Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Design And Synthesis Of Nanoparticle-Encapsulated Drug Delivery Systems For Targeted Cancer Therapy: Exploring The Role Of Green Chemistry And AI-Driven Molecular Modelling

Manisha Masih Singh*[1], B. Vasuki[2], Suchitra Mishra[3], Nidhi Mittal[4], Tamalika Chakraborty [5], Navdeep Kaur[6], Neha Sharma[7]

*[1]**Corresponding Author Email: manishamasih85@gmail.com**

*[1]School of Pharmacy, Chouksey Engineering College, Lal Khadan, Bilaspur, Chattisgarh-495004

[2]PGP College of Pharmaceutical Science & Research Institute, Namakkal-637207

[3]Dadasaheb Balpande College of Pharmacy, Besa, Nagpur- 440037

[4]S R College of Pharmacy, Ambabai, Gwalior Road, Jhansi- 284002

[5]Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata-700114

[6]Rayat Institute of Pharmacy, Lamrin Tech Skill University, SBS Nagar, Punjab- 144533

[7]Rayat Institute of Pharmacy, Lamrin Tech Skill University, SBS Nagar, Punjab- 144533

Cite this paper as: Manisha Masih Singh, B. Vasuki, Suchitra Mishra, Nidhi Mittal, Tamalika Chakraborty, Navdeep Kaur, Neha Sharma, (2024) Design And Synthesis Of Nanoparticle-Encapsulated Drug Delivery Systems For Targeted Cancer Therapy: Exploring The Role Of Green Chemistry And AI-Driven Molecular Modelling. *Frontiers in Health Informatics*, *Vol.13,(8)*, 2462-2471

ABSTRACT

In order to determine the localisation in the breast cancer model system, the newly created polymeric Poly-ε-caprolactone (PCL)/pluronic F108 nanoparticles loaded with an anticancer medication and near infrared (NIR) dye are prepared, characterised, and applied in this study. The nanoprecipitation approach was used to create poly-ɕ-caprolactone (PCL)/pluronic F108-based nanoparticles loaded with docetaxel and NIR dye,"1,1′-dioctadecyl-3,3,3′,3′-tetra methyl indotri carbocyanine iodide (DiR)". Numerous physicochemical techniques, such as size distribution, zeta potential, surface morphology, loading capacity, drug entrapment efficiency, differential scanning calorimetry, in vitro release, and kinetics, were then used to characterise this formulation. The anticancer effectiveness of the nanoparticles was assessed using in vitro cell proliferation tests of human breast cancer BT-474 cells. The contribution of pluronic F108 to the uptake of the nanoparticles by cells was also assessed. Noninvasive infrared dye imaging was used to investigate the localisation of drug- and NIR dye-loaded nanoparticles at the tumour location. The optimised nanoparticle formulation had a zeta potential of -7.37 mV ± 4.93 mV, a particle size of 100-300 nm, and a polydispersity index of 0.156. Diffusion-mediated drug release was suggested by the nanoparticle system's drug release, which followed Higuchi kinetics. According to DSC measurements, the medication in the nanoparticle formulation is either in an amorphous or disordered crystalline state. The potential of PCL/F108 nanoparticles as an enhanced targeted drug delivery method is indicated by their higher accumulation in vivo.

Keywords: Polycaprolactone nanoparticles, Nanoprecipitation, Targeted delivery, Non-invasive imaging, Docetaxel, Pluronic F108, Breast cancer.

INTRODUCTION

The European yew tree (Taxus baccata) yields docetaxel, a semisynthetic cytotoxic taxane counterpart of paclitaxel (Taxol®). An effective chemotherapeutic medication with clinical efficacy against a variety of tumours, including advanced and metastatic breast cancers, gastric cancer, prostate cancer, nonsmall cell lung carcinomas, etc., is its commercial formulation, Taxotere® (Sanofi Aventis), which is administered via intravenous (IV) infusion [1]. Cell cycle arrest is the

result of docetaxel's inhibition of mitotic spindle assembly [2]. In the commercial formulation, docetaxel, which is otherwise poorly water soluble (approximately 10-20μM), is dissolved using the nonionic surfactant Tween 80 (Polysorbate 80) and 13% ethanol in saline.

