

## Formulation and Characterization of a Pluronic F127 Polymeric Micelle as a Nanocarrier for Berberine Delivery

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**Abstract:** Berberine's (Ber's) lower water solubility, which leads to low bioavailability, poses substantial delivery-related barriers to its therapeutic efficacy. Thus, a new approach to improving Ber's delivery and bioavailability is required. In this study, a Pluronic F127 micelle containing Ber (mBer) was formulated using thin-film hydration technique with the intention of resolving challenging issues associated with Ber delivery. The micelle was tested for drug loading and retention efficiency, size, zeta potential, shape, in vitro release, and in vitro toxicity. The spherical micelles that were made had an average encapsulation efficiency of 85%, a hydrodynamic size of 82.2 nm, a polydispersity of 0.176, and a zeta potential of -47.4 mV. The results of Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) indicated that Ber was physically entrapped and in an amorphous state within the synthesized micelles. Compared to the free Ber solution, the in vitro release of Ber from micelles exhibited both short-term rapid release and sustained release. The mBer was shown to be relatively non-toxic to blood cells via an in vitro hemolysis assay. Our findings showed that polymeric F127 micelles could be a simple nanocarrier for Ber delivery, which can be used to enhance the therapeutic efficiency of Ber.

**Keywords:** Berberine; Pluronic F127; micelles; polymeric drug delivery systems; nanoparticles

### ■ INTRODUCTION

Berberine (Ber) is a natural isoquinoline alkaloid extracted from the stem barks, rhizomes, and roots of many herbs, such as *Coptis chinensis*, *Berberis aristata*, *Coptis rhizome*, and *Hydrastis canadensis* [1]. Ber is well recognized as a prominent natural product with extensive global usage. It has been stated that over 25 billion Ber pills are consumed annually in various regions of Asia and Africa [2]. A growing number of studies have linked Ber to several pharmacological effects, such as anti-inflammatory, anti-diabetic, antibacterial, neuroprotective, anti-mutagenic, cholesterol-lowering, and anti-tumor effects [3]. However, the clinical application of Ber is limited by its very low water solubility and permeability across biological membranes [4]. Therefore, Ber's delivery to the target of interest in the

body has become quite challenging [2]. With the continued development of Ber delivery systems, problems like targeting specific tissues, cellular internalization, and controlling drug release at the targeted site in the body have been challenged [5].

To address these issues, nanotechnology-based therapeutic approaches have been implemented in an effort to enhance the efficacy of Ber in the treatment of disease. One example is liposomes as nano-delivery carriers, which were used to improve Ber pharmacokinetic issues; however, their clearance remained high [6]. In addition, silver-, magnetic-, and chitosan-based nanoparticles were used to improve Ber delivery [2]. However, problems with the biocompatibility of nanocarriers, which were not all completely safe, issues with Ber release from some of

these nanocarriers, the rapid efflux of Ber from cells, low to medium retention efficiency, and low stability were not solved with a single nano approach mentioned above. One way to solve such issues is to load these photochemical molecules into amphiphilic polymeric nanocarriers known as polymeric micelles. A thiolated polymer micelle was used successfully to treat skin injury. However, the thiolated micelles bind to keratin through a disulfide bond, preventing Ber from achieving the desirable therapeutic concentration in the plasma that is required for systemic treatment. Given the hydrophobic nature of Ber, we hypothesize that it could be entrapped within the hydrophobic core of polymeric micelles via hydrophobic interactions, thereby improving its solubility, stability, permeability, and distribution in the body.

Amphiphilic polymeric micelles are core/shell nanostructures that are formed by spontaneous self-assembly of amphiphilic block polymers in an aqueous solution when their concentration exceeds a particular level known as the critical micellar concentration [7-8]. Polymeric micelles are composed of a hydrophobic core surrounded by a hydrophilic shell. The hydrophobic core possesses the capability to incorporate hydrophobic agents, thereby serving as an efficacious delivery vehicle that enhances the stability of poorly soluble pharmaceutical compounds [9]. Many advantages exist to using polymeric micelles, such as their small size, between 5 and 150 nm [9], increasing the half-life of loaded drugs, providing sustainable release of drug molecules, and improving the stability and therapeutic effect of low water-soluble drugs by protecting them from degradation and metabolism [10]. Due to their biocompatibility and biodegradability, they can be safer alternatives to conventional solubilizers such as alcohol or Cremophor EL, which are commonly used and associated with toxic effects [11].

