NATURALISTA CAMPANO
ISSN: 1827-7160
Volume 28 Issue 1, 2024

Fabrication, Characterization and Cytotoxic Evaluation of Docetaxel Loaded Plga Nanoparticles Using Novel Polymer Pluronic F127 as Surfactant

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Abstract: This study aims to prepare polymeric nanoparticles using PLGA. A double emulsion solvent evaporation method was used to formulate the nanoparticles using Pluronic F127 as the surfactant. In order to encapsulate the hydrophobic drug Docetaxel, a variety of formulations were designed and tested in terms of particle size, zeta potential, polydispersity index, entrapment efficiency, and drug loading. Scanning electron microscopy was used to observe morphology of the formulations. MTT assay was used to determine whether nanoparticles are more cytotoxic than free drugs. It was shown that nanoparticles performed better than free drugs in terms of cytotoxicity as a result of Successful fabrication of the nanoparticles.

Keywords: Docetaxel, Nanoparticle, Pluronic F127, Anticancer, Surfactant

1. Introduction

As a pragmatic approach, the development of nanosized polymeric particles can be used in the formulation of hydrophobic drugs in an efficient manner to achieve a better tolerance to their hydrophobic nature. These systems' quick dissolving rate is a plus because it increases the dosage's bioavailability following oral administration. These systems' quick rate of dissolution is one of their main features. Due to their simplicity and advantages over alternative approaches, they have demonstrated a great deal of promise in addressing the difficulties related to the delivery of pharmaceuticals that are poorly soluble in water as well as poorly soluble in lipids. In order for such colloidal dispersions of nanosized drug particles to maintain their colloidal nature and function, a suitable method must be used to make them and a suitable stabilizer must be applied to maintain the colloidal nature and function of the dispersion. The nanoparticles were prepared using Docetaxel as a model drug. Several beneficial effects can be attributed to Docetaxel in cancer patients [1, 2] as it got an powerful anticancer efficiency including antiproliferative effect on cancer cell lines of breast and prostate [2-4]. Because of the low solubility and instability of Docetaxel, its potential activity is limited, as it is not readily bioavailable and prone to metabolism due to its low solubility [5-9]. Despite our literature review, there are few well-documented efforts to formulate Docetaxel in a way that increases its efficacy and overcomes its related problems. The purpose of our study was to determine whether a new amphiphilic polymer, Pluronic F127® has the potential to stabilise nanoformulations

for the purpose of designing and assessing the efficacious dosing of Docetaxel, a model medication with low solubility. Pluronic F127 ® is an amphiphilic graft copolymer made by BANFP specifically for formulation of poorly soluble drugs, and is derived from polyvinyl caprolactam, polyvinyl acetate, and polyethene glycol [10, 11]. It is anticipated that because of its dual functionality, it will operate well as a matrix to dissolve medications in aqueous solutions. Specifically designed for fourth-generation solid solutions to enhance dissolution, Pluronic F127 is a novel polymer. Poorly soluble drugs can be made more soluble and bioavailable with Pluronic F127 [10]. This work aims to evaluate the drug loading and entrapment efficiency, particle size, polydispersity index, zeta potential, and surface morphology of Pluronic F127-based nanoparticles. Additionally, it investigates the possibility of synthesising nanoparticles with improved wetting characteristics while reducing nanoparticle agglomeration. Cell viability was also evaluated through cytotoxicity testing.

2. Material and Methods

Drugs and Chemicals

The PLGA was obtained from Sigma Aldrich. Fresenius Kabi Oncology Limited provided Docetaxel as a gift sample, while BANFP (USA) provided Pluronic F127. Analytical-grade chemicals were used for all other purposes.

Drug Excipient Compatibility study

Drug degradation may occur as a result of the interaction between the drug and the excipients. For a stable and effective dosage form, the excipients must be compatible. Through FTIR spectroscopy, the possibility of drug interactions was explored.

FTIR Spectroscopy

When comparing the FTIR spectra of the physical mixture of the drug and excipients over wave numbers between 4000 cm-1 and 400 cm-1 to the FTIR spectra of the pure drug and individual excipients obtained from Fourier Transform infrared (FTIR) spectroscopy (Bruker, Germany), the IR spectra obtained were analysed to see if there was any interaction.

