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Evaluation and investigation of micellar structure of pluronic copolymer nanoaggregates of Docetaxel

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Abstract

The study was undertaken to develop and characterize docetaxel-loaded lyophilized block co-polymeric micelles for cancer therapy treatment. The study demonstrates that micellar size, shape, zeta potential, and other characteristics of lyophilized polymeric micelles are appropriate; furthermore, because of the polymer's encapsulation and capacity to maintain the drug release, significantly reduces toxicity and adverse effects. Block co-polymeric micelles offer improved solubility and a high capacity for drug loading, perhaps enhancing the bioavailability. As per this research, the idea of employing block co-polymeric micelles to enhance the solubility of hydrophobic medications holds excellent promise as an intravenous drug delivery system. While the outcomes are encouraging, the docetaxel-loaded block co-polymeric micelles must be further evaluated for In-Vivo studies to check the targeted drug delivery.

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INTRODUCTION

Lung Cancer is the second leading cause of cancer in both men and women, with the highest mortality rate. 19 % of all cancer deaths are only due to lung cancer. Early-stage diagnosis is also a hurdle that typically leads to progress if the disease is not controlled. Treatment causes additional symptoms [1]. Lung cancer is the most common and leading cause of death worldwide, both in men and women. In men, it holds an 8% risk of developing lung cancer in both way of incidence and mortality. In women, it is third highest after breast cancer, which is second highest, holding 6% of developing lung cancer. Lung cancer has been more common amongst men as compared to women in the UK, with incidences of 77 and 66 per 1,00,000 population [2]. This depends on the differences in smoking behavior, with 21.1% males and 16.5 % females. Healthcare

Table 1 Preliminary batches of Pluronic F68

S. No	Batches	Polymer Concentration (mM)	Ultrasonication Time (min)	Methods
1	Pluronic F68	0.5	10	Direct dissolution
2	Pluronic F68	0.5	10	Sonication
3	Pluronic F68	0.5	10	Evaporation

Table 2 Preliminary batches of polymeric micelles

S. No	Batches	Polymer Concentration (mM)	Ultrasonication Time (min)	Methods
1	Pluronic F-127	0.5	10	Direct dissolution
2	Creamophor	0.5	5	Direct dissolution
3	SLS	5	10	Sonication

providers divide lung cancers into small-cell (SCLC) and non-small-cell (NSCLC). Lung cancers are also referred to as bronchogenic carcinomas since they develop from the airways inside the lungs. The microscopic appearance of the cancer cells, precisely their size, is the basis for this classification. Given the distinct ways these two types of cancer manifest, spread, and potentially respond to different treatments, it's important to distinguish between them [3]. SCLCs make up about 20% of all lung cancers and are the most aggressive and fast-growing of them all. SCLC is linked to cigarette smoking, with non-smokers accounting for just 1% of all cases. These cancers are often called oat cell carcinomas because of a particular cell appearance seen while analyzing samples of SCLC under the microscope [4].

MATERIALS AND METHODS

MATERIALS

Docetaxel is the API from BDR Pharmaceuticals Ltd, and other selective polymer mixtures such as Pluronic F-127, Sodium lauryl sulfate, Tween 80, TPGS, Creamophor were utilized along with organic solvents Ethanol, and Methanol.

METHODS

Pre-formulation Study for Drug

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectrophotometer (Alpha II, Bruker) was used to get the Infrared spectra to verify the Drug's purity. To prepare 10% of the mixture, the technique involved dispersing a sample (drug alone or drug mixture with excipients) in KBr. The sample was then commonly pulverized in a mortar pestle using KBr before being compacted into pellets [5]. The spectrum for this pellet was collected at a resolution of 2 cm-1 throughout a frequency range of 4000 to 400 cm-1 after it was placed in the light path. The reference point for the determination was the background spectrum of KBr [6].

Preformulation study for polymer

Preliminary screening of different polymers and different process variables for Blank micelles [7]

Six different types of polymers were used for screening, i.e., Pluronic F68, Polycaprolactone (PCL), Pluronic F127, Creamophor EL, tween 80, and sodium lauryl sulfate (SLS). While different methods are used to prepare polymeric micelles (Table 1 & 2).