Its high volume of distribution and high lipophilicity cause a number of adverse effects, such as peripheral neuropathy, anaemia, mouth sores, hair loss, fluid retention, and hypersensitivity reactions [3]. As a result, patients must get corticosteroid pretreatment [4]. Additionally, it is incompatible with administration systems for polyvinyl chloride (PVC). Additionally, cellular efflux pumps for cancer therapy drugs may be produced as a result of docetaxel treatment [5]. Alternative formulations are therefore required in order to reduce the excessive toxicity linked to the excipients and to provide a desired therapeutic response at the tumour site. Due to their similar size to the constituents of human cells, nanoparticles (NPs) and microparticles (MPs) have become more and more popular in drug delivery [6]. Polymers, lipids, viral nanoparticles, and organometallic compounds are some of the sources of nanoparticles and microparticles used as drug delivery vehicles [7]. They are distinct and aid in getting beyond the drawbacks of traditional diagnostic and therapeutic moieties because of their small size, high surface area to mass ratio, and high reactivity [8]. Polymeric nanoparticles that are both biocompatible and biodegradable have become a viable strategy in anticancer treatment. By (1) making the anticancer drug more soluble, which eliminates the need for organic solvents, (2) shielding the drugs from enzymatic breakdown in the blood [9], and (3) keeping the drugs in the carriers, which extends the blood's circulation time and enables them to passively accumulate at tumour sites through the enhanced permeability and retention (EPR) effect [10], the nanomedicines help to overcome the drawbacks of intravenous (IV) administered anticancer agents. Once accumulated, they can be engineered to serve as a local drug reservoir, delivering therapeutic medicines into the tumour tissue constantly and sustainably [11]. By adjusting the surface chemistry and polymer properties, they can be made to transport drugs to specific tumours and give controlled release [12]. Furthermore, active targeting moieties can be added to the surface of nanoparticles to target cells specifically and encourage endocytosis, which is a process that promotes cellular uptake. Drug accumulation in the target locations is improved because, upon uptake, the nanoparticles are typically wrapped by endosomes by receptor-mediated endocytosis, avoiding recognition by the p-glycoprotein [13]. Therefore, it is possible to design nanoparticles to deliver drugs with a maximum payload, protect the drug's bioactivity with a controlled release rate and a prolonged circulation time, minimise nonspecific biodistribution to normal tissue, and maximise the drug's therapeutic potential by preferentially accumulating at tumour sites [14]. For the formulation of docetaxel in the nanoparticles, Poly-ε-caprolactone (PCL) was selected as the preferred polymer. For many years, poly-caprolactone, a biodegradable polyester, has been utilised in tissue engineering and drug delivery systems as biomaterials [15]. In comparison to polyglycolic acid and other polymers, it is a semicrystalline polymer with a low rate of degradation, high permeability to small molecules, and no creation of an acidic environment during the degradation process. However, due to its hydrophobic nature, PCL can be phagocytosed by the mononuclear phagocyte system (MPS) and eliminated from the body. Because blood serum proteins on hydrophobic particles' surfaces are more absorbable than those on hydrophilic particles, hydrophobic particles often opsonise more quickly [16]. In order to prevent opsonisation and effectively camouflage the nanoparticles, coating materials or shielding groups such as polysaccharides, polyacrylamide PEG, and PEG-containing polymers like pluronic® polymers can help evade the MPS's recognition and extend the systemic circulation period. In this work, we describe the nanoprecipitation process used to generate, characterise, and assess docetaxel-loaded PCL/Pluronic® F108 nanoparticles. The size of the nanoparticles is greatly influenced by formulation and process parameters [17]. In order to design the nanoparticle formulation, the concentrations of polymers, stabilisers, and the ratio of solvent to antisolvent volumes were optimised. The in vitro release characteristics, size distribution, surface charge, antiproliferative activity against cancer cells, cellular uptake, and passive targeting potential in vivo were all assessed. In order to eliminate the use of hazardous solvents required for nanoprecipitation techniques, the overall goal of this work was to assess the usage of pluronic acid as a surfactant during the synthesis of NPs. Additionally, the first time a theranostic agent has been incorporated in this way, it enables tumour localisation and monitoring of the drug-loaded nanoparticles at the tumour locations.

MATERIALS AND METHODS

Materials

Sigma-Aldrich, St. Louis, MO, supplied the paclitaxel and docetaxel, which were used without additional purification. Acetone, coumarin-6, and poly-~-caprolactone were acquired from Sigma-Aldrich in St. Louis, Missouri. The gift from BASF Corporation, NJ, was Pluronic® F108. 1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide (DiR), a near-infrared dye, was acquired from Biotium in Hayward, California. From the American Type Culture Collection (ATCC), located in Manassas, Virginia, human breast cancer BT-474 cells were acquired. Atlanta Biologicals, GA supplied Dulbeccos Modified Eagle Medium (DMEM) and Dulbeccos phosphate buffer saline (PBS) for the in vitro cell culture investigations. The MTS cell proliferation assay kit was acquired from Madison, Wisconsin's Promega Corporation. Every additional reagent was analytical or reagent grade.

