RESEARCH PAPER

Formulation Docetaxel Encapsulated Nanocarriers By Boxbehnken Design Of Experiments And Determination Of Efficacy In Mcf-7 Cell Line

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Abstract

Docetaxel (DTX) is a strong chemotherapy medicine that is used to treat many types of cancer, such as ovarian, breast, and non-small cell lung cancer. However, it is hard to employ in clinical settings since it doesn't dissolve well in water, has serious side effects, and may cause multi-drug resistance. Researchers are exploring for innovative ways to distribute drugs to get around these problems, and docetaxel-loaded hyaluronic acid (HA) micelles are getting a lot of interest. HA is a naturally occurring polymer that is very soluble in water, safe for living things, and breaks down easily. It is found in the extracellular matrix.HA can self-assemble into micelles in water when it is chemically changed with hydrophobic substances. After that, hydrophobic medicines like docetaxel can be put in the midst of these HA micelles. Docetaxel-loaded hyaluronic acid Pluronic (DTX-HA-PF127) micelles represent a significant advancement in cancer nanomedicine, offering advantages over conventional chemotherapy through targeted distribution, enhanced efficacy, and reduced unwanted effects. This study seeks to enhance the efficacy of docetaxel by encapsulating it within hyaluronic acid micelles, thereby prolonging its half-life in the bloodstream. The Box-Behnken experimental design was utilised to enhance the formula.

Keywords: Docetaxel, docetaxel-loaded hyaluronic acid Pluronic micelles, Box-Behnken experiment design

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1.Introduction

Docetaxel (DTX) is a powerful chemotherapy treatment that is often used to treat tumours of the ovaries, breasts, and lungs. However, it is hard to use in the clinic since it doesn't dissolve well in water, has serious side effects, and can cause multi-drug resistance. To address these limitations, researchers are investigating novel drug delivery systems, with docetaxel-loaded hyaluronic acid (HA) micelles receiving considerable focus [1-3]. HA is a natural polysaccharide that is present in the extracellular matrix. It dissolves easily in water, is safe for living things, and breaks down easily. When HA is chemically changed to include hydrophobic parts, it can make micelles on its own in solutions that are mostly water. You can then put hydrophobic medicines like docetaxel into these HA micelles [4].

HA micelles have a lot of benefits over other ways to deliver drugs. Micelles can make medications that don't dissolve in water, like DTX, much more soluble in water. This makes them more available to the body and more effective as a treatment. HA can specifically bind to CD44 receptors, which are overly expressed on the surface of some cancer cells. This particular connection enables HA micelles to actively seek out tumour cells and distribute DTX to them. This increases the amount of the drug that builds up at the tumour location and may protect healthy tissues from damage. HA micelles can make the medicine stay in the bloodstream longer, which could mean that the tumour is exposed to it for a longer time and that the treatment doesn't need to be given as often. Studies show that DTX-loaded HA-QU polymeric micelles (DTX/HA–QU PMs) had AUC0–∞ and t1/2 values that were 3.0 times and 5.51 times larger than those of the commercial formulation (Taxotere®), respectively. HA micelles can distribute DTX to specific parts of the body, which can lower the amount of the drug that gets

into the body as a whole. This means less adverse effects and a greater maximum tolerated dose than regular DTX formulations. One study discovered that DTX-loaded poly(2-oxazoline) micelles had an IC50 as low as 40% of the commercial DTX injection in MCF-7 cells in vitro and a twofold maximum tolerated dose in vivo. In certain investigations, DTX/HA–QU PMs have demonstrated the capacity to downregulate P-glycoprotein (P-gp) expression in neoplastic cells, a principal mechanism of multidrug resistance (MDR) in cancer [5].

HA micelles can be synthesized using many techniques, including solvent evaporation or the incorporation of water into a thin film. Numerous studies, both in vitro and in vivo, have demonstrated the efficacy of HA micelles against many malignancies, including breast, skin, lung, ovarian, and prostate cancers. However, further study, especially clinical trials, is necessary to thoroughly assess their efficacy and safety before widespread practical application. Docetaxel-loaded hyaluronic acid Pluronic (DTX-HA-PF127) micelles signify a significant advancement in cancer nanomedicine, providing benefits over traditional chemotherapy through targeted distribution, enhanced efficacy, and diminished adverse effects. Subsequent study and clinical advancement may improve their successful clinical application [6-8].

This research project was created to improve the efficacy of docetaxel by encapsulating in hyaluronic acid micelles which improve the drug's half-life in the bloodstream, according to pharmacokinetics. Hence the formula was refined through the application of Box-Behnken design of experiment.

Table 1: Novel drug delivery system widely used for delivery of anticancer agents and their effectiveness

Aspect	**HA Micelles**	**Liposomes**	**Polymeric Nanoparticles**	**Dendrimers**	**Other Micelles**
Targeting	Yes (via CD44)	No (passive or surface targeting)	Yes (via surface modification)	Yes (via surface groups)	Varies
Biocompatibility	High	High	High	Moderate to high	Varies
Stability	Moderate	Good	Good	Variable	Varies
Drug Loading	Moderate	High	High	High	Variable
Ease of surface functionalization	Yes	Yes	Yes	Yes	Yes
Clinical approval	Limited	Yes (e.g., Doxil)	No	No	Varies

2. Material and methods

Sun Pharmaceutical Pvt. in Baroda gave us a sample of docetaxel as a gift. We got hyaluronic acid from BFC Lab. Loba Chemi Pvt. Ltd. gave us ethanol, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF). The rest of the substances were of analytical grade.

