Future Journal of Pharmaceutical Sciences

RESEARCH

Open Access

Development of docetaxel-loaded (Soluplus®–PF108) mixed micelles vacuum foam-dried product for improved stability and melanoma treatment by QbD approach

Rutuja Chougale[1,2], Kiran Patil[2*], John Disouza[3], Ashok Hajare[4], Namdeo Jadhav[5] and Popat Kumbhar[3]

Abstract

Background Docetaxel (DTX) finds extensive use in treating various cancers, but its limited solubility, side effects, and multi-drug resistance (MDR) hinder its effectiveness. To enhance DTX's properties, the study aimed to formulate DTX-loaded mixed micelles (MMs) and evaluate their anticancer potential using Quality by Design (QbD) approach. Using solvent evaporation, DTX-loaded MMs were prepared and optimized via a 3^2 full factorial design.

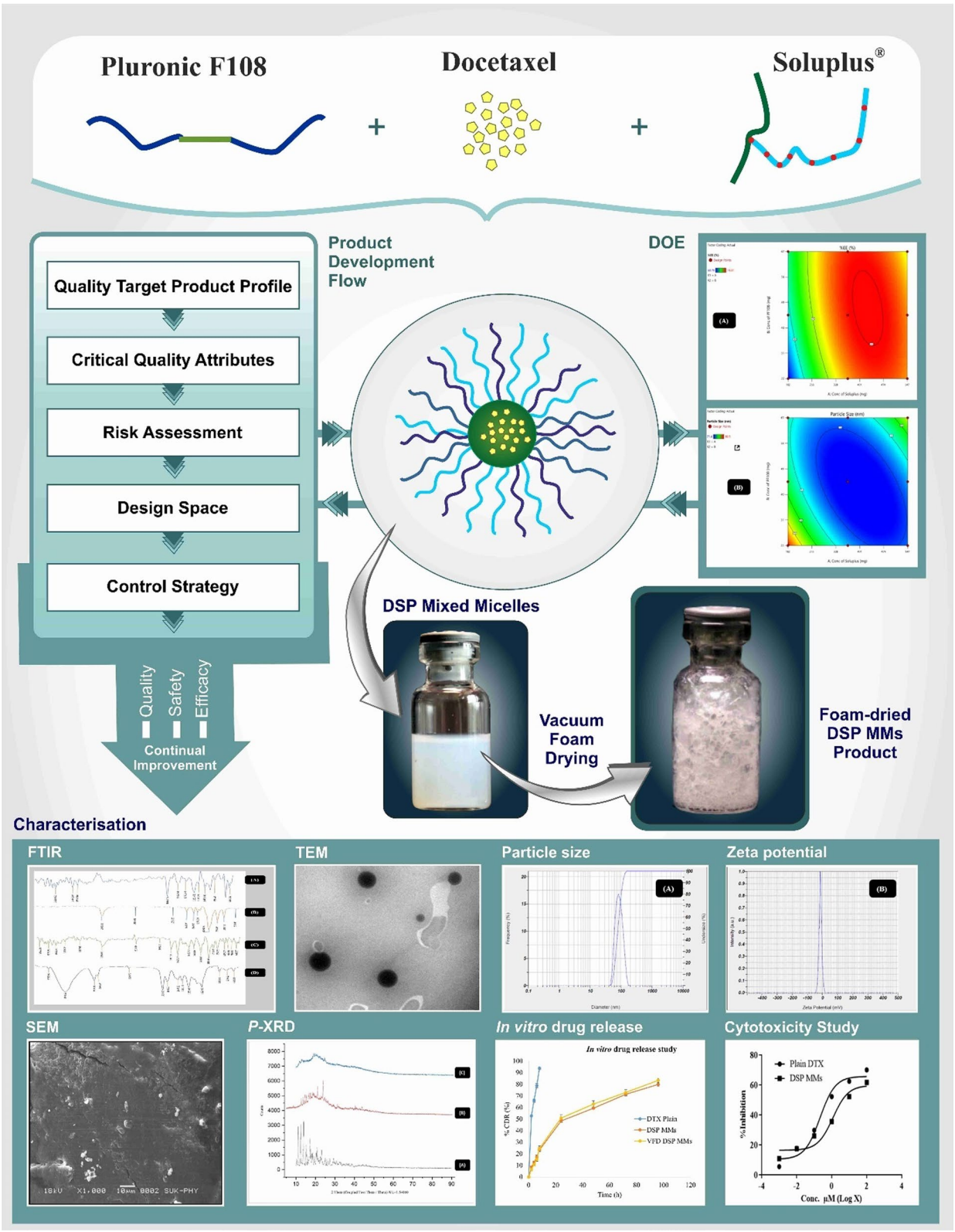
Results The optimized formulation (R5) displayed a % entrapment efficiency (%EE) of 74.81 ± 4.27%, % drug loading capacity (%DLC) of 29.27 ± 0.70%, and mean particle size (MPS) of 71.4 ± 1.24 nm. TEM images confirmed well-dispersed spherical MMs. Analytical studies (IR, DSC, and *P*-XRD) showed no adverse drug-excipient interactions. The MMs were converted into vacuum foam-dried (VFD) products for enhanced stability. The optimized VFD products exhibited low residual moisture, rapid reconstitution, consistent drug content, and high %EE. Notably, sustained drug release from the VFD product reduced hemolysis and in vitro cytotoxicity against B16F10 melanoma cells.

Conclusion This study creatively tackled DTX's challenges through targeted MM development, transformed them into VFD products, demonstrating the potential for melanoma treatment. The QbD approach ensures the formulation's safety, efficacy, and quality, underscoring the promising VFD technology and multifunctionality of mixed micelles.

Keywords Docetaxel, Quality by design, Vacuum foam drying, Stability, Melanoma, Mixed micelles

*Correspondence:
Kiran Patil
kspatil.tkcp@gmail.com
Full list of author information is available at the end of the article

Graphical abstract



Background

Docetaxel (DTX), a BCS Class IV drug, possesses challenges in oral absorption due to its low solubility and low permeability, leading to poor bioavailability [1]. Its narrow therapeutic index and potential side effects necessitate cautious formulation to minimize toxicity and enhance patient tolerability [2]. Improving efficacy with reducing adverse effects on healthy tissue are difficult aspects of targeting cancer cells. Besides, large-scale production transition poses difficulties in maintaining consistency and quality. These considerations are crucial in optimizing docetaxel pharmaceutical properties for effective cancer treatment [3]. Efforts to enhance drug effectiveness have led to advancements in drug delivery technologies. Targeted medication delivery entails selective and effective localization of a pharmacologically active ingredient at a preselected target in therapeutic concentration while limiting its access to nontarget areas, hence increasing the treatment's effectiveness [4]. Mixed micelles (MMs) refer to micellar systems composed of multiple amphiphilic components that offer targeted drug delivery, enhanced drug loading capacity, and better control over drug release kinetics [5]. Many researchers have formulated DTX MMs to enhance the properties of DTX in various applications. However, these formulations pose challenges due to certain limitations these include MPP, TPGS, and CSO-SA copolymers by thin film hydration method for enhanced oral absorption [6]. However, difficulties with achieving constant film thickness and managing the size and stability of MMs occur when employing the thin film hydration approach for the formulation. A PF127 and Tween 80 MMs gel to address stability and solubility challenges [7]; DTX MMs with TPGS and Poloxamer 188 [8]; a Pluronic P105 and F127 polymeric MMs against taxol-resistant lung cancer [9]; a monomethylol poly(ethylene glycol)-poly(d,l-lactic acid) (MPP) and Pluronic copolymer MM for enhanced bioavailability and overcoming multidrug resistance [10]; and DTX MMs with TPGS and Pluronic F108 for overcoming MDR in cancer [11]. But, TPGS, a surfactant, and solubilizer, have limitations regarding stability, efficacy, and solubility. Besides, Tween 80, another surfactant, may pose concerns about cytotoxicity and adverse effects [12]. Compatibility and phase separation issues should be addressed when employing multiple Pluronic copolymers, like Pluronic P105 and F127. Moreover, it is crucial to note that the MPS of TPGS and Pluronic F108 MMs was observed to be 233 ± 3 nm which exceeds 100 nm, which hinders their ability to leverage the Enhanced Permeability and Retention (EPR) effect for tumor targeting [13]. Hence, careful selection and optimization of MMs composition and method of preparation help to tailor the properties of drug delivery systems to specific cancer treatment needs.

The scalability of solvent evaporation for mixed micelles (MMs) is vital for large-scale production, offering ease of optimization for reproducibility [14]. The cost-effectiveness of this method, coupled with the novel combination of Soluplus® and PF108, enables the modulation of drug release kinetics, addressing challenges associated with maintaining a constant film thickness and managing particle size [15, 16]. By avoiding the use of Tween 80, concerns regarding cytotoxicity and adverse effects are minimized, while ensuring that the MMs achieve a size below 100 nm [17]. Furthermore, in cancer treatment product development, the application of Quality by Design (QbD) principles is pivotal, offering a systematic approach to optimize formulation, manufacturing, and analytical methods for consistent and high-quality cancer therapeutics, ensuring proactive identification and mitigation of risks for safer and more effective treatments [18].

Previous literature shows that nanoparticles, including micelles, often face long-term stability issues, such as aggregation and chemical degradation [19]. To enhance micelle stability, methods like lyophilization, spray drying, membrane extrusion, and coacervation are employed [20], but they face challenges. Lyophilization and spray drying may lead to changes in micelle size [21] and structure, while membrane extrusion can result in the loss of active ingredients [22]. Coacervation may pose difficulties in controlling particle size and stability [23]. Addressing these challenges is crucial for advancing micellar formulations with improved stability and functionality. To overcome these challenges and further enhance the stability of the developed MMs, novel methods like vacuum foam drying (VFD) have been explored in product development. VFD involves the use of foam structures, formed by mixing drug-loaded micelles with a foaming agent, which are then subjected to a VFD [24]. This method allows for the production of solid, porous materials that maintain the structure and stability of micelles, preventing drug leakage and preserving their therapeutic properties [25]. Retaining drug encapsulation effectiveness, improving stability, extending shelf life, and being simple to handle and store are some benefits of VFD.