Methods

Preparation of PCL/pluronic® F108–based nanoparticles

According to earlier reports, the nanoprecipitation process was successfully used to create nanoparticles [10,18]. The preferred solvent for poly-caprolactone (PCL) was acetone. In short, a poly-caprolactone solution of 15 mg/ml in acetone was made. At the same time, an aqueous solution of pluronic® F108 was made and mixed at 800 rpm with weight ratios of 1:2 with regard to poly-caprolactone. Drop by drop, acetone containing PCL was added to the aqueous solution containing F108, causing nanoparticles to form. The volume ratios of acetone to pluronic® solution (solvent/antisolvent) must be kept at 1:6. At 1 ml/min, the flow rate was kept constant. To ensure that all of the acetone evaporated, the suspension was stirred all night.

Nanoparticles filled with drugs and dyes were made similarly. Together with the PCL polymer, docetaxel (10% w/w), coumarin-6 (0.1% w/w), and DiR (0.5% w/w) were added to acetone to create drug-loaded nanoparticles, fluorescent dye-loaded nanoparticles, and NIR dye-loaded nanoparticles, respectively. Subsequently, the aqueous solution containing pluronic® F108 at room temperature is stirred and the medication and/or dye-loaded acetone-based solution containing PCL polymer is added dropwise. For the acetone to completely evaporate and to encourage the precipitation of PCL/F108 nanoparticles, the resultant nanosuspension was agitated throughout the night. For fifteen minutes, the suspension of nanoparticles was centrifuged at 13,000 g. The pellet was given two washes, and the supernatant was disposed of. Until they were used or lyophilised, the produced formulations were kept at 4°C. The suspension of nanoparticles was lyophilised using a Labconco freeze dryer (Labconco corporation, Missouri, USA) after trehalose (2% w/v) was added as a cryoprotectant. In short, the sample was frozen for six hours during the lyophilization procedure, which involved lowering the shelf temperature to -40°C. The material was then dried first for 24 hours at -10°C and then again for 4 hours at 20°C. The vacuum was kept at 10 to 100µm Hg during the lyophilization process.

Characterization of docetaxel-loaded PCL/pluronic F108 nanoparticles

Particle size distribution and surface charge measurements

Using a Malvern Zetasizer Nano–ZS (Malvern Instruments Inc., UK), dynamic light scattering was used to measure the size distribution of the nanoparticles. The study was conducted at a 90° scattering angle after a 50-µl aliquot of either the drug-loaded nanoparticles (3 mg/ml) or the blank nanoparticles (3 mg/ml) were diluted to 1 ml using deionised water. Every measurement was carried out three times.

Surface morphology

Scanning electron microscopy was used to analyse the nanoparticles' surface morphology (Phenom Pure desktop SEM, Phoenix, AZ). To create a consistent coating of particles, the lyophilised nanoparticles were adhered to an aluminium stub using double-sided carbon tape. After that, the particles were photographed at an acceleration voltage of 5 kV and a magnification of × 14,000.

Encapsulation efficiency and loading capacity

Based on a previously published technique, reverse phase high-performance liquid chromatography (RP-HPLC) was used to quantify the amount of docetaxel loaded in the nanoparticles [19]. The Waters Alliance 2695 system (Waters Corporation, Milford, MA) with a 996-photodiode array detector made up the HPLC system. A reverse phase C18 column (ZORBAX Eclipse XDB-C18, 4.6×150 mm, 5µm) kept at room temperature was used for the chromatographic separation. Methanol and 25 mM phosphate buffer (70:30 v/v) were combined to create the isocratic mobile phase, which was administered at a rate of 1 ml/min. The ratio of the amount of drug encapsulated in the nanoparticles to the initial amount of drug employed in the synthesis of NP is known as the drug encapsulation efficiency (EE%). To put it briefly, DMSO and dichloromethane (DCM) were mixed vigorously to dissolve 5 mg of drug-loaded nanoparticles. To ensure that DCM was completely removed from the nanoparticles, the solution was stirred for two hours. After centrifuging the mixture, the drug content of the supernatant was determined. As the internal standard, paclitaxel was utilised at a concentration of 0.2µM. 230 nm was the wavelength of detection. Between 0.25 and 7.5µM, the docetaxel standard curve was linear. Paclitaxel and docetaxel had retention durations of roughly 7.5 and 9.0 minutes, respectively.

Differential scanning calorimetry

The thermal behaviour of the drug docetaxel's interaction with poly-ε-caprolactone was investigated using differential scanning calorimetry (DSC). A TA DSC-Q100 device with a Liquid Nitrogen Cooling System (LNCS) add-on was used to conduct the DSC measurements. In hermetically sealed aluminium pans, samples weighing around 5 mg were heated between 0 and 250°C at a scan rate of 5 °C/min. For the analysis, the nitrogen flow rate was kept at 50 millilitres per minute.

