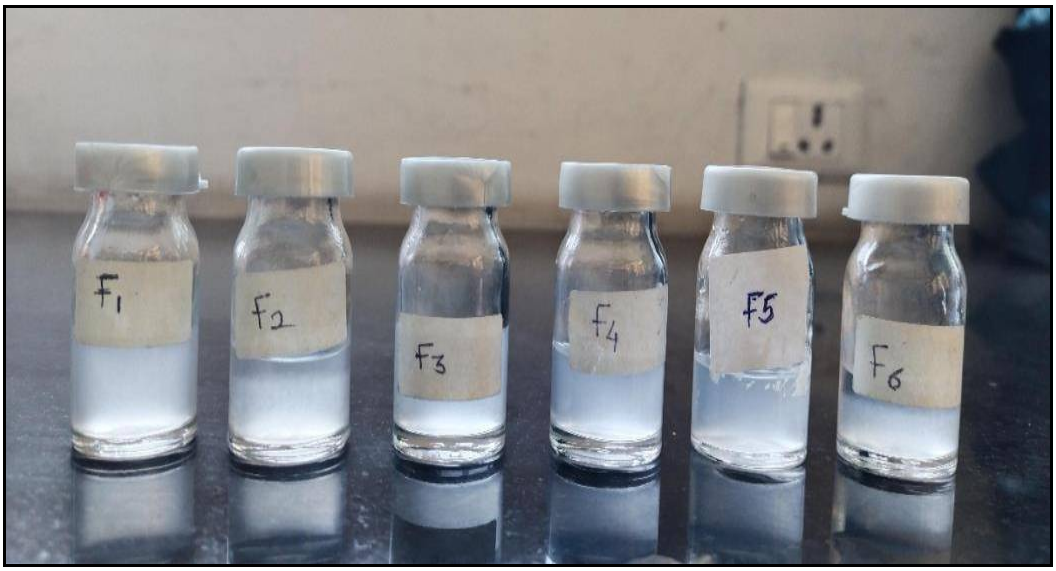

Figure 1: Image of PTX-CS NPs containing 1 mg / mL CS and 1 mg/ mL Na-TPP with drug: Polymer ratio on [Formulation]

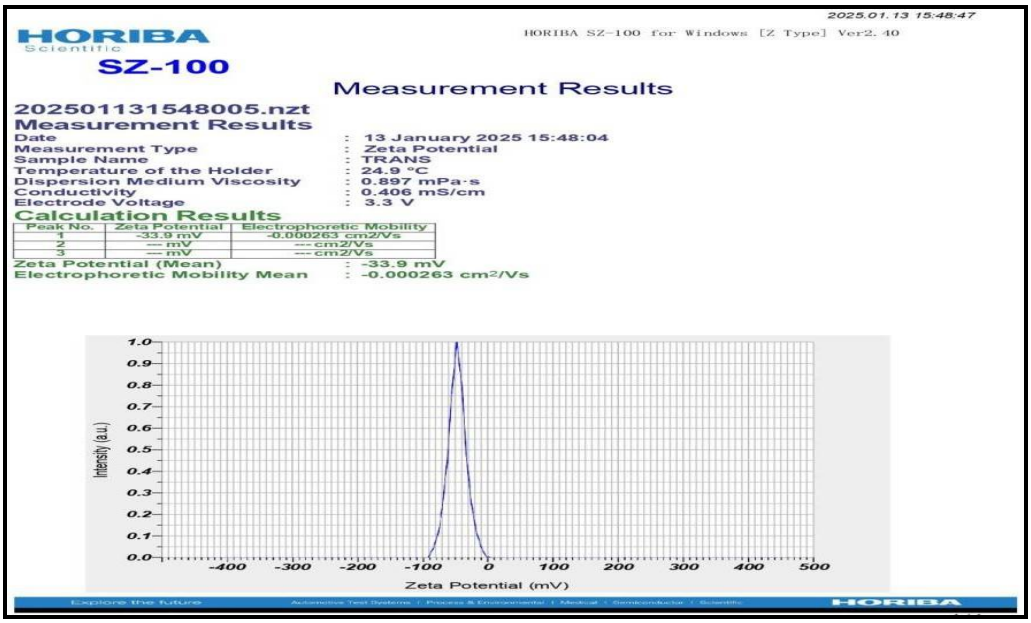

Figure 2: (A) Zeta potential of PTX- Loaded Chitosan Nanoparticles (CS-NP's)