The objective of this research was to develop DSP-MMs loaded with novel pluronic polymers Soluplus® and PF108 using the solvent evaporation method, for enhanced treatment against melanoma. To accomplish this goal, a QbD approach was employed for the formulation process, focusing on optimizing the MM's quality, efficacy, and stability as given in the Additional file 1: Supplementary Data S1 - Quality by Design Parameters

for Formulation Optimization (see Additional file 1 for details). Additionally, the research was focused on exploring the application of VFD as a means to further improve the stability of the DSP MMs, ensuring their suitability for long-term storage and sustained effectiveness in melanoma treatment.

Methods

Materials

Docetaxel was purchased from IQGEN-X Pvt. Ltd, Mumbai, India. Soluplus® was graciously gifted by BASF, India. PF108 was sourced from Sigma-Aldrich, Mumbai. Other chemicals were obtained from Molychem, Mumbai.

Culture medium and cell line

In this study, the B16F10 murine melanoma cell line (ATCC, USA) was obtained from the American Tissue Culture Collection. The stock cultures were then maintained at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum, and streptomycin (100 µg/mL) under a 5% CO_2 atmosphere. The cells were seeded in a microplate within an artificial womb with 5% CO_2 and incubated at 37 °C for 24 h [9].

Critical micelle concentration determination

The CMC of Soluplus® and PF108, both individually and in combination, was determined using the Iodine (I_2) UV-visible spectrophotometric method. A standard KI/I_2 solution was prepared by dissolving 2 gm of potassium iodide (KI) and 1 gm of iodine (I_2) in deionized water. Solutions of Soluplus®, PF108, and their mixtures at concentrations of 0.00001, 0.00005, 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1 mM were prepared in double-distilled water (DDW), to which the KI/I_2 solution was added. The samples were stored in the dark at room temperature for 12 h. Subsequently, the absorbance at 366 nm was measured using an Agilent 1800 UV spectrophotometer [12].

Development and optimization of MMs

The development of DTX-loaded Soluplus® with PF108 MMs (DSP-MMs) involved the utilization of the most efficient and scalable solvent evaporation method. Sonication was employed for 5 min to dissolve a combination of DTX and copolymer in methanol, which was gradually added drop by drop to distilled water under continuous stirring. The evaporation of methanol at room temperature was facilitated through continued stirring until a clear supernatant of DSP-MMs was obtained after centrifugation. Blank MMs were also prepared without the addition of DTX.

For optimizing the DSP-MMs, a highly efficient 3^2 full factorial design was employed. The design focused on two independent variables the concentration of Soluplus® (X_1) and PF108 (X_2). The study considered two dependent variables, namely % entrapment efficiency (%EE; Y_1) and mean particle size (MPS; Y_2). To determine suitable levels of the independent variables, a preliminary screening was conducted, and each factor was assigned values of − 1, 0, and + 1, as for the concentration of Soluplus® (X_1), levels were set at 182 mg, 364.5 mg, and 547 mg. Likewise, for the concentration of PF108 (X_2), levels were defined as 22 mg, 44.5 mg, and 67 mg. The statistical analysis was carried out using Design Expert® VR software. Analysis of variance (ANOVA) was employed to assess the significance of the model. The coefficients of magnitude were calculated based on the polynomial equations, and p values were used to determine the statistical significance ($p < 0.05$) [9].

Evaluation of DSP-MMs

Entrapment efficiency and drug loading capacity (DLC)

The concentration of DTX in DSP-MMs was measured by diluting 1 mL of DSP-MMs supernatant with methanol to 10 mL and sonicating for 5 min. The resulting solutions were spectrophotometrically scanned at 230 nm using a UV–visible spectrophotometer (Model UV-1900, Shimadzu) equipped with a high-quality quartz UV cell designed to accommodate standard cuvettes with a 1 cm path length, facilitating accurate estimation of the DTX concentration. Substituting values in the following formulae, the %EE and %DLC of DSP-MMs were determined [26]. The %EE and %DLC of DSP-MMs were calculated by substituting values in Eq. 1 and Eq. 2 respectively [26].

$$\%\ \mathrm{EE} = \frac{\text{Weight of drug in micelles}}{\text{Weight of drug taken initially}} \times 100 \tag{1}$$

$$\%\ \mathrm{DLC} = \frac{\text{Weight of drug in micelles}}{\text{Weight of drug fed initially} + \text{Weight of copolymers}} \times 100. \tag{2}$$

Mean particle size (MPS) and zeta potential (ZP):

The Horiba particle size analyzer (Horiba SZ-100, ver. 2.40) was used to determine the MPS and ZP of DSP MMs. The dynamic light scattering method was employed to analyze particle size, and

Laser Doppler Anemometry technology was used to measure zeta potential. Each experiment was carried out in triplicate at 25 ± 5 °C [26].

Drug-excipient compatibility study

Fourier transform infrared spectroscopic analysis (FTIR) FTIR analysis was conducted to investigate the compatibility of DTX with Soluplus® and PF108. FTIR spectra were recorded for plain DTX, Soluplus®, PF108, and a physical mixture of DTX, Soluplus®, and PF108. Samples for FTIR were prepared following a methodology based on the principles outlined by Patravale et al. This involved maintaining a specific 1:0.5 ratio of active pharmaceutical ingredient (API) to solubilizers, mainly Soluplus® and PF108. Each unit of the API was combined with 0.5 units of Soluplus® and PF108. The FTIR measurements were performed using an Agilent Alpha 100508 instrument over a wave number range of 4000 to 400 cm^{-1} [27].

Differential scanning calorimetry (DSC) analysis The thermal characteristics and interactions between DTX and the co-polymer were examined through DSC analysis. Both plain DTX and optimized DSP-MMs were subjected to the analysis using a DSC instrument Thermo Gravimetric Analysis–Differential Thermal Analysis–Differential Scanning Colorimetric (TGA–DTA–DSC) instrument with make TA instruments and model SDT Q600 V20.9 build 20. The samples were heated from 0 to 500 °C at a scan rate of 10 °C/min. An empty aluminum pan was used as the reference material for the measurements [9].

Processing of mixed micelles by vacuum foam drying

The product development process involved the careful transfer of precise 1 mL portions of DSP-MMs formulations into 3 mL glass vials. These vials contained a solution with 15% sucrose and 3% citric acid. The vials were partially sealed and then placed on the shelf of a Labconco lyophilizer (FreeZone 2.5® model). The lyophilizer condenser temperature was set to − 50 °C. Vacuum concentration of the DSP-MMs compositions took place for 4 h below 10 °C, maintaining a vacuum above 100 mBar. During this step, the composition's viscosity and surface area were carefully adjusted to ensure foam stability and efficient removal of water through evaporation during bubble formation. Subsequently, further drying of the viscous solutions occurred under vacuum conditions ranging from 1.650 to 0.030 mBar, with varying holding times to complete a 24 h cycle. Secondary drying was conducted at a temperature between 10 and 20 °C and 0.024 mBar for 2 h. As a result of this process, the dried products exhibited a highly porous thin bubble film inside the vials. The next step involved fully stoppering the vials under vacuum, and the finished product, known as the vacuum foam-dried DSP-MMs (DSP-MMs VFD product), was stored in a refrigerator at 4–8 °C until further analysis [25].

Characterization of DSP-MMs VFD product

After processing, the final product was further subjected to characterization to assess PS, ZP, PDI, %EE, %DLC, moisture content, and reconstitution time [25].

Comparative analysis of DSP-MMs and DSP-MMs VFD product

Morphological characterization using TEM and SEM analysis

The surface morphology of optimized DSP-MMs and DSP-MMs VFD products was analyzed using TEM and SEM. The SEM was done on the Scanning Electron Microscope with Jeol Ltd., Japan, and model JSM 6360 A. For TEM analysis, two drops of each sample were placed on a nitrocellulose-coated copper grid and air-dried for over 12 h at room temperature. Following this, the samples were stained with a 2% w/v phosphotungstic acid solution and examined using a Transmission Electron Microscope with Jeol and model JM 2100 to analyze morphological characteristics [28]. For SEM, samples were fixed on a brass stub, made electrically conductive by platinum coating in a vacuum using a Hitachi Ion Sputter, and analyzed with ImageInside Ver. 2.32 [29].

Powder X-ray diffraction analysis

The crystallinity behavior of the formulations was assessed using *P*-XRD analysis. Diffractograms of standard DTX, optimized DSP-MMs, and DSP-MMs VFD products were obtained using an X-ray Diffractometer (Bruker D8 Advance). The X-ray diffractometer operated with Cu-K radiation ($\lambda = 1.54$) at a voltage of 40 kV and 50 mA, incrementing in steps of 0.02° from 5° to 60° diffraction angle ($2°\theta$) at 1 s/step. A zero background was used during the scanning of all samples [9].

In vitro drug release study

In vitro drug release behavior of DTX from DSP-MMs, plain DTX, and DSP-MMs VFD products was investigated using the dialysis tube method. Dialysis bags containing DTX dispersion and DSP-MMs equivalent to 2.5 mg DTX were immersed in phosphate buffer solution (PBS) pH 7.4 with tween 80, maintained at 37 °C, and stirred at 150 rpm. At pre-determined intervals (0, 2, 4, 6, 8, 24, 48, 72, and 96 h) samples were extracted, replaced with fresh medium, and analyzed using a UV–visible spectrophotometer at wavelength 230 nm. The drug release study results were analyzed by correlating the percentage cumulative drug release against time and; mathematical kinetic models viz. zero-order, first-order, Higuchi, and Korsemeyer-Peppas to understand the drug release mechanism of the MMs formulations [30].

In vitro hemolysis study

The hemolytic effect of pure DTX, blank DSP-MMs, DSP-MMs (optimized R5 formulation), and DSP-MMs VFD products were evaluated using human blood. Fibrinogen was removed from a 5 mL blood sample through centrifugation, and the RBC pellets were cleaned and redispersed in 0.9% NaCl injection water. A 2% erythrocyte pellet solution was prepared and mixed with the above samples in flasks, with the volume adjusted to 10 mL using 0.9% NaCl. Besides, negative and positive controls were also prepared. The flasks were incubated at 37 °C for 2 h, followed by centrifugation and absorbance measurement at wavelength 420 nm. The percentage hemolysis was calculated using Eq. 3 [31]:

$$\%\text{Hemolysis} = \frac{\left(\text{Abs of sample} - \text{Abs of negative control}\right)}{\left(\text{Abs of positive control} - \text{Abs of negative control}\right)} \times 100. \quad (3)$$

In vitro cytotoxicity

The cytotoxicity of plain DTX, blank DSP-MMs, and DSP-MMs was evaluated using the murine melanoma cell line (B16F10) through a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dye reduction test. The stock cultures were stored for 18 h at 2–8 °C before the cells were seeded. Cells were seeded in a 96-well microtiter plate, and after the formation of a partial monolayer, various test drug doses were added. Following a 48 h incubation period, MTT reagent was added, and the formazan was dissolved using DMSO. Absorbance was measured at wavelength 590 nm, and the percentage growth inhibition was calculated. The IC_{50} values were determined using dose–response curves for each cell line [9].