In vitro drug release

A dialysis technique was used to assess the amount of docetaxel released from the nanoparticles [20]. Theoretically, 10%

w/w docetaxel to polycaprolactone was injected into the drug-laden nanoparticles. It was investigated how docetaxel was released from nanoparticles in phosphate buffered saline (PBS, 20 mM, pH 7.4). In short, a dialysis bag containing 5 mg of nanoparticles suspended in 1 mL of PBS with a MW cutoff of 15,000 Da was used. To dissolve the released docetaxel, each dialysis bag was placed in a sink media that contained PBS (20 mM, pH -7.4) and 0.1% Tween 80. The bags were then continuously shaken (80 rpm) at 37 °C. At specified intervals, 200 µl of the sink medium was taken out for analysis and replenished with an equivalent volume of PBS. Using HPLC, the amount of docetaxel in each sample was measured.

In vitro cytotoxicity/antitumoral studies

Using the MTS assay, the anticancer effectiveness of the medication in the nanoparticles was investigated against the BT-474 cell line. BT-474, HER2+ human breast cancer cells, acquired from ATCC (Manassas, VA), were cultured in DMEM: F12 medium (Atlanta Biologicals, GA) enhanced with 1% penicillin–streptomycin sulphate (Cellgro) and 10% foetal bovine serum (Atlanta Biologicals, GA). In a 96-well plate, approximately 7.5×103 cells were planted per well and allowed to grow for 24 hours. The cells were cultured for 72 hours at 37°C after being treated with varying quantities of docetaxel solution and equivalent amounts of docetaxel-loaded nanoparticles for 24 hours. The MTS assay was used to assess the vitality of the cells. The ability of the cells to produce formazan product was used to assess their viability. Using a microplate reader, the absorbance of the formazan dye generated by the viable cells was measured at 490 nm. Media alone was used as the blank control, while untreated cells were used as the control.

Cell uptake

Fluorescence microscopy

In order to facilitate cell adhesion, 1×105 human breast cancer BT-474 cells were sown in 35 mm petri plates and incubated for 24 hours at 37 °C. Fresh media containing the coumarin-6-loaded nanoparticles (20µg/ml) was added to the media after 24 hours, and it was then incubated for one hour at 37°C. Following incubation, the nanoparticles were eliminated, and the dishes underwent four rounds of ice-cold PBS (pH 7.4) washing. After being fixed for 15 minutes at room temperature with a 4% paraformaldehyde solution, the cells were twice rinsed with PBS and then water. After that, the dishes were examined using an Olympus fluorescence microscope. Untreated cells were used as a control for autofluorescence.

Flow cytometry

This study set out to assess the nanoparticles' formulation-dependent uptake. F108's contribution to PCL nanoparticle cellular uptake was assessed. In order to facilitate cell adhesion, human breast cancer BT-474 cells were first seeded at a density of 1×105 in 35-mm petri plates and then incubated for 24 hours. Following a 24-hour period, the cells were treated with 20 µg/ml of PCL/F108 nanoparticles loaded with coumarin-6, PCL nanoparticles loaded with coumarin-6 (without pluronic® F108), and an equivalent amount of dye in solution. They were then incubated for one and two hours. The cells were rinsed five times with ice-cold PBS (pH 7.4) after the nanoparticles and dye solution were withdrawn at predetermined intervals. After harvesting the cells with 0.25% trypsin, they were centrifuged at 130 g for 10 minutes at 4°C. The cells were then resuspended in PBS and subjected to flow cytometry analysis. The BD Accuri C6 flow cytometer (Accuri® cytometers) was used to measure the fluorescence intensities and individual fluorescence of 15,000 cells in the samples.

In vivo passive targeting potential

Two groups of four female BALB/C mice, ages 4-6 weeks, were assigned. A total of 106 murine breast cancer T07 cells were subcutaneously injected into the mice after being suspended in 200µl of serum-free DMEM medium. For two weeks, the tumour was left to grow until its mean tumour volume was 250 cubic millimetres. Subsequently, DiR dye solution was given to one group and DiR dye-loaded nanoparticles to the other. In short, 200µl of PBS (pH 7.4) was used to suspend 5 mg of the nanoparticles carrying 1µg of dye. Mice were given intravenous injections of the suspended DiR nanoparticles/dye solution via the tail vein. The mice were then imaged using an Odyssey bio-imager to map the biodistribution of the particles/dye solution, and the distribution was tracked at pre-established intervals. Higher dye solution concentrations (10µg) were used to optimise NIR imaging, and the results were compared to DiR dye nanoparticles loaded with 2.5 µg.