Pluronic F127<sup>®</sup> are tri-block amphiphilic synthetic copolymers consisting of a poly(propylene oxide) chain flanked by two chains of poly(ethylene oxide) (PEO-PPO-PEO) [12]. The PEO block is hydrophilic and water-soluble, while the PPO block is hydrophobic and water-insoluble. In aqueous solutions, these polymers form micelles with a hydrophobic PPO core and a hydrophilic

PEO corona that interface with water [13]. They improve the circulation time of loaded molecules as well as enhance the intracellular uptake of encapsulated molecules via endocytosis rather than simple diffusion [14]. Pluronic polymers are approved by the Federal Drug Administration as safe and biodegradable compounds [15].

Thus far, the applicability of Ber as an effective therapeutic agent has been substantially restricted due to its major shortcomings: low water solubility [16], low bioavailability as it has low cell permeability and poor intestinal solubility, leading to a very low bioavailability of 0.68% [17], and rapid efflux of Ber because it is a substrate for P-glycoprotein [18]. Considering this, achieving the desired plasma concentration of Ber is challenging. Therefore, there is a need to develop a new approach to overcome the low efficacy and bioavailability of Ber. The objective of this study is to synthesize and characterize Ber-loaded, physically entrapped Pluronic F127<sup>®</sup> micelles (mBer) and assess the *in vitro* release of Ber from prepared micelles, with the aim of resolving Ber solubility issues and increasing its cellular permeability.

## ■ EXPERIMENTAL SECTION

### Materials

Materials used were berberine hydrochloride (Ber) ( $\geq 98\%$  purity) and Pluronic F127 ( $\geq 99\%$  purity) were all purchased from Sigma Aldrich (USA). Defibrinated rabbit blood was purchased from Thermo Fisher Science (USA). Chemicals of analytical grade were used in this research. All experiments were conducted using deionized water.

### Instrumentation

The Instruments used in this study were rotary evaporator (Labtech, Korea), UV-visible spectrophotometer (Sunny, Japan), Zetasizer-3000 instrument (Malvern Instruments, UK), JEM-100CX electron microscope (JEOL, Japan), Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific, USA), X-ray diffractometer (XRD, Bruker, USA), and dialysis cassettes (Thermo Fisher Scientific, USA).

## Procedure

### Preparation of Ber-loaded micelles

Ber in Pluronic F127 micelles (mBer) was prepared by a slight modification of the thin film hydration method [19]. In order to get better encapsulation efficiency of the mBer, we prepared different micelle compositions by using different weights of Ber (1, 2, 3, 4, 5.6, 7, 8, 9, and 10 mg), while the other conditions in the micelle preparation are fixed. Shortly, a fixed amount of Pluronic F127 (100 mg) and one of the variable weights of Ber mentioned above were co-dissolved in 20 mL of methanol (99% purity) in a round-bottom flask, followed by sonication to completely dissolve the chemicals. Next, to remove alcohol, the flask was placed on a rotary evaporator (45 °C) for a few min until a homogenous film was formed. To further remove the residual alcohol, the resultant film was placed in a vacuum dryer overnight at room temperature. The film was then rehydrated in 30 mL of deionized water at 37 °C for 1 h with gentle agitation, followed by mild sonication for 2 min. To remove undissolved agents or contaminants, the resultant micellar solution was filtered through a 0.22 µm nylon filter.

### Characterization of Ber-loaded micelles

**Determination of drug loading content and retention efficiency.** The Ber loaded within polymeric micelles was first extracted with 100% ethanol, and then the concentrations were measured using a UV-vis spectrophotometer at 350 nm. Standard solutions of Ber in 100% ethanol at concentrations ranging from 2–10 g/mL were used to calibrate the content determination method. Freshly prepared mBer was assessed to determine the initial drug loading (DL) capacity and retention efficiency of the micelle. The data was recorded as four replicates. The entrapment efficiency (EE) and DL content DL were calculated using Eq. (1) and (2) [20].

$$EE(\%) = \frac{\text{mass of drug loaded in micelles}}{\text{mass of drug initially added}} \times 100 \quad (1)$$

$$DL(\%) = \frac{\text{mass of the drug loaded in micelles}}{\text{mass of the feeding polymers and drug}} \times 100 \quad (2)$$