Stability study of MMs and DSP-MMs VFD product

According to the ICH stability testing requirements for biologicals short-term stability studies on the improved DSP-MMs and DSP-MMs VFD products were conducted for 6 months. The optimized MMs composition was filled in vials and stored at 2–8 °C and 60% RH. The product appearance, MPS, and %EE of the dried composition were assessed [25].

Results

This research aimed to prepare DSP MMs containing Soluplus® and PF108 by leveraging the essential properties of the excipients. The additional application of VFD was employed to overcome various challenges associated with previous methods. Soluplus®, a novel polymer renowned for its exceptional solubilization properties, particularly for poorly soluble APIs, was combined synergistically with PF108. The selection of PF108 was based on its outstanding foaming capacity, strategically intended for use in the novel VFD process. Simultaneously, Soluplus® was chosen strategically to facilitate the solubilization of the drug and achieve the formation of mixed micelles (MMs) with a size below the accepted threshold of 100 nm.

CMC of polymers and mixture

In this study, the iodine UV–visible spectrophotometric method was used to determine the CMC. Iodine served as a hydrophobic probe, and its conversion from I_3 to I_2 in the solution indicated the formation of micelles. This method is preferred over the cloud point method, because the reliability of the cloud point method is compromised by temperature sensitivity, making it less robust without strict control [32]. Additionally, its subjective nature, relying on visual observation, can introduce variability in results due to individual interpretation. The CMC values of pure Soluplus®, PF108, and Soluplus® + PF108 mixture were 0.0031 mg/mL, 0.01047 mg/mL, and 0.001 mg/mL, respectively, as shown in Fig. 1. The lower CMC of the Soluplus® + PF108 mixture is indicative of enhanced drug loading capacity, drug delivery efficiency, and significant in vivo stability which is crucial for pharmaceutical applications [12].

Development and optimization of MMs

In the pursuit of product development, preliminary experiments were conducted, followed by formulation optimization using a 3^2 full factorial design. Two independent variables, Soluplus® concentration (X_1) and PF108 concentration (X_2), were investigated at three different levels, resulting in nine possible combinations of DSP-MMs as presented in Table 1. The impact of independent variables was assessed with two dependent variables %EE (Y_1) and MPS (Y_2). Through optimization, batch R5 emerged as the most promising, exhibiting %EE of 74.81 ± 1.35%, MPS of 71.4 ± 0.2 nm, and drug loading of 29.27%.

Effect of formulation variables on %EE

The effect of Soluplus®(X_1) and PF108 (X_2) concentrations on the %EE was estimated using a contour plot, Fig. 2A, and a 3D surface response plot Fig. 2B.

The %EE of DTX in MMs was observed to be increased with an increase in the concentration of both PF108 and Soluplus®. However, at a medium Soluplus®

concentration (365 mg), a substantial increase in %EE was observed that ranged from 72 to 74.81%. The %EE of the DSP-MMs also increased with an increase in concentrations of both the components up to medium concentrations. Beyond this point, further increases in Soluplus® and PF108 concentrations led to a decrease in %EE [33]. This can be attributed to establishing a saturation point within the carrier system at elevated concentrations, which diminished the progressive advantages of additional Soluplus® and PF108. Moreover, apprehensions about potential aggregation or precipitation of these components emerged as concentrations increased, significantly compromising their efficacy in enhancing encapsulation efficiency. The simultaneous increase in solution viscosity due to heightened concentrations was also recognized as a pertinent factor contributing to this phenomenon, potentially obstructing the unobstructed diffusion of the DTX into the MMs.

The final %EE equation in terms of coded components is given below in Eq. 4:

$$\%EE = +74.59I + 4.21A + 0.8633B - 1.82AB - 5.95A^2 - 1.13B^2 \quad (4)$$

Equation (4) shows a positive value indicating an increase in %EE owing to an increase in the concentration of Soluplus® and PF108. The Model F-value of 153.92 and a p value of 0.0008 being < 0.05 indicates that the model terms are significant.

Effect of formulation variables on particle size

The effect of Soluplus® (X_1) and PF108 (X_2) concentrations on the MPS was revealed using a contour plot, Fig. 2C, and a 3D surface response plot, Fig. 2D.

The MPS of DSP-MMs formulations were in the range of 71.4 ± 0.2 nm to 80.5 ± 1.4 nm. The concentration of PF108 and Soluplus® significantly affected MPS. The MPS of the DSP-MMs goes on decreasing with the increase in the concentration of both variables up to a certain extent after which it further goes on increasing with the increase in the concentration [34].

$$\text{MPS} = +70.76I - 1.18A - 0.1500B + 2.80AB + 3.32A^2 + 2.12B^2 \quad (5)$$

The model F-value 41.77 and a p value 0.0056 < 0.0500 indicates that the model terms are significant [31].

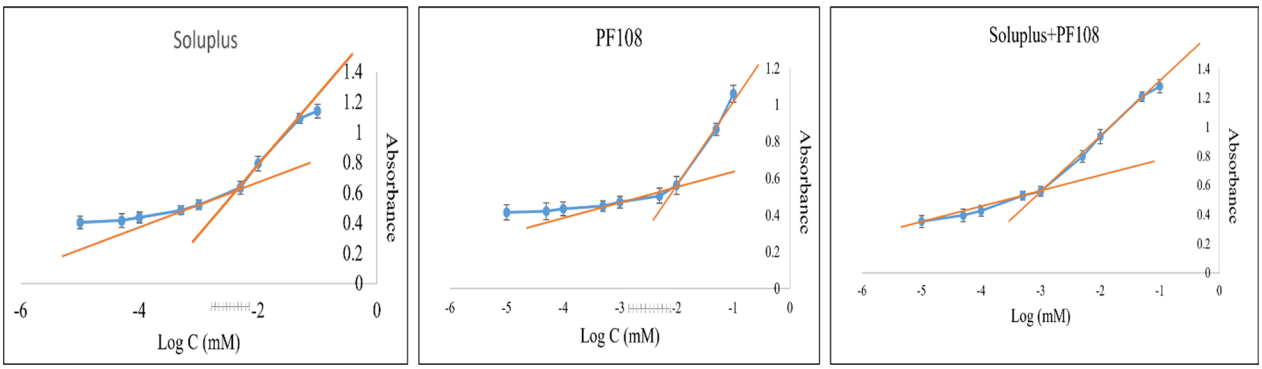


Fig. 1 CMC of Soluplus®, PF108, and Soluplus® + PF108 mixture

Table 1 Formulation compositions using 3^2 full factorial design and results of their characterization

Batch code	Drug (mg)	Soluplus® (mg)	PF108 (mg)	% EE (%)	MPS (nm)	DLC (%)
R1	2.5	182	22	60.79 ± 2.23	80.5 ± 1.40	20.43 ± 0.75
R2	2.5	182	45	64.51 ± 1.22	74.8 ± 2.89	23.75 ± 0.91
R3	2.5	182	67	65.73 ± 3.56	74.7 ± 4.59	26.14 ± 1.27
R4	2.5	365	22	72.06 ± 2.27	72.8 ± 03.27	28.02 ± 1.41
R5	2.5	365	45	74.81 ± 4.27	71.4 ± 1.24	29.27 ± 0.70
R6	2.5	365	67	74.64 ± 1.82	72.3 ± 3.81	25.11 ± 0.69
R7	2.5	547	22	73.04 ± 2.43	72.4 ± 4.78	21.23 ± 0.34
R8	2.5	547	45	72.54 ± 4.65	72.7 ± 3.25	19.46 ± 1.25
R9	2.5	547	67	70.70 ± 1.28	77.8 ± 4.7	18.12 ± 0.89

The results are the mean ± SD (n = 3)

