Preparation, Characterization and Cytotoxic Evaluation of Paclitaxel loaded PLGA Nanoparticles Using Novel Polymer Soluplus® as Surfactant

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Abstract

This study aims to prepare polymeric nanoparticles using PLGA. A double emulsion solvent evaporation method as well as a single emulsion solvent evaporation method were used to formulate the nanoparticles using Soluplus® as the surfactant. In order to encapsulate the hydrophobic drug Paclitaxel, a variety of formulations were designed and tested in terms of particle size, polydispersity index, zeta potential, drug loading, and entrapment efficiency. Morphology was observed using scanning electron microscopy. 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: Paclitaxel, Soluplus®, Anticancer, Surfactant, Nanoparticle

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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. A beneficial feature of these systems is their rapid dissolution rate, which improves the bioavailability of the dosage after oral administration. One of the biggest advantages of these systems is their rapid dissolution rate. In addition to their simplicity and their advantages over other strategies, they have shown a great deal of promise when it comes to dealing with the challenges associated with the delivery of poorly water-soluble as well as poorly water- and lipid-soluble drugs due to their simplicity. 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 stabiliser must be applied to maintain the colloidal nature and function of the dispersion. The nanoparticles were prepared using Paclitaxel as a model drug. Several beneficial effects can be attributed to paclitaxel in cancer patients (Koziara et al., 2004, Spencer and Faulds, 1994)as it got an powerful anticancer efficiency including antiproliferative effect on cancer cell lines of breast and prostate (Sultan Alvi et al., 2017, Spencer and Faulds, 1994, Barbuti and Chen, 2015). Because of the low solubility and instability of Paclitaxel, its potential activity is limited, as it is not readily bioavailable and prone to metabolism due to its low solubility (Lee and Foo, 2013, Panchagnula, 1998, Ma and Mumper, 2013, Skwarczynski et al., 2006, Long, 1994). Despite our literature review, there are few well-documented efforts to formulate Paclitaxel in a way that increases its efficacy and overcomes its related problems.

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The purpose of our study was to determine whether a new amphiphilic polymer, Soluplus ®®, could be used to stabilize nano formulations for the design and evaluation of effective drug administration of Paclitaxel, a poorly soluble model drug. Soluplus® ® is an amphiphilic graft copolymer made by BASF specifically for formulation of poorly soluble drugs, and is derived from polyvinyl caprolactam, polyvinyl acetate, and polyethene glycol(Linn et al., 2012, Shamma and Basha, 2013). As a result of its bifunctional nature, it is expected to act as an excellent matrix to dissolve drugs in aqueous solutions. Specifically designed for fourth-generation solid solutions to enhance dissolution, Soluplus® is a novel polymer. Poorly soluble drugs can be made more soluble and bioavailable with Soluplus® (Linn et al., 2012). Using Soluplus® as a surfactant, this study seeks to determine if it is possible to synthesize nanoparticles that have improved wetting characteristics while reducing nanoparticle agglomeration and to evaluate their drug loading and entrapment efficiency, particle size, polydispersity index, zeta potential, and surface morphology. Cell viability was also evaluated through cytotoxicity testing.

Materials and Methods

Drugs and Chemicals

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

Drug Excipient Compatibility studies

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.

FTIRSpectroscopy

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

Formulation of nanoparticles

Double Emulsion Solvent Evaporation (DESE) using Soluplus® as stabilizer

DESE method was used to prepare Paclitaxel loaded nanoparticles from PLGA polymer using different concentrations and volumes of Soluplus® as a surfactant. Approximately 10 mg of Paclitaxel were taken and dissolved in 2.5 ml of dichloromethane. About 20 mg of polymers (PLGA) were dissolved in the drug and dichloromethane solution (drug: polymer ratio of 1:2). The first primary emulsion was made by adding 2.5 ml of 1.5 % w/v Soluplus® dropwise and homogenising for 20-30 minutes at high speed 3,000 rpm until a rich creamy emulsion was obtained. Using the creamy foam consistency of the primary emulsion, it was then combined with 25 ml of 0.5 % w/v Soluplus® and homogenised 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. To discard the large particles formed in the double emulsion, it was centrifuged at 5,000 rpm for 5 minutes. For obtaining nanoparticles, the supernatant was centrifuged again at 7,000 rpm for 30 minutes, washed three times with distilled water to remove the surfactant, and finally freeze-dried. This first formulation was code named as SF1. As with formulation SF1, formulation SF2 was prepared using Soluplus® at the same concentrations (2.0 % w/v and 1.0 % w/v) in both primary and secondary emulsions, but by increasing its volume to 5 ml in primary emulsion and 50 ml in secondary emulsion.(Dian et al., 2014, Jog et al., 2016).