**Assessment of micelle zeta potential, particle size, and polydispersity index.** They were assessed based on the dynamic light scattering (DLS) technique. The tested sample formulations were first diluted with deionized

distilled water so the final F127 concentration was 200 µg/mL, and the pH was 6.8, then placed within the electrophoretic cell of the Zetasizer-3000 instrument. The equilibration of the instrument was done at room temperature for 2 min, and then the He-Ne laser was fixed at 90° and adjusted at 633 nm [21]. The mean values of the zeta potential, which is a parameter that assesses the electrochemical equilibrium at the interface between a particle and a liquid, were estimated. It allows for the evaluation of nanoparticles' surface charge and predicts the physical stability of particles in dispersion systems and their tendency to interact with other molecules as it measures the magnitude of electrostatic repulsion or attraction between particles [22]. The hydrodynamic size of the particles and the polydispersity index (PDI) were also measured. All measurements were conducted on four replicates.

### Morphological analysis of the created micelle by transmission electron microscopy.

Transmission electron microscopy (TEM) was used to examine the morphology of the formulation. TEM images of the micelles were captured at 120 kV. Before examination, micelles were diluted to a concentration of approximately 1000 µg/mL with deionized water. A drop of diluted micelles was dropped on a copper grid coated with carbon, and excess liquid was collected with fine filter paper. The sample was then negatively stained with a drop of phosphotungstic acid at 2% (w/v). Before viewing under the TEM, the grid was properly washed with deionized water to remove excess discoloration and air-dried at room temperature for approximately 10 min [19].

**Fourier transform infrared spectroscopy.** To characterize the status of Ber in polymeric micelles. The infrared spectrograms were recorded based on the KBr method in the range of 4000–400 cm<sup>-1</sup>. FTIR spectra of Ber, mBer, and corresponding empty blank micelles were recorded at room temperature [19].

**X-ray diffraction.** Diffraction patterns of Ber, mBer, and corresponding empty blank micelles were recorded using an XRD. The conditions of the analysis were set as follows: the voltage was 40 kV, the current was 30 mA, and diffraction patterns were collected with 2θ at an angle range of 5–70° [23].

### Assessment of *in vitro* release of Ber from prepared micelles.

Ber release from loaded micelles was assessed at pH 7.4 to mimic physiological conditions. A known amount of mBer was dispersed in 4 mL of phosphate-buffered saline (PBS). Then, 2 mL of mBer was placed into a dialysis cassette with a molecular weight cut-off (MWCO) of 14 kDa to ensure that both free Ber and the free polymer were diffused out of the cassette. Next, both ends of the bag were sealed and then immersed in 50 mL of PBS (pH 7.4). The samples were maintained at 37 °C and shaken at 100 rpm. At the appropriate intervals (0, 0.5, 1, 2, 3, 6, 10, 12, 24, and 48 h), a volume of 100 µL was taken out of the release medium, followed by the addition of an equivalent volume of fresh medium, which is the same as the release medium. The Ber concentration in each sample was determined using UV-vis spectrophotometry.

### Evaluation of micelles' toxicity via *in vitro* hemolysis assay.

A hemolysis test was performed on empty micelles, free Ber, and mBer samples. The micelles' toxicity was measured based on the principle of hemoglobin release from erythrocytes, as previously reported [24-25]. The defibrinated blood of a rabbit was diluted tenfold with phosphate buffer solution and centrifuged at 2,000 rpm for 15 min. The supernatant was drained, and the precipitate was washed three times with PBS, followed by 15 min of centrifugation at 2,000 rpm. Adjusting the concentration of the resulting blood cells to 2% (v/v), 50 µL of test samples were combined with 500 µL of blood cells, and the resulting suspensions were incubated at 37 °C for 3 h, simulating a physiologically appropriate dilution setting for intravenous bolus injection with a concentration of 10 g/mL of Ber. As a negative control for this experiment, red blood cells suspended in physiological saline indicated no hemolysis, but red blood cells suspended in distilled water produced 100% hemolysis and were considered a positive control. The Eq. (3) was used to calculate the hemolysis percentage [25].

Hemolysis(%) =

$$\frac{\text{Test sample abs.} - \text{Negative control abs.}}{\text{Positive control abs.} - \text{Negative control abs.}} \times 100 \quad (3)$$

### Statistical analysis

In this study, we used a one-way analysis of variance (ANOVA) and least significant differences (LSD) as a post

hoc test to analyze normally distributed data and identify statistically significant differences. GraphPad Prism 9.5.0 was utilized with a significance level of  $P < 0.05$  [26].