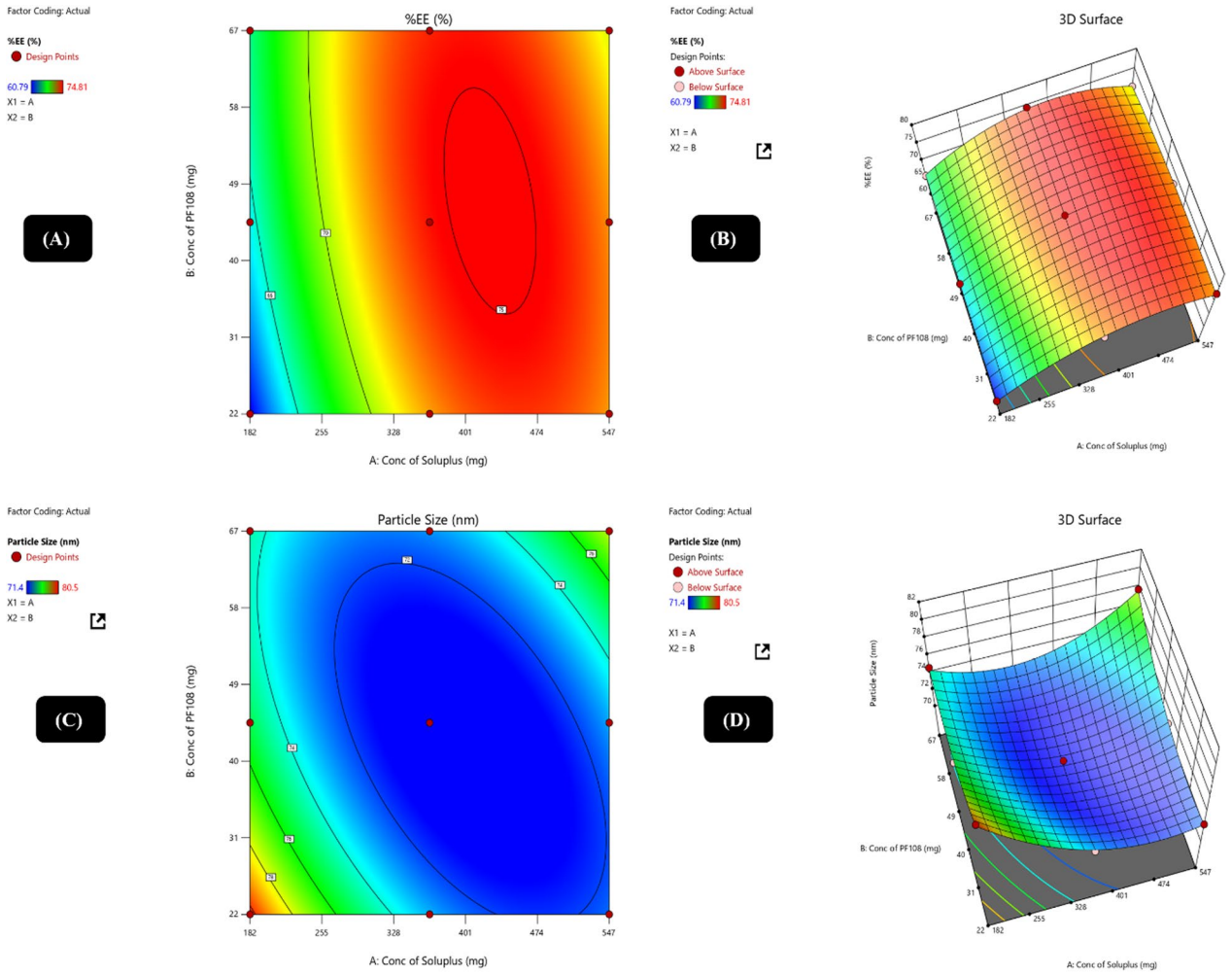


Fig. 2 **A** Contour plot of %EE, **B** 3D surface plot of %EE, **C** contour plot of particle size, **D** 3D surface plot of particle size

Mean particle size and zeta potential:

The MPS of the MMs in the absence of DTX was found to be 74.4 nm, as shown in Fig. 3A. The MPS and zeta potential of DSP-MMs optimized batch R5 are presented in Fig. 3B and C were found to be 71.4 ± 0.2 nm and − 17.6 mV, respectively. TEM analysis confirmed the MPS estimated by dynamic light scattering [9].

Drug-excipient compatibility study

FTIR analysis

Figure 4 displays the overlain FTIR spectra of DTX, PF108, Soluplus®, and the optimized formulation DSP MMs. In product development, FTIR analysis is pivotal for studying drug-excipient interactions and assessing formulation stability. DTX FTIR peaks include O–H, N–H stretching at 3630, 3444, and 3355 cm^{-1}, aromatic C=C stretching at 1699 cm^{-1}, and C–O–C stretching at 1157, 1251 cm^{-1}. PF108 shows C–H stretching at 2875 cm^{-1}, C–H bending at 1462 cm^{-1}, and C–H bending at 840 cm^{-1}. Soluplus® exhibits O–H, N–H stretching at 3656 cm^{-1}, C–H stretching at 2886 cm^{-1}, C=O at 1731 cm^{-1}, and C–O, C–O–C stretching at 1102 cm^{-1}[34].

DSC analysis

DSC analysis is vital in product development, offering insights into thermal transitions and formulation properties. The overlain DSC thermograms in Fig. 5I depict DTX and DSP MMs VFD products. DTX exhibits an endothermic peak at 178–188 °C, while DSP MMs VFD product shows a peak at 308.16 °C, indicating polymer melting and a solid-to-liquid transition. Notably, the DTX melting point peak disappears in DSP MMs VFD products, suggesting a transition from a crystalline to a partially amorphous state, crucial for optimizing drug delivery properties in pharmaceutical development [35].

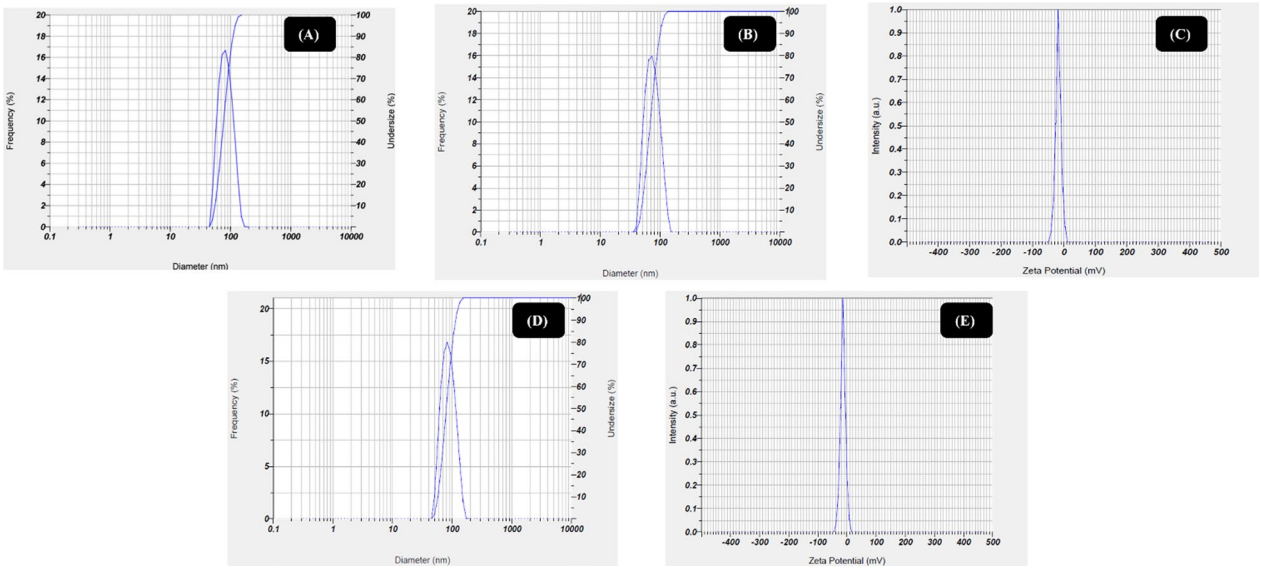


Fig. 3 **A** MPS of MMs in the absence of DTX, **B** MPS of DSP MMs, **C** zeta potential of DSP MMs, **D** MPS of DSP-MMs VFD product, **E** zeta potential of DSP-MMs VFD product

Characterization of DSP-MMs VFD product

The DSP-MMs VFD products were a lightweight, porous material with a high surface area that was obtained under reduced pressure conditions. The product formed facilitates rapid drying by removing moisture at lower temperatures than traditional drying methods. The resulting product typically exhibits characteristics such as enhanced porosity, improved rehydration capabilities, and a unique texture, as shown in Fig. 6A. The DSP-MMs VFD products underwent characterization to evaluate MPS, zeta potential, PDI, EE, drug loading, moisture content, and reconstitution time. The MPS was 82.7 ± 0.98 nm (Fig. 3D), zeta potential was − 14.1 ± 0.9 (mV) (Fig. 3E), PDI was 0.073 ± 0.011, %EE was 79.98 ± 0.97%, DLC was 98.2 ± 1.3%, moisture content was 2.25 ± 0.36%, and reconstitution time was 46 ± 5 s (Fig. 6B). These findings provided valuable insights into the physical properties and performance of the final product.

Morphological characterization using TEM and SEM analysis

The morphological characteristics of the optimized R5 formulation DSP-MMs structure and DSP-MMs VFD product are validated by TEM analysis Fig. 7A and B. The self-assembled MMs were observed to be spherical in shape. TEM images confirmed the nanoscale of generated MMs, which was comparable with the results, obtained using Zetasizer [36]. The SEM images revealed a highly porous structure of DSP-MMs VFD product (Fig. 7C and D). This porous nature facilitates the rapid reconstitution of DSP-MMs VFD products into micellar solutions [37].

Powder X-ray diffraction analysis

The *P*-XRD patterns of the plain DTX, DSP-MMs, and DSP-MMs VFD products are displayed in Fig. 5II. Plain DTX showed characteristic strong, high-energy diffraction peaks, indicating crystallinity. In the case of optimized DSP-MMs, these peaks were dramatically broadened with reduced peak intensities indicating partial amorphization. These characteristic peaks disappeared in the diffractogram of VFD products, indicating the amorphization of DSP-MMs VFD products [38].

In vitro drug release study

Illustrated in Fig. 8I are the release profiles of DTX from different formulations: plain DTX DSP-MMs and DSP-MMs VFD products. When dissolved in methanol, plain DTX exhibited an impressive release rate of over 90% after 8 h in PBS (pH 7.4) with 0.5% w/v tween 80, maintaining the sink condition. Comparatively, the DTX release from DSP-MMs after 96 h was measured to be 81.95 ± 1.3%. However, the DSP-MMs VFD product showcased even more remarkable sustained release capabilities, reaching 83.23 ± 1.5%, outperforming plain DTX in terms of sustained release performance [9].