Screening of polymer ratio for self-assembled mixed micelles

Pluronic F127 and TPGS were selected based on the ratio selection 1:1, 2:3, 3:7, 4:1, and 9:1 for Pluronic F127 and TPGS. All the batches were prepared using the solvent evaporation method [8].

The optimized ratio was selected from all the ratios based on micellar size and Entrapment efficiency [9].

Preparation of self-assembled mixed micelles

Docetaxel-loaded mixed micelles were prepared using a solvent evaporation method. Docetaxel has low water solubility, so an organic solvent is selected, which is expected for both the polymers and the Drug (such as Ethanol, dimethyl sulfoxide,

Table 3 Formulation of Micellar Structure Of Pluronic Copolymer Nanoaggregates

Formulation Code	Amt of Pluronic F 127 (mg)	Amt of TPGS (mg)	Solvent volume (ml)
F1	45	7.5	7.5
F2	45	7.5	10
F3	45	10	7.5
F4	67.5	7.5	7.5
F5	67.5	7.5	10
F6	67.5	10	7.5
F7	90	7.5	7.5
F8	90	7.5	10
F9	90	10	7.5

N, N-dimethyl formamide, acetonitrile, THF, acetone or dimethyl acetamide). The solvent removal process determines the mechanism by which micelles self-assemble [10]. The requisite amount of Docetaxel is added in different mass ratios of Pluronic F 127 and TPGS. A water-miscible organic solvent, Ethanol, was used to dissolve the resulting combinations ultrasonically [Table 3]. Using a micropipette, the dispersion was stirred for three hours until the Methanol evaporated after the complete dissolution of the polymers. Finally, a 0.2µM filter removed large aggregates from the solution [11].

Self-assembly Mixed Micelle Characterization

Size determination

A photon correlation spectrometer in the Zetasizer NanoZS, manufactured by Malvern Instruments Ltd. in the UK, was used for DLS measurements in every instance. The average of three different samples was used for all measurements, which were done in triplicate at 25°C following five minutes of equilibration. To prevent losing particles such as more extensive vesicles, the generated samples were often examined without dilution or filtration to get information on all species that appeared during sample preparation. The polydispersity index was analyzed to ascertain how the molecular mass was distributed inside the polymer [12].

Encapsulation Efficiency and drug loading

The drug concentration of Docetaxel in micelles was determined using HPLC analysis and a maximum absorption wavelength of 332 nm. The unentrapped drugs were extracted from docetaxel-loaded micelles by passing them through a 0.22-micron syringe filter [13]. Docetaxel was released after Methanol was added to Docetaxel mixed micelles, dissolving the core-shell structure. After pipetting out 1 milliliter of the filtered micellar solution, 10 milliliters of Methanol were added, and the mixture was sonicated for 15 minutes to shatter the micelles further. The EE percent and drug loading (DL percent) were calculated using the following formulas [14].

$$EE\% = \frac{\text{Weight of encapsulated drug}}{\text{Weight of feeding drug}} \times 100$$

$$DL\% = \frac{\text{Weight of encapsulated drug}}{\text{Weight of Polymer}} \times 100$$

Critical micelles concentration

Zeta sizer was used to measure micelles' essential micelle concentration, employing the dynamic light scatter approach. Different ratios have been picked from the design matrix. Samples with concentrations ranging from 0.01 mM to 0.05 mM were diluted for a 4:1 ratio in Ethanol and deionized water, and each sample's variations in light intensity were recorded [15]. The light intensity and the sample's molar concentration were plotted on a graph. The point at which the intensity slope sharply rose, suggesting micelle formation, was used to compute the CMC [16].

Surface Morphology

Using a transmission electron microscope (Philips, Philips XL 30 ESEM), the surface morphology of drug-containing polymeric micelles, drug dispersion-containing polymer, and pure drug powder were recorded. Samples will be mounted on an aluminum stub using conductive double-sided adhesive tape. The samples will then be coated with gold in an argon environment (50 Pa) at 50mA for 50 seconds [17].