Formulation of nanoparticles: Double Emulsion Solvent Evaporation (DESE) using Pluronic F127 as stabilizer

Docetaxel-loaded nanoparticles containing PLGA polymer were synthesized by the DESE process, employing varying volumes and concentrations of Pluronic F127 as a surfactant. 2.5 milliliters of dichloromethane were used to dissolve around 20 milligrams of docetaxel. In the drug and dichloromethane solution, about 20 mg of polymers (PLGA) were dissolved (drug: polymer ratio: 1:2). To create the initial primary emulsion, Pluronic F127 was added dropwise, and the mixture was homogenized for 20–30 minutes at 3,000 rpm high speed, resulting in a rich, creamy emulsion. Using the creamy foam consistency of the primary emulsion, it was then combined with Pluronic F127 and homogenized at 18,000 rpm for 20-30 minutes to form the secondary emulsion. A magnetic stirrer was used overnight to evaporate the organic solvents from the secondary emulsion that was formed after being sonicated for 45 minutes. It was centrifuged at 5,000 rpm for five minutes in order to remove the big particles that had developed in the double emulsion. The supernatant was centrifuged once more for 30 minutes at 7,000 rpm in order to extract nanoparticles. Following three rounds of distilled water washing to get rid of surfactant, the supernatant was freeze-dried. The code for this initial formulation was NFP1. As with formulation NFP1, formulation NFP2 was prepared using Pluronic F127 at the same concentrations (Table 1) in both primary and secondary emulsions, but by increasing its volume to 5 ml in primary emulsion and 50 ml in secondary emulsion. [12, 13].

Characterization of PLGA nanoparticles

Percentage Yield

Using the following formula, Yields (%) of nanoparticle batches were calculated after they had been prepared by both methods DESE [14]:

$$Yield\ (\%) = \frac{\text{Weight (nanoparticles obtained)}}{\text{Weight (drug and polymer used for nanoparticles preparation)}} x100$$

Drug Loading and Entrapment Efficiency

In order to determine the entrapment and loading efficiency of Docetaxel loaded nanoparticles, 2 mg of Docetaxel loaded nanoparticles were weighed accurately and placed in a centrifuge tube with 2mL of dichloromethane. A shaker incubator was used to continuously shake the mixture for 3–4 hours at 37°C. Centrifugation was used to

separate the dispersed phase from the continuous phase. Spectrophotometric measurements at 227 nm were then performed on the collected supernatant to determine the release of the drug. The following equations were used to calculate the percentage of drug loading and entrapment efficiency [15, 16]:

$$Drug\ loading\ efficiency\ (\%) = \frac{\text{Drug Amount present in nanoparticles}}{\text{Drug Amount loaded nanoparticles}} x100$$

$$Entraapment\ efficiency\ (\%) = \frac{\text{Drug Amount present in nanoparticles}}{\text{Intial Drug Amount added}} x100$$

Particle Size and Zeta Potential

A solid-state laser was used to measure the particle size and size distribution of the nanoparticles using a Malvern Nano ZS90 equipped with a dynamic light scattering (DLS) system [17]. Prior to measurement, appropriate volumes of dried nanoparticles were suspended in double-distilled water and sonicated for an appropriate amount of time. Next, for the homogeneous suspension, the average hydrodynamic particle size, size distribution, and polydispersity index were calculated. Zeta potential measurements were also performed using the Malvern NANO ZS90. Prior to measurement, the dried nanoparticles from each formulation were suspended in double-distilled water and sonicated for an appropriate amount of time; the ZP offers details on the particle surface charge and long-term stability.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (Hitachi SEM, S-3600N) was used to examine the shape and surface morphology of the nanoparticles [18]. To create an acceptable sample of nanoparticles, a sample was mounted on metal stumps and broken with a razor blade with the help of double-sided adhesive carbon tape. The samples were sputter-coated with gold under an argon environment, and secondary electron emissive microscopy (SEM) was used to examine the morphology of the samples.

In Vitro Drug Release Study

Drug release studies of formulated nanoparticles were conducted in phosphate buffer pH 7.4. For the goal of drug release investigations, this was accomplished by utilizing Eppendorf tubes that contained 5 mg of freeze-dried nanoparticles and 2 ml of phosphate buffer. The tubes were then maintained at 37°C in an incubator. After shaking the samples for zero, one, three, six, nine, twelve, twenty-four, thirty-six, and forty-eight hours at a speed of 125 revolutions per minute, we centrifuged them. After that, 0.6 milliliters of the produced supernatant were saved. 0.6 milliliter of the removed samples were replaced with brand-new phosphate buffer solution to keep the conditions the same. Drug release from the samples was measured using a spectrophotometer at 227.4 nm [19].

In Vitro Drug Release Kinetic Study

In order to understand the pharmacokinetic models of nanoparticles, an assessment of their kinetics and the process by the drugs are released from them is necessary. Data from in vitro drug release investigations were analysed using a variety of kinetic equations, including zero, first order, and others. Based on the results of these equations, graphs were created. To determine r2 and k, a regression analysis of the linear plots was carried out [20].

Cytotoxicity evaluation using MTT assay

The cytotoxicity of the free drug and PLGA NPs on breast cancer cells (MCF7) was determined by MTT assay as described elsewhere [21-23].