The drug release pattern was analyzed by fitting the %CDR data from the release profile into various models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. Among these models, the Higuchi model demonstrated the highest correlation

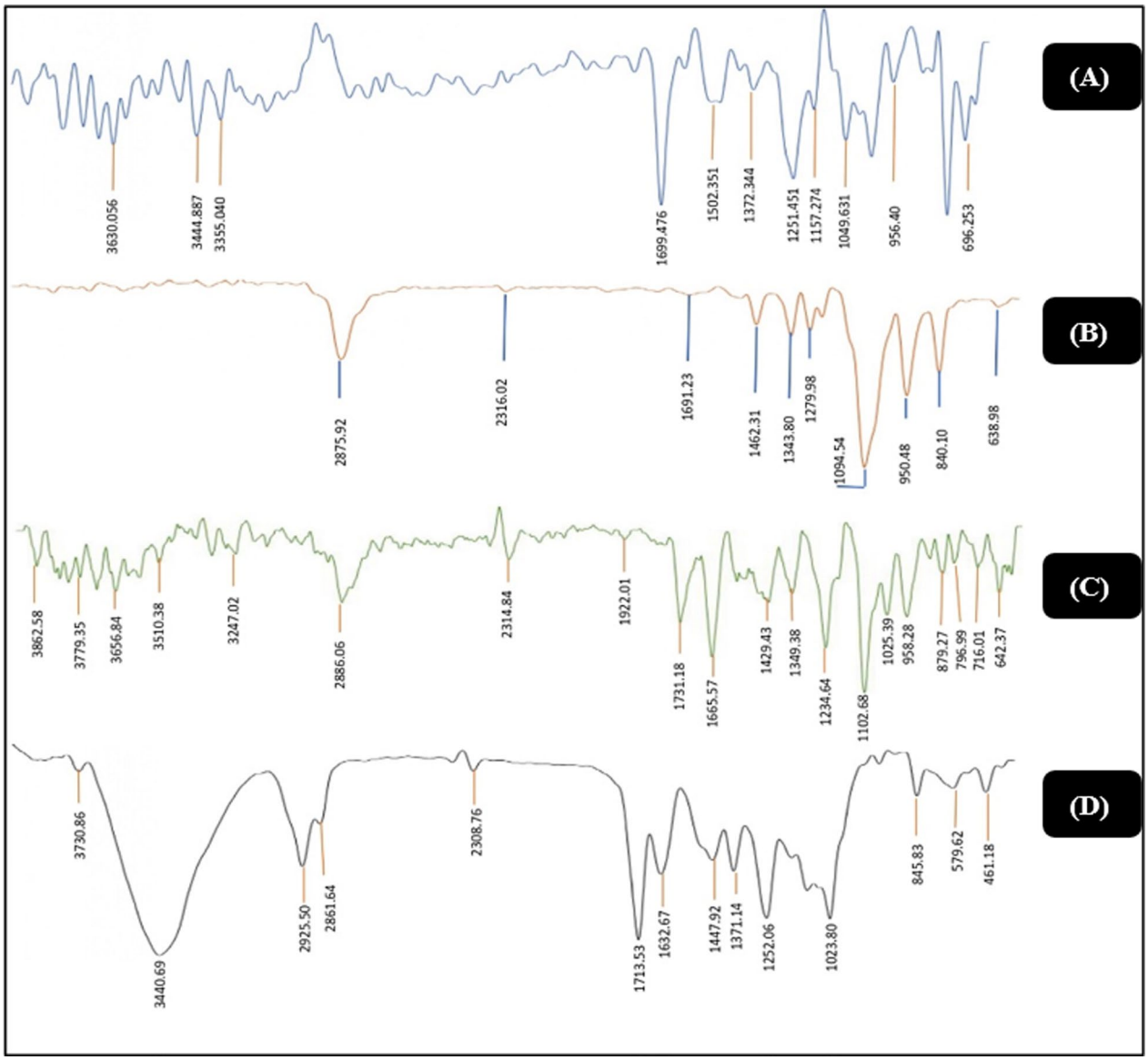


Fig. 4 FTIR spectrums: **A** Docetaxel, **B** PF108, **C** Soluplus® and **D** DSP MMs

coefficient (R^2), as depicted in Fig. 7II. Consequently, this model was selected as the best fit for the data, indicating that the main drug release mechanisms from DSP-MMs involve dissolution and diffusion [39].

In vitro hemolysis study

The hemolysis assay was performed to assess the biocompatibility and safety of the formulations. The results of in vitro hemolysis in plain DTX, blank Soluplus®/PF108 MMs, DSP-MMs, and DSP-MMs VFD products are presented in Fig. 9. The positive control displayed complete hemolysis, while the negative control showed minimal hemolysis. Plain DTX exhibited a higher percentage of hemolysis compared to DSP-MMs and DSP-MMs VFD products at the same dose. Compared to Taxotere, a marketed formulation of the DTX and previously prepared docetaxel nanoformulations, DSP-MMs VFD product exhibits reduced hemolysis, providing evidence of the enhanced quality of the developed product. This indicates that encapsulation of DTX in DSP-MMs and the subsequent DSP-MMs VFD product significantly reduced the hemolytic potential of the formulation. The reduced hemolytic activity indicates a decreased risk of red blood cell damage and supports the potential for safe and effective delivery of DTX using DSP-MMs and DSP-MMs VFD products intravenously [40].

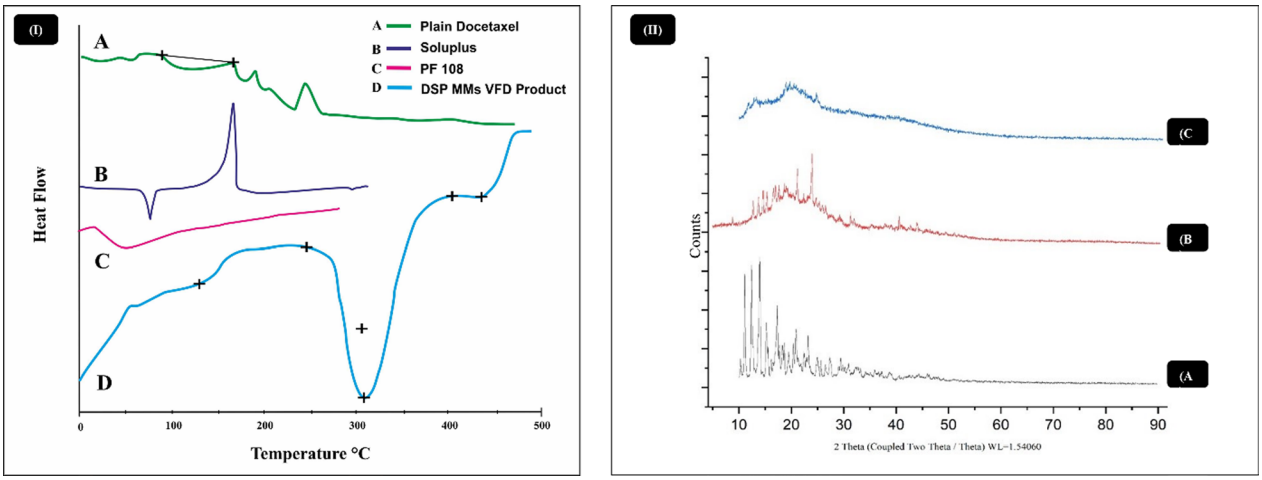


Fig. 5 (I): DSC thermograms: **A** DTX, **B** DSP-MMs (R5), and **C** VFD product and (II) *P*-XRD: **A** Plain DTX, **B** DSP-MMs, and **C** DSP-MMs VFD product

In vitro cytotoxicity study

All the test samples showed a cytotoxic effect on the B16F10 cell line that was concentration-dependent (Fig. 8III and Table 2). At the same doses, both plain DTX and DSP-MMs demonstrated virtually comparable cytotoxicity against cancer cells. On the B16F10 cell line, the simple DTX displayed a lower IC_{50} value of 0.2155 µM than DSP-MMs with an IC_{50} value of 1.170 µM. In comparison to conventional DTX, DSP-MMs displayed a slightly higher IC_{50} value, indicating lesser cytotoxicity against the B16F10 cell line. The sustained release of DTX from DSP-MMs, as seen in the in vitro release study, can be attributed to reduced cytotoxicity. The sustained release of DTX from MMs corresponded to a previous study described by Patil et al. [9].

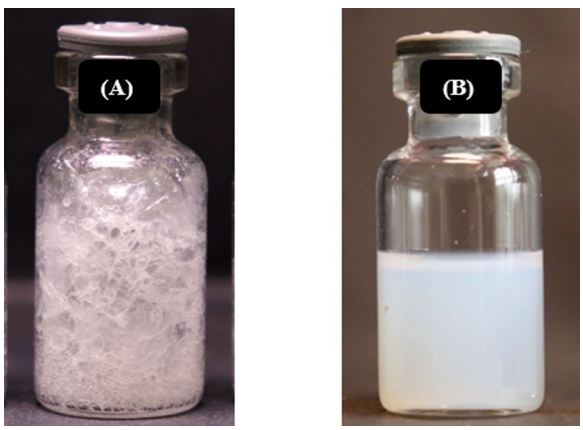


Fig. 6 DSP-MMs product: **A** VFD product and **B** reconstituted VFD product

Stability study of DSP-MMs and DSP-MMs VFD product

The comparison between the stability of the developed formulations, DSP-MMs, and DSP-MMs VFD products under accelerated storage conditions is depicted in Table 3. The table showcases key physical properties at two distinct time junctures: initial time (time zero) and following a 3-month storage period. This evaluation provides valuable insights into the endurance of the formulations over time and in the face of adverse storage conditions [25].

Discussion

Soluplus®, a versatile polymer in drug delivery, offers advantages like high solubilization capacity, biocompatibility, and improved drug bioavailability [41]. In this current study, a composite formulation comprising PF108 and Soluplus® was employed. The inclusion of PF108 was driven by its foaming capacity. Nonetheless, prior research revealed that PF108 single micelles commonly exhibited a moderately higher particle size. In light of this limitation and with the objective of achieving a reduced particle size, the collaborative incorporation of PF108 with Soluplus® was employed. As a result, the resultant mixed micelles exhibited a decrease in particle size compared to the micelles composed of PF108 alone. When combined with PF108, these benefits are further amplified in MMs. The combination of Soluplus® and PF108 in MMs results in smaller MPSs compared to individual micelles of either polymer. This reduction in MPS facilitates the extravasation of MMs through the leaky tumor vasculature [42], leading to enhanced accumulation at the tumor site through the EPR effect [43]. Additionally, the mixed micelles formed by Soluplus® and PF108 can accommodate a