## RESULTS AND DISCUSSION

### Drug Loading and Retention Efficiency

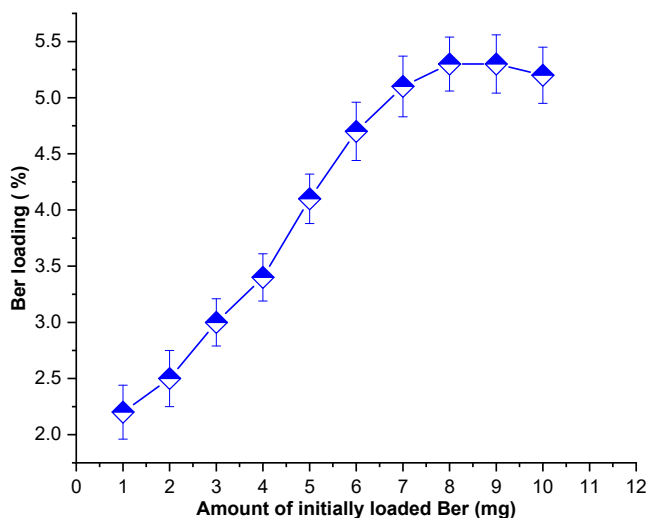
Ber was intended to be encapsulated within a Pluronic F127 micelle to enhance its delivery. The results indicated that Ber was successfully loaded into Pluronic F127 micelles. The feeding weight of Ber can influence the DL and retention efficiency (RE) of micelles. Consequently, for maximum encapsulation efficiency of Ber, we investigated the effects of varying Ber feeding weights on loading efficiency, while other variables, such as temperature, pH, and solvent type, remained constant during the creation of micelles. As the amount of Ber utilized in the preparation rose from 1 to 10 mg, the DL increased from  $2.02 \pm 0.24\%$  to  $5.30 \pm 0.25\%$  (Fig. 1), but the RE of the micelles considerably dropped because of this change (Fig. 2).

To top it all off, when the feeding Ber weight was 7 mg, the RE achieved its maximum value of  $85.0 \pm 2.60\%$  (Fig. 2). Micelle formulations exhibited an increase in DL but a reduction in RE in response to increasing Ber feeding (Table 1). The concentration of 7 mg of Ber was chosen as a suitable amount for making mBer in subsequent experiments as it achieved the maximum value of RE as compared with other different weights of Ber used in the preparation of the micelle.

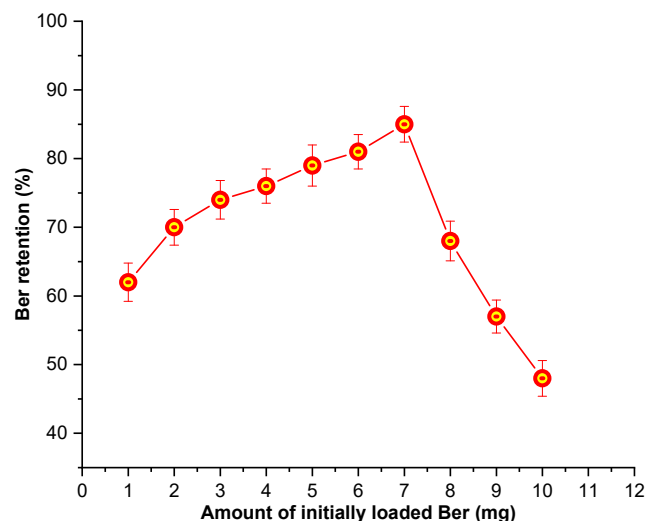
According to the findings of this study, the observed pattern of increasing DL but decreasing EE with increasing Ber addition during micelle formulations may be due to the saturation of the hydrophobic micelle core with Ber during micelle production. This finding is consistent with what was believed to occur when creating a different type of polymeric micelle, where the core was so saturated with drug molecules that precipitation of these molecules could occur on the exterior of the micelle [27].

### Size and Zeta Potential of Micelles under Hydrodynamic Conditions

DLS was used to measure the mean hydrodynamic particle size, size distribution, and zeta potential of the



**Fig 1.** The relationship between the amount of initially loaded Ber and the loading capacity (mean  $\pm$  SD% with  $n = 4$ ) of the created mBer.



**Fig 2.** Effect of different amounts of loaded Ber on the retention capacity (mean  $\pm$  SD% with  $n = 4$ ) of Ber-loaded micelles.