Single Emulsion Solvent Evaporation (SESE) using Soluplus® as stabilizer

In 2.5 ml of dichloromethane, 10 mg of Paclitaxel were dissolved. A total of 20 mg polymer was added to this (drug to polymer ratio of 1:2). Dropwise addition of the drug-polymer solution was performed to 50 ml 3.0 % w/v Soluplus® and homogenization at 15000 rpm for 10-15 minutes produced an emulsion.

The presence of a creamy emulsion indicates the formation of an emulsion. To remove the organic solvent, dichloromethane, the solution was sonicated for 45 minutes followed by gentle magnetic stirring for 12-14 hours. Afterward, the solution was centrifuged at 15000 rpm for 30 minutes to obtain the nanoparticles, which were then repeatedly washed three times with distilled water to remove the surfactant, and then freeze-dried. The formulation was then code named as SF3. Furthermore, formulation SF4 was prepared using 2.0 % w/v of 50 ml of Soluplus® (Dian et al., 2014, Jog et al., 2016).

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 and SESE (Mukerjee and Vishwanatha, 2009):

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

Drug Loading and Entrapment Efficiency

In order to determine the entrapment and loading efficiency of Paclitaxel loaded nanoparticles, 2mg of Paclitaxel 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 (Ling and Huang, 2008, Gupta et al., 2016):

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

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

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(Marsalek, 2014). Suitable amounts of dried nanoparticles were suspended in double distilled water and sonicated for a suitable period before measurement. The average hydrodynamic particle size, the size distribution, and the polydispersity index were then determined for the homogeneous suspension. The Malvern NANO ZS90 was also used for zeta potential measurements. The dry nanoparticles from each formulation were suspended in double distilled water and sonicated for a suitable period before measurement; the ZP provides information about long-term stability and the particle surface charge.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (Hitachi SEM, S-3600N) was used to examine the shape and surface morphology of the nanoparticles(Radice et al., 2005). With the assistance of double-sided adhesive carbon tape, a sample of nanoparticles was mounted on metal stubs and fractured by using a razor blade to produce an appropriate sample of nanoparticles. Under an argon atmosphere, the samples were sputter-coated with gold and observed for the morphology of the samples using secondary electron emissive SEM.

In Vitro Drug Release Study

Drug release studies of formulated nanoparticles were conducted in phosphate buffer pH 7.4. This was done by using Eppendorf tubes containing 5 mg of freeze-dried nanoparticles and adding 2 ml of phosphate buffer to the tubes, which were then kept at 37°C in an incubator for the purpose of drug release studies. We centrifuged the samples after shaking them at 120 rotations/minute for 0 hours, 1 hours, 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, 36 hours, and 48 hours. Hereafter, 0.5 ml of the obtained supernatant was collected. In order to maintain the same conditions, 0.5 ml of the withdrawn samples were replaced with fresh phosphate buffer solution in order to maintain the same conditions. Drug release from the samples was measured using a spectrophotometer at 227.4 nm(Maji et al., 2014).

In Vitro Drug Release Kinetic Study

An evaluation of the mechanism by which drugs are released from nanoparticles, as well as their kinetics, is essential for understanding their pharmacokinetic models. A range of kinetic equations were used to analyse data collected from in vitro drug release studies, such as zero order, first order, etc., and graphs were compiled based on these equations. A regression analysis of the linear plots was performed to find r^2 and k (Jana et al., 2014).

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(Cao et al., 2016, Ahmed and Kaur, 2017, Lupu and Popescu, 2013).

Statistical analysis

Data are displayed as a mean ± SD. Data were scrutinized by one-way ANOVA and Tukey-Kramer test as *post hoc* analyses based on $p<$ 0.05 statistical differences between the groups. This study employed GraphPad Prism software (Version 8.01, GraphPad Software, San Diego, USA).

Results and Discussion

Compatibility studies

Based on the individual FTIR spectrum of PLGA, Paclitaxel and Soluplus® and its comparison to the spectrum of PLGA-Paclitaxel-Soluplus® physical mixture, it is evident that major as well as the characteristic peaks of the drug and the excipients were found to be retained in the spectra, indicating that there is no incompatibility between the excipients, suggesting that they are perfectly stable.