In vitro release

The above-discussed validated HPLC method investigated the in-vitro drug release behavior. The optimal batch of drug-loaded mixed micelles was assessed for in vitro drug release in phosphate buffer (pH 7.4 and 5.5) using a Franz diffusion cell via dialysis bag methods. After precisely weighing the lyophilized mixed micelles to equal 75 mg of the drugs, they were reconstituted in 10 ml of phosphate buffer (pH 7.4 and 5.5). The diffusion cell was placed in a shaker incubator at 37.0±0.5°C and moderate shaking at 100 rpm [18]. One milliliter of the sample was taken out, and the incubation medium was changed out for a freshly made release medium at predetermined intervals to maintain the sink conditions. The distribution strategy was selected to complement the in vivo state of the receiver chamber's site of action and administration [19]. The cumulative drug release vs. time curve was plotted to assess the release profile of Docetaxel. To ensure reproducibility, three duplicates of the experiment were conducted.

RESULT AND DISCUSSION

FTIR Study:

Using an FTIR spectrophotometer and KBr pellets, FTIR spectra were obtained for the Drug alone (as shown in Figure 1, 2,3,4) and the excipients to determine the identity of the Drug and excipients and investigate their compatibility. The observed values are near the standard value, representing that the Drug is pure, as shown in Table 4.

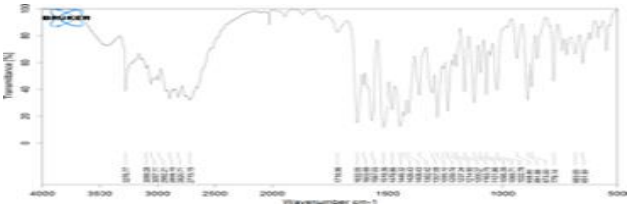

Figure 1 Identification of Docetaxel using FTIR spectroscopy

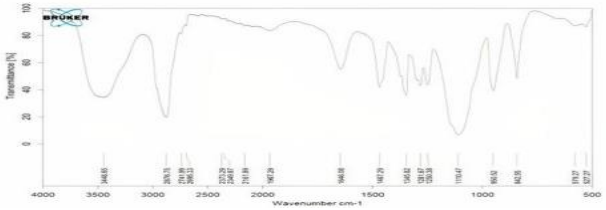

Figure 2 Identification of Pluronic F-127 using FTIR spectroscopy

Table 4 FTIR spectra Interpretation of Docetaxel

S. No	Functional group	Standard wave number (cm^{-1})	Observed wave number (cm^{-1})
1	NH Secondary Amine	3500-3300	3276
2	CH Aromatic	3300	3099
3	CH Aliphatic	3100-3000	2898
4	C=C Aromatic	1600-1400	1362

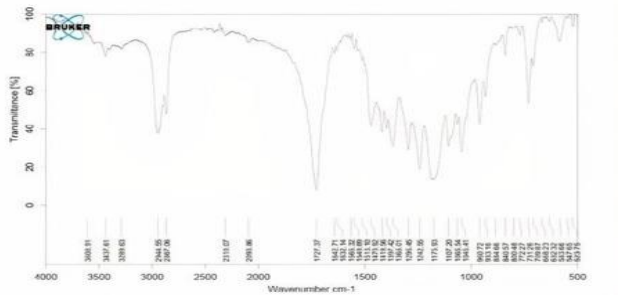

Figure 3 Identification of TPGS using FTIR spectroscopy

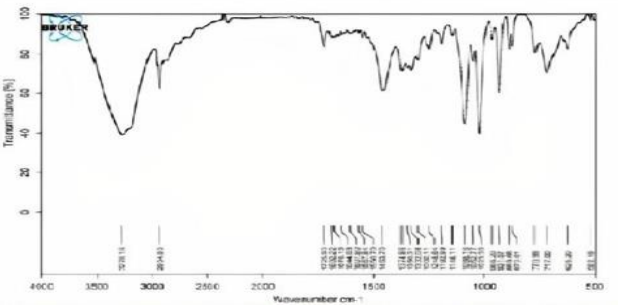

Figure 4 FT-IR of Drug and excipients

From the results, it was evident that there is no interaction between drugs and excipients.