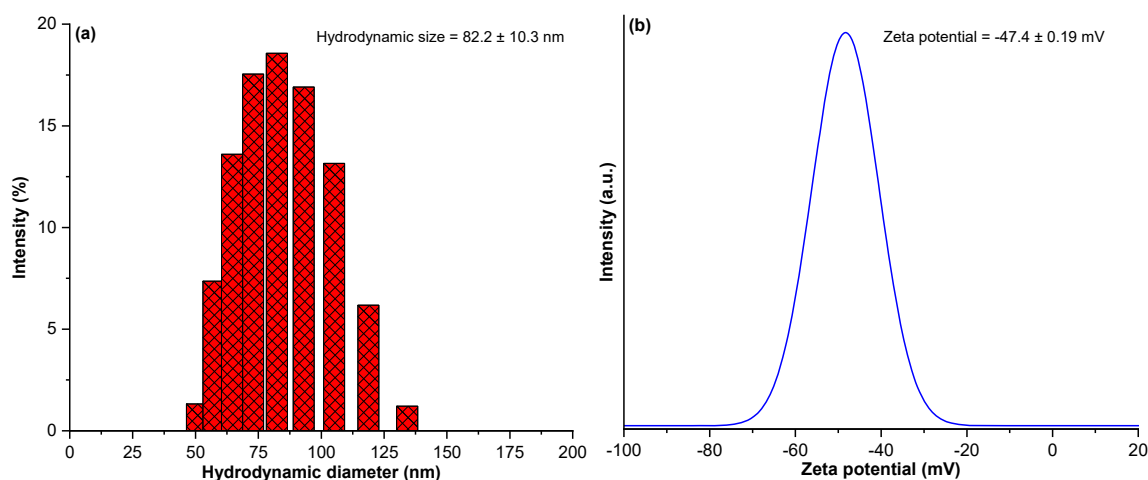
**Table 1.** The retention efficiency and loading capacity percentages of Pluronic F127 micelles loaded with Ber. The values indicate the mean  $\pm$  SD;  $n = 4$

Weight of feeding Ber (mg)	Loading efficiency (%)	Retention efficiency (%)
1	2.20 $\pm$ 0.24	62.0 $\pm$ 2.80
2	2.50 $\pm$ 0.25	70.0 $\pm$ 2.60
3	3.00 $\pm$ 0.21	74.0 $\pm$ 2.80
4	3.40 $\pm$ 0.21	76.0 $\pm$ 2.50
5	4.10 $\pm$ 0.22	79.0 $\pm$ 3.00
6	4.70 $\pm$ 0.26	81.0 $\pm$ 2.50
7	5.10 $\pm$ 0.27	85.0 $\pm$ 2.60
8	5.30 $\pm$ 0.24	68.0 $\pm$ 2.90
9	5.30 $\pm$ 0.26	57.0 $\pm$ 2.40
10	5.20 $\pm$ 0.25	48.0 $\pm$ 2.60

created micelles. The hydrodynamic size of the produced mBer was around  $82.2 \pm 10.3$  nm. The mBer size distribution closely resembles a normal distribution, as depicted in Fig. 3(a). This result suggests that the predicted formation of monodisperse nanoscale micelles has occurred. The PDI value in the created mBer was  $0.176 \pm 0.029$ , indicating that the created micelles had a relatively confined size distribution. The zeta potential of a micelle dispersion system is a crucial characteristic for predicting its physical storage stability as well as measuring particle aggregation tendency. Following what is depicted in Fig. 3(b), the micelles that had been loaded with Ber had a zeta potential of  $-47.4 \pm 0.19$  mV.

A dimensionless number, PDI, expresses the particle size distribution in the system under study. The closer the polydispersity value approaches zero, the more uniform the particle population [28]. The created micelles in this study had PDI values of less than 0.3, indicating that the measured micelles possessed a narrow size distribution [29]. The zeta potential is a commonly employed metric for assessing and forecasting the physical stability of particle dispersion systems. A zeta potential exceeding 20 mV generates electrostatic repulsive energy that impedes particle aggregation and growth [30]. As evidenced by the measured zeta potential value, the analysis conducted in





**Fig 3.** Physical properties of mBer: (a) hydrodynamic particle size and distribution of mBer as measured by DLS; (b) zeta potential the mBer. Data presented as mean  $\pm$  SD.  $n = 4$