Drug-excipient compatibility studies

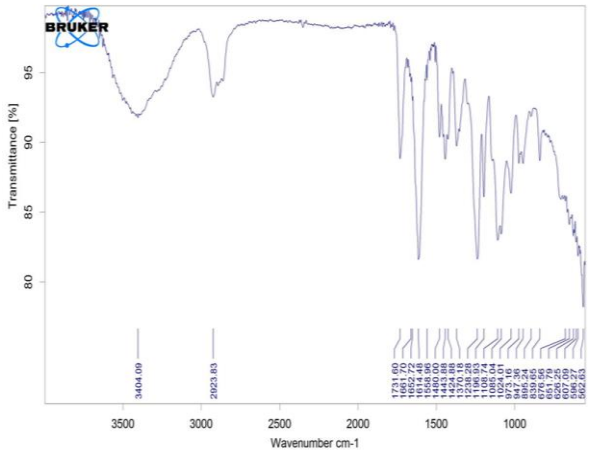

Figure 1. FTIR spectrum of Soluplus®

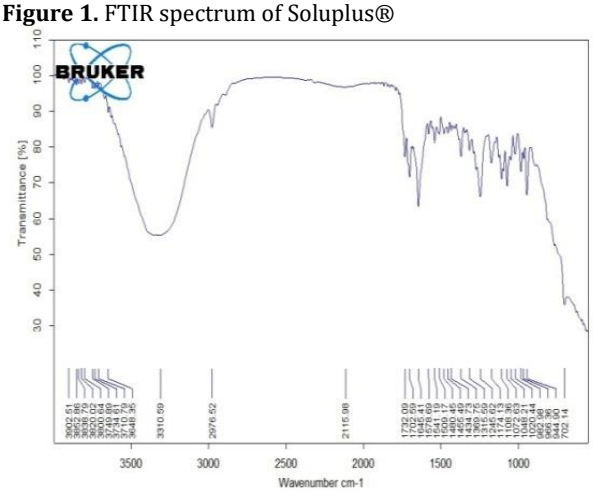

Figure 2. FTIR spectrum of Paclitaxel

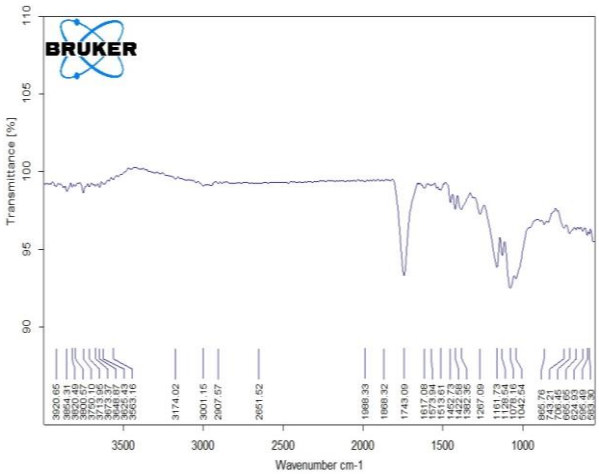

Figure 3. FTIR spectrum of PLGA

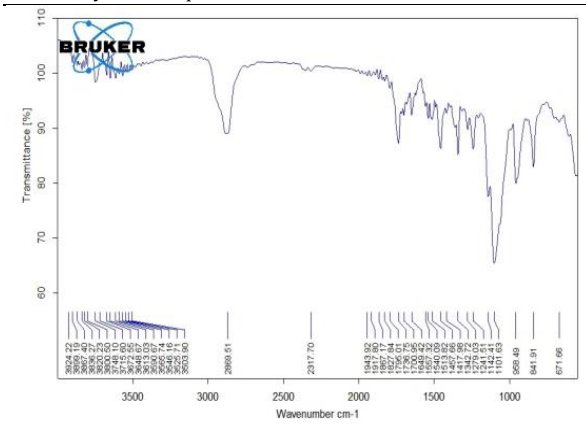

Figure 4. FTIR spectrum of Paclitaxel, PLGA and Soluplus®
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Nanoparticle preparation

There were two methods that were used in this study for the preparation of Paclitaxel loaded nanoparticles. The first method was Double Emulsion Solvent Evaporation (DESE) and the second method was Single Emulsion Solvent Evaporation (SESE) as shown in Table 1. It is possible to achieve formulations with the required size, encapsulation, and surface properties with either of the methods. A complete emulsification of both organic and aqueous phases is the most critical aspect of emulsion technique. It is common practice to use PVA as a stabilizer, despite different stabilizers being used. Instead of PVA, Soluplus® was used in this study. As shown in Table 1, the particles prepared by both the methods have sufficient yield which revealed a better method of formulation.