2.1 Preparation of DTX-HA-PF127 micelles

The DTX-HA-PF127 micelles were made using a modified version of the previously described thin film hydration approach (TFH) [9]. Briefly, 4 milligrammes of docetaxel (DTX) diluted with 3 millilitres of acetonitrile., and 50 mg of HA disintegrated in 10 mL of water separately. Then both the solution subject to ultrasonication for 10 cycles of three minutes (30 minutes). About 10 mg of Pluronic F127 was added to HA solution with heating to get homogeneous solution. Both the solution then transferred to rotary evaporator for further evaporation of sol vent at 50°C for 30 minutes. This results into the thin film formation which was subject to hydration using miliQ water. After that, the DTX-HA-PF127 micelles were made by stirring them constantly on a magnetic stirrer at 40°C for an hour. After that, it was passed through a membrane filter with 0.22 µm pores. Prepared micelles were then subject to further analysis.

2.2 Quality by Design (QbD) based design of experiment

The QbD-based experiment design is the most effective way to create experiments for the systematic formulation technique. Therefore, a trial with a QbD-based design was employed to accomplish the study's suggested aim, and the related reaction characteristics

were used to gauge the influence of the formulation and process components.

2.3 Experimental Design

In the current study, the Box-Behnken type of experimental strategy technique was used to systemically enhance the effectiveness of the formula and processing parameters [10]. Independent variables including drug concentrations (X1, %w/v), hyaluronic acid concentrations (X2, %w/v), and temperature (X3, °C) have all been considered in order to improve the formula. Their influence on trapping effectiveness was also quantified. As shown in Table 4, seventeen distinct batches were made with every conceivable combination of medication and hyaluronic acid at the ideal temperature in order to get an ideal formulation with the maximum entrapment efficiency.

Equation (1), which uses a statistical framework with polynomial and interaction factors, was used to evaluate the response.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

Eq. (1)

In this case, b0 reflects the average result from all 17 trials, b1 is the expected coefficient for the factor X1, and Y is the dependent variable. The primary impacts (X1, X2, and X3) reflected the mean results of altering each element individually from a minimal to maximal number. The terms of interaction (X1X2, X2X3, and X1X3) showed that the answer varied when all three variables were altered all at once. Terms involving polynomials (X12, X22, and X32) were introduced in order to explore non-linearity.

The three factors' values at the level and the overall make-up of both Box-Behnken design batches were as seen in tables 2 and 3.

Table 2: The study's factors, together with their coded levels and "Real" values

Variables	Levels		
	-1	0	+1
Independent	**Real values**		
Conc. of Drug (X_1, %w/v)	0.1	0.5	1
Conc. of Hyaluronic Acid (X_2, %w/v)	1	2	3
Temperature (°C)	40	50	60
Dependent			
Entrapment efficiency (Y, % w/w)			

Table 3: Box-Behnken design of experiment formulation batches

Sr. No.	Batches	X1	X2	X3
1.	F1	0.55	3	60
2.	F2	0.55	2	50
3.	F3	0.55	2	50
4.	F4	1	1	50
5.	F5	0.1	2	40
6.	F6	0.1	1	50
7.	F7	0.55	1	40
8.	F8	0.55	1	60
9.	F9	1	3	50
10.	F10	0.1	2	60
11.	F11	0.1	3	50
12.	F12	1	2	40
13.	F13	0.55	2	50
14.	F14	0.55	3	40
15.	F15	0.55	2	50
16.	F16	1	2	60
17.	F17	0.55	2	50

2.4 Characterization of HA-DTX micelles

2.4.1 Particle Size and zeta potential analysis

The particle sizes of the DTX-HA-PF127 micelles were examined using photon correlation spectroscopy (PCS) and dynamic light scattering on a Zetasizer® nano (Model: Zen 3600, Malvern Instruments, Malvern, UK) equipped with a 5-mW helium neon laser with an output wavelength of 633 nm [11, 12]. The evaluations were taken at a 90° angle, at 25 °C, and for a minimum of 40 to 80 seconds. Water served as the dispersant. Micelles derived from phospholipids have an electrophoretic mobility-based zeta potential that was calculated using Smoluchowski's equation [13]. Three duplicates of each measurement were made.

2.4.2 DSC analysis

DSC was used to analyse the polymorphism state of docetaxel, hyaluronic acid, pluronic and their physical combination. Dried nitrogen gas was supplied at a flow rate of 80 mL/min as a purging agent. Inside the instrument chamber was an aluminum pan with around 5 mg of powdered material. a single heating cycle that increased the temperature by 10 °C per minute from 40 °C to 400 °C [13]. Their highest points, found examined using TA software.

2.4.3 FTIR

The interaction between the ingredients needed to make DTX-HA-PF127 was investigated, and the physical mixing infrared spectra of docetaxel and hyaluronic acid were acquired. An FTIR spectrophotometer (FTIR-8300) was used to record the FTIR spectra of docetaxel, HA, pluronic and their physical mixture. Pretreatment was briefly administered to the docetaxel, HA, pluronic to prepare them for Fourier transform infrared (FTIR) analysis, as well as their physical mixes. At a ratio of 1:100, these substances were mix together evenly in the presence potassium bromide (KBr) of FTIR quality. Then leter, they had 45 scans with a 4 cm–1 resolution and analyses between 4,500 and 400 cm–1 [14].