Screening of polymers and different preparation methods for micelles

Pluronic F68 polymeric micelles were prepared using three different methods: direct dissolution, evaporation, and dialysis. From the dialysis method, the micellar size observed is 1084nm and PDI 0.483. By evaporation and direct dissolution, the micellar size was 493 and 353nm, with PDI 0.163 and 0.112, respectively. Also, Other polymers like Pluronic F127 PCL were used for screening, where the Micellar size of Pluronic F127 was found to be 22.06 nm and PDI 0.2. PCL and Pluronic F68 mixed micelles were prepared,

Table 5 Screening of polymer ratio (Pluronic F127: TPGS)

S. No	Trial Batches With polymer ratio (Pluronic F127:TPGS)	Particle Size (nm)	Polydispersity Index	%EE (%)
1	9:1	231.1	0.568	84.33 %
2	4:1	256.0	0.629	80.69 %
3	7:3	290.4	0.563	81.23 %
4	3:2	128.1	0.445	73.21 %
5	1:1	111.8	0.566	56.34 %

Table 6 Micellar Size and %EE of Docetaxel loaded self-assembled micelles

F. Code	Amt of Pluronic F 127 (mg)	Amt of TPGS (mg)	Solvent volume (ml)	Micellar size (nm)	%EE (%)
F1	45	7.5	7.5	316.2±2.25	59.21±0.12
F2	45	7.5	10	205±1.23	68.71±0.15
F3	45	10	7.5	200.2±1.66	62.45±0.35
F4	67.5	7.5	7.5	185.33±2.3	72.83±0.22
F5	67.5	7.5	10	185.33±1.34	72.83±0.21
F6	67.5	10	7.5	113.6 ± 2.76	73.33±0.25
F7	90	7.5	7.5	162.84±1.23	71.32±0.32
F8	90	7.5	10	148.36±3.1	79.3±0.15
F9	90	10	7.5	103.5 ± 1.45	79.12±0.17

showing micellar size of 564nm and PDI 0.7. Surfactants like Creamophor, Tween 80, and SLS were used to prepare micelles, giving micellar size 68.88nm, 396.1nm, and 250.2nm with PDI 0.3, 0.6, and 0.8, respectively.

Screening of polymer ratio for self-assembled mixed micelles

The optimized ratio was selected based on micellar size and Entrapment efficiency from 1:1, 2:3, 3:7, 4:1, and 9:1 ratios. As the amount of Pluronic F127 and TPGS increases, the entrapment of the Drug decreases, and the micelle size also decreases. From the entire ratio, 9:1 is selected for better entrapment and micellar size, as shown in Table 5.

Docetaxel loaded self-assembled micelles.

The micellar size and percent EE of docetaxel-loaded self-assembled micelles were examined. Table 6 displays the size range of 103-316 nm for batches F1 through F9. which is appropriate for being targeted at the malignant site; for this reason, the crucial factors pH 7, solvent type and volume, polymer concentration, and sonication time were chosen, in that order, to produce a range of particle sizes with uniform distribution and high drug entrapment [Table 6]. EE% from F1-F9 ranges from 59% - 79 %.

From the results, we can interpret that micellar size decreases as the polymer increases. The influence of TPGS is more significant than that of other factors. At high TPGS concentrations, different analyses reveal a reduction in core radii. Together with this, the number density of micelles (N) increased, and the aggregation number decreased. These findings suggest a micellization process involving fewer surfactant units, whereby residuals join to form new micelles. A higher concentration of TPGS polymers may be responsible for more than reduction, as this could improve the interaction between the hydrophobic chains and the constituents of both polymeric mixtures, leading to a more compact structure. Particle size is influenced by solvent volume and polymer concentration, showing that particle size decreases as polymer concentration increases. The reason for this is that while increased solvent volumes improve the polymer's solubility, which also results in smaller particle sizes, increasing concentrations lengthens the hydrophobic chains in the natural micelle building blocks.