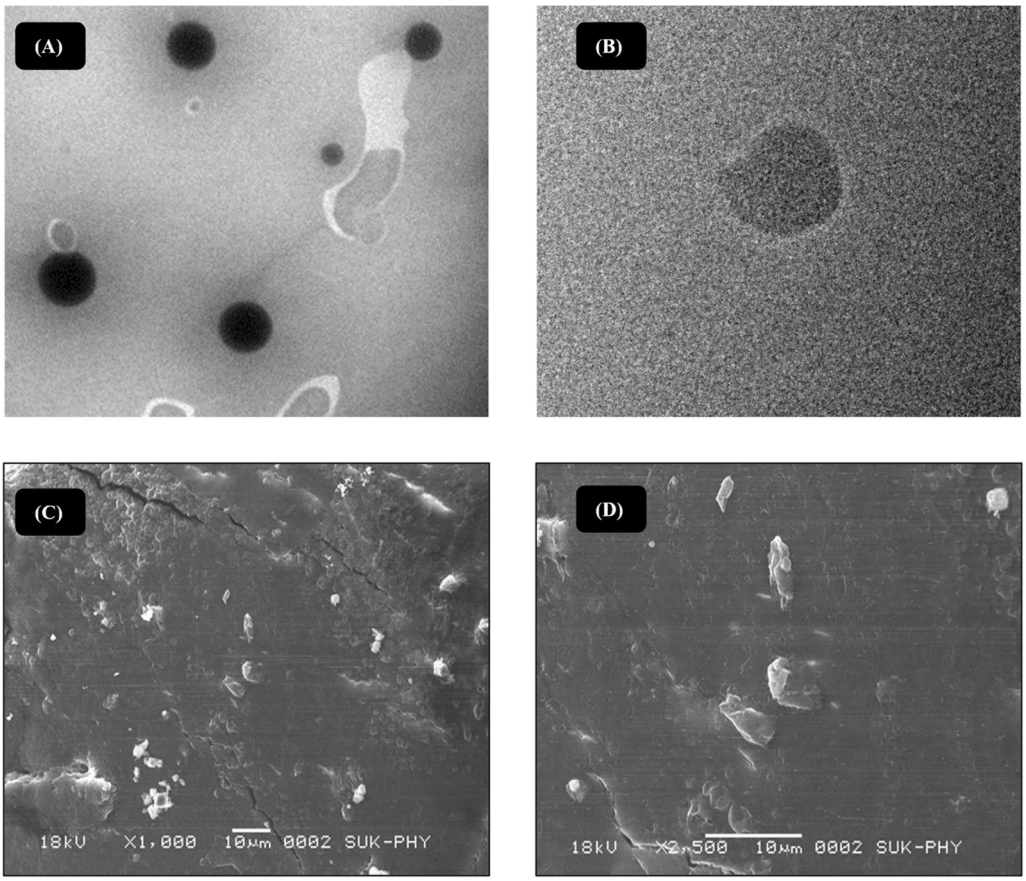


Fig. 7 TEM images: **A** DSP-MMs, **B** DSP-MMs VFD product and SEM images: **C** DSP-MMs, **D** DSP-MMs VFD product

higher drug payload, maximizing the amount of drug delivered to the target site, further improving the therapeutic effect [44]. Previously, Patil KS et al. developed DTX-MMs using TPGS and PF108, achieving a MPS of 233 ± 3 nm and %DLC of 2.06 ± 0.08 [12]. In contrast, this research demonstrated that MMs formed by combining Soluplus® and PF108 resulted in a significantly smaller MPS, measuring 71.4 ± 0.2 nm, and a substantially higher %DLC of 29.27%. Hence, the combination of Soluplus® and PF108 proves to be a superior choice for the development of docetaxel MMs. Further, QbD principles were crucial in product development, starting with defining the QTPP and identifying CQAs through pre-formulation studies [45] as given in the Additional file 1. A factorial design was employed to systematically vary critical factors, including polymer concentrations and other formulation parameters, through multiple experimental runs [46]. The effects of these variations on the MPS and %EE were evaluated. The objective of this optimization phase was to determine the optimal formulation that would yield MMs with the desired attributes, ultimately resulting in a high-quality product. Through this optimization R5 batch demonstrated promising characteristics, indicating an efficient encapsulation of the drug within the micellar structure, a desirable MPS for enhanced drug delivery, and an appropriate drug loading content; hence, it was used for further analysis [47].

In the previous research by Patil et al., DTX micelles had a zeta potential of − 7.4 mV, while the product developed in this research achieved a higher zeta potential of − 17.6 mV [9]. This increase can be attributed to the ionizable hydroxyl (–OH) groups present in both Soluplus® and PF108. These groups dissociate, releasing H+ ions, and maintaining the negative charge. The elevated zeta potential in the new research offers several advantages in continuous flow. Firstly, it enhances electrostatic repulsion between particles, preventing aggregation and ensuring uniformity during storage and circulation. Secondly, the increased zeta potential provides better control over drug release kinetics. The higher repulsive force between MMs slows down drug release, resulting in sustained and controlled drug delivery over an extended period [48].

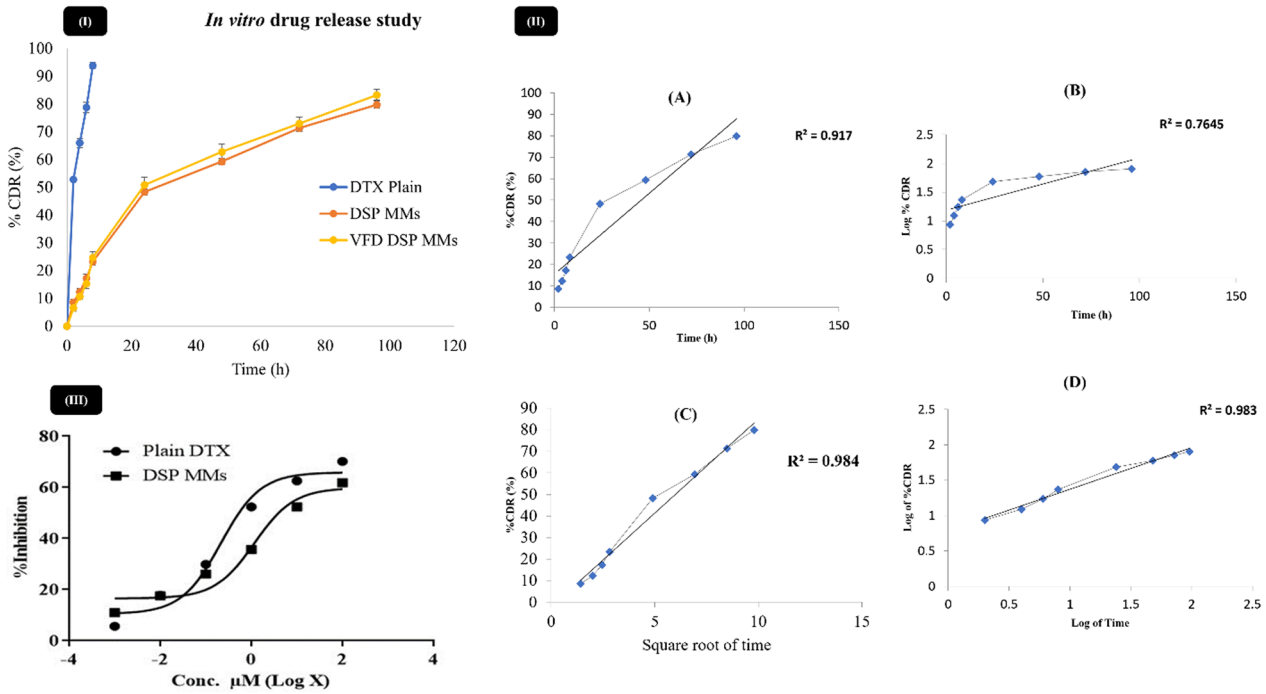


Fig. 8 (I)In vitro drug release study from DTX, DSP MMs (R5), and DSP-MMs VFD product, (II) Drug release kinetics for DSP-MMs VFD Product, and (III) In vitro cytotoxicity against B16F10 cell line

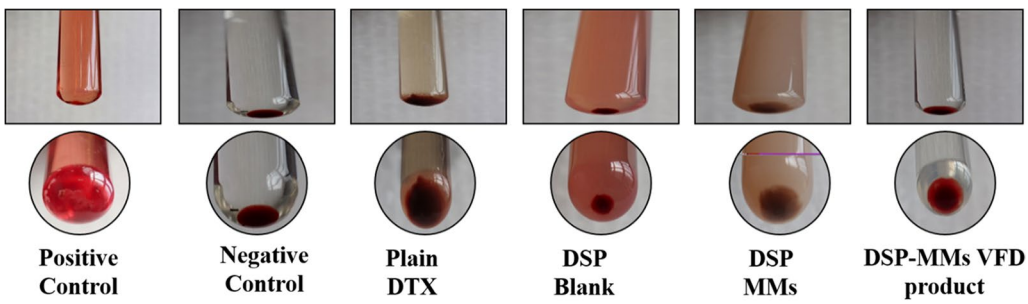


Fig. 9 Hemolysis study of DTX, DSP blank, DSP-MMs, and DSP-MMs VFD product

Table 2 In vitro cytotoxicity study of formulations

Formulation	IC_{50} value against B16F10 cell lines (µM)
Plain DTX	0.2155 ± 0.005
DSP blank MMs	0.7100 ± 0.007
DSP MMs	1.170 ± 0.028

FTIR spectroscopic studies were conducted to investigate potential drug-excipient interactions between DTX, TPGS, and PF108. The DSP MMs VFD product displayed characteristic peaks of the drug, suggesting no significant evidence of chemical interaction between the drug and excipients. This finding ensures the compatibility of the drug with its MMs formulation. DSC studies revealed a transformation from a crystalline to a partially amorphous state.

Further for enhancing the stability of the liquid product, converting it into a solid form is a common approach. Traditionally, lyophilization and spray drying were used, but they presented drawbacks, such as lengthy processing times, potential loss of bioactivity, and limited control over MPS [49]. A drawback of lyophilization is the potential formation of a compacted cake at the bottom of the product container during the

Table 3 Stability study of DSP-MMs

Sampling time	DSP MMs			DSP MMs VFD product				
	%EE (%)	MPS (nm)	%DLC (%)	%EE (%)	MPS (nm)	Moisture content (%)	Reconstitution time (sec)	%DLC (%)
0 Day	74.81 ± 0.27	71.4 ± 0.52	29.27 ± 0.70	79.98 ± 0.97	82.70 ± 0.98	2.25 ± 0.36	46 ± 5	98.2 ± 1.3
1 Month	72.5 ± 0.16	75.8 ± 1.12	29.07 ± 1.80	79.51 ± 1.12	82.84 ± 0.75	2.40 ± 0.51	49 ± 4	98.1 ± 0.9
2 Months	70.19 ± 0.32	79.2 ± 2.26	28.87 ± 0.90	78.87 ± 0.58	82.84 ± 0.75	2.80 ± 0.76	52 ± 3	97.4 ± 1.1
3 Months	66.28 ± 0.57	89.1 ± 1.48	28.77 ± 0.80	78.24 ± 0.72	84.79 ± 0.87	2.96 ± 0.84	55 ± 4	96.7 ± 0.7

drying process. This cake can be difficult to reconstitute as it may resist the absorption of water or solvent. As a result, reconstitution might require more vigorous shaking or mixing to break down the cake and achieve a uniform solution. This can be time-consuming and lead to inconsistencies in the reconstituted product, affecting its usability and quality. Hence, as an innovative alternative, this research tried using novel VFD for DSP-MMs [50]. VFD's ability to operate under mild conditions helps maintain the stability and efficacy of the final solid product. The solid product formed after lyophilization is a cake form whereas VFD gives a porous product hence reconstitution becomes easy as well as there is less percentage of moisture content. The product formed after VFD was further used for different in vitro tests.