2.4.4 Entrapment efficiency

The DTX-HA-PF127 micelles' entrapment efficiency was assessed by means of a range of techniques that were documented across published works[15, 16]. One milliliter of DTX-HA-PF127 was added to the Centricon® reservoir (Model: YM-100, Amicon, Millipore, Bedford, MA, USA). The filtrate containing free docetaxel was collected after the DTX-HA-PF127 micelles were through a centrifuge 40 minutes at 15,000 rpm. To ascertain the quantity of docetaxel, The purified diffusion was further mixed with methanol and put through a UV analysis. The UV-spectrophotometric analysis was employed to ascertain the docetaxel concentration in the filtrate following centrifugation (Cf) and the total concentration of docetaxel (Ct).

The entrapment efficiency was calculated using the following equation (2);

$$\text{Entrapment efficiency (\%)} = \frac{C_t - C_f}{C_t} \times 100$$

Eq. (2)

2.4.5 Drug content

DTX-HA-PF127 micelles loaded with docetaxel were detected using the UV technique, which was first described by Rarokar et al. in 2022 [13]. For 20 minutes, DTX-HA-PF127 micelles containing 10 mg of docetaxel in 100.0 mL of ethanol were dissolved through shaking strongly. Five minutes were spent sonicating the solution. 1.0 mL of the filtrate was taken out after the the solution through a filter using a 0.45 µ filter. It was then reduce the concentration with 10 mL of distilled water and put through a spectrophotometric analysis at 230 nm.

2.4.6 Scanning electron microscopy

Following the formation of the spheres, little bit of the DTX-HA-PF127 micelle formulation that had been tuned stood on a microscope slide and let lack of moisture [17]. The sphere's surface area on microscope slide was covered together with a modest coating of palladium using an auto fine coater (Model: JFC1600, Jeol Ltd., Tokya, Japan). A scanning electron microscope (Model: JSM-6390LV, Jeol Ltd., Tokyo, Japan) with a digital camera and a 10 KV accelerating voltage was used to investigate the palladium-coated samples.

2.5 *In-vitro* docetaxel release study

The docetaxel discharge from the DTX-HA-PF127 micelles was assessed by calculating the drug's diffusion utilising a Franz diffusion cell through a cellophane membrane. The cell comprised two compartments.: two separate compartments, one for donors and one for receptors. A semipermeable barrier, earlier on Already enabled, divided these two compartments. The donor compartment above the membrane was filled with the DTX-HA-PF127 micelle formulation. A release medium of about 18 milliliters of phosphate buffer saline solution (PBS) pH 7.4 was maintained at 37±0.5°C inside the receptor compartment. At at regular intervals, partial solutions of the releasing agent were removed and swapped out with an equivalent amount of new-release material. The amounts of drugs released media at several periods were examined using UV light [17].

2.6 Statistical analysis

The data was expressed using the mean±standard deviation (SD). A two-way analysis of variance (ANOVA) and a Bonferroni post-test were employed for the statistical evaluation, which was conducted using GraphPad® Prism® software version 5.03 (San Diego, CA). The P value was considered less than 0.05 if there were significant differences between the means.

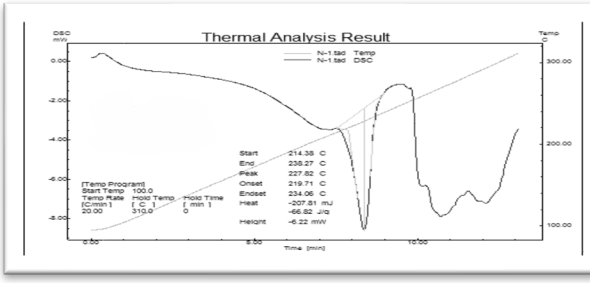
Results and discussion

Drug-Excipient Compatibility Studies

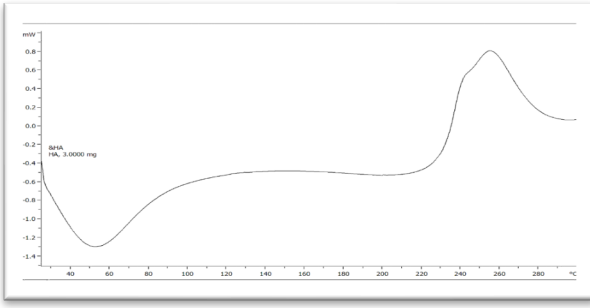
DSC is a popular and well-recognized analytical method for examining how different materials interact chemically. It is also helpful for obtaining data on stability, compatibility, melting, and deterioration. Peak alterations, peak start time, peak shape, and relative area all reveal information about the interaction between the medication and the excipient and the creation of new entities. As illustrated in Figure 1A, Pure DTX's DSC thermogram revealed a

prominent the peak of endothermic activity observed at around 169 °C, which corresponds to the fusion temperature of DTX. At around 54 °C, hyaluronic acid (HA) displayed distinct endothermic peaks (see Figure 1B). At at 57 °C, the Pluronic® F127 displayed distinct endothermic peaks (see Figure 1C). A little and insignificant alteration within the thermal behaviour of DTX when there is HA and Pluronic® F127 was found by the physical mixtures' DSC analysis, DTX, HA, and Pluronic F127 (1:1) (see Figure 1D). with In the physical preparations of the corresponding polymers with DTX, Pluronic® F127's melting signals (endotherm) were easily discernible. Therefore, any physical contact or evident conflicting nature of the medication as well as polymers was ruled out due to the lack of all other types of heat transfer events across the whole ambient temperature. So, the findings from the DSC showed these polymers were appropriate for use in the composition that were produced.

