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Fabrication, Characterization and Assessment of Polymeric Nanoparticles as a Nanomedicine Approach for Paclitaxel Delivery for Enhanced Cancer Treatment

Mayuri Ninad Deshmukh[1], Ramdas Baburao Rode[2*], Shaikh Amir Afzal[3], Landge Dhananjay Ashok[4], M. Keerthi[5], Bhavik Sharma[6], Ankur Patel[7], Rajarshi Nath[8]

[1]Nagpur College of Pharmacy, Nagpur, Datta Meghe Institute of Higher Education and Research, Wardh.441110
[2]Shri Swami Samarth Institute of Pharmacy, Malwadi (Bota), Sangamner, Ahemadnagar, Maharashtra. 422602*
[3]Department of Pharmaceutics, SCES's Indira College of Pharmacy,Tathwade, Pune, Maharashtra. 411033
[4]HSBPVTS GOI Faculty of Pharmacy, Kashti, Ahmednagar, Maharashtra. 414701
[5]MB school of pharmaceutical sciences, A. Rangampeta, Tirupati, Andhrapradesh. 517102
[6]Department of Pharmacy, Madhav University, Pindwara (Sirohi), Rajasthan.
[7]Department of Pharmaceutics, Sardar Patel College of Pharmacy, Vidyanagar-Vadtal Road, Bakrol, Gujarat. 388315
[8]Department of pharmaceutical chemistry, Bharat Technology, Uluberia, Howrah, & JIS University, 81, Nilgunj Rd, Jagarata Pally, Deshpriya Nagar, Agarpara, Kolkata, West Bengal. 700109

[*]Corresponding Author: Ramdas Baburao Rode[2*]
[2]Shri Swami Samarth Institute of Pharmacy, Malwadi (Bota), Sangamner, Ahemadnagar, Maharashtra. 422602*

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Abstract

The effectiveness of paclitaxel as a cancer treatment is widely recognized. However, its solubility issue can be addressed by blending it with ethanol and Cremophor EL, a product marketed under the trade name Taxol. Nevertheless, to enhance the anticancer efficacy of Cremophor EL and reduce adverse effects, alternative delivery methods and strategies must be explored. The objective of this work was to synthesize PLGA nanoparticles (PNF) loaded with paclitaxel and evaluate a number of characteristics, including in vitro drug release, drug loading, polydispersity index, zeta potential, and particle size. Finding the best formulation, PNF4, based on its in vitro drug release properties, was the main goal of the study. The surface morphology of PNF4 was then investigated by means of scanning and transmission electron microscopy after that (SEM and TEM). The delivery method follows the Korsmeyer-Peppas model, according to analysis of the in vitro drug release kinetics, indicating a "Fickian diffusion" mechanism. Furthermore, the in vitro cytotoxicity assessment demonstrated that the PNF4 formulation exhibited superior cytotoxicity compared to free paclitaxel.

Keywords: *Osphronemus. Gouramy, Insulin-Like Growth factor-I (IGF-I), Growth*

1. INTRODUCTION

Over the years, paclitaxel, a renowned anti-cancer agent, has been used widely to treat a variety of solid tumours and cancers. One of the commercially available products that contains Paclitaxel is Taxol, which was initially derived from the bark of *Taxus brevifolia* (the Pacific Yew Tree). It is noteworthy that the crude form of paclitaxel faces solubility challenges, which can be effectively addressed by incorporating Cremophor EL into the drug formulation. However, it's worth mentioning that Cremophor EL has been associated with reports of allergic reactions and potential toxicity concern [1]. For this reason, it is crucial to choose different ways of administering Paclitaxel instead of Taxol while treating cancer [1]. Paclitaxel is delivered to solid tumours using a variety of nanoparticulate systems, such as micelles, niosomes, self-emulsifying delivery, liposomes, polymeric nanoparticles, and lipid emulsions. In the arena of targeted disposition of drugs, PLGA (polylactic-co-glycolic acid) polymeric nanoparticles are preferred because of their biodegradability, compatibility, and remarkable efficacy. It's also significant that the FDA has approved PLGA nanoparticles, enhancing its legitimacy as a medication delivery method. These nanoparticles can be effectively directed to tumors by taking advantage of the Enhanced Permeation and Retention (EPR) effect in solid tumors. This phenomenon enables PLGA nanoparticles to be preferentially absorbed by the tumor due to the leaky vasculature and impaired lymphatic drainage often found at the tumor site, facilitating their penetration into the tumor tissue [2, 3]. The primary objective of the current study is to develop Paclitaxel nanoparticles utilizing PPF as the polymer. This approach seeks to provide an alternative means of delivering Paclitaxel while circumventing the inclusion of Cremophor EL, which has been associated with toxicity concerns. The resulting nanoparticles hold potential for cancer treatment [1].

To accomplish this objective, we employed the Double Emulsion Solvent Evaporation Method (DESE) to fabricate nanoparticles. We utilized a combination of PVA (Polyvinyl Alcohol) and PVA-SDS (Polyvinyl Alcohol-Sodium Dodecyl Sulfate) blend as stabilizers during the nanoparticle preparation process. These nanoparticles' characteristics were carefully assessed, accounting for factors like drug loading, surface charge, polydispersity index, in vitro drug release profiles, and particle size in addition to encapsulation efficiency. Additionally, MCF7 cell lines were used in in vitro cytotoxicity investigations.