Table 1: Nanoparticles compositions (SF1-SF4) and percentage yield of the nanoparticles

Formulation code		SF1	SF2	SF3	SF4
Paclitaxel (mg)		10	10	10	10
Polymer Used		PLGA 85:15	PLGA 85:15	PLGA 85:15	PLGA 85:15
Amount of Polymer (mg)		20	20	20	20
Method used		DESE	DESE	SESE	SESE
Stabilizer Soluplus® (% w/v) & Volume (ml)	Primary	2 & 2.5	2 & 5	3 & 50	2 & 50
	Secondary	1.0 & 25	1.0 & 50	---	---
Yield (%)		74.41	74.67	73.66	74.75

Nanoparticles Characterization

Figure 5 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 Paclitaxel were of submicron size and were homogeneously distributed in the experimental conditions as shown in table 2.

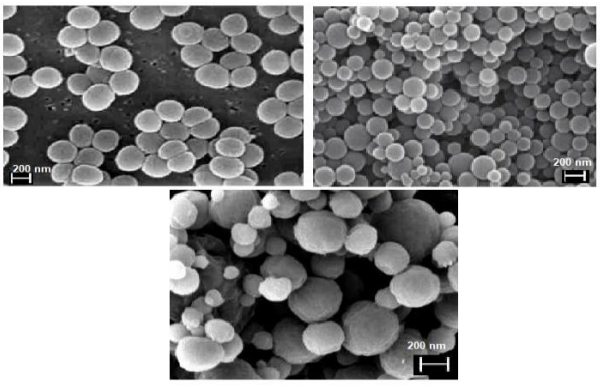

Figure 5. SEM images of prepared PLGA nanoparticles (SF2)

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 Paclitaxel Loaded Polymeric nanoparticles using Soluplus® as surfactant

Formulation code	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)	Drug loading (%) (Mean ± SD)*	Entrapment efficiency (%)
SF1	560.5	0.706	-25.4	14.77 ±0.17	34.68±0.16
SF2	336.1	0.998	-19.7	17.06 ± 0.48	39.60±0.20
SF3	513.4	0.939	-17.6	15.33 ±0.16	32.1`0±0.42
SF4	504.5	1.000	-14.6	14.1±0.16	34.29±0.22

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 (Mozafari et al., 2017, Bulbake et al., 2017). 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)(Dong et al., 2019, Nobbmann, 2014). 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 (Malvern, 2011).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 SF2 demonstrated a comparatively better profile in comparison with other nano formulations based on particle size, PDI (close to 0.7) and zeta potential. An analysis of the zeta potential (ZP) of Paclitaxel loaded nanoparticles was performed with the goal of determining the surface charge of the particles. Besides affecting the biodistribution and pharmacokinetics of nanoparticles in the physiological environment, the zeta potential also has an effect on their biodistribution. It has been demonstrated that negatively charged nano emulsions are excreted more rapidly and are taken up more readily by the reticuloendothelial system than neutrally or positively charged nanoparticles(Xu, 2008). In addition, the zeta potential of nanoparticles and the type of binding between drugs and nanoparticles determine the efficiency of drug loading and the rate in which drugs are resorbable from the nanoparticles. Additionally, it can be used to determine the location of active ingredient/drug in the nanoparticles, as if it is encapsulated at the centre or if it has adsorption on its surface if it is encapsulated at the centre. 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 (Honary and Zahir, 2013). 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 (Honary and Zahir, 2013). All the formulations were also found to have the ZP values of polymeric nanoparticles that indicated that they were stable. The zeta potential profiles of nanoparticles of PLGA prepared with Soluplus® as surface active agent indicate that they could be a promising delivery system for encapsulating hydrophobic drugs such as Paclitaxel. However, considering all the findings together, it can be concluded that

Soluplus® 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.