RESULTS

Blank nanoparticles have a polydispersity value of 0.141 and an average particle size of 166 ± 2.8 nm. Drug-loaded nanoparticles had an average size of 216±3.4 nm and a polydispersity index of 0.156. The blank nanoparticles had a surface charge of approximately -0.252 mV± 5.49. The particles had a neutral charge since PCL is a neutral polymer. The drug-loaded nanoparticles had a charge of around -7.37 mV± 4.93 (Figure 1). The nanoparticles were smooth and spherical in shape, according to the SEM image (Figure 2). It is computed that docetaxel has an entrapment efficiency of 86.0 ± 3.9% and a percentage drug loading capacity of 5.5% w/w. PCL displayed a melting endotherm peak at 65.98°C based on DSC data. Similarly, the nanoparticle made with PCL and F108 showed an endotherm at 104.87 °C, indicating the presence of interaction between the polymers, whereas pure F108 had a melting endotherm at 59.61 °C. The melting point of anhydrous docetaxel in crystalline form is 164.98 °C, and deterioration peaks at 186.91 °C, according to the thermogram. Nevertheless,

the formulation of nanoparticles does not exhibit this peak (Figure 3). This implies that the drug is present in the nanoparticles in either an amorphous or disordered crystalline or solid solution state. The dialysis method was used to study the in vitro release of docetaxel. The docetaxel in vitro release profile from PCL/pluronic F108 nanoparticles as a function of time is shown in Figure 4a. According to the literature, the medication is released from nanoparticles in a continuous manner, with a 30% release in as little as six days [21]. The release data was fitted against well-known models, including zero order (Figure 4b), first order (Figure 4c), and Higuchi (Figure 4d), using a model-dependent methodology. The PCL/F108 nanoparticles' release kinetics were assessed using the Higuchi, Korsemeyer Peppas, zero order, and first order models. The best-fitting models were indicated by the correlation coefficient (R2). The release of docetaxel from the PCL matrix is consistent with the Higuchi model (R2=0.9596), according to the R2 values of various models. Human breast cancer BT-474 cells were used in a cell proliferation assay to test the docetaxel-loaded PCL/F108 nanoparticles' anticancer effectiveness. The proliferation of human breast cancer BT-474 cells was inhibited by docetaxel-loaded PCL/F108 nanoparticles in a manner similar to that of free docetaxel, as shown in Figure 5. There were no statistically significant differences between the two treatments at concentrations of 2.5 to 250 nM docetaxel (2 to 202 ng/ml), and a significant inhibition was observed at 750 nM (606 ng/ml). These findings suggest that the cytotoxicity of free docetaxel is preserved by the docetaxel nanoparticles. The blank nanoparticles' cytotoxicity and safety profile were also examined using RAW 264.7 mouse macrophage cells. Following a 72-hour incubation period with cells, the percentage of cell viability was plotted at different nanoparticle doses ranging from 0.5 to 500µg/ml. Over the course of three days, the measured concentrations of the blank PCL/F108 nanoparticles (0.5-500 µg/ml) were determined to be noncytotoxic (data not shown). Fluorescence microscopy was used to examine the uptake of PCL/F108 nanoparticles into human breast cancer BT-474 cells. As early as 0.5 hours, the uptake of fluorescently laden particles was seen. After one hour of treatment, the human breast cancer BT-474 cells internalised the coumarin-6-loaded NPs, as seen by the green fluorescence in Figure 6. The FITC channel was used to stain the cells' fluorescence. Using flow cytometry, the cellular absorption of coumarin-6 in PCL nanoparticles (no F108) was contrasted with that of coumarin-6-loaded PCL/F108 nanoparticles and coumarin-6 solution (Figure 7). When the cells are synthesised in nanoparticles, the mean fluorescence intensities of the cells significantly rise, indicating a higher absorption of the nanoparticles than in solution. Additionally, compared to particles with PCL alone, the presence of F108 produced improved absorption. Five hours after the mice were injected with the nanoparticle formulation and a 1-µg dye solution, they were scanned. Although there was no discernible dye solution localisation in the tumour area, nanoparticles began to collect in the tumours as early as five hours. Whereas the dye solution was removed from the animals (low signal intensity), the nanoparticles localised in the tumour (high signal intensity). As a result, it is clear that mice given the nanoparticle injection had the dye remain in their bodies for a longer period of time than mice given the free dye solution. It is clear from the drug release data in vitro that the PCL possesses a slow-releasing matrix. In order to assess the PCL/F108 nanoparticles' capacity for in vivo passive targeting and sustained release, 2.5µg of DiR was entrapped in the nanoparticles. In the meantime, the nanoparticles began to localise in the tumours in 4.5 hours and persisted until the final time point that was recorded, which was 48 hours. As the dye intensity rose, the localisation followed an increasing pattern. This implies that the nanoparticles might offer a regulated release and passively build up in the tumour in vivo. Using Odyssey Bioimager at 780 nm, Figure 8 illustrates the buildup of DiR dye in the tumour when given as a solution and in nanoparticle formulation, assessed after 0.5, 4.5, 12, 24, and 48 hours, respectively. Interestingly, despite injecting four times less dye than the solution group, the nanoparticle group showed higher signal intensities. This is the best-case situation to back up our claim that, in terms of passive targeting capability, nanoparticles are superior to free solutions. We regrettably missed the time point for analysis since the mice given the free DiR group injection could not be fully sedated throughout the experiment. Initial studies show that, in comparison to the dye solution, the formation of dye nanoparticles increases with time.