Statistical analysis

The data was presented as mean ± SD. One-way ANOVA and the Tukey-Kramer test were used as post hoc analysis on the data, with $p < 0.05$ being the threshold for statistical differences between the groups. GraphPad Prism (Version 8.0, GraphPad Software, San Diego, USA) was used in this work.

3. Results and Discussion

Compatibility studies: **Drug-excipient compatibility study**

The major and distinctive peaks of the drug and the excipients were found to be retained in the spectra, indicating that there is no incompatibility between the excipients and suggesting that they are perfectly stable. This is based on the individual FTIR spectra of PLGA, Docetaxel, and Pluronic F127 and its comparison to the spectrum of PLGA-Docetaxel-Pluronic F127 physical mixture (Figure 1).

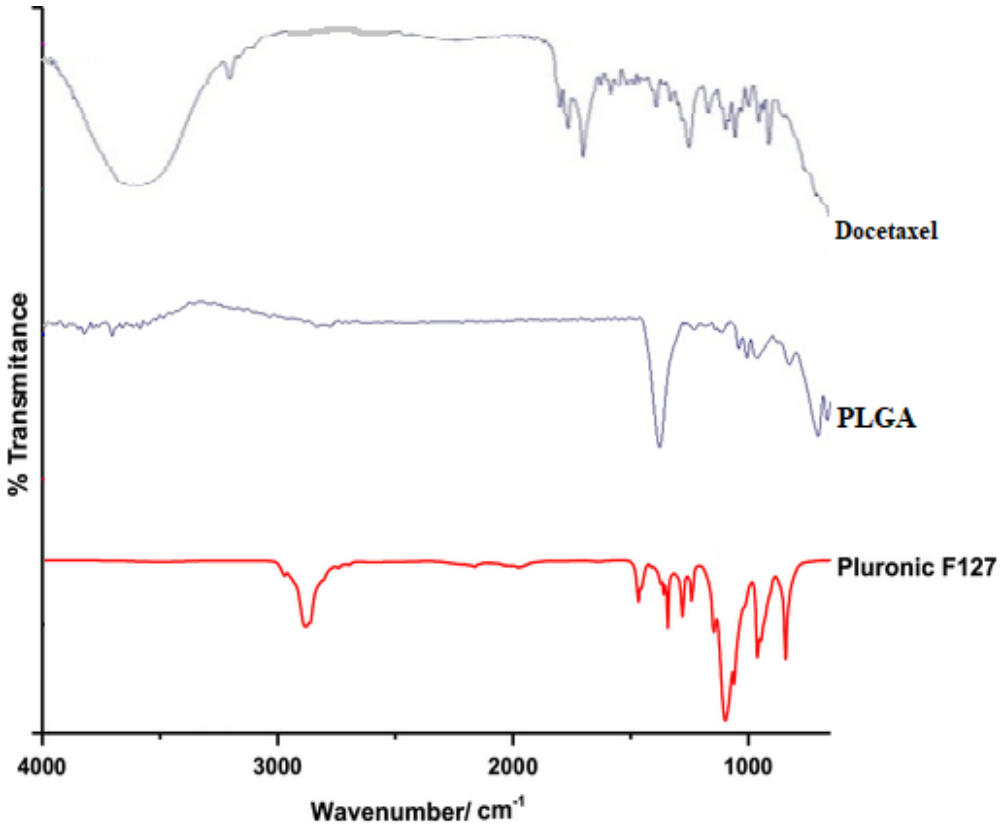

Figure 1. FTIR spectrum of Docetaxel, PLGA and Pluronic F127.

Nanoparticle preparation

There is one method that was used in this study for the preparation of Docetaxel loaded nanoparticles. Table 1 illustrates the Double Emulsion Solvent Evaporation (DESE) technique. Using one of the approaches, formulations with the necessary size, encapsulation, and surface qualities can be produced. The most important component of emulsion technology is full emulsification of both organic and aqueous phases. PVA is frequently used as a stabilizer, even if other stabilizers are also employed. In this investigation, Pluronic F127 was utilized in place of PVA. Table 1 illustrates that the particles produced by DESE have a satisfactory yield, indicating an effective formulation technique.

Table 1: Nanoparticles compositions (NFP1-NFP4) and percentage yield of the nanoparticles

Formulation code		**NFP1**	**NFP2**	**NFP3**	**NFP4**
Docetaxel (mg)		20	20	20	20
Polymer Used		PLGA 85:15	PLGA 85:15	PLGA 85:15	PLGA 85:15
Amount of Polymer (mg)		20	20	20	20
Stabilizer Pluronic F127 (% w/v) & Volume (ml)	**Primary**	1 & 1.5	1 & 2	1.5 & 2.5	2 & 3
	Secondary	1.0 & 25	1.0 & 50	1.5 & 25	1.5 & 50
Yield (%)		78.53	78.83	77.59	78.91

Nanoparticles Characterization

Figure 2 shows SEM images of NPs with smooth surfaces. Based on the results of evaluating its polydispersity index, it can be concluded that the particles loaded with Docetaxel were of submicron size and were homogeneously distributed in the experimental conditions as shown in table 2.