The compatibility between polymers and drugs is demonstrated by the three thermograms of the drug, polymer, and the combination of both.



(A)



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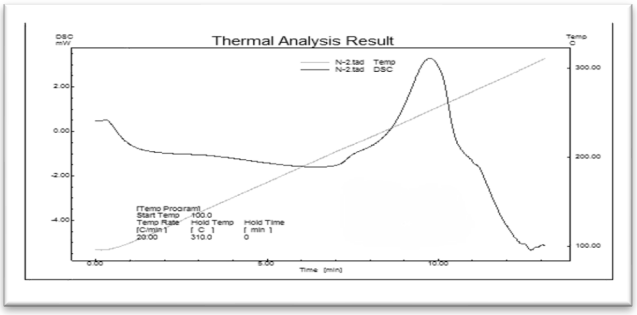
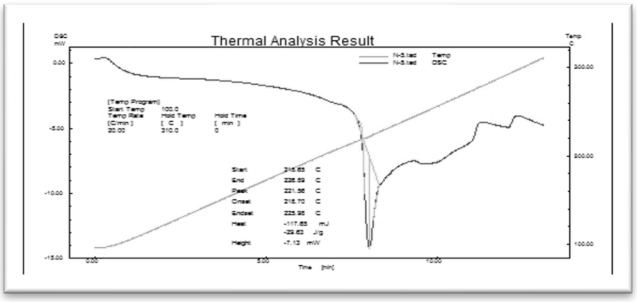


(C)



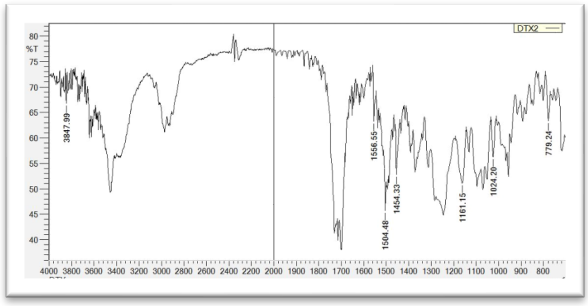
(D)

Figure 1: DSC thermogram of DTX (A) HA (B) Pluronic F127 (C) and physical mixture of DTX, HA and Pluronic F127 (D)

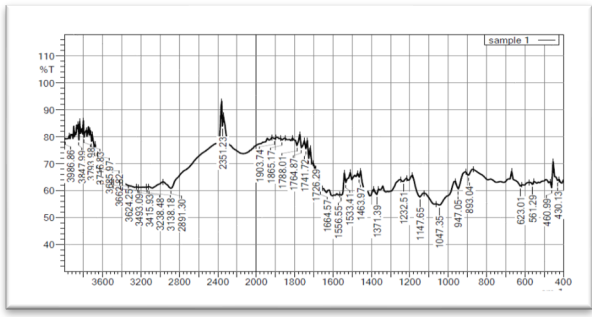
FTIR

Figure 2 depicted the FTIR spectra of DTX (2A), HA (see Figure 2B), Pluronic® F127 (see Figure 2C) or the binary combination of it (see Figure 2D). The distinctive peaks in the pure DTX spectrum are roughly located at wave numbers 779, 950, 1024.20, 1161.15, 1454.33, 1504.48, 1700 and 3847.99 (cm-1), which correlate to the hydroxyl group (-OH), ester (C=O), and amino group (-NH). The peaks were discovered to be comparable to the results that had been previously published [18, 19]. The distinctive peaks in HA's FTIR spectra are located at 476.42, 947.05, 1147.65, 1232.51, 1463.97, 1664.57, 2351.23, and 3847.99 (cm-1). The distinctive peaks of the Pluronic® F127 were typically located at 840, 1240, 1278, 2164.13, and 2235.50 cm-1. The physical mixture of DTX, HA, and Pluronic® F127 was subjected to FTIR analysis, which showed that C=O stretching peaks, amino groups (-NH), and hydroxyl groups (-OH) were all conserved in the spectrum (see Figure 2D). The distinct spectra as well as those derived from their physical composition did not, therefore, exhibit any discernible differences. Accordingly, the FTIR study's results showed no conclusive proof of a reaction between DTX and the polymer in question.

Accordingly, the FTIR study's results showed no conclusive proof of a reaction between the used polymers and docetaxel trihydrate.