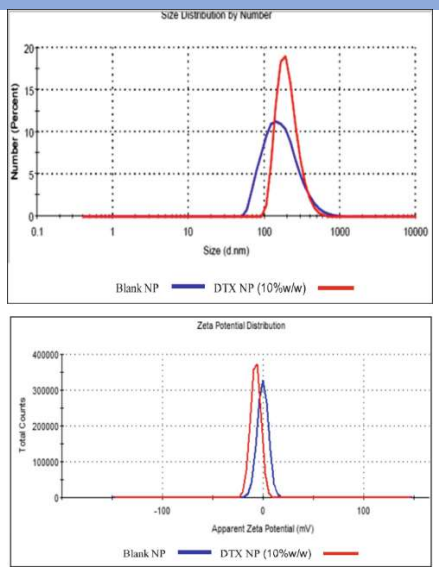

Figure 1: Size distribution and Zeta potential measurements of 10% w/w docetaxel loaded PCL/F108 nanoparticles

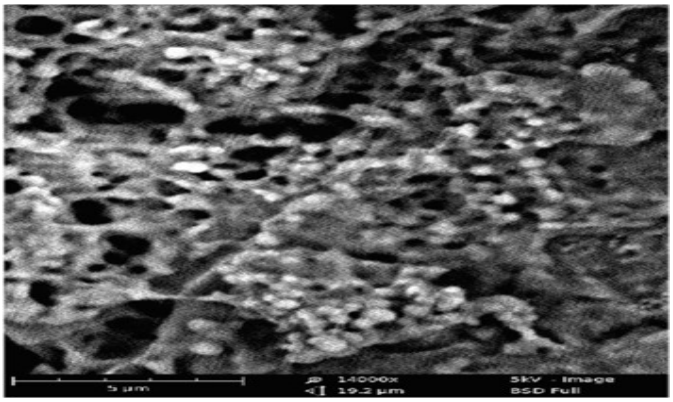

Figure 2: SEM image of drug loaded nanoparticles represent the surface morphology of the nanoparticles (scale-5 µm)

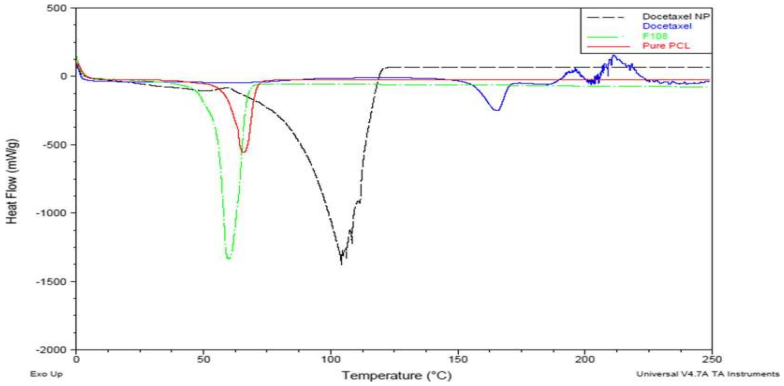

Figure 3: DSC thermograms of docetaxel (shown in blue), PCL (shown in red), F108 (shown in green) and PCL/F108 docetaxel loaded nanoparticles (shown in black) In vitro drug release

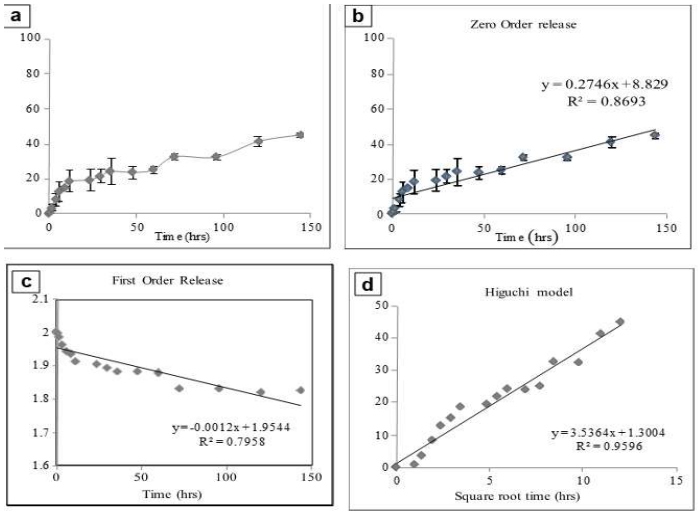

Figure 4: a Release of docetaxel from nanoparticles with time measured at 37 °C in PBS, pH -7.4. b, c, d, the zero order, First order, Higuchi models respectively