Interpretation for % Entrapment Efficiency

According to the results, entrapment efficiency would rise with the polymeric amount. Due to the increased availability of carriers to entrap the necessary fraction of the Drug, drug loading

Table 7 Assembled micelles

S. No	Amt. of Pluronic F127(mg)	Amt. of TPGS (mg)	Micellar size (nm)	% EE (%)
F6	67.5	10	113.6 ± 2.76	73.33±0.25
F9	90	10	103.5 ± 1.45	79.12±0.17

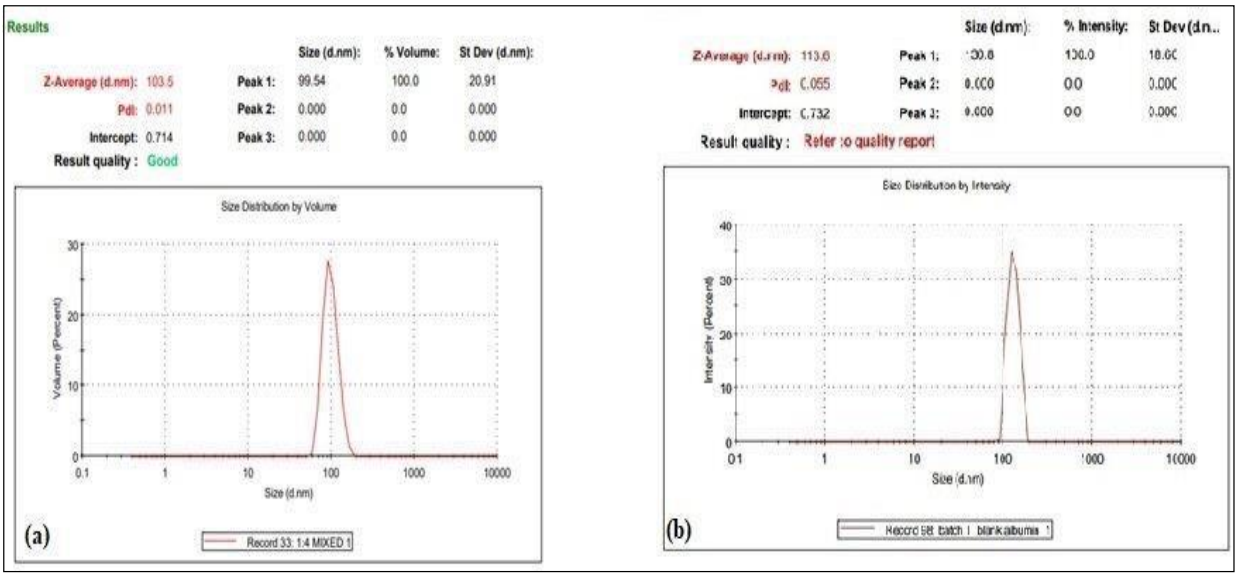

Figure 5 Micellar size of the optimized batch of self-assembled mixed micelles

increases as polymer concentration rises. Hydrophobic: One of the mechanisms that promotes entrapment is the hydrophobic contact between the Drug and the polymer. However, the response (%EE) will be negative when this factor interacts with solvent volume. The combined effect of solvent volume and polymer concentration on entrapment efficiency demonstrates that entrapment efficiency rises with both variables. The optimized batches of Docetaxel-loaded Pluronic F127 and TPGS mixed micelles are shown in Table 7.

Characterization of Docetaxel loaded mixed micelles.

Size determination

The optimized batch's Z averages were 103.5 and 113.6 nm, respectively, with PDI of 0.011 and 0.055, as shown in Figure 5.