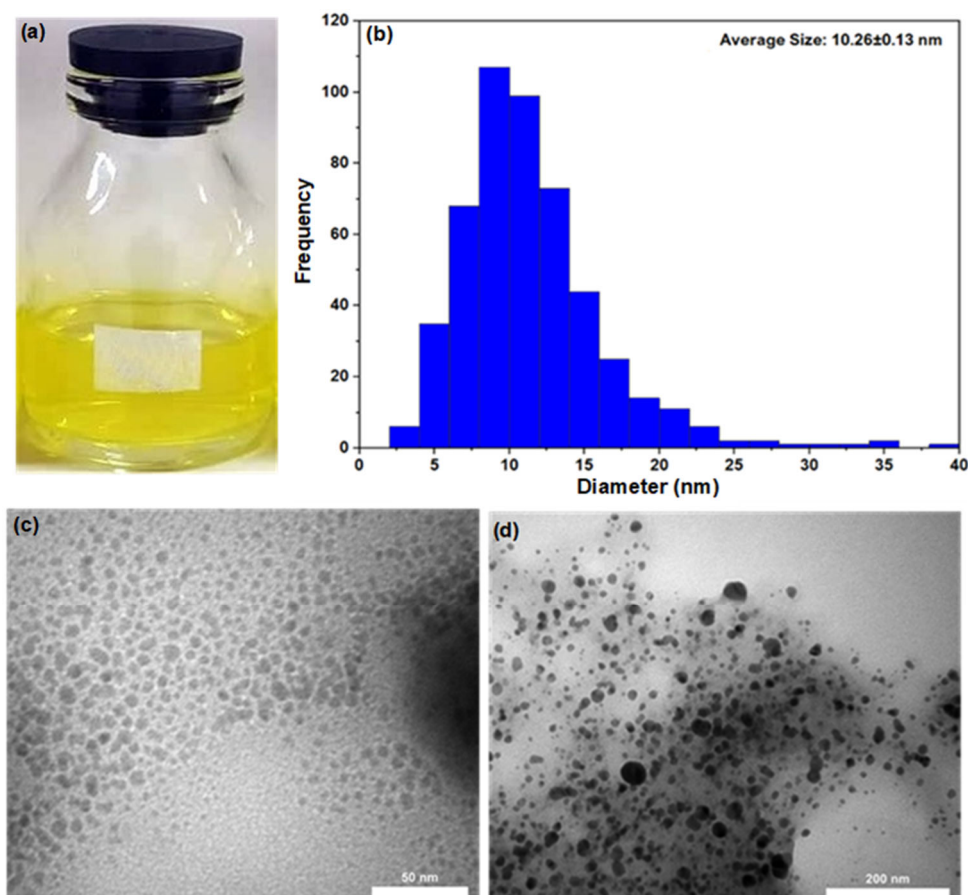
this study indicates a low probability of aggregation and particle growth for the created mBer.

#### Analysis of Morphological Characteristics with TEM

The mBer solution visually appeared transparent and yellowish (Fig. 4(a)). TEM results showed the formed micelles loaded with Ber have a moderately uniform particle size with an average of  $10.26 \pm 0.13$  nm (Fig. 4(b)). Our results exhibited a polydispersed, spherical morphology of grains-like mBer, and upon closer inspection of the TEM image, it became clear that these micelles had both bright and dark regions (Fig. 4(c) and 4(d)).

The majority of the Pluronic polymer micelles created in this study were found to have spherical morphological characteristics, which is in accordance with the previous report [31]. TEM images of the produced micelles displayed bright and dark regions. The PEO block of the Pluronic copolymers may be responsible for the light regions, while the Ber-loaded hydrophobic interior of the PPO chains may be responsible for the dark regions. The average particle size of the mBer, as measured by DLS or TEM, shows clear differences between the two methods, but it can be used to show that the micelles we made were small enough to pass through the cell membranes. In light of the literature review to date, it is evident that the optimal nanoparticle size is approximately less than 100 nm. At this size, the particle could pass through cellular barriers such as the blood-brain barrier, where smaller particles are believed to