2. EXPERIMENTAL

Drugs and chemicals

A gift of a paclitaxel sample was kindly given by Fresenius Kabi Oncology Limited. PVA-SDS was a gift, and the polymer compounds, PLGA, were procured from Himedia Laboratory Pvt. Ltd. We purchased cold water-soluble polyvinyl alcoholfrom Sigma Aldrich. Notably, every other chemical utilised in the study was of the caliber of a reagent grade.

FTIR and DSC Study for assessing compatibility of Paclitaxel with the excipients

Using DSC (Differential Scanning Calorimetry) with a Perkin DSC 4000 instrument and FTIR (Fourier Transform Infrared Spectroscopy) with an Alpha instrument from Bruker in Ettlingen, Germany, the compatibility between Paclitaxel and the other important excipients used in the nanoparticle fabrication process was evaluated. The resulting DSC thermograms and FTIR spectra were carefully analysed to look for any possible interactions or incompatibilities.

Paclitaxel loaded PLGA nanoparticles: Fabrication process

After making some adjustments to an earlier publication [12, 13], a new method for making Paclitaxel-loaded PLGA nanoparticles was created by modifying the DESE (Double Emulsion Solvent Evaporation) procedure. The nanoparticles were made using different ratios of Polyvinyl Alcohol (PVA) and a PVA-sodium dodecyl sulphate (SDS) combination. In the formation of the primary emulsion, PVA was used at a concentration of 3.5% w/v as a stabilizer, while PVA-SDS was used at 1.25% w/v. For the secondary emulsion, PVA was employed at 2.5% w/v, and PVA-SDS at 1.03% w/v. For the formulations PNF2 and PNF5, a combination of PVA and PVA-SDS was utilized as stabilizers. The concentration of PVA used was 1.25 percent w/v for the primary emulsifier and 2.5 percent w/v for the secondary emulsifier. The procedure was to dissolve the medication and PLGA in 3 ml of dichloromethane, then gradually add 1.5 ml of 3.5 percent w/v PVA. In order to create a double emulsion and produce nanoparticles, homogenization was done at 16,000 rpm. After homogenising the primary emulsion once more at 16,000 rpm, 80 millilitres of PVA at a 2.5 percent w/v concentration was added to create the secondary emulsion. To aid in the evaporation of the organic solvent and the solidification of the nanoparticles, the double emulsion was sonicated for 55 minutes and stirred gently all night. The supernatant was purified from larger nanoparticles using. Centrifugation was used to extract larger nanoparticles from the supernatant for eight minutes at 4,000 rpm. For 20 minutes, the recovered supernatant was centrifuged again at 17,000 rpm to produce nanoparticles of the required size. The nanoparticles were centrifuged after being resuspended in distilled water toeliminate any remaining free-form drug from their surface. This washed procedure was carried out twice. After being separated, the nanoparticles were placed in a deep freezer at -45°C and then lyophilized to preserve them. For a comprehensive breakdown of the components of each of the six PNF nanoparticle formulations, refer to Table 1 below.

Table 1: The configuration of PNF.

Formulation code							
		PNF1	**PNF2**	**PNF3**	**PNF4**	**PNF5**	**PNF6**
Paclitaxel (mg)		15	15	15	15	15	15
PLGA (50:50) (mg)		120	120	120	60	60	60
Polyvinyl alcohol (PVA) (%w/v)	**Primary**	3.5	---	---	3.5	---	---
	Secondary	2.5	2.5	---	2.5	2.5	---
PVA-sodium dodecyl sulfate mixture (SDS) (%)	**Primary**	---	1.25	1.9	---	1.25	1.9
	Secondary	---	---	1.03	---	---	1.03

Characterization of PNF

Drug Loading and Entrapment Efficiency

A separator tube for centrifugationcomprising 2.5 mg Paclitaxel-loaded nanoparticles and 2.5 ml of acetonitrile had beenfashionedto measure the efficiency of drug loading and trapping. The tube was then allowed to cool to room temperature after being kept in an incubator shaker at 37°C for four to five hours. Centrifugation was used as the following step to detached the incessant phase from the dispersed phase. The supernatant collected after the completion of the reaction was subjected to spectrophotometric analysis at a wavelength of 228.2 nm. This analysis allowed for the quantification of the released drug, providing valuable information regarding drug loading and entrapment efficiency [4, 5]. The

entrapment efficiency and drug loading percentages were calculated with the following formulas:

$$Drugloading(\%) = \frac{X}{Y} x100$$

X =Drug content of the nanoparticles,Y = amount of PNF sample subjected to assay

$$Entrapmentefficiency(\%) = \frac{A}{B} x\ 100$$

A =Real drug loading − Actual, B= Drug loading – Theoretical

Measurement of Zeta Potential (ZP) and Analysis of Particle Size:

A Malvern NANO ZS90 analyzer was used to analyse the zeta potential, distribution, and size of the Paclitaxel-loaded PNF nanoparticles. With the use of a solid-state laser and dynamic light scattering (DLS), this device measures the size, distribution, and zeta potential of the nanoparticles. The results of the investigation showed that various important parameters may be ascertained by suspending freeze-dried nanoparticles in double-distilled water and sonicating them. Among these were the particle's zeta potential, the particle size distribution, the average size of the hydrodynamic particles, and the polydispersity index, which measures the particle size distribution.For the purpose of identifying and comprehending the characteristics of the PNF, these measures are essential [6, 7].