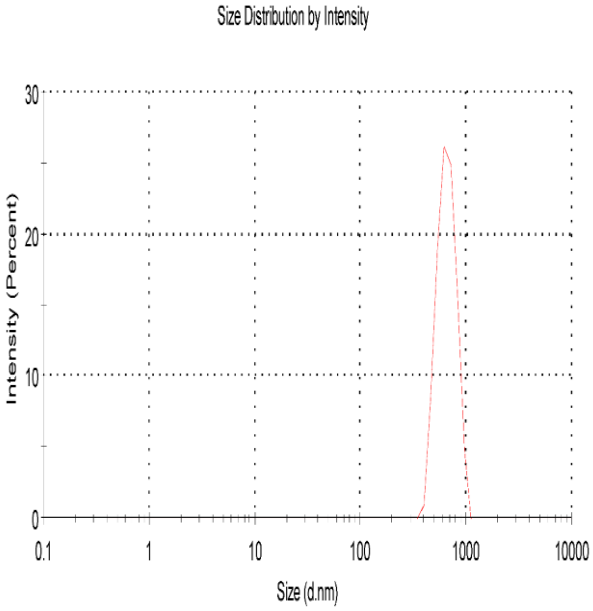

Figure 6. Particle size distribution curve of SF1

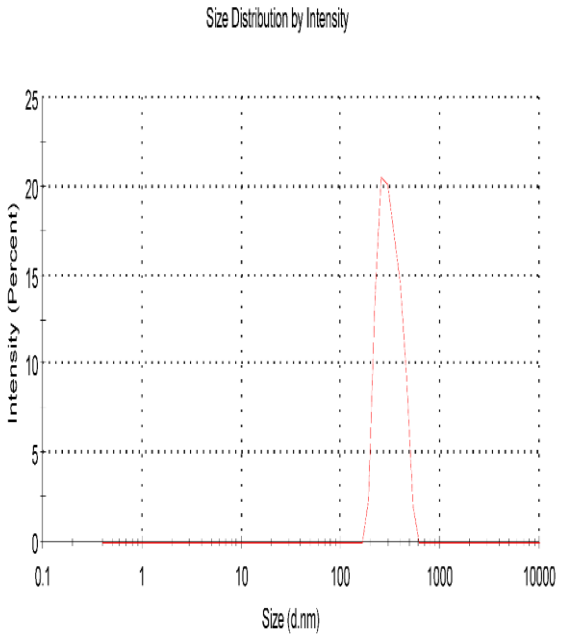

Figure 7. Particle size distribution curve of SF2

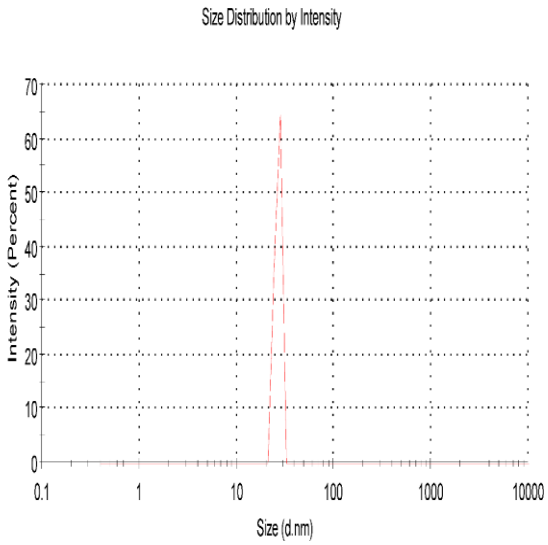

Figure 8. Particle size distribution curve of SF3

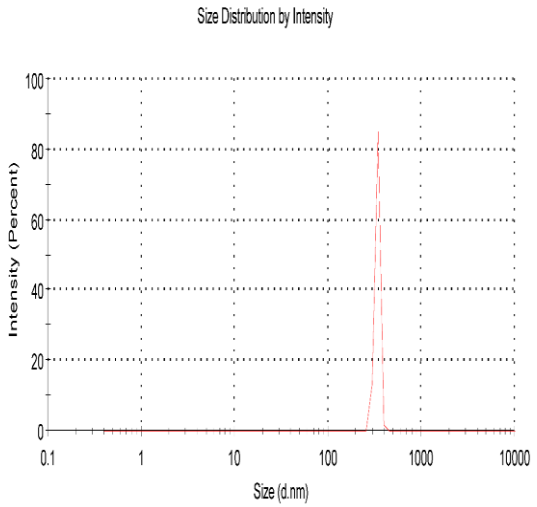

Figure 9. Particle size distribution curve of SF4

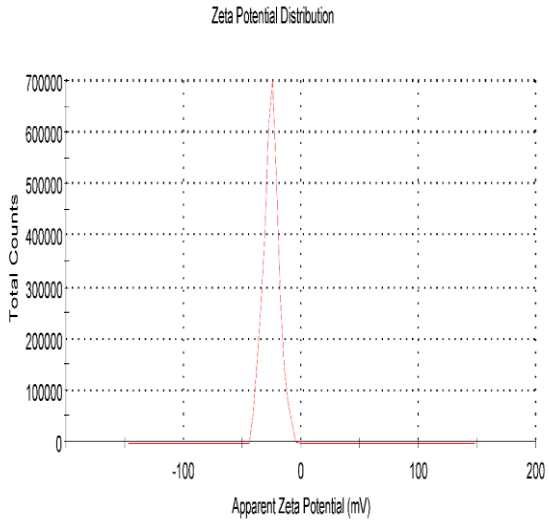

Figure 10. Zeta potential of SF1

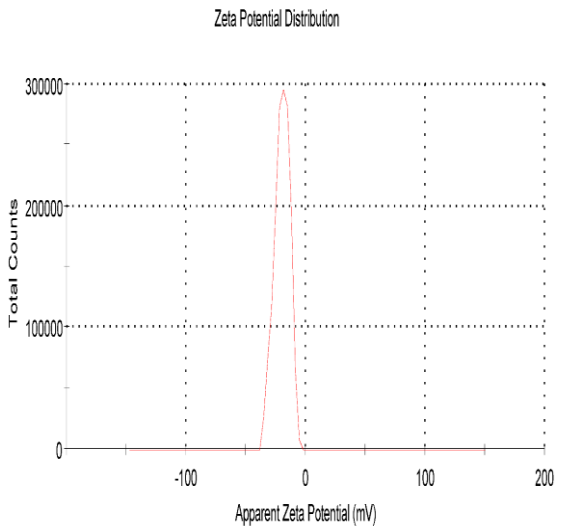

Figure 11. Zeta potential of SF2

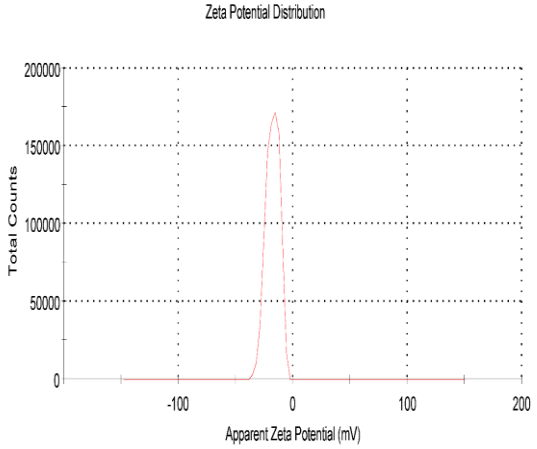

Figure 12. Zeta potential of SF3

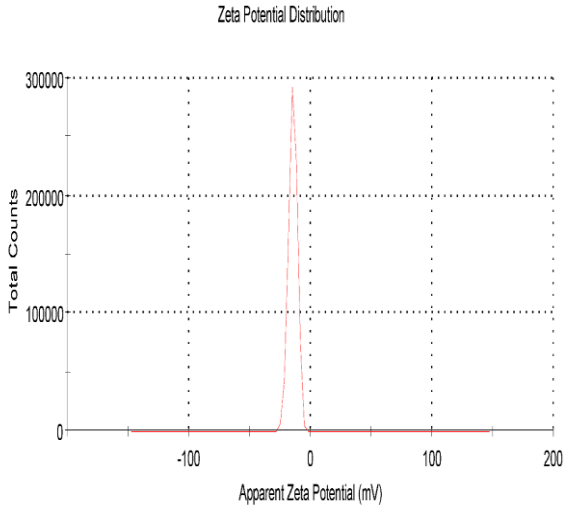

Figure 13. Zeta potential of SF4

Assessment of entrapment efficiency and drug loading

The percentage entrapment efficiency of all the formulation was varied in the range from 32.10±0.42% to 39.60±0.20% and percentage drug loading was found in the range from 14.1±0.16% to 17.06± 0.48%. According to the values of entrapment efficiency and drug loading, drug polymer ratio (1:2) and stabilizer concentration significantly affected entrapment efficiency and drug loading. Nanoparticles stabilized with Soluplus® showed decent entrapment efficiency. A formulation's polymer content does not directly influence drug loading and entrapment, according to these findings. In order for this process to be successful, a number of factors need to be taken into consideration, including the optimum ratio between the drug and the polymer to be used, the stabiliser, and the speed of the homogenization. Previous studies using PLGA polymer also found that when the drug to polymer ratio was 1:1, drug loading was seven times higher than when it was 1:3(Maji et al., 2014).