(A)



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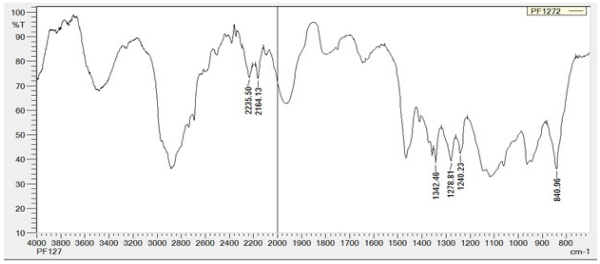
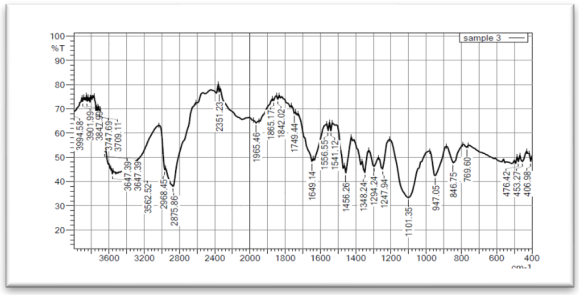


(C)

EE = +82.77-8.09A+2.73B-0.4563C+0.5375AB-0.3250AC+0.4425BC +2.28A²+0.5480B²-0.6695C²



(D)

Figure 2: FTIR spectra for DTX (A) HA (B) Pluronic F127 (C) and physical mixture of DTX and Pluronic F127 (D)

Optimization of formula

Three-dimensional surface plots of entrapment efficiency (Y, %) against drug and HA concentration (X1), drug concentration and temperature (X2), and HA concentration and temperature (X3) are shown in Figure 3 (D, E, and F). Figure 3 (A, B, and C) shows a contour plot of entrapment efficiency (Y, %) against drug and hyaluronic acid concentration, drug concentration and temperature, and hyaluronic acid concentration and temperature. Figure 3 (A, B, C, D, E, and F) and Equation 3 demonstrated that while X2 had a positive effect on Y, independent variables X1 and X3 had a negative effect on %EE.

An F-value for the model of 478.28 is indicative its importance. There is a 0.01% chance that noise might contribute to a high F-value.

If the p-value was less than 0.0500, the parameters used in the model were considered Relevant. Here, A, B, C, AB, A2, B, and C2 are crucial model parameters. If the values exceeded 0.1000, No meaningful results were obtained from the model terms.. If your model possesses numerous superfluous terms (aside from individuals needed to manage hierarchy), Reducing the model could make it better.

A 2.25 F-value indicates a lack of fit indicated it did not constitute a substantial Lack of Fit when compared as a result of the inherent mistake. Then there was a 22.48% possibility of noise would cause a higher Discordant F-value. Good lack of fit that is insignificant.

The Adjusted R2 of 0.9963 and the Predicted R2 of 0.9827 are somewhat in sync, with a discrepancy of under 0.2.

Precision is used to measure of signal-to-noise ratio. The optimal ratio is more than four. The percentage of 74.804 is indicative a sufficient signal. You can use this model to find your way around the design area.

A Final-Ditch Formula Based on Coded Variables

Eq (3)

For specific values forecasts for each component on the response can be derived from the coded factors by applying equation 3. By default, the numbers +1 and -1 represent the elevated and decreased levels of the factors, in that order.The relative relevance of the elements might be determined using the equation with codes by comparing the component values.

Table 4: Seventeen batches of DTX-HA-PF127 micelles by employing Box-Behnken Design

Batch	Factor 1 A: Concentration of drug (%w/v)	Factor 2 B: Concentration of HA (%w/v)	Factor 3 C: Temperature (°C)	Response 1 Entrapment Efficiency (%)
1	0.55	3	60	85.69
2	0.55	2	50	82.8
3	0.55	2	50	82.71
4	1	1	50	74.23
5	0.1	2	40	92.78
6	0.1	1	50	91.65
7	0.55	1	40	80.5
8	0.55	1	60	79.22
9	1	3	50	80.62
10	0.1	2	60	92
11	0.1	3	50	95.89
12	1	2	40	77.41
13	0.55	2	50	82.96
14	0.55	3	40	85.2
15	0.55	2	50	82.3
16	1	2	60	75.33
17	0.55	2	50	83.1

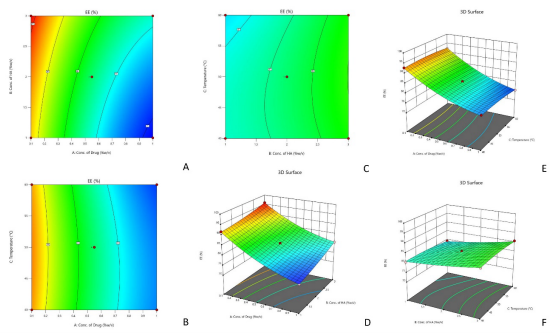


Figure 3: Contor plot of entrapment efficiency (%) against A) concentration of DTX and HA B) concentration of DTX and temperature and C) concentration of HA and temperature; 3D surface plots of entrapment efficiency (%) against D) concentration of DTX and HA, E) concentration of DTX and temperature and F) concentration of HA and temperature.