Scanning electron microscopy (SEM) for defining surface morphological observations

SEM was used to examine the produced nanoparticles' shape and structure in order to assess their surface morphology (Hitachi SEM S-3600N). Metal stubs were affixed with the nanoparticle sample using double-sided carbon tape adhesive. A razor blade was used to score the carbon tape in order to obtain the appropriate number of nanoparticles for review. The samples were then sputter-coated with gold in an argon environment. This coating process enhances the conductivity of the sample and allows for effective imaging. The morphology and structure of the nanoparticles were then observed using secondary electron emissive SEM, providing valuable insights into their surface characteristics and physical properties [6, 8].

Transmission electron microscopy (TEM)

TEM, specifically the JEM instrument, model CX-100 functioning at 200 kV, which offers a p-2-pevaluation and firmness capable of visualizing as well as depicting the nanoparticles' intricate features, was an effective method of visualizing and illustrating the nanoparticles' detailed surface features and attributes. The PNF sample were dried on carbon based glazedlattices, and after they were completely dry, they were negative stained using a 2 percent aqueous uranyl acetate solution. This staining process enhances the contrast and clarity of the nanoparticle images under TEM. The results of this analysis revealed that PPF nanoparticles exhibited a similar formation and size distribution as observed in other nanoparticle types. This conclusion was drawn from the amalgamation of multi-mode tomography and lighted-field imaging steered at various amplifications, providing comprehensive insights into the morphology and characteristics of these nanoparticle [6, 8]

Drug Release Study: *In Vitro*

Drug release tests of the prepared PNF nanoparticles were carried out in phosphate buffer pH 7.4 [16]. For the release study, Six milligrams of freeze-dried nanoparticles were used in Eppendorf tubes. After adding 2.5 ml of phosphate buffer to each of these tubes, the tubes were put in an incubator that was preheated to 37°C. The specimens underwent a series of

shaking periods (0, 1, 3, 6, 9, 12, 24, 36, and 48 hours) at a speed of 130 revolutions per minute. Following the designated times, the samples underwent centrifugation, and 0.6 milliliters of the supernatant were meticulously extracted for examination and at the same time replaced with the same quantity of the buffer. Using a spectrophotometer to measure the absorbance at 228.2 nm, the drug's release from the samples was ascertained. This examination made it possible to appraise the drug's steady release from the PNF nanoparticles.

***In Vitro* Drug Release Kinetic Study:**

Analyzing the mechanism and kinetics of drug release from nanoparticles is crucial to understanding the pharmacokinetic models. Many kinetic equations, for instance0 order, 1 storder, & so on, had been used to analyze the numbers derived from the in vitro drug release investigations. Following that, diagrams were created using these equations. On the corresponding linear plots, a regression analysis was performed to find the values of r^2 and k. This analytical approach helps in quantifying the release kinetics and understanding the behavior of drug release from the Paclitaxel-loaded nanoparticles [9].

MTT assay: Cytotoxicity assessment

According to the previously published accounts, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was used to measure and assess the cytotoxicity of both free Paclitaxel and PNF on MCF7 breast cancer cell lines [10-12].

Statistical analysis

The mean ± Standard Deviation is how the data is displayed (SD). To find statistical differences between the groups, one-way ANOVA was used in the statistical analysis, which was then followed by post hoc Tukey-Kramer tests at a significance level of $p < 0.05$. The statistical analysis in this study was conducted using GraphPad Prism software program (Version 7, GraphPad Software, USA).

3. RESULTS

Assessment of compatibility of Paclitaxel with the excipients

An FTIR study was carried out on a number of samples, including the pure & untaintedAPI, individual excipient employed, physical mixture of the excipients and the API (Drug), and polymeric nanoparticles loaded with paclitaxel, in order to evaluate the possible interactions among the ingredients particularly between the Paclitaxel and the excipients. In Figure 1, displayed the spectra of FTIR.