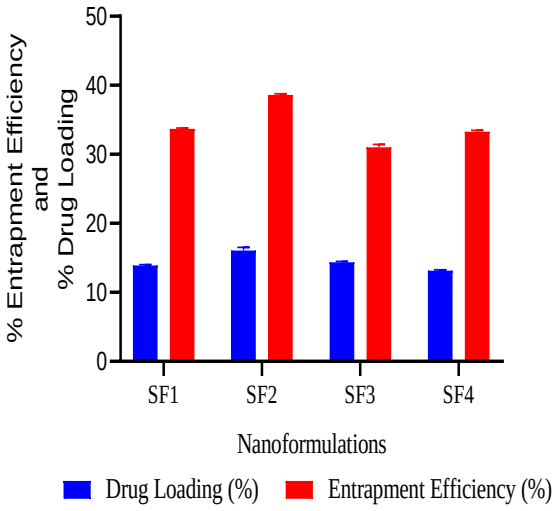

Figure 14. Entrapment efficiency and drug loading of nanoformulations SF1 - SF4.

In vitro drug release and pharmacokinetic modeling of Paclitaxel loaded PLGA nanoparticles

In vitro drug release at 1hr ranges from 16.45±0.10to 20.01±0.06for all the formulations and at 3hr, release was 38.03±0.09to 45.76±0.12for all formulations. 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 initial erosion of PLGA may have caused the pattern of release, followed by slow diffusion to 72.93±0.09% after 48 hours. Formulation (SF2) showed the highest drug release of 72.93±0.09% at 48 hours. When the kinetic pattern of this *in vitro* drug release was analysed for its linearity, the results showed very good linearity in the Korsmeyer-Peppas plot, followed by a zero-order kinetics based on the calculated R^2 values. Detailed kinetic modelling of the *in vitro* release data depicting its release mechanism using the Korsmeyer-Peppas model confirms the release behavior of the polymeric formulation based on the *in vitro* data. Formulation SF2, which had the highest release, also had an 'n' value of 0.161, 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 following table shows a comparison of the various rate constants and exponents that have been calculated from drug release data using different kinetic models.

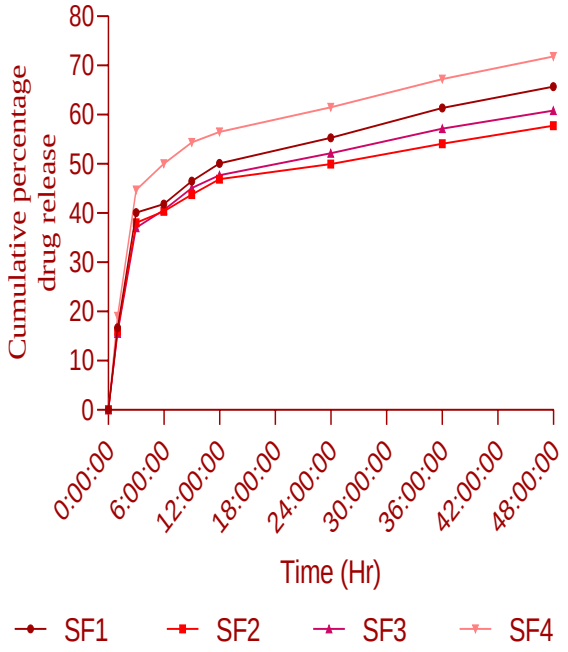

Figure 15. Plot of cumulative percent drug released vs time

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

Formulation Code	Zero Order Model	First Order Model	Higuchi Model	Hixon-Crowell Model	Korsmeyer-Peppas Model	
	R^2_Z	R^2_F	R^2_H	R^2_{HC}	R^2_{KP}	n
SF1	0.970	0.655	0.831	0.297	0.969	0.120
SF2	0.977	0.667	0.796	0.299	0.978	0.161
SF3	0.954	0.666	0.821	0.301	0.973	0.157
SF4	0.975	0.592	0.790	0.283	0.958	0.111`

Evaluation of cytotoxicity using MTT assay

A MTT assay was performed to determine the IC 50 (50% growth inhibition) of SF2 against MCF7 cells at different concentrations. There are several concentrations of SF2 that were used in the experiments, and the results are shown in figure 16. It was found that the SF2 concentrations between 100 nM and 2000 nM had significant effects on MCF7 cells on MTT assays when compared with control and free drug concentrations. Among the concentrations of SF2 that showed the most cytotoxicity against the MCF7 cell, the highest was found to be 2000 nM when it was found to have 15.37±0.78 percent of cell viability. As SF2 concentration increased, growth inhibition percentage increased, and IC 50 value of this assay was 119µg/ml.