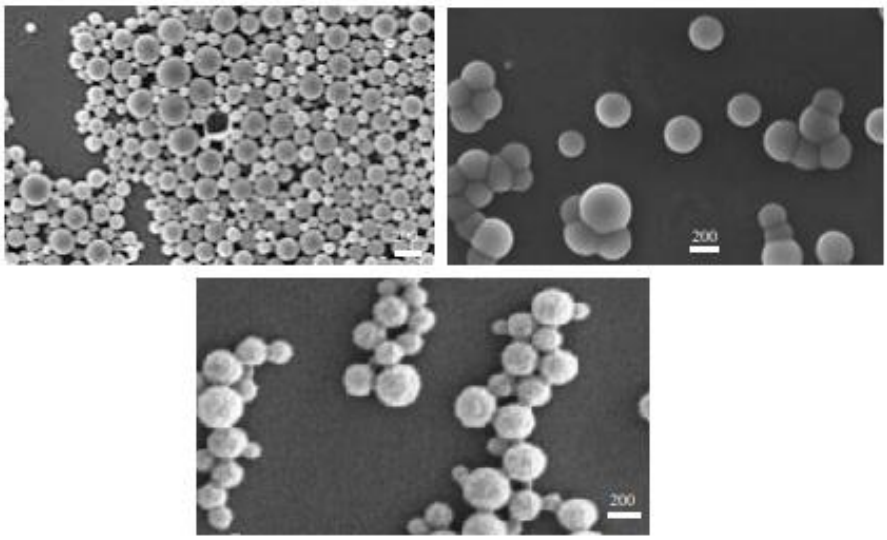

Figure 2. SEM images of prepared PLGA nanoparticles (NFP4)

There are several factors that have an adverse effect on the effectiveness and safety of therapeutic compounds, including inadequate delivery of the drug to the target tissue or undesired side effects such as severe toxicities in healthy tissue and organs. Enhanced bioavailability and minimal side effects can be achieved by encapsulating a drug in nanocarriers with defined and predictable characteristics. Physicochemical characteristics of nanocarriers, such as the distribution of particle size in the nanocarrier, determine their tendency to accumulate in the target tissue. Therefore, for the formulation of safe, stable and efficient nanocarriers to be Successful, homogeneous (monodisperse) populations of nanocarriers of a certain size must be prepared in a homogeneous manner.

Table 2: Characteristics of Docetaxel Loaded Polymeric nanoparticles using Pluronic F127 as surfactant

Formulation code	**Particle size (nm)**	**Polydispersity index (PDI)**	**Zeta potential (mV)**	**Drug loading (%)**	**Entrapment efficiency (%)**
				(Mean ± SD) *	
NFP1	554.4	0.613	-26.2	16.68 ±0.89	36.87±0.86
NFP2	496.1	1.000	-15.8	16.73±0.88	36.71±0.99
NFP3	507.2	0.825	-17.9	17.47 ±0.98	34.54±0.91
NFP4	330.3	0.874	-18.4	19.91 ± 0.99	41.91±0.76

In spite of this, it is difficult to control particle size distribution without considering the composition of nanocarriers as well as the nature of the solvents and cosolvents used during their preparation [24, 25]. After they have been prepared, nanocarriers need to be characterised in order to ensure that they are suitable for *in vitro* and *in vivo* applications. An important parameter used in particle size distribution characterization is the polydispersity index (PDI). An IUPAC-recommended term for non-uniformity in particle size distribution is polydispersity (or dispersity) [26, 27]. PDI, also called the heterogeneity index, is a number that can be calculated when a two-parameter fit is applied to the correlation data (known as the cumulants analysis). This index is dimensionless and scaled in such a way that values lower than 0.05 are mainly observed in standards that have highly monodisperse distributions. When the PDI value exceeds 0.7, the sample probably has a very broad particle size distribution and cannot be further improved. It is possible to use different size distribution algorithms with data that falls between these two extreme values of PDI (0.05–0.7). ISO standard documents 13321:1996 E and ISO 22412:2008 define the calculations used to determine size and PDI parameters [28]. According to the present study, all PDI values exceeded 0.7, indicating broad particle size distributions. Further investigation is needed to determine