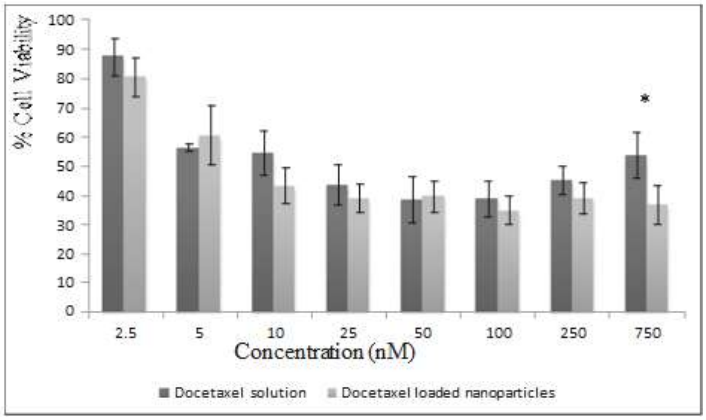

Figure 5: In vitro assessment of antiproliferative effects of docetaxel-loaded polycaprolactone/pluronic®F108 nanoparticles in human breast cancer BT-474 cells

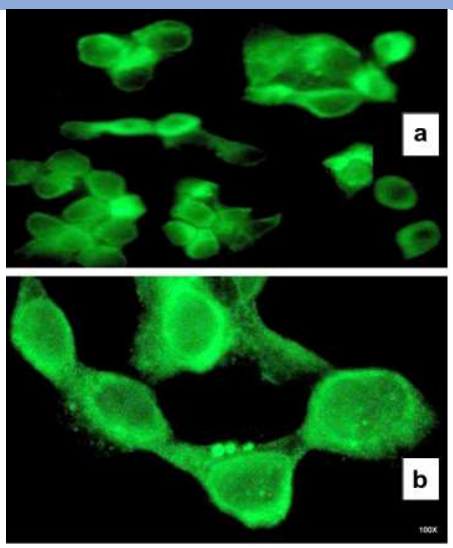

Figure 6: Fluorescence microscopy images studying uptake of Coumarin-6-loaded nanoparticles into human breast cancer BT- 474 cells. The fluorescence in the cells was stained in the FITC channel and a, b represents magnifications at × 40 and × 100 respectively

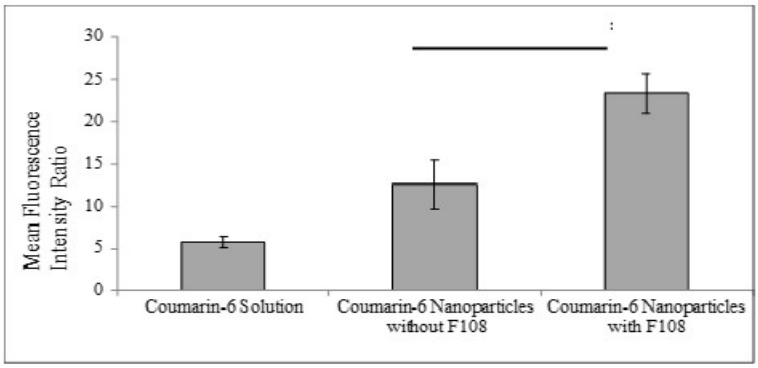

Figure 7: Flow cytometry analysis of Coumarin-6-loaded nanoparticles and Coumarin-6 solution into human breast cancer BT-474 cells

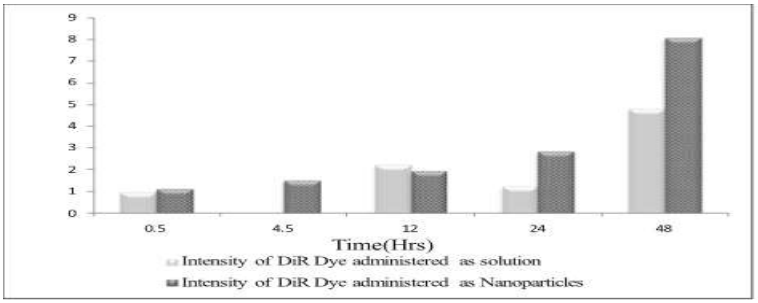

Figure 8: Comparison of near infrared dye DiR intensities in the tumor when administered as

solution and in nanoparticles

DISCUSSION

Because of their many benefits and adaptability, nanoparticles have become more and more popular as a medication delivery