Validation of Model

For assessing the optimisation capacity of models produced in accordance with the findings of the central composite design, DTX-HA-PF127 micelles were made with an ideal concentration of drug (DTX), concentration of HA and temperature were 0.1 %w/v, 3 %w/v and 50 °C respectively. The % Bias when it comes to the estimated and real entrapment efficiency values for for DTX-HA-PF127 micelles were determined to be below ±3.0%, confirming that significancy of the model.

$$Bias\ (\%) = \frac{predicted\ value - observed\ value}{predicted\ value} \times 100 \quad \text{Eq (4)}$$

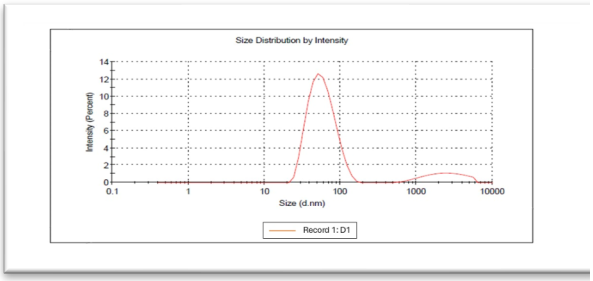
Physicochemical characterization of HA-PF127-DTX micelles

Particle size, polydispersity index (PDI) and the zeta potential

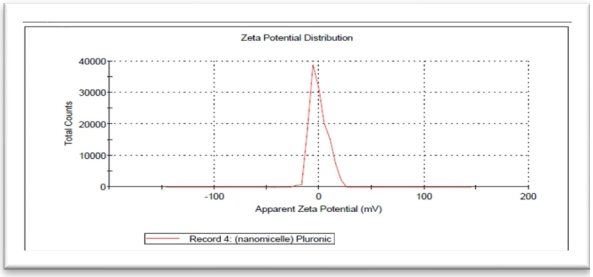
Since they had increased surface area/volume ratio, smaller particles facilitate the release of the encapsulated medication from the DTX-HA-PF127 micelles through surface erosion and diffusion. Additionally, this has the benefit of allowing the DTX-HA-PF127 micelles to enter and pass past the physiological drug barriers. Prior research has proposed that the lymphatics would absorb the larger particles (less than 5 mm), whereas endocytosis would allow the smaller particles (less than 500 nm) to pass past the membrane of epithelial cells [20]. The hydrodynamic diameter of the nanocarriers affects the effectiveness of passive targeting as well [21].

Figure 5 (A and B) displays the zeta potential, polydispersity index (PDI), and particle size of DTX-HA-PF127 micelles. As shown in Figure 5A, about the typical size of DTX-HA-PF127 micelles was determined to be 149.9±2.58. Therefore, the average particle dimension of the DTX-HA-PF127 micelles was observed to be only slightly increased by the DTX loading. DTX-HA-PF127 micelles were discovered to have a polydispersity index of 0.152±0.017. Referring to Figure 5B, the zeta potential of DTX-HA-PF127 micelles was determined to be -18 mV.

Furthermore, DTX-HA-PF127 micelles' low polydispersity index value suggested a limited particle size distribution [22]. Another crucial indicator of the DTX-HA-PF127 micelles' stability is their zeta potential. In a buffer solution, A high zeta potential indicates that the surface of the material is heavily charged electrically DTX-HA-PF127 micelles, which can prevent the micelles from aggregating and create strong repelling forces between particles [23, 24, 25]. While smaller particle sizes are more likely to have a lengthy circulation half-life and a higher likelihood of tumor localization, a lowest possible zeta potential of more than -30 mV is generally regarded as appropriate and suggestive of strong physical stability [26].



(A)

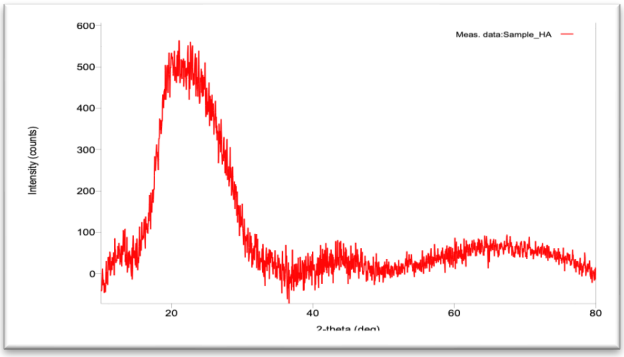


(B)

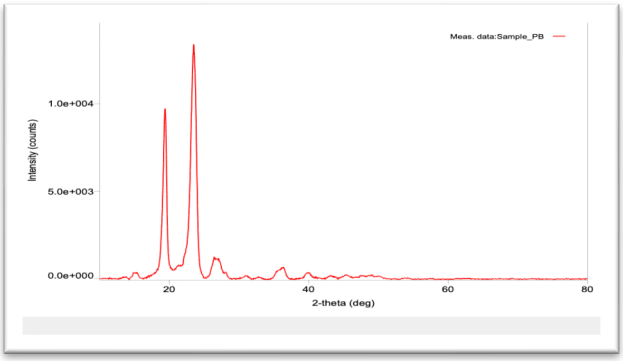
Figure 5: Particle size (A) and zeta potential (B) of DTX-HA-PF127 micelles