whether the formulations are adequate. Formulation NFP4 demonstrated a comparatively better profile in comparison with other nanoformulations based on particle size, PDI (close to 0.7) and zeta potential. An analysis of the zeta potential (ZP) of Docetaxel loaded nanoparticles was performed with the goal of determining the surface charge of the particles. The zeta potential influences not only the pharmacokinetics and biodistribution of nanoparticles in the physiological environment, but also their biodistribution. It has been demonstrated that negatively charged nanoemulsions are excreted more rapidly and are taken up more readily by the reticuloendothelial system than neutrally or positively charged nanoparticles [29]. Furthermore, the efficacy of drug loading and the rate at which medicines can be resorbable from the nanoparticles are determined by the zeta potential of the nanoparticles and the kind of binding that occurs between the medications and the nanoparticles. Furthermore, it can be utilised to ascertain the position of the medicine or active ingredient within the nanoparticles, including if it is encapsulated at the centre or whether adsorption occurs on its surface if it is. According to studies conducted on negatively charged nanoparticles, after intravenous administration of the particles, they are cleared from the bloodstream at a slower pace than positively charged nanoparticles, and they remain in the bloodstream for longer periods of time than positively charged nanoparticles [30]. Studies have also found that nanoparticles with negative zeta potentials or cationic charges are more cytotoxic. The reason for this might lie in enhancing the interaction between the oppositely charged cell membrane and nanoparticles, which in turn can result in membrane destabilization and destruction as a result of the interaction [31]. All the formulations were also found to have the ZP values of polymeric nanoparticles that indicated that they were stable. PLGA nanoparticles produced with Pluronic F127 as the surface-active agent have zeta potential profiles that suggest they could be a suitable delivery strategy for encapsulating hydrophobic drugs like docetaxel. However, considering all the findings together, it can be concluded that Pluronic F127 is a good surface-active agent but in this present study it demonstrated to be a mediocre choice while formulating PLGA nanoparticles. The particle size and broad particle size distribution might be improved in further studies for better stability of the nanoparticles.