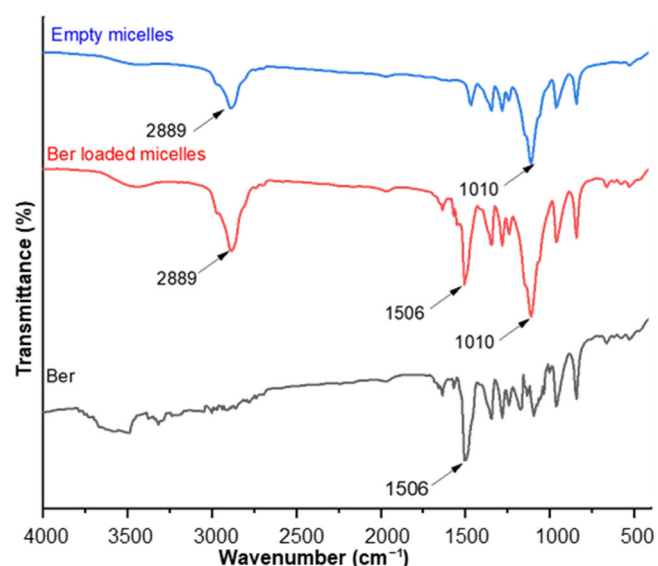
penetrate deeper into tissue layers [32]. The micelle size is inversely proportional to the interface area; therefore, small micelles increase the contact area with the body's targets, delivering a sufficient quantity of loaded drug due to the high ratio of surface area that comes into contact with nanoparticles [33]. Also, the small size of nanoparticles prevents quick clearing via the lymphatic system, where it has been demonstrated that particulates 200 nm or larger tend to activate the lymphatic system and are quickly removed from circulation [34]. It is important to note that there is a difference in the nanoparticle size reported from DLS and TEM images. Size estimation by TEM, which is a number-based observation, requires the examination of a sample in its dry state, whereas size estimation by DLS, which is an intensity-based observation, permits the examination of a sample in its solvated state, where solvent molecules will surround a micelle. DLS measures the hydrodynamic size. Under the effect of Brownian motion, the hydrodynamic diameter gives information about the core of a micelle, any material around it, and the molecules of the solvent that interact with a micelle in different ways that do not involve covalent bonds. Using TEM to estimate the size of a nanoparticle is based on an estimation of the size of the compact particle without the presence of a hydration layer. Consequently, the hydrodynamic diameter measured by DLS remains relatively greater than the TEM-estimated size [35].



**Fig 4.** Morphological characteristics of mBer: (a) photographic images of mBer, (b) particle size distribution of mBer measured by TEM, (c) TEM image of the mBer formulation, and (d) TEM micrograph of the mBer formulation

### FTIR Analysis

The FTIR spectra of Ber-loaded micelles were compared to those of blank micelles made of Pluronic F127 and free Ber (Fig. 5). In the FTIR spectrum, pure Ber can be shown to exhibit a variety of distinct absorption bands. The characteristic absorption band is typically located at  $1506\text{ cm}^{-1}$ . The blank F127 micelles sample exhibits distinctive absorption bands at  $2889$  and  $1010\text{ cm}^{-1}$ . The absorption bands of Ber-loaded micelles were quite comparable to those of blank micelles, except for the Ber-specific peaks. Based on these findings, it is possible to conclude that Ber was successfully localized and contained within the hydrophobic core of the micellar particle. Interestingly, the micelles that had been loaded with Ber did not show any additional peaks or variations in the location of the characteristic peak. This



**Fig 5.** FTIR spectra of Ber, empty Pluronic F127 micelles, and Pluronic F127 micelles loaded with Ber

demonstrates that no new chemical compound was created throughout the procedure. Micelles loaded with Ber did not display any new peaks or shifts in the typical peak location (Fig. 5). This is evidence that no new chemical compound was produced.

The significant absorption peak at  $2889\text{ cm}^{-1}$  that has been seen in the FTIR spectrum of Pluronic F127 has been prominently linked to C–H stretching vibrations [36]. The characteristic peak at  $1506\text{ cm}^{-1}$  in Ber has been attributed to furyl group C=C bond vibrations [37]. The resultant mBer spectra show all of the unique absorption bands of the polymer and the Ber, albeit with slightly less prominent peaks than those seen in the spectra of the pure components. During the process of mBer synthesis, there was no discernible peak shift, which suggests that no new chemical bonds were formed and that there were only some physical interactions that took place between the Ber and the polymer.