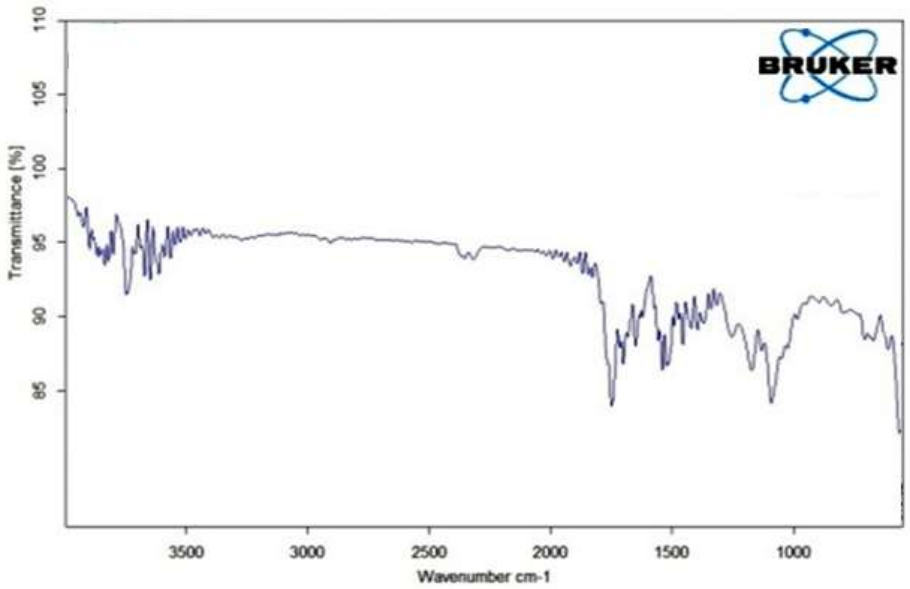

Figure 1. FTIR spectra of the drug sample mix containing the excipients (Paclitaxel, PLGA, PVA, PVA-SDS)

The DSC thermograms for the drug in its pure form, drug in physical mixes with polymers, and lyophilized polymeric nanoparticles were compared. In Figure 2, this comparison is illustrated.

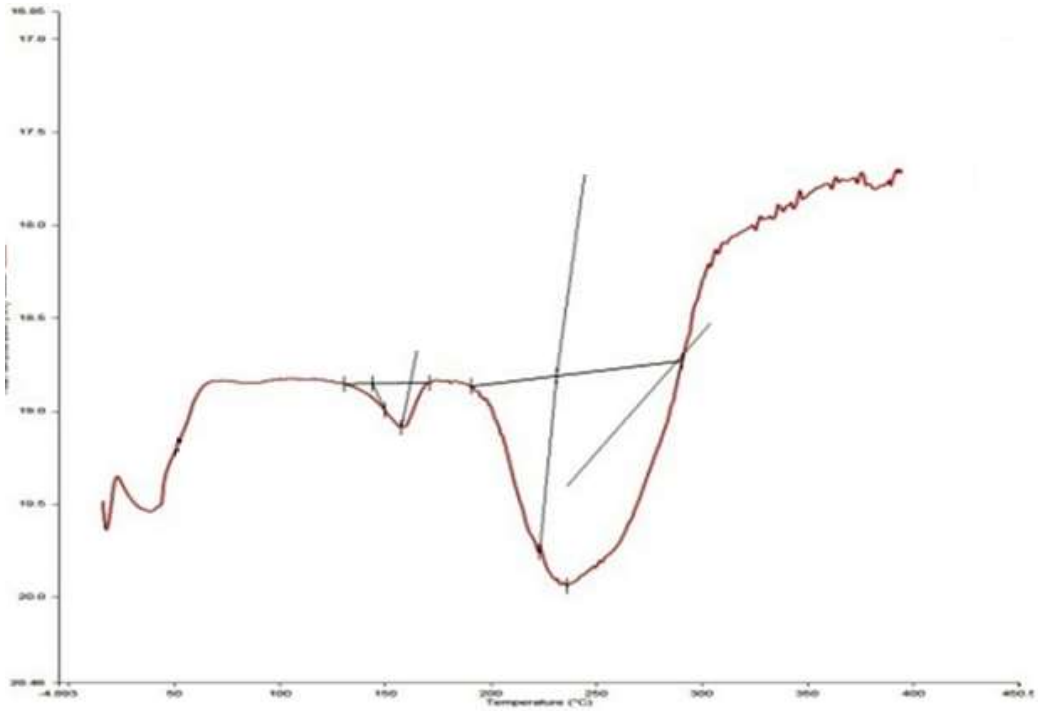

Figure 2. DSC thermograms of a physical drug mixture including the excipients (Paclitaxel, PLGA, PVA, and PVA-SDS).

Fabrication of PNF

A double-emulsion solvent evaporation method was used to produce polymeric nanoparticles encapsulating Paclitaxel.As the matrix, the PLGA polymer was utilised, and various types and ratios of stabilizers, including PVA and a PVA-SDS blend, were employed for

stabilization. In the formulation process, diverse concentrations of drug-to-polymer ratios and varying stabilizer concentrations were explored. Subsequent to the preparation of these formulations, a thorough evaluation was conducted to assess their size, surface properties, and release characteristics. This comprehensive analysis aimed to determine the effectiveness of different formulations in delivering Paclitaxel while considering their physical attributes and drug release profiles.

Drug loading and entrapment efficiency determination:

The entrapment efficiency (%)and drug loading (%) for the nanoparticle formulations PNF1-PNF6 fall within the range of 61.03±0.16% to 74.15±0.12%and 5.37±0.15% to 11.65±0.24%, respectively. Table 2 and Table3 presents comprehensive outcomes for each formulation.

Table 2: Polymeric nanoparticles' (PNF) characteristics including Particle size (nm), Polydispersity index (PDI) and Zeta potential (mV).