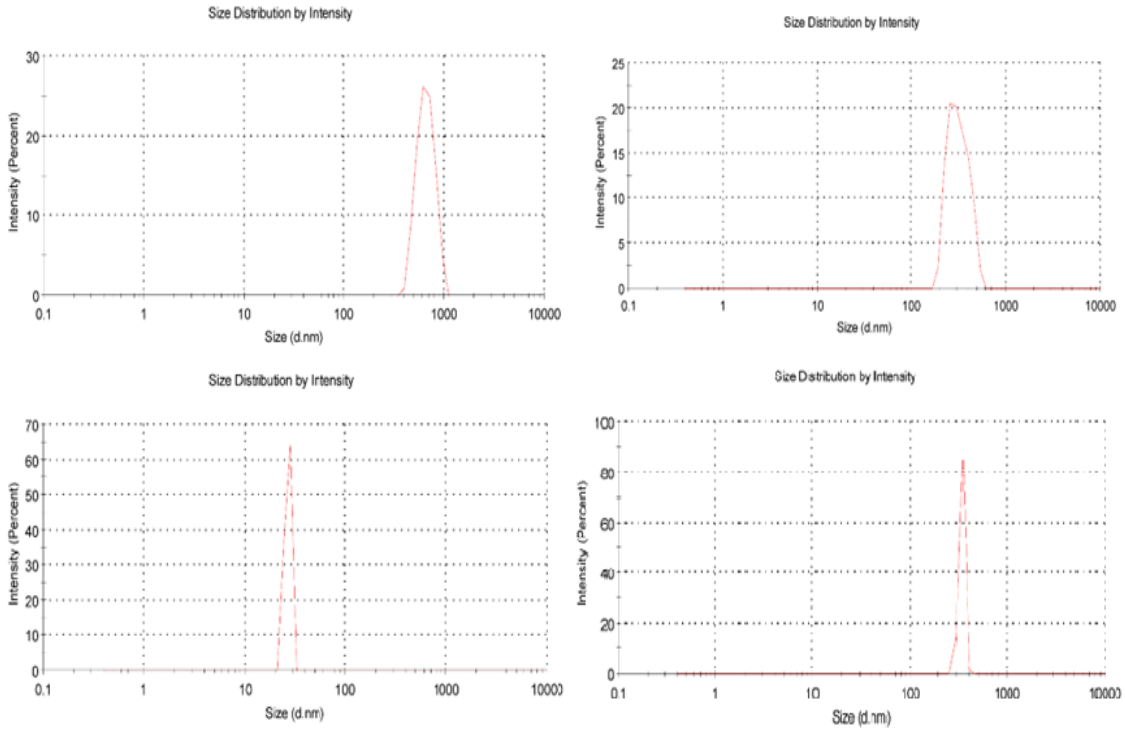

Figure 3. NFP1–NFP4 particle size distribution curve

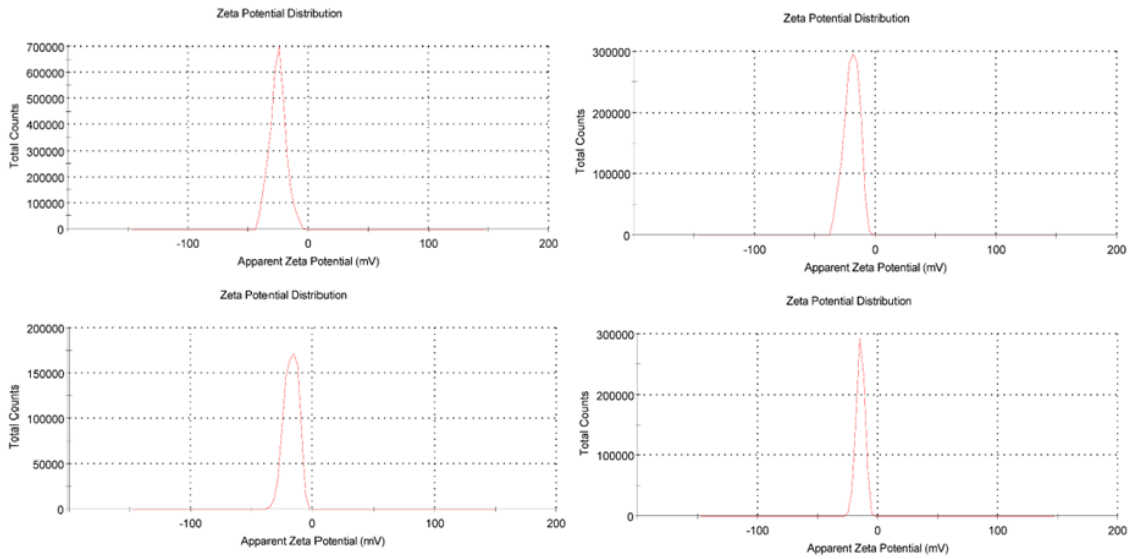

Figure 4. Zeta potential of NFP1-NFP4

Assessment of entrapment efficiency and drug loading

All formulations showed varying percentages of entrapment efficiency, ranging from 34.54±0.91% to 41.91±0.76%, and percentages of drug loading, ranging from 16.68 ±0.89% to 19.91 ± 0.99%. The drug polymer ratio (1:2) and stabilizer concentration had a substantial impact on both drug loading and entrapment efficiency, based on the values of these parameters. Nanoparticles stabilized with Pluronic F127 showed decent entrapment efficiency. A formulation's polymer content does not directly influence drug loading and entrapment, according to these findings. A number of variables must be taken into account for this procedure to be successful, including the stabilizer, the speed of homogenization, and the ideal ratio between the drug and the polymer to be utilized. Previous studies using PLGA polymer also found that when the drug to polymer ratio was 1:1, drug loading was seven times higher than other ratio [19].

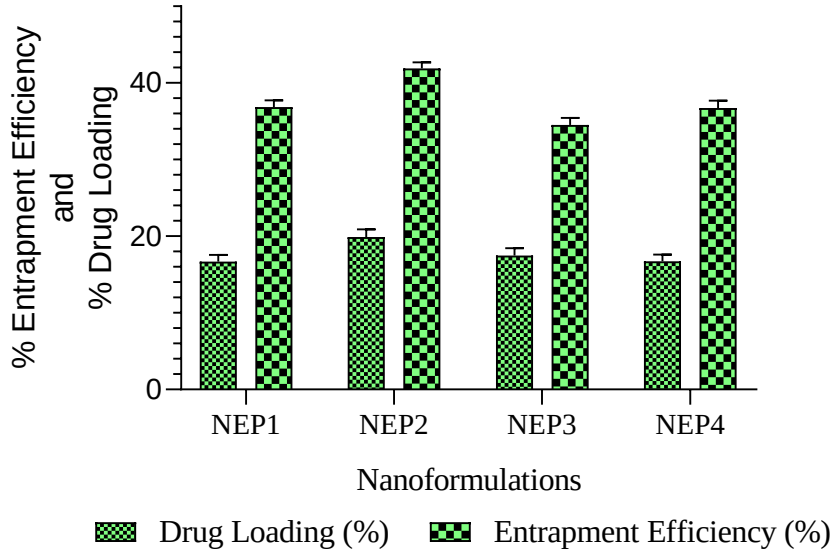

Figure 5. Entrapment efficiency and drug loading of nanoformulations NFP1 - NFP4.