### XRD Analysis

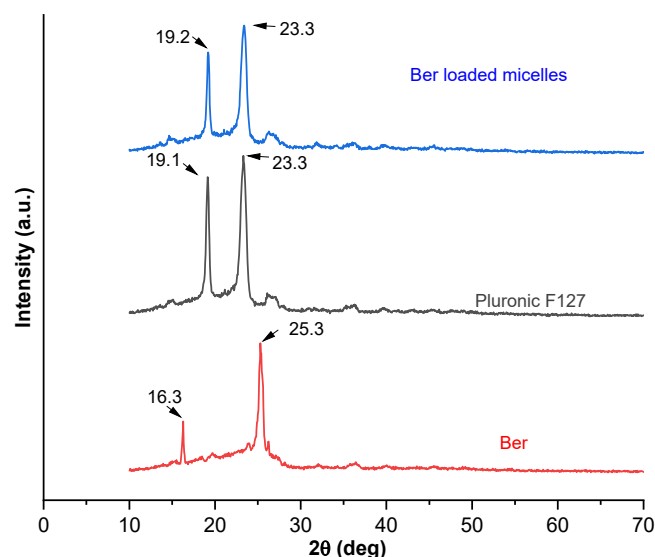
Ber, Pluronic F127, and mBer were examined using XRD in order to confirm the physical existence of Ber within the synthesized micelles. The results obtained from the XRD spectra are depicted in Fig. 6. Ber showed two sharp peaks with maximum intensities at  $16.3^\circ$  and  $25.3^\circ$ , while Pluronic F127 showed two sharp peaks at  $19.1^\circ$  and  $23.3^\circ$ . In addition to this, the diffraction patterns of Ber and Ber-loaded micelles were completely different from one another. To be more specific, the distinct Ber peaks that were previously observed in the diffraction patterns of the free Ber are nowhere to be detected in the diffraction pattern of the Ber-loaded micelles, revealing that Ber had been encapsulated within the polymeric micelles in an amorphous form and that there was relatively no free Ber present on the surface of the micelles.

The two sharp peaks that Ber exhibited in XRD data in this study are consistent with what has been previously documented about the XRD peaks of Ber [19]. The results of the current study showed that micellar formulations have broken up the crystalline structure of Ber, leaving behind amorphous particles in the micellar core compartment, and this is consistent with reports that Ber

molecules were transformed from their crystalline state into an amorphous one [19]. Our study shows that the polymer and Ber work well together, and it suggests that Pluronic F127 is a good carrier for loading Ber. Less crystalline and amorphous substances have larger free energies and more solubility capacity than their crystalline counterparts [38]. Thus, physical modification of Ber may increase its solubility and dissolution rates compared to crystalline Ber. Thus, a micellar formulation may improve Ber's solubility and release by modifying its crystalline nature.

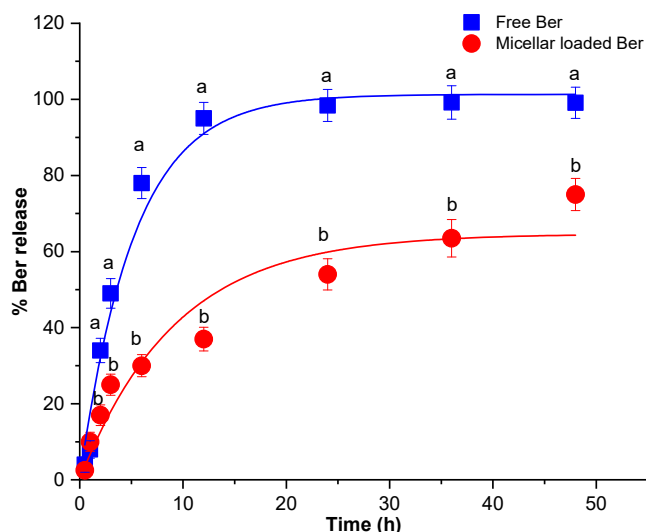
### Ber Release from Micelles *In Vitro*

At a pH of 7.4, the behavior of mBer's release was studied via *in vitro* testing. This was done to mimic the release that happens in the normal physiological environment of the body. Fig. 7 depicts the shift that occurs in the Ber release rate from both the free stock solution of Ber and mBer throughout the course of time. Ber was released from polymeric micelles at a much slower rate than it was from the free solution. After 3 h, it was determined that just 25% of the Ber had been released from the mBer, whereas around 50% of the Ber had been found to be released from the PBS solution over the same time period. After 12 h, almost 95% of the



**Fig 6.** XRD diffractogram patterns for Ber, empty Pluronic F127 micelles, and Ber-loaded Pluronic F127 micelles





**Fig 7.** Ber release from Ber stock solution and mBer over 48 h at pH 7.4 Data depicted as mean Ber released (%)  $\pm$  SD.  $n = 4$ . Comparing the release of Ber from the stock solution to that of mBer at the same time point reveals significant differences ( $P < 0.05$ ), denoted by the different letters