The sustained release behavior of DSP-MMs and DSP-MMs VFD products hinders premature drug release, reducing exposure to healthy tissues and potentially enhancing DTX accumulation in tumors via the EPR effect for improved treatment efficacy [51]. The finding of reduced hemolysis demonstrates that the DSP-MMs VFD product formulation is a promising candidate for further development and potential clinical application. It highlights the positive impact of the vacuum foam drying process on the quality and safety of the developed DSP-MMs, reinforcing the importance of this manufacturing approach in producing improved drug delivery systems for melanoma treatment.

The stability of DSP-MMs and DSP-MMs VFD products was assessed at regular intervals. Over time, the MPS of the MMs increased from 71.4 to 90.7 nm due to slight aggregation of the cores of the hydrophobic micelles [52]. After 3 months, the liquid MMs showed precipitation and a significant decrease in %EE. In contrast, DSP-MMs VFD products exhibited stability with only a slight increase in MPS and a minor reduction in %EE. This comparison highlights the superior stability of DSP-MMs VFD products, as they demonstrated minimal changes in MPS and %EE compared to the liquid formulation.

Conclusion

In this research, DSP-MMs loaded with Soluplus® and PF108 were successfully developed to enhance melanoma treatment. Employing QbD principles, the formulation optimization process yielded an efficient encapsulation of the drug, desirable MPS, and appropriate drug loading content. The nanoscale size of the MMs facilitated drug accumulation in tumors through the EPR effect, improving therapeutic efficacy. IR spectroscopic studies indicated no significant drug-excipient interactions, ensuring the stability of the drug within the MM formulation. DSC studies revealed a transformation from a crystalline to a partially amorphous state, indicating potential changes in drug properties. VFD significantly improved the stability of DSP-MMs, reducing MPS growth and maintaining encapsulation efficiency over time. Moreover, DSP-MMs VFD products demonstrated superior stability compared to liquid MMs, exhibiting minimal changes in MPS and encapsulation efficiency. The sustained release behavior of DSP-MMs VFD product minimized premature drug release and enhanced drug accumulation in tumors, contributing to improved treatment efficacy. The applied QbD approach will ensure the safety, efficacy, and quality of the formulation improve the stability of docetaxel-loaded mixed micelles, and enhance their efficacy for melanoma treatment. Further, a novel VFD technique was applied to liquid mixed micelles formulation and converted to a foam-dried product that exhibits improved stability and therapeutic potential. The study of novel vacuum foam drying techniques is a budding phase for the pharmaceutical industry as a whole. This section of the study is interesting and acts as a lamppost for numerous researchers on the stability perspective of nanoformulations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-024-00619-z.

Additional file 1. Supplementary Data S1. Quality by Design Parameters for Formulation Optimization.

Acknowledgements
We extend our gratitude to SWVSM's Tatyasaheb Kore College of Pharmacy, Warananagar and Bharati Vidyapeeth College of Pharmacy, Kolhapur, for providing essential facilities and equipment for our research. Special thanks to BASF for the generous gift of Soluplus®, and to Shivaji University, Kolhapur for granting access to SEM, *P*-XRD, and DSC facilities, enhancing the comprehensive analysis of our study.

Author contributions
KP and RC: conceptualization, investigation, data curation, software, methodology, visualization, formal analysis, supervision, writing—original draft, writing—review and editing. JD, AH, NJ, and PK: formal analysis, writing—review and editing.

Funding
This research was conducted without any external funding support.

Availability of data and materials
The data will be made available on request.

Declarations

Ethics approval and consent to participate:
Not applicable.

Consent for publication
I, Dr. Kiran S. Patil, hereby grant permission to the Editor-in-Chief of the Future Journal of Pharmaceutical Sciences (FJPS) to publish the manuscript titled "Development of Docetaxel-Loaded (Soluplus®–PF108) Mixed Micelles Vacuum Foam-Dried Product for Improved Stability and Melanoma Treatment by QbD Approach."

Competing interests
The authors declare that there are no conflicts of interest related to this work.

Author details
[1]Department of Pharmaceutical Quality Assurance, Bharati Vidyapeeth College of Pharmacy, Shivaji University, Near Chitranagari, Kolhapur, Maharashtra 416013, India. [2]Department of Pharmaceutical Quality Assurance, SWVSM's Tatyasaheb Kore College of Pharmacy, Shivaji University, Warananagar, Kolhapur, Maharashtra 416 113, India. [3]Department of Pharmaceutics, SWVSM's Tatyasaheb Kore College of Pharmacy, Shivaji University, Warananagar, Kolhapur, Maharashtra 416 113, India. [4]Department of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Dr. Babasaheb Ambedkar Technological University, Palus, Sangli, Lonere, Maharashtra 416 310, India. [5]Department of Pharmaceutics, Krishna Vishwa Vidyapeeth, Krishna Institute of Pharmacy, Karad, Maharashtra 415539, India.

Received: 12 February 2024 Accepted: 18 March 2024
Published online: 02 April 2024

References

1. Gala UH, Miller DA, Williams RO III (2020) Harnessing the therapeutic potential of anticancer drugs through amorphous solid dispersions. Biochim Biophys Acta Rev Cancer 1873(1):188319. https://doi.org/10.1016/j.bbcan.2019.188319
2. Rijcken CJ, De Lorenzi F, Biancacci I, Hanssen RG, Thewissen M, Hu Q, Atrafi F, Liskamp RM, Mathijssen RH, Miedema IH, Menke-van der Houven CW (2022) Design, development and clinical translation of CriPec®-based core-crosslinked polymeric micelles. Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2022.114613
3. Jing Z, Du Q, Zhang X, Zhang Y (2022) Nanomedicines and nanomaterials for cancer therapy: Progress, challenge and perspectives. Chem Eng J 446:137147. https://doi.org/10.1016/j.cej.2022.137147
4. Pople PV, Singh KK (2006) Development and evaluation of topical formulation containing solid lipid nanoparticles of vitamin A. AAPS PharmSciTech 7:E63–E69. https://doi.org/10.1208/pt070476
5. Majumder N, Das G, Das SK (2020) Polymeric micelles for anticancer drug delivery. Ther Deliv 11(10):613–635. https://doi.org/10.4155/tde-2020-0057
6. Dou J, Zhang H, Liu X, Zhang M, Zhai G (2014) Preparation and evaluation *in vitro* and *in vivo* of docetaxel loaded mixed micelles for oral administration. Colloids Surf B Biointerfaces 114:20–27. https://doi.org/10.1016/j.colsurfb.2013.09.052
7. Yang Y, Wang J, Zhang X, Lu W, Zhang Q (2009) A novel mixed micelle gel with thermo-sensitive property for the local delivery of docetaxel. J Control Release 135(2):175–182. https://doi.org/10.1016/j.jconrel.2009.02.013
8. Patil KS, Hajare AA, Manjappa AS, More HN, Disouza JI (2022) Design, development, *in silico*, and *in vitro* characterization of camptothecin-loaded mixed micelles: *in vitro* testing of verapamil and ranolazine for repurposing as coadjuvant therapy in cancer. J Pharm Innov. https://doi.org/10.1007/s12247-022-09552-1
9. Chen L, Sha X, Jiang X, Chen Y, Ren Q, Fang X (2013) Pluronic P105/F127 mixed micelles for the delivery of docetaxel against Taxol-resistant non-small cell lung cancer: optimization and *in vitro*, *in vivo* evaluation. Int J Nanomed 8:73–84. https://doi.org/10.2147/IJN.S39041
10. Mu CF, Balakrishnan P, Cui FD, Yin YM, Lee YB, Choi HG, Yong CS, Chung SJ, Shim CK, Kim DD (2010) The effects of mixed MPEG–PLA/Pluronic® copolymer micelles on the bioavailability and multidrug resistance of docetaxel. Biomaterials 31(8):2371–2379. https://doi.org/10.1016/j.biomaterials.2009.11.099
11. Patil KS, Hajare AA, Manjappa AS, More HN, Disouza JI (2021) Design, development, *in silico* and *in vitro* characterization of Docetaxel-loaded TPGS/Pluronic F 108 mixed micelles for improved cancer treatment. J Drug Deliv Sci Technol 65:102685. https://doi.org/10.1016/j.jddst.2021.102685
12. Sanarova EV, Lantsova AV, Nikolaeva LL, Oborotova NA (2022) Using polysorbates to create parenteral dosage forms of hydrophobic substances (a review). Pharm Chem J 56(7):974–978. https://doi.org/10.1007/s11094-022-02765-3
13. Tao Y, Yan C, Li D, Dai J, Cheng Y, Li H, Zhu WH, Guo Z (2022) Sequence-activated fluorescent nanotheranostics for real-time profiling pancreatic cancer. JACS Au 2(1):246–257. https://doi.org/10.1021/jacsau.1c00197
14. Pham DT, Chokamonsirikun A, Phattaravorakarn V, Tiyaboonchai W (2021) Polymeric micelles for pulmonary drug delivery: a comprehensive review. J Mater Sci 56:2016–2036. https://doi.org/10.1007/s10853-020-05452-w
15. Kadhim ZJ, Rajab NA (2022) Formulation and characterization of glibenclamide nanoparticles as an oral film. Film Int 12(1):387–394. https://doi.org/10.15406/filmij.2022.12.00556
16. Hardy IJ, Windberg-Baarup A, Neri C, Byway PV, Booth SW, Fitzpatrick S (2007) Modulation of drug release kinetics from hydroxypropyl methyl cellulose matrix tablets using polyvinyl pyrrolidone. Int J Pharm 337(1–2):246–253. https://doi.org/10.1016/j.ijpharm.2007.01.018
17. Chaudhuri A, Ramesh K, Kumar DN, Dehari D, Singh S, Kumar D, Agrawal AK (2022) Polymeric micelles: a novel drug delivery system for the treatment of breast cancer. J Drug Deliv Sci Technol. https://doi.org/10.1016/j.jddst.2022.103886
18. Beg S, Rahman M, Kohli K (2019) Quality-by-design approach as a systematic tool for the development of nanopharmaceutical products. Drug Discov Today 24(3):717–725. https://doi.org/10.1016/j.drudis.2018.11.020
19. Susmitha A, Rajitha G, Eri GK (2023) A comprehensive review on QbD driven analytical procedures developed for the analysis of various drugs. J Liq Chromatogr Relat Technol. https://doi.org/10.1080/10826076.2023.2041685
20. Franco D, Antequera T, Pinho SC de, Jiménez E, Pérez-Palacios T, Fávaro-Trindade CS, Lorenzo JM (2017) The use of microencapsulation by spray-drying and its aplication in meat products. In: Strategies for obtaining healthier foods. New York: Nova Science Publishers
21. Pardeshi SR, Deshmukh NS, Telange DR, Nangare SN, Sonar YY, Lakade SH, More MP (2023) Process development and quality attributes for the freeze-drying process in pharmaceuticals, biopharmaceuticals and nanomedicine delivery: a state-of-the-art review. Future J Pharm Sci 9(1):99. https://doi.org/10.1186/s43094-023-00242-8
22. Dietel L, Kalie L, Heerklotz H (2020) Lipid scrambling induced by membrane-active substances. Biophys J 119(4):767–779. https://doi.org/10.1016/j.bpj.2020.07.036