XRD

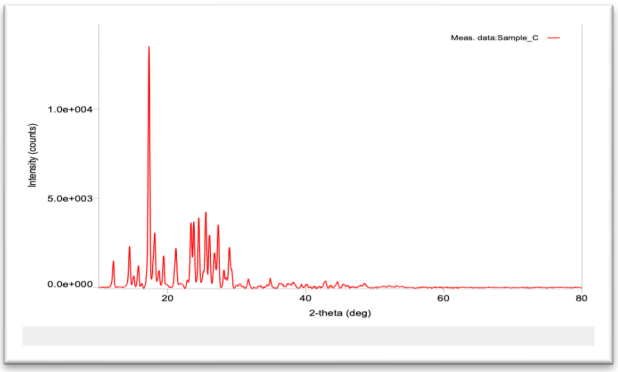
The crystalline nature of the chemical conjugated with the other polymer structure is determined using X-ray diffraction (XRD). The XRD spectra of HA, PF127, and DTX-HA-PF127 micelles are shown in Figs. 6A, 6B, and 6C. The characteristic peaks at the Bragg angle, 2_ h = 12.40, 15.724, 18.122, 20.01, 22.67, and 24.991 with intensity 654, are seen in the pluronic® diffractogram.Peaks at Bragg angle 2 h = 19.42, 21.99, 31.02 (with a higher intensity of 5879), and 34.210 (intensity = 3108) were visible in the XRD diffractogram of HA-PF127. The XRD study's findings showed that DTX-HA-PF127 micelles were formed, which was consistent with earlier research [27]. The XRD pattern of DTX-HA-PF127 micelles (see Fig. 4C) showed that the distinctive HA peaks had vanished, leaving only a few peaks remaining. The XRD of the DTX-HA-PF127 micelles, on the other hand, showed the distinctive peaks of PF127.



(A)



(B)



(C)

Figure 6: XRD of DTX (A), HA (B) and physical combination of DTX and HA-Pluronic F127 (C)

Entrapment efficiency

Important measures for drug delivery systems include drug entrapment efficiency. Especially with pricey medications, this is true. DTX-HA-PF127 micelles were discovered to have an entrapment efficiency of 95.89±2.5 (% w/w), meaning that the majority of DTX was encapsulated in these micelles.

Drug content

The drug content for optimized DTX-HA-PF127 micelles was found to be 97.28 ± 1.2.

***In-vitro* DTX Release from** DTX-HA-PF127 micelles

Figure 7 illustrates the DTX release pattern from the DTX-HA-PF127 micelles as assessed by the Franz diffusion cell. Within one hour, the DTX-HA-PF127 micelles showed their first burst release (30.56%). After then, DTX was gradually released from the DTX-HA-PF127 micelles over a 12-hour period. Over 95.66% of DTX was released from the DTX-HA-PF127 micelles after 12 hours, whereas over 93.85% of DTX was liberated in just 6 hours from the DTX hydroalcoholic solution. According to the findings, DTX-HA-PF127 micelles the possibility of being put to use in controlled medication delivery systems.

Table 5: Drug release of DTX from DTX solution and DTX-HA-PF127 micelles

Time (h)	DTX-solution	DTX-HA-PF127 Micelles
0	0	0
2	55.65	30.56
4	81.46	48.392
6	93.85	64.558
8	-	78.34
10	-	86.168
12	-	95.66

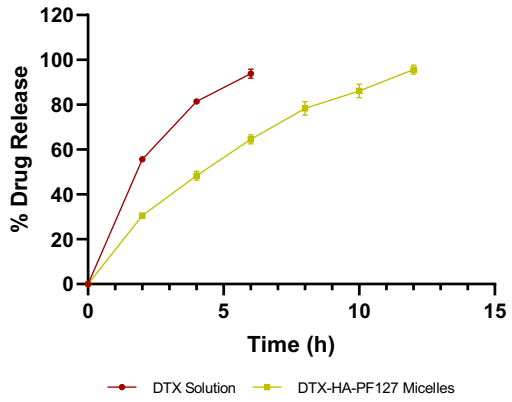
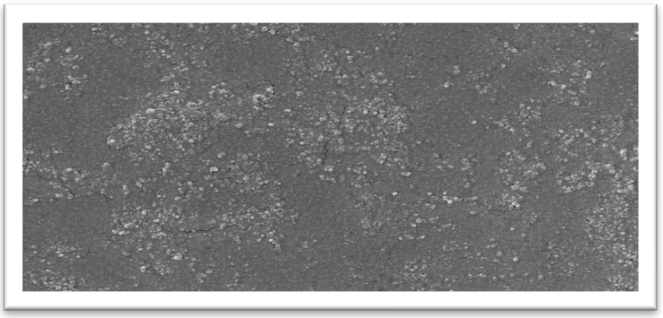


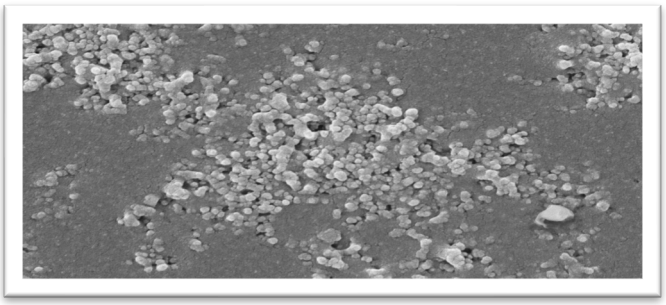
Figure 7: % drug release of DTX from DTX solution and DTX-HA-PF127 micelles

Scanning Electron Microscopy (SEM)

The DTX-HA-PF127 micelles were analyzed by SEM for surface morphology. The electron micrographs at different magnifications at 100x, 700x and 5000x revealed the formation of some spherical shape DTX-HA-PF127 micelles formation as shown in figure 8A and 8B.