***In vitro* drug release study and mathematical/pharmacokinetic modeling**

In vitro drug release ranges for all formulations from 16.68±0.05 to 21.00±0.05 at one hour, and for all formulations from 39.09±0.09 to 46.88±0.09 at three hours. Furthermore, obtaining these data shows that the drug release slowly increases over the first three hours of the study, rather than a sudden explosion of release during the first three hours of the study. The pattern of release may have been generated by the initial erosion of PLGA, which was followed by a slow diffusion to 73.94±0.17% after 48 hours. At 48 hours, formulation (NFP4) exhibited the maximum drug release, measuring 73.94±0.17%. Upon analyzing the linearity of the in vitro drug release kinetic pattern, the findings revealed excellent linearity in the Korsmeyer-Peppas plot, which was succeeded by zero-order kinetics as determined by the computed R2 values. The release behavior of the polymeric formulation based on the in vitro data is confirmed by thorough kinetic modelling of the in vitro release data using the Korsmeyer-Peppas model, which depicts its release mechanism. Formulation NFP4, which had the highest release, also had an 'n' value of 0.158, indicating 'Fickian diffusion' as the mechanism underlying its release. As a result, this finding clearly indicates that drugs are released from polymeric systems in a zero-order manner coupled with diffusion. The rate constants and exponents that have been determined from drug release data using several kinetic models are compared in the following table.

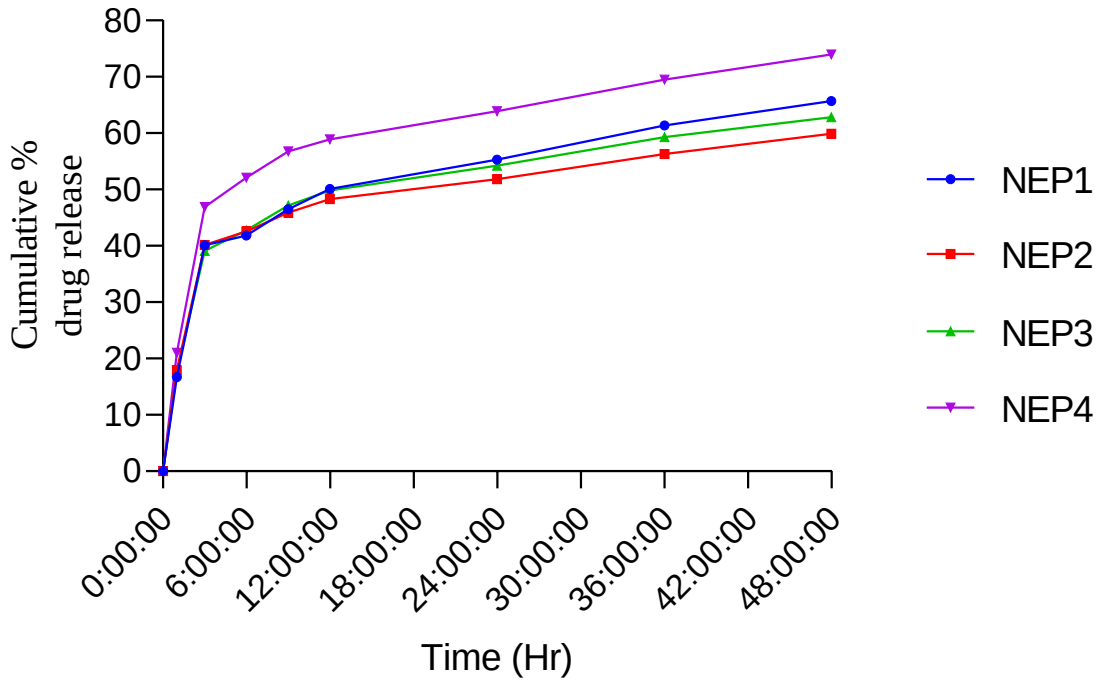

Figure 6. Plot of cumulative percent drug released vs time

Table 3: *In vitro* drug release data of different kinetic models

		Formulation Code			
Model	**R^2**	**NFP1**	**NFP2**	**NFP3**	**NFP4**
Zero Order Model	R^2_Z	0.981	0.976	0.965	0.987
First Order Model	R^2_F	0.665	0.582	0.675	0.675
Higuchi Model	R^2_H	0.821	0.789	0.832	0.798
Hixon-Crowell Model	R^2_{HC}	0.296	0.298	0.3	0.284
Korsmeyer-Peppas Model	R^2_{KP}	0.963	0.967	0.962	0.969
	n	0.12	0.111`	0.147	0.158

Evaluation of cytotoxicity using MTT assay

A MTT assay was performed to determine the IC50 (50% growth inhibition) of NFP4 against MCF7 cells at different concentrations. There are several concentrations of NFP4 that were used in the experiments, and the results are shown in figure 7. When compared to control and free drug concentrations, it was discovered that MCF7 cells responded significantly to NFP4 doses ranging from 200 nM to 2100 nM on MTT assays. The concentration of NFP4 that exhibited the greatest cytotoxicity against the MCF7 cell was determined to be 2100 nM, at which point 13.98±0.91% of cell viability was observed. Growth inhibition % rose with increasing NFP4 concentration, and the assay's IC_{50} value was 121 µg/ml.