	Formulation codes					
Parameters	**PNF1**	**PNF2**	**PNF3**	**PNF4**	**PNF5**	**PNF6**
Particle size (nm)	386.3	307	280	320	320	212.6
Polydispersity index (PDI)	0.501	0.35	0.332	0.437	0.227	0.617
Zeta potential (mV)	-3.06	-6.17	-5.54	-13.2	-1.06	-4.76

*n=3

Table 3: Drug loading (%) and Entrapment efficiency (%) of PNFs.

Drug loading (%)	**Entrapment efficiency (%)**
5.59±0.14	67.38±0.19
5.37±0.15	63.56±0.24
5.83±0.18	67.56±0.17
11.65±0.24	74.15±0.12
9.72±0.31	61.03±0.16
10.54±0.19	70.62±0.17

Appraisal of particlesize and surface morphology

The produced nanoparticle formulations, PNF1–PNF6 have a particle size distribution that spans from 212.6 nm to 386.3 nm, as shown in Table 4. The PNF were discovered to be round as well as spherical in shape and to have even surfaces, as demonstrated by the SEM photomicrographs and TEM pictures (Figures 3 to 4).

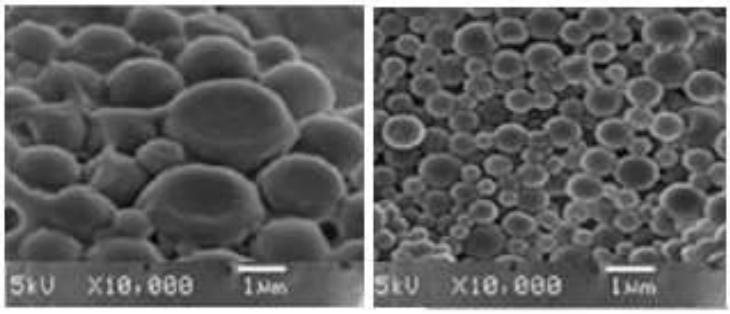

Figure 3. TEM pictures of PNF4

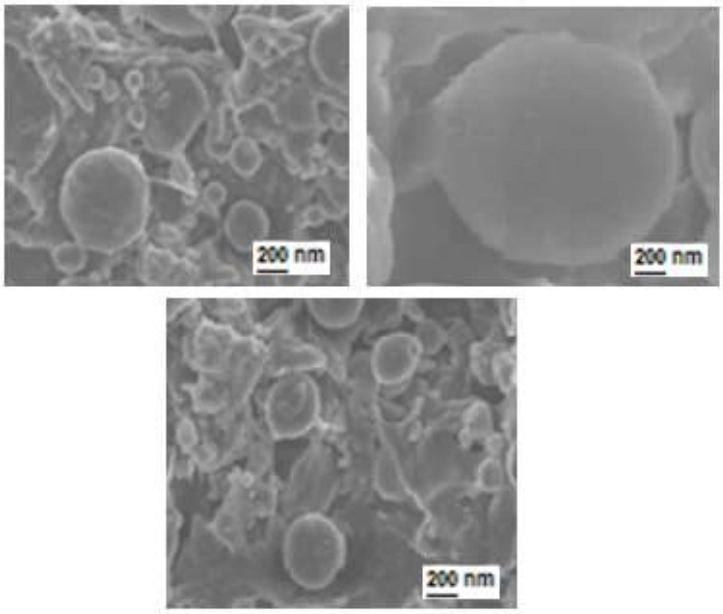

Figure 4. SEM pictures of PNF4

***In vitro* drug release and mathematical modelling for pharmacokinetics**

The release study for Paclitaxel from PNF was investigated in phosphate buffer, which has a pH of 7.4. The computation and presentation of the cumulative percentage of drug released over time are included in the results, as shown in Table 4. Additionally, Figure 5 shows a graphical depiction of the cumulative proportion of medication released over time. The drug release behaviour of the polymeric nanoparticles loaded with paclitaxel is visually profiled in this graph.

Table 4. Data on drug release from PNF nanoparticles *in vitro*

Time (hours)	**Drug release (Cumulative percentage) (Mean ±SD) ***					
	PNF1	**PNF2**	**PNF3**	**PNF4**	**PNF5**	**PNF6**
0	0	0	0	0	0	0
1	22.83±0.08	22.43±0.16	21.92±0.13	25.63±0.09	20.68±0.16	24.54±0.09
3	46.23±0.08	44.83±0.12	43.73±0.14	50.79±0.13	41.71±0.09	50.41±0.09
6	48.08±0.11	46.17±0.13	46.97±0.19	56.24±0.09	42.64±0.19	53.57±0.09
9	52.62±0.08	50.21±0.09	51.35±0.17	60.17±0.15	45.63±0.16	56.66±0.09
12	55.28±0.13	53.32±0.09	53.68±0.19	62.58±0.18	48.24±0.19	59.24±0.12
24	61.65±0.10	56.01±0.19	58.15±0.18	67.58±0.13	51.46±0.18	63.56±0.17
36	67.54±0.09	60.54±0.12	63.46±0.12	73.09±0.19	55.43±0.19	68.76±0.15
48	71.83±0.12	64.21±0.18	67.91±0.13	78.19±0.16	58.54±0.17	75.84±0.19