(A)

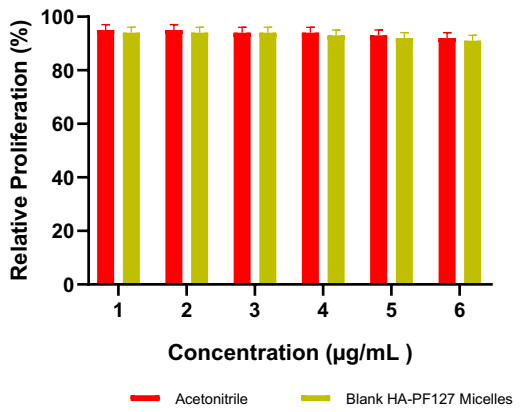


(B)

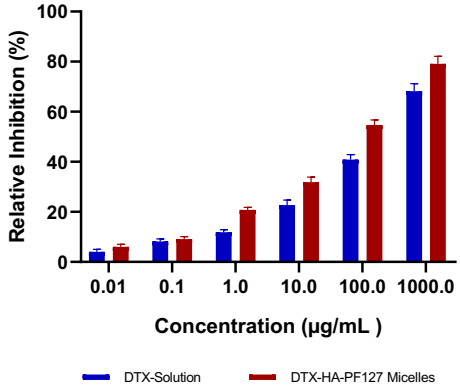
Figure 8: SEM image of DTX-HA-PF127 micelles at various magnification shows somewhat spherical shape micelles

Cytotoxicity studies (MTT Assay)

We utilized the MTT test to perform a cytotoxicity of cells investigation using human breast adenocarcinoma cell lines (MCF-7) applicable to both the DTX hydroalcoholic solution and the (DTX HA Sol.) and DTX-HA-PF127 micelles at different doses. Relative proliferation after treated with acetonitrile as well as blank-HA-PF127 micelles and relative inhibition after treatment with DTX-HA-PF127 micelles and DTX HA solution were determined (**Figure 9A**). According to the findings of the cell cytotoxicity investigation, for every concentration of 0.01, 0.1, 1, 10, 100, and 1000 µM, the percentage of human breast cancer cells that were inhibited considerably higher for DTX-HA-PF127 micelles compared to DTX HA (**Figure 9B**).. As a result, DTX-HA-PF127 micelles exhibit poorer cellular viability than DTX HA solution, indicating higher cytotoxicity of the formulation towards cells. The IC50 values for DTX-HA-PF127 micelles and DTX HA solution were determined to be 0.51µM and 0.89µM, respectively.



(A)



(B)

Figure 9: *In vitro* cytotoxicity of acetonitrile and blank-HA-PF127 micelles on human breast adenocarcinoma cells (MCF-7) after 72 h. A) Effect on relative proliferation of MCF-7 cell line after treatment with different concentration of samples. The bars represent mean±SD (n = 3). *In vitro* cytotoxicity of DTX-HA-PF127 micelles and DTX solution (free drug) on human breast adenocarcinoma cells (MCF-7) after 72 h, B) showing effect of different concentration of DTX solution and DTX-HA-PF127 micelles. The bars represent mean±SD (n = 3)

Stability study

The development of formulation requires a stability study, which is useful in demonstrating the product's quality under the influence of changing environmental conditions over time. According to ICH recommendations, the stability investigation of the optimised DTX-HA-PF127 micelle formulation was conducted for three months at room temperature, freezing temperature, and 40 ± 2 °C with 75 ± 5% relative humidity (RH) as quoted in **Table 6, 7** and 8**,** respectively.

Table 6: Stability study data for DTX-HA-PF127 micelles at 40 ± 2 °C, 75 ± 5% RH

Sr. No.	Stability study parameters	Initial	After 3 months
1	Phase separation	No Phase separation	No Phase separation
2	Drug content (%)	94.70 ± 1.2	95.34 ± 0.67
3	Particle size (nm)	149.9±2.58	154.3±1.0

Table 7: Stability study data for DTX-HA-PF127 micelles at room temperature

Sr. No.	Stability study parameters	Initial	After 3 months
1	Phase separation	No Phase separation	No Phase separation
2	Drug content (%)	94.70 ± 1.2	96.86 ± 0.83
3	Particle size (nm)	149.9±2.58	152.2±1.25

Table 8: Stability study data for DTX-HA-PF127 micelles at freezing temperature

Sr. No.	Stability study parameters	Initial	After 3 months
1	Phase separation	No Phase separation	No Phase separation
2	Drug content (%)	94.70 ± 1.2	96.0 ± 0.57
3	Particle size (nm)	149.9±2.58	150.5± 1.0

Conclusion

Based on the overall findings from the cytotoxicity study in human breast adenocarcinoma cells (MCF-7), zeta potential, scanning electron microscopy, entrapment efficiency, and in vitro drug release study, it was determined that the DTX-HA-PF127 micelles have a greater potential for use as a administering medication vehicle owing to their distinctive capacity to incorporate hydrophobic drugs into their own personal space, their less sized particles at the at the nanoscale, and their medication delivery under strict oversight. Strong experimental support is provided by this study for the creation of DTX-HA-PF127 micelles as a successful strategy for creating a regulated drug delivery system for anticancer medications with enhanced stability.

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