technology for a variety of hydrophilic and hydrophobic compounds. By localising them in the target area, they prevent the medicine from degrading and increase its effectiveness. They are also a desirable drug delivery method due to their huge payload capacity and capacity to sensitise cells to drug resistance. Fessi et al. [22] created and patented the nanoprecipitation process, which was used in this investigation to generate polymeric nanoparticles. Rapid polymer desolvation through the addition of the polymer solution to the nonsolvent is the fundamental idea behind nanoprecipitation. Due to their high miscibility, acetone and deionised water were selected as PCL's solvent and antisolvent, respectively. A hydrophobic polypropylene block (PPO) sits between two hydrophilic blocks (PEO) in Pluronic F018, a triblock, nonionic surfactant that can form micelles as long as the pluronic® polymer concentration is higher than the critical micellar concentration (CMC). However, compared to more hydrophobic pluronic® derivatives, F108 is less effective at solubilising hydrophobic pharmaceuticals at low concentrations due to its greater PEO weight percentage [23]. Additionally, the final formulation's F108 concentration is significantly lower than the CMC; as a result, they are adsorbed on the PCL surface rather than forming micelles.

The hydrophilic arms (PEO) of F108 stretch outward from the particle surface, providing stability to the nanosuspension through steric hindrance and repulsion. The assembly of pluronic F108 with the PCL results in a strong interaction between the hydrophobic component (PPO) of F108 and the PCL matrix. It gives the hydrophobic PCL nanoparticles a hydrophilic coating, which reduces the likelihood that the RES system will recognise the nanoparticles. Due to substantial cellular absorption through strong nonspecific contacts with the plasma membrane, the drug-loaded particles had a slightly negative charge, which could be a useful metric for cellular internalisation of the particles [24]. The stability of the formulation is greatly impacted by the drug's physical state in the nanoparticles. The absence of the drug peak when entrapped in nanoparticles indicates that the drug loaded in the nanoparticles is more stable than the free drug. Therefore, the long-term stability of the drug in nanoparticle suspensions can be improved by managing crystallisation and eventually avoiding Ostwald ripening. Stability of nanosuspensions can be useful for nanoparticles which have prolonged lyophilization cycle periods and difficult resuspension of the lyophilized particles. Polymeric nanoparticles that are kept in the systemic circulation for prolonged period with controlled release are recommended as a chemotherapeutic drug delivery technology. Higuchi square root kinetics were followed by the nanoparticle. The Higuchi model was confirmed by similar release patterns, where nanoparticles displayed continuous drug release with a modest burst effect. In chitosan nanoparticles, Banerjee et al. [25] observed the Higuchi model release pattern without the free medicines' burst effect. The fluorescent coumarin-6 nanoparticles were employed to better comprehend cell absorption because their surface charge was comparable to that of the drug-loaded nanoparticles. Consequently, it can be said that pluronic®F108 facilitates nanoparticle internalisation and significantly contributed to PCL/pluronic F108 nanoparticle cellular uptake. According to in vitro antitumoural investigations, the medications may be able to kill cancer cells when they become trapped in a polymer matrix that resembles the free drug. In order to assess the effectiveness of a therapeutic intervention, researchers can employ noninvasive optical imaging to visualise a variety of biological processes in vivo, including disease development, medication pharmacokinetics, and vaccination trafficking. Near-infrared dyes are becoming more and more popular since they provide a number of benefits over conventional visible light dyes. It is possible to employ DiR, a lipophilic carbocyanine dye with an infrared excitation/emission spectrum, as an in vivo tracer. DiR's absorption and fluorescence maxima are 750 and 782 nm, respectively, suggesting that the region's low autofluorescence has minimised background interference. For in vivo imaging, light may enter the tissues more deeply [26] with little to no interference, which is why they are becoming more and more common. Therefore, DiR dye was added to the nanoparticles, and the near-infrared DiR-loaded nanoparticles' passive accumulation was assessed in vivo. For the entrapped anticancer medicines to effectively cure the tumour, polymeric nanoparticles must be able to passively collect in the tumour through the EPR effect. The potential of PCL/F108 nanoparticles as a targeted agent is suggested by their enhanced accumulation during the course of the in vivo investigation. The stability of pluronic F108 in the systemic circulation and its capacity to passively accumulate in tumours may be the reasons for its presence on the surface of the nanoparticles [27]. However, the nanoparticles also accumulated non-specifically in the renal system and the lymphatics. The early time points indicate the localization of the nanoparticles in the lymph nodes as previously established (Reddy et al. 2007). While, the nanoparticles drain into the lymphatics, the nonspecific buildup can be avoided by utilising active targeting ligands [28].

CONCLUSION

In conclusion, the polycaprolactone/pluronic® F108-designed nanoparticles demonstrated encouraging outcomes as a drug delivery system through the effective entrapment of hydrophobic docetaxel molecules, their delayed and sustained release, and their preferential homing in solid tumours with compromised lymphatic systems, all while accompanied by NIR dye for visualisation. These nanoparticles therefore show potential for the targeted administration of docetaxel in the treatment of breast cancer. Future publications will examine and report on additional research on the system's therapeutic efficacy.

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