*n=3

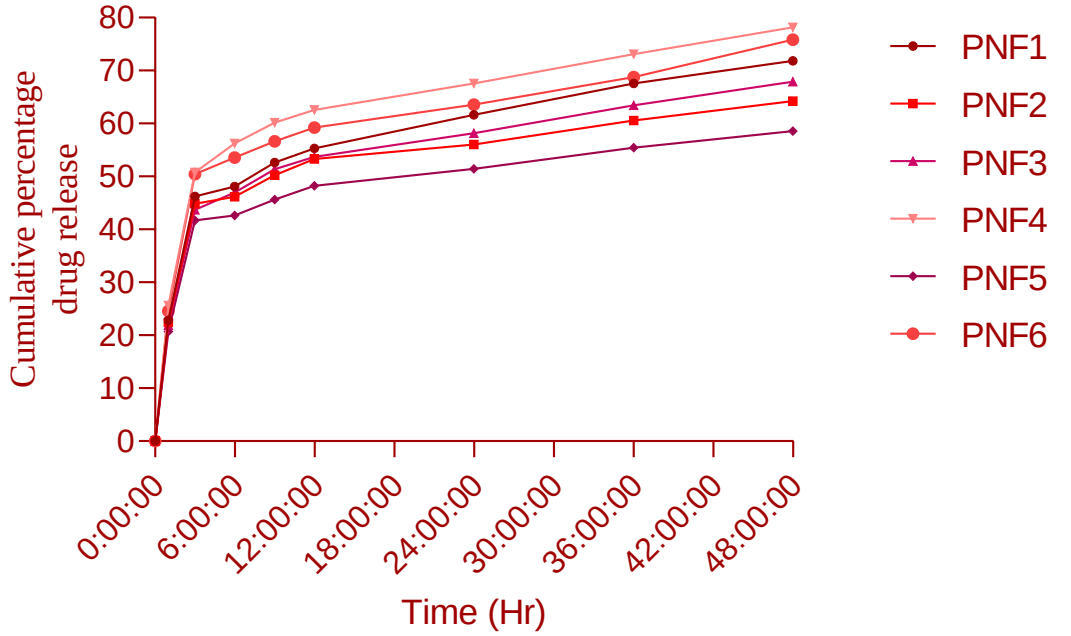

Figure 5. A graph showing the total amount of drug released over time

Table 4 presents a comparative assessment of the dissociation constants and release rate exponents that were calculated using data from release study using various kinetic mathematical models. This table compares the computed parameters obtained from several kinetic models, shedding light on the drug's release kinetics from the nanoparticles.

Table 5. Data on drug release *in vitro* using several kinetic models

Formulation Code	Zero Order	First Order	Hixon-Crowell	Higuchi	Korsmeyer-Peppas	
	R^2				R^2	n
PNF1	0.965	0.642	0.287	0.819	0.957	0.115
PNF2	0.965	0.579	0.269	0.779	0.946	0.107
PNF3	0.944	0.654	0.288	0.804	0.968	0.152
PNF4	0.965	0.653	0.283	0.796	0.968	0.156
PNF5	0.934	0.569	0.269	0.763	0.889	0.047
PNF6	0.974	0.608	0.282	0.783	0.785	0.115

***In vitro* cytotoxicity study using MTT assay**

This investigation used a number of PNF4 concentrations, and the findings are shown in Table 6 and Figure 6. PNF4 concentrations between 150 nM and 2050 nM were shown to significantly affect MCF7 cells when compared to free Paclitaxel group, as evidenced by the MTT assay.

Table 6. Comparing the cytotoxicity of PLGA nanoparticles to that of free Paclitaxel

Concentration (nM)	Paclitaxel	PLGA NPs
0	99.96±3.71	99.98±3.45
150	96.37±2.18	78.44±1.76

250	95.47±1.89	65.65±1.91
350	96.62±1.91	46.47±1.51
450	84.67±1.51	43.96±1.72
550	63.64±1.72	37.96±1.94
800	45.73±1.94	33.57±0.99
1050	38.70±1.92	23.58±1.91
1350	30.57±1.86	21.41±1.51
1550	29.33±1.83	8.69±1.72
2050	17.89±1.08	8.68±1.94