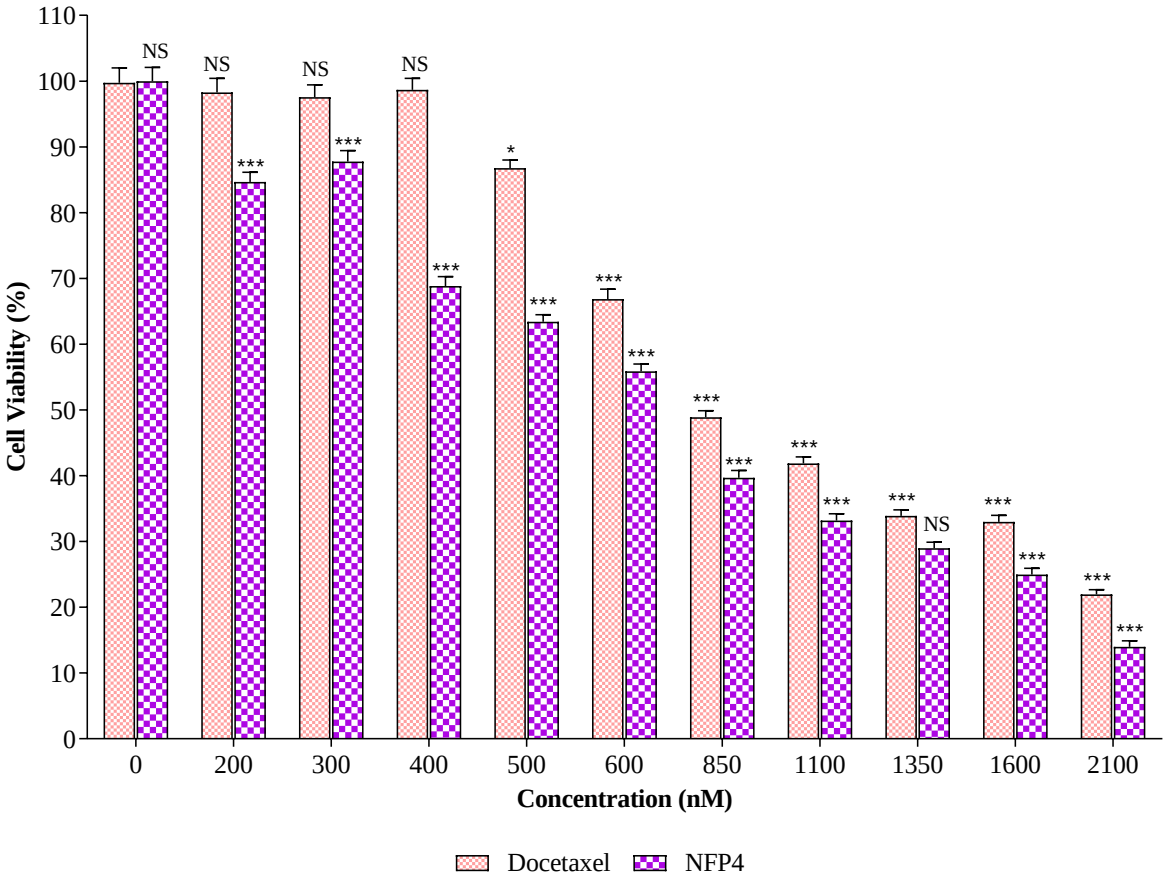

Figure 7. Assessing the PLGA nanoparticles' cytotoxicity in relation to the free drug

4. Conclusions

This study assessed the physicochemical properties of Docetaxel-loaded PLGA nanoparticles prepared by double emulsion-solvent evaporation method. After characterization, formulation (NFP4) was determined to be the best formulation. The morphological properties of the selected formulation were further characterized using SEM. Several spherical polymeric nanoparticles were visible in the SEM images. In formulation NFP4, the cumulative amount of Docetaxel released by the lyophilized polymeric nanoparticles loaded with Docetaxel was higher than that released by the comparison formulations. The Korsmeyer-Peppas plot revealed that the drug release kinetics for formulation NFP4 were studied in vitro. The results showed that the R^2 values were more linear (0.969) and followed by zero order kinetics (0.987). 'Fickian diffusion' from matrix-type nanoparticles is suggested by the drug release exponent (n value) in the Korsmeyer-Peppas plot being less than 0. 5.. NFP4 was found to be the most effective and optimal formulation in *in vitro* drug release studies. The delivery of Docetaxel through PLGA nanoparticles may therefore be an effective and promising method for treating cancer. Docetaxel nanoparticles were successfully designed and evaluated using Pluronic F127 as a surfactant. Pluronic F127 has proven to be a successful surfactant and a potentially useful carrier for encapsulating and delivering poorly water-soluble chemicals as nanoparticles with the right loading, encapsulation, size, and shape. It is necessary to conduct further

studies to investigate the application and usefulness of Pluronic F127 as a sole surfactant in successful and efficient nanoparticle fabrication.

Declaration of Interest
None
Funding
None

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