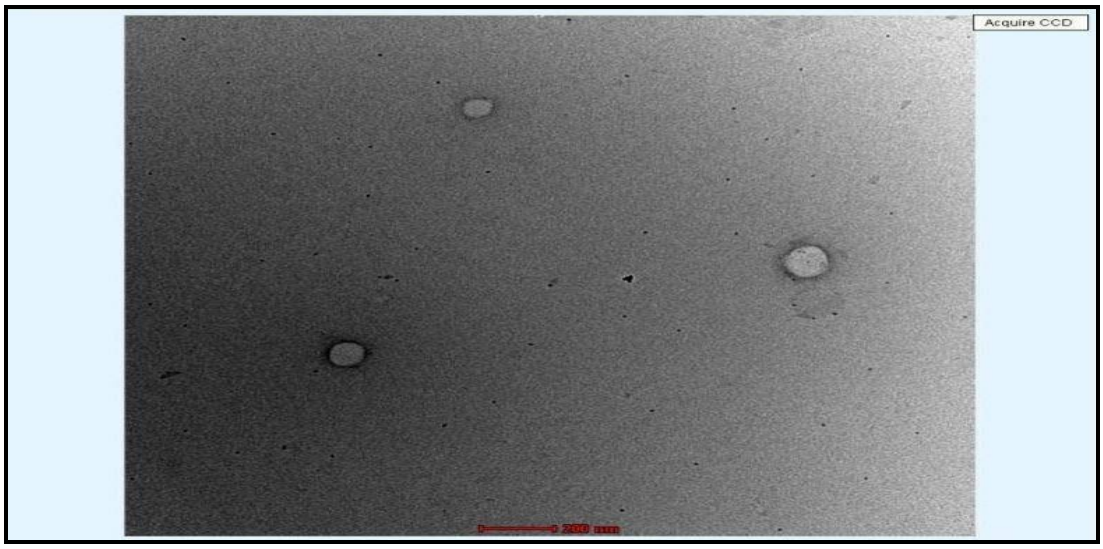

Figure 3 : [TEM] image of PTX- loaded CS NPs containing 1 mg / mL CS and 1 mg/ mL Na-TPP with drug: Polymer ratio on formulation

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analysis was performed to investigate the crystalline characteristics and potential drug-polymer interactions in the PTX-loaded chitosan nanoparticles (PTX-PNP). The thermal behavior of **p**ure paclitaxel (PTX)**,** placebo chitosan nanoparticles (PNP without drug)**,** and the optimized PTX-PNP formulation was evaluated[26-28].

As shown in Figure 3, the DSC thermogram of pure PTX exhibited a sharp endothermic peak **at** 220.6 °C**,** corresponding to its melting point, which is indicative of its crystalline nature. In contrast, the thermogram of the placebo PNP displayed a broad endothermic peak

around **61 °C**, which is characteristic of the thermal behavior of the chitosan polymer matrix.

Interestingly, the DSC thermogram of the PTX-PNP formulation also displayed a broad endothermic peak at approximately **61 °C**, similar to that of the placebo PNP, while the characteristic melting peak of pure PTX at 220.6 °C was absent. This suggests that paclitaxel was completely encapsulated within the chitosan matrix **and** transformed from a crystalline to an amorphous or molecularly dispersed state during nanoparticle formation[30].

These findings confirm the successful encapsulation of PTX in the nanoparticle system and suggest improved solubility and bioavailability due to the drug's amorphous state.

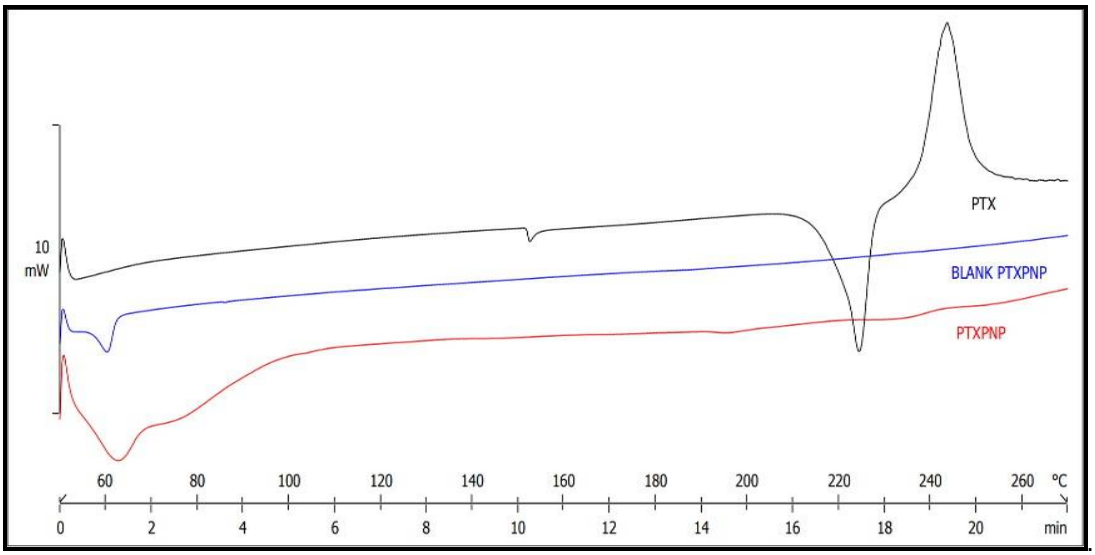

Figure 4: physical mixture (Drug + Excipient) PTX PNPs

In-Vitro Drug Release Study

A comparative in-vitro drug release study of PTX-loaded chitosan nanoparticles (PTX-PNP) was conducted in phosphate-buffered saline (PBS**)** at three different pH conditions—5.5, 6.5, and 7.4 to simulate the acidic tumor microenvironment**,** slightly acidic intracellular and compartment**s**, physiological pH, respectively. The study was carried out over a 72-hour period, and the results are shown in Figure 4 [32-34].