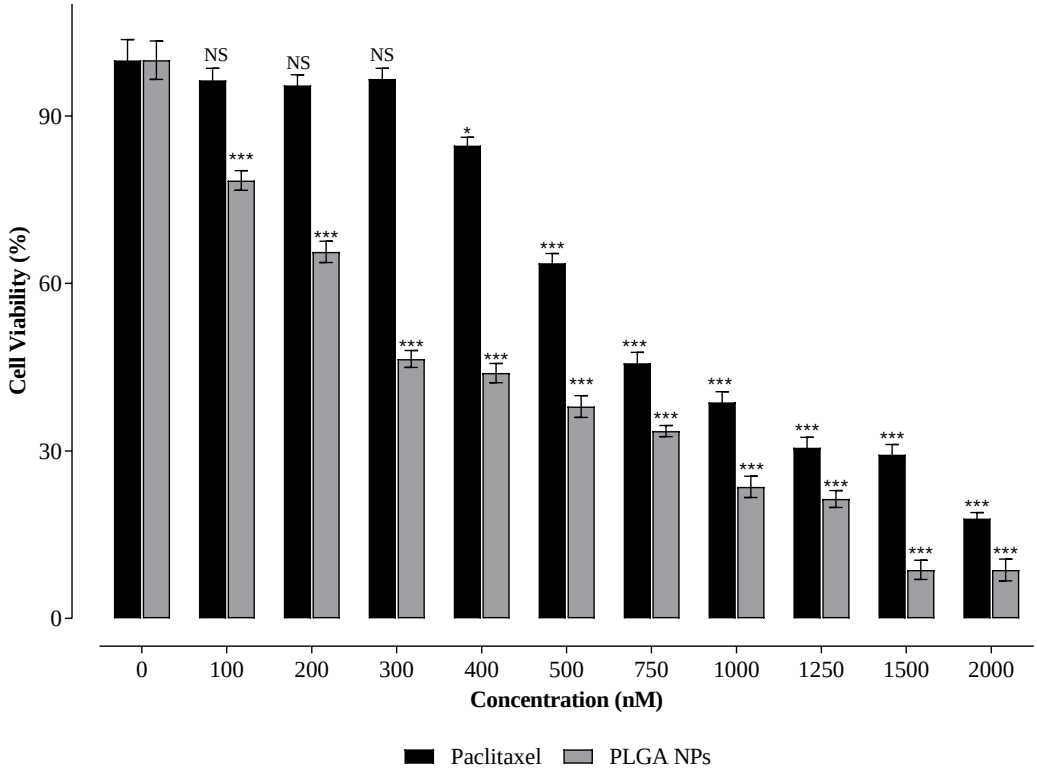

Figure 6. Comparing the cytotoxicity of PNF and free Paclitaxel in terms of cell viability (%)

4. DISCUSSION

The interpretation of the FTIR spectra, which included the spectra of each individual drug, the spectra of the excipients, their physical mixes, and the final formulation, revealed no chemical interactions between the drug and excipients. This result was reached because the polymeric nanoparticles containing Paclitaxel and the drug and excipient mixture both contained all of the important drug peaks. Paclitaxel and PNF (paclitaxel-loaded polymeric nanoparticles) had broad endotherms at 222.4°C and 52.49°C, respectively, according to analysis of their DSC thermograms.The drug's compatibility with the polymer was indicated by the drug peak that was present at the same temperature in the DSC thermograms of the drug-loaded formulation and the physical combination. To sum up, there were no interactions produced by the formulations that would have changed the drug's characteristics, demonstrating the component parts' compatibility with one another in the finished product. Six distinct formulations of PNF (Paclitaxel-loaded PLGA nanoparticles) nanoparticles were prepared to create PNFs with ideal characteristics, including size, polydispersity index,

entrapment efficiency, zeta potential, and in vitro drug release features. These formulations used PVA and PVA-SDS as stabilizers in the double emulsion solvent evaporation process, aiming to attain the desired specifications. Among these formulations, it was observed that the nanoparticles formulated with PVA as the stabilizer, specifically PNF4, showed much greater drug loading and entrapment efficiency than the other formulations. This suggests that PNF4 may be an optimal choice for achieving the desired drug delivery characteristic.

The percentage entrapment efficiency across all formulations ranged from 61.03±0.16% to 74.15±0.12%, while the percentage drug loading ranged from 5.37±0.15% to 11.65±0.24%. Notably, the values for entrapment efficiency and drug loading demonstrated a significant dependence on the drug-to-polymer ratio and stabilizer concentration. Using a 1:5 ratio (drug: PLGA) was found to result in higher drug loading and entrapment efficiency than a 1:10 ratio.Furthermore, compared to those generated with PVA-SDS, nanoparticles fabricated using PVA as the stabiliser confirmed higher percent entrapment efficiency of the drug. This implies that drug loading and entrapment efficiency are not directly correlated with the amount of polymer used in the formulation. Multiple factors, including the optimal drug-to-polymer ratio, stabilizer type, and homogenization speed, among others, contribute to the final outcomes. This observation aligns with previous studies involving PLGA polymer, whereas at a drug to polymer ratio of 1:1 as opposed to 1:2, the drug loading was eight times higher. These findings emphasize the importance of carefully optimizing the formulation parameters to achieve the desired drug loading and entrapment efficiency [4].