23. Devi N, Sarmah M, Khatun B, Maji TK (2017) Encapsulation of active ingredients in polysaccharide–protein complex coacervates. Adv Colloid Interface Sci 239:136–145. https://doi.org/10.1016/j.cis.2016.08.007
24. Pisal S, Wawde G, Salvankar S, Lade S, Kadam S (2006) Vacuum foam drying for preservation of LaSota virus: effect of additives. AAPS PharmSciTech 7:E30–E37. https://doi.org/10.1208/pt070476
25. Patil KS, Hajare AA, Manjappa AS, Dol HS (2023) Vacuum foam drying of docetaxel mixed micelles for improved stability and ovarian cancer treatment. J Drug Deliv Sci Technol 86:104747. https://doi.org/10.1016/j.jddst.2022.104747
26. Kumbhar PS, Sakate AM, Patil OB, Manjappa AS, Disouza JI (2020) Podophyllotoxin-polyacrylic acid conjugate micelles: improved anticancer efficacy against multidrug-resistant breast cancer. J Egypt Natl Cancer Inst 32(1):1–8. https://doi.org/10.1186/s43046-020-0016-x
27. Patravale VB, Disouza JI, Rustomjee M (2016) Pharmaceutical product development: insights into pharmaceutical processes, management and regulatory affairs. CRC Press
28. Ramya AR, Sudheer P, Mohameid AS, Das AK (2019) Design and evaluation of a self-emulsifying drug delivery system of aripiprazole. Indian J Pharm Sci 81(6):1089–1098
29. Dol HS, Hajare AA, Patil KS (2022) Statistically designed novel ranolazine-loaded ethosomal transdermal gel for the treatment of angina pectoris. J Drug Deliv Sci Technol 75:103574. https://doi.org/10.1016/j.jddst.2022.103574
30. Patil K, Patil J, Bharade S, Disouza J, Hajare A (2023) Design and development of sodium alginate/carboxymethyl cellulose in situ gelling system for gastroretentive delivery of lisinopril. J Res Pharm 27(2):825–836
31. Shi L, Song XB, Wang Y, Wang KT, Liu P, Pang B, Wei FC (2016) Docetaxel-conjugated monomethoxy-poly (ethylene glycol)-b-poly (lactide) (mPEG-PLA) polymeric micelles to enhance the therapeutic efficacy in oral squamous cell carcinoma. RSC Adv 6(49):42819–42826. https://doi.org/10.1039/c6ra04047c
32. Wu S, Liang F, Hu D, Li H, Yang W, Zhu Q (2019) Determining the critical micelle concentration of surfactants by a simple and fast titration method. Anal Chem 92(6):4259–4265
33. Senthilkumar M, Sheelarani B, Joshi RG, Dash S (2019) Solubilization and interaction of ciprofloxacin with pluronics and their mixed micelles. New J Chem 43(42):16530–16537. https://doi.org/10.1039/c9nj03217k
34. Kumbhar PS, Birange S, Atavale M, Disouza JI, Manjappa AS (2018) d-Gluconic acid-based methotrexate prodrug–loaded mixed micelles composed of MDR reversing copolymer: *in vitro* and *in vivo* results. Colloid Polym Sci 296:1971–1981. https://doi.org/10.1007/s00396-018-4381-1
35. Paranthaman S, Uthaiah CA, Osmani RA, Hani U, Ghazwani M, Alamri AH, Gowda DV (2022) Anti-proliferative potential of quercetin loaded polymeric mixed micelles on rat C6 and human U87MG glioma cells. Pharmaceutics 14(8):1643. https://doi.org/10.3390/pharmaceutics14081643
36. Abdelwahed W, Degobert G, Fessi H (2006) Investigation of nanocapsules stabilization by amorphous excipients during freeze-drying and storage. Eur J Pharm Biopharm 63(2):87–94. https://doi.org/10.1016/j.ejpb.2006.02.009
37. Hajare AA, Velapure PD, Rathod PN, Patil KS, Chopade SS (2020) Formulation and evaluation of solid lipid nanoparticle gel for topical delivery of clobetasol propionate to enhance its permeation using silk sericin as permeation enhancer. Int J Pharm Sci 11(5):2356–2365
38. Morteza-Semnani K, Saeedi M, Akbari J, Eghbali M, Babaei A, Hashemi SM, Nokhodchi A (2022) Development of a novel nanoemulgel formulation containing cumin essential oil as skin permeation enhancer. Drug Deliv Transl Res. https://doi.org/10.1007/s13346-022-01213-7
39. Jindal N, Mehta SK (2015) Nevirapine loaded Poloxamer 407/Pluronic P123 mixed micelles: optimization of formulation and *in vitro* evaluation. Colloids Surf B Biointerfaces 129:100–106. https://doi.org/10.1016/j.colsurfb.2015.03.047
40. Ghezzi M, Pescina S, Padula C, Santi P, Del Favero E, Cantù L, Nicoli S (2021) Polymeric micelles in drug delivery: an insight of the techniques for their characterization and assessment in biorelevant conditions. J Control Release 332:312–336. https://doi.org/10.1016/j.jconrel.2021.01.025
41. Attia MS, Elshahat A, Hamdy A, Fathi AM, Emad-Eldin M, Ghazy FE, Ibrahim TM (2023) Soluplus® as a solubilizing excipient for poorly water-soluble drugs: recent advances in formulation strategies and pharmaceutical product features. J Drug Deliv Sci Technol. https://doi.org/10.1016/j.jddst.2023.104519
42. Mehanny M, Hathout RM, Geneidi AS, Mansour S (2016) Bisdemethoxycurcumin loaded polymeric mixed micelles as potential anti-cancer remedy: preparation, optimization and cytotoxic evaluation in a HepG-2 cell model. J Mol Liquids 214:162–170. https://doi.org/10.1016/j.molliq.2015.12.018
43. Chen L, Zang F, Wu H, Li J, Xie J, Ma M, Zhang Y (2018) Using PEGylated magnetic nanoparticles to describe the EPR effect in tumor for predicting therapeutic efficacy of micelle drugs. Nanoscale 10(4):1788–1797. https://doi.org/10.1039/c7nr08417g
44. Kong X, Qi Y, Wang X, Jiang R, Wang J, Fang Y, Hwang KC (2023) Nanoparticle drug delivery systems and their applications as targeted therapies for triple negative breast cancer. Prog Mater Sci. https://doi.org/10.1016/j.pmatsci.2023.101070
45. Namjoshi S, Dabbaghi M, Roberts MS, Grice JE, Mohammed Y (2020) Quality by design: development of the quality target product profile (QTPP) for semisolid topical products. Pharmaceutics 12(3):287. https://doi.org/10.3390/pharmaceutics12030287
46. Singh B, Dahiya M, Saharan V, Ahuja N (2005) Optimizing drug delivery systems using systematic "design of experiments." Part II: retrospect and prospects. Crit Rev Ther Drug Carrier Syst 22(3):215–292
47. Patil SS, Chougale RD, Manjappa AS, Disouza JI, Hajare AA, Patil KS (2022) Statistically developed docetaxel-laden mixed micelles for improved therapy of breast cancer. OpenNano 8:100079. https://doi.org/10.1016/j.onano.2022.100079
48. Manjappa AS, Kumbhar PS, Khopade PS, Patil AB, Disouza JI (2018) Mixed micelles as nano polymer therapeutics of docetaxel: increased *in vitro* cytotoxicity and decreased *in vivo* toxicity. Curr Drug Deliv 15(4):564–575. https://doi.org/10.2174/1567201814666170505104453
49. Salazar J, Müller RH, Möschwitzer JP (2014) Combinative particle size reduction technologies for the production of drug nanocrystals. J Pharm. https://doi.org/10.1155/2014/896287
50. Fonseca F, Meneghel J, Kilbride P, Passot S, Morris GJ (2019) Physical events during cryopreservation: consequences on cells' post-thaw performance and on cryobiological protocols optimisation. In: ISLFD 2019—9th international symposium on lyophilization of pharmaceuticals
51. Li T, Yang Y, Jing W, Yan Z, Che J, Xu H, Zhang R (2022) Melanin-gelatin nanoparticles with both EPR effect and renal clearance for PA/MRI dual-modal imaging of tumors. Biomater Adv 134:112718. https://doi.org/10.1016/j.bioadv.2022.100003
52. Sipos B, Szabó-Révész P, Csóka I, Pallagi E, Dobó DG, Bélteky P, Katona G (2020) Quality by design based formulation study of meloxicam-loaded polymeric micelles for intranasal administration. Pharmaceutics 12(8):697. https://doi.org/10.3390/pharmaceutics12080697

Publisher's Note