Additionally, the design batches had a range of 43.22 to 133.7 nm. The particle size increase was correlated with an increase in Pluronic F127 concentration. PEO's longer chain length contributes to its higher hydrophilicity, which raises micellar size and is related to the PEO to PPO block ratio. In contrast to Pluronic F127, TPGS has the opposite effect on the size of micelles. One of the explanations for this is that TPGS interacts with the PEO block through its hydrophobicity, creating a more compact structure. Tiny micellar agglomerate more effectively within tumors, allowing for more reliable drug release.

Encapsulation Efficiency and drug loading

Table 8 provides the efficiency of entrapment. The drug loading and entrapment fraction increases with polymer quantity; this is correlated with generating extra micelles over the critical micelle concentration (CMC) threshold. At 79.12 percent, F9 has the most excellent entrapment rate. The F9 batch had the highest quantities of TPGS and plinonic F127. Increased Pluronic alone is insufficient to enhance medication loading. Hydrophobicity is not something Pluronic F127 is good at. The percentages of DL and EE were increased when TPGS was added to the mixed micelles. The PPO segment of Pluronic, the Docetaxel, and the aromatic ring of TPGS have hydrophobic and van der Waals interactions. More polymer means more interaction with the medication and the production of micelles.

Table 8 *In vitro* drug release

Time in Hrs	% Cumulative Drug Release of Docetaxel mixed micelles				
	Docetaxel Solution	F6 (pH 5.5)	F9 (pH 5.5)	F6 (pH 7.4)	F9(pH 7.4)
2	18.65±2.5	8.65±4.8	11.49±5.8	10.38±6.8	12.85±0.5
4	21.49±1.1	12.49±3.9	18.65±4.5	18.58±5.4	15.47±4.5
8	26.48±3.8	32.48±7.5	31.15±6.6	31.85±7.2	29.45±5.3
12	30.19±4.8	42.15±3.6	44.25±7.8	49.49±4.7	47.35±2.5
16	39.58±4.8	54.19±2.5	55.28±5.7	57.48±3.2	54.19±5.8
20	41.19±2.7	65.19±5.8	66.48±3.6	64.55±3.7	62.06±2.5
24	44.19±4.6	82.48±3.6	87.49±5.4	79.74±3.5	83.19±8.9

Critical Micelles Concentration

Sample dilutions ranging from 0.01 mM to 0.05 mM were made. Light intensity was measured for each sample. The combined polymer's CMC value was determined as the point at which intensity increased. 3.3 × 10^-5 M It was discovered that the crucial micellar concentration for the 4.5:1 ratio in Figure 6 was concentration.

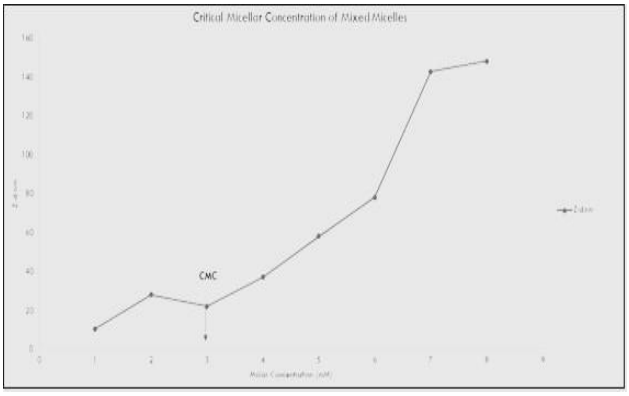

Figure 6 CMC plot of self-assembled mixed micelles

As the TPGS fraction rose, the CMC value decreased. The mixed micelles may, therefore, have more physical stability when diluted. Increased hydrophobic interactions between the vitamin E component of TPGS and the Pluronic® PPO segment in the inner core of micelles most likely caused this.

Surface morphology

Surface morphology was assessed for optimized checkpoints. It was discovered that docetaxel-loaded self-assembled micelles had a spherical form. This is the morphology in Figure 7.