The analysis of the particle size data revealed that the drug-to-polymer ratio and surfactant concentration are significant determinants of the size of the nanoparticles.Consequently, the average diameter of each formulation loaded with Paclitaxel, from PNF1 to PNF6, showed a range in average size between 212.6 and 386.3 nm.These results imply that PVA may be a better stabiliser or surfactant when it comes to creating nanoparticles of the appropriate size. Moreover, the examination of particle size revealed that for the drug-polymer formulations, a rise in polymer concentration was accompanied by an increase in particle size. When comparing formulations with a drug-to-polymer ratio of 1:10 (PNF1-PNF3) to those with a ratio of 1:5, this impact was very apparent (PNF4-PNF6). Prior studies have indicated that an increase in the organic phase's polymer content during homogenization could lead to a decrease in shear stress.This is because the viscosity of the organic phase increases with increasing polymer concentration. Consequently, as the viscosity of the organic phase decreases, so does its capacity to disperse with the aqueous phase, leading to an increase in particle size [13].The drug-containing formulations' average polydispersity index (PDI) was found to range from 0.227 to 0.617. The PDI results showed that the distribution of the nanoparticles was quite uniform during the production process. Furthermore, zeta potential (ZP) measurement was used to evaluate the surface charge of the PLGA nanoparticles loaded with Paclitaxel. It was found that the zeta potentials of the nanoparticles PNF1 through PNF6 ranged from -1.06 to -13.2. With zeta potentials between -30 and +30 mV, these nanoparticles have a low propensity to aggregate or cluster quickly and can sustain their initial size for a considerable amount of time. This stability is a crucial feature for applications involving the delivery of drugs[4].After being synthesised and shaped to the desired sizes, the nanoparticles are clearly capable of staying intact and not aggregating, as shown by the obtained zeta potential values. This property is very advantageous since it facilitates medication absorption in biological systems, guaranteeing the stability of the nanoparticles and efficient drug delivery [14].The zeta potential of PLGA nanoparticles was measured in order to analyse their stability and comprehend their destiny in vivo. This investigation sheds light on the nanoparticles' surface charge, which may have an impact on

how they behave and interact with biological systems [15]. The presence of negative carboxylic groups on the PLGA polymer is most likely the cause of the observed negative charge or zeta potential. These carboxylic groups might have dissociated hydrogen ions during the creation of the nanoparticles, which would have contributed to the negative zeta potential that resulted. This polymer's chemical property can affect the nanoparticles' surface charge, which in turn affects how stable and behaves in biological systems [16].After analysing the Paclitaxel-loaded nanoparticles' scanning electron microscopy (SEM) pictures, it was discovered that the particles' size was submicron and that their distribution was uniform. Images obtained using transmission electron microscopy (TEM) demonstrated that the medication was dispersed throughout the entire nanoparticle in a particulate state. This finding aligns with the findings regarding the polydispersity index and highlights the drug's uniform and uniform & homogenous distribution throughout the nanoparticles.
The in vitro drug release results at 1 hour ranged from 20.68±0.16 to 25.63±0.09 for all the formulations, and at 3 hours, the release varied from 41.71±0.09 to 50.79±0.13. The data indicated a gradual increase in drug release after the initial 3 hours of the in vitro study, rather than an abrupt burst release. This release pattern may be attributed to the initial erosion of PLGA, followed by slow diffusion, eventually reaching 78.19±0.16% release at 48 hours. Notably, Formulation PNF4 exhibited the highest drug release of 78.19±0.16% at 48 hours, surpassing other formulations. Upon analysing the in vitro drug release data for release kinetics, the R2 values revealed zero-order kinetics, which were followed by strong linearity in the Korsmeyer-Peppas plot. The release from a polymeric formulation was verified by the mathematical kinetic modelling of the release data*in vitro*, as represented by the Korsmeyer-Peppas model. Furthermore, Formulation PNF4, which produced the largest release, had a n value of 0.156, indicating a release mechanism compatible with Fickian diffusion. This result strongly suggests that the medication is being released from the polymeric system in conjunction with a zero-order release.

The MTT experiment revealed that PNF4's IC_{50}, or the concentration at which 50% of cell growth is inhibited, differed at different reagent concentrations against MCF7 cells. After analysing the PNF4 concentrations that showed the greatest cytotoxicity against MCF7 cells, the maximum concentration of 2050 nM produced a cell viability rate of 8.68±1.94%. As the PNF4 concentration rose, the growth inhibition percentage also increased. The IC_{50} value of this experiment was found to be 84 µg/ml. These findings highlight the effectiveness of PNF4 in inhibiting the growth of MCF7 cells, with higher concentrations leading to greater cytotoxic effects.

5. CONCLUSION

The double emulsion-solvent evaporation approach was used in this study to carefully manufacture paclitaxel-loaded nanoparticles, with PVA and PVA-SDA blend serving as stabilisers. The physicochemical characteristics of these nanoparticles were extensively studied. Based on its characterisation, the formulation PNF4 was subsequently determined to be the most promising formulation. The selected formulation, PNF4, underwent further characterization of its morphological properties, utilizing transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The images obtained from both TEM and SEM revealed that the polymeric nanoparticles displayed a spherical shape. In terms of drug release, it was found that, after 48 hours, 78.19±0.16 percent of the drug had been cumulatively released from lyophilized polymeric nanoparticles loaded with paclitaxel in PNF4. Comparing this release to other formulations, it was noticeably higher. R^2 values showed a more linear behaviour in the Korsmeyer-Peppas plot (0.968) in the drug release

kinetic modellingin vitro investigations for PNF4, which was followed by zero-order kinetics (0.963). These findings suggested that the matrix-type nanoparticle formulation's "Fickian diffusion" was the release mechanismof Paclitaxel. The thorough drug release in-vitro study directed to the conclusion that formulation PNF4 was the most effective and ideal nanoformulation. In conclusion, PLGA nanoparticles loaded with paclitaxel have the potential to be a novel and effective drug delivery technique for the treatment of cancer.

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