The cumulative drug release at the end of 72 hours was found to be:

96.3% at pH 5.5
91.3% at pH 6.5
86.7% at pH 7.4

These results indicate that drug release was significantly influenced by ambient pH**,** with higher release observed under acidic conditions. This behavior can be attributed to the protonation and swelling of chitosan in acidic media, leading to faster degradation of the polymer matrix and increased drug diffusion. Additionally, stabilizers may undergo structural relaxation in acidic conditions, further facilitating drug release[35].

The release profile exhibited a biphasic pattern, characterized by an initial burst release within the first hour, followed by a sustained release over the remaining 72-hour period. The initial burst may be due to the surface-associated drug, while the sustained phase corresponds to the gradual diffusion and polymer erosion of the encapsulated drug[36].

This enhanced and controlled release pattern, especially in acidic environments, suggests that the optimized formulation **(**F2**)** has the potential for improved bioavailability and effective drug delivery in tumor-targeted therapies. The improved solubility and extended release behaviour confirm the successful formulation of PTX-PNPs with promising characteristics for brain-targeted intranasal delivery [37].

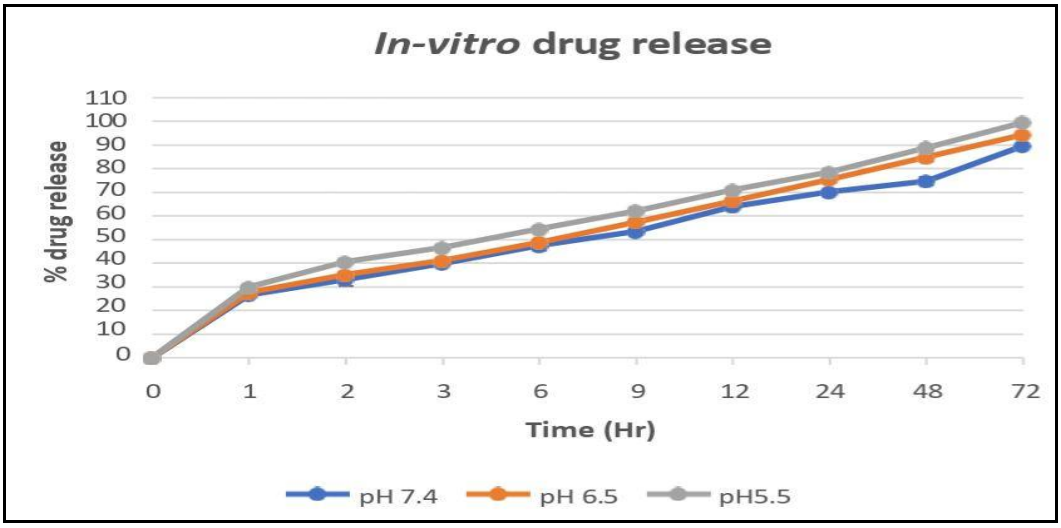

Figure 5: *In vitro* release profile of PTX-CS NPs

CONCLUSION

The present research successfully developed a novel nanoparticle formulation for the intranasal delivery of paclitaxel (PTX) using the ionic gelation method. Various formulation parameters, including polymer, stabilizer, and drug concentration, were optimized to achieve nanoparticles with desirable characteristics.

The optimized PTX-loaded chitosan nanoparticles (PTX-PNPs) exhibited **s**pherical morphology**,** high entrapment efficiency**,** and favorable particle size and PDI, making them suitable for brain targeting via the nasal route. The formulation demonstrated stability and a sustained release profile, particularly under acidic conditions that mimic the tumor microenvironment.

This study confirms the potential of PTX-PNPs as an effective delivery system for site-specific brain targeting**,** potentially improving therapeutic outcomes while minimizing systemic side effects commonly associated with conventional chemotherapy. The developed nanoparticles provide a promising strategy for active targeting of brain tumors**,** enhancing the clinical utility of paclitaxel for central nervous system malignancies.

LIST OF ABBREVIATION

Abbreviation	Full Form
FTIR	Fourier-Transform Infrared Spectroscopy
UV	Ultra-Violet Spectroscopy
CS	Chitosan
NA-TPP	Sodium Tripolyphosphate
DSC	Differential Scanning Calorimetry
BCS	Biopharmaceutics Classification System
TEM	Transmission Electron Microscopy
ZP	Zeta Potential
RPM	Revolutions Per Minute
PS	Particle Size
PDI	Polydispersity Index
HPLC	High Performance Liquid Chromatography
NPs	Nanoparticles

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHT
Human or animals were sued for studies that are the basis of this research

CONSENT FOR PUBLICATION
Not applicable

AVAILAVILITY OF DATA AND MATERIAL
All the data and supportive information are provided within the article.

FUNDINGS
None

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise

ACKNOWLEDGMENT
I am grateful to prof. Dr. Khanderao Jadhav arousing my interest in scientific research and providing with my project.

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