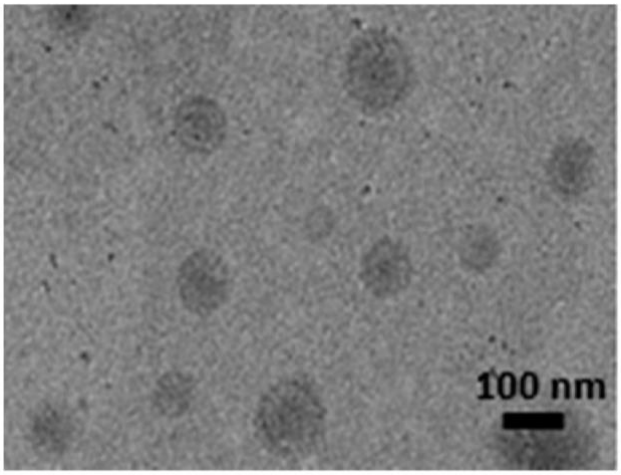

Figure 7 TEM image of self-assembled mixed micelles

In vitro drug release

As seen in Figure 8, we have dissolved the docetaxel solution and the mixed micelles checkpoint batch Table 8.

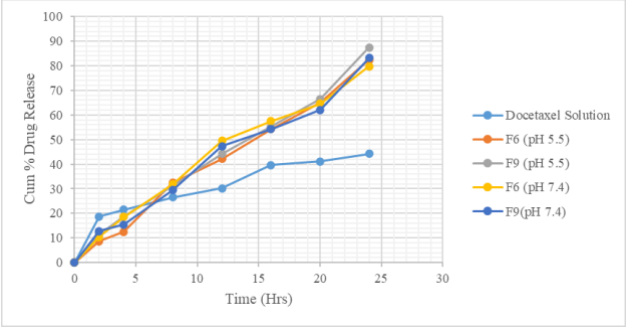

Figure 8 *In-vitro* drug release of Drug, Drug loaded mixed micelles at pH 7.4 and 5.5

Docetaxel's hydrophobicity causes the solution to release only 25.32 percent of the Drug, which is a shallow level of drug release. Docetaxel-loaded polymeric micelles release 87.39% of the

medication at pH 5.5. Docetaxel-loaded polymeric micelles release 83.19% of the medication at a pH of 7.4. An initial burst discharge occurred. In 12 hours, polymeric micelles release 44.25 percent at initial. The burst release of Docetaxel from mixed micelles may be explained by the rapid disintegration of the micellar system brought on by cohesiveness, a more significant concentration gradient, and sink conditions in the system. This release could reach the therapeutic concentration, and the steady rise might be able to keep it there.

The lowered drug release at pH 7.4 may be beneficial since delayed and sustained release of Docetaxel from micelles at physiological pH may minimize the adverse effects associated with nonspecific absorption of Docetaxel. The improved drug release at pH 5.5 (87.49 percent) may be explained by the higher partition coefficient of Docetaxel in the acidic medium relative to the micellar core. However, due to EPR effects and improved drug release in the tumor environment, docetaxel-loaded mixed micelles may have higher therapeutic effectiveness because of their extended mean residence duration in tumors. However, polymeric micelles prepared by Pluronic F127 and TPGS do not form micelles by covalent solid bonds between polymers. Hence, burst release was initially observed due to drug diffusion from the polymer matrix. A copolymer is synthesized, covalently bonds the hydrophobic and hydrophilic portion, tightly encapsulates the Drug, and resists burst release to overcome this type of release.

CONCLUSION

The study demonstrates that micellar size, shape, zeta potential, and other characteristics of lyophilized polymeric micelles are appropriate, which further minimizes the toxicity and side effects due to the encapsulation by polymer and the ability to sustain the drug release. Block co-polymeric micelles have a high drug-loading capacity and superior solubility, which could improve bioavailability. This study suggests that employing block co-polymeric micelles to enhance the solubility of hydrophobic medications has excellent potential as a workable platform for intravenous drug delivery. Although the results are promising, the docetaxel-loaded block co-polymeric micelles must be further evaluated for In-Vivo studies to check the targeted drug delivery.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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