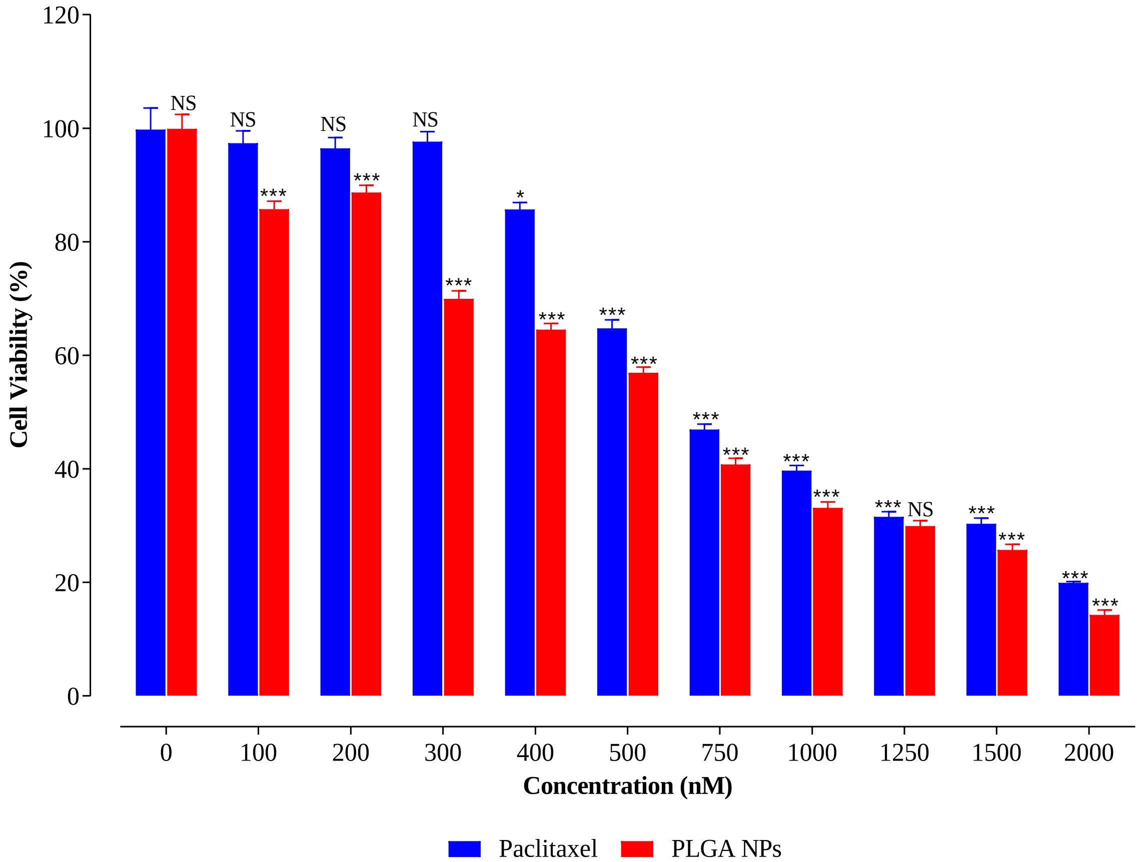

Figure 16. Evaluation of cytotoxicity of the PLGA nanoparticles as compared to free drug

Conclusion

A This study assessed the physicochemical properties of paclitaxel-loaded PLGA nano-particles prepared by double emulsion-solvent evaporation and single emulsion-solvent evaporation methods. After characterization, formulation (SF2) was determined to be the best formulation. The morphological properties of the selected formulation were further characterised using SEM. Several spherical polymeric nanoparticles were visible in the SEM images. In formulation SF2, the cumulative amount of paclitaxel released by the lyophilized polymeric nanoparticles loaded with paclitaxel was higher than that released by the comparison formulations. As a result of the *in vitro* studies of the drug release kinetics for formulation SF2, Korsmeyer-Peppas plot showed the R^2 values to be more linear (0.978) followed by zero order kinetics (0.977). In the Korsmeyer-Peppas plot, the drug release exponent (n value) was less than 0.5, which suggests 'Fickian diffusion' from matrix-type nanoparticles. SF2 was found to be the most effective and optimal formulation in *in vitro* drug release studies. The delivery of Paclitaxel through PLGA nanoparticles may therefore be an effective and promising method for treating cancer. Paclitaxel nanoparticles were successfully designed and evaluated using Soluplus® as a surfactant. It has been demonstrated that Soluplus® is an effective surfactant and a promising vehicle for the delivery of poorly water-soluble compounds as nanoparticles with desired particle size, shape, loading, and encapsulation characteristics. It is necessary to conduct further studies to investigate the application and usefulness of Soluplus® as a sole surfactant in successful and efficient nanoparticle fabrication.

Declaration of interest

The authors declare that there is no conflict of interest in the paper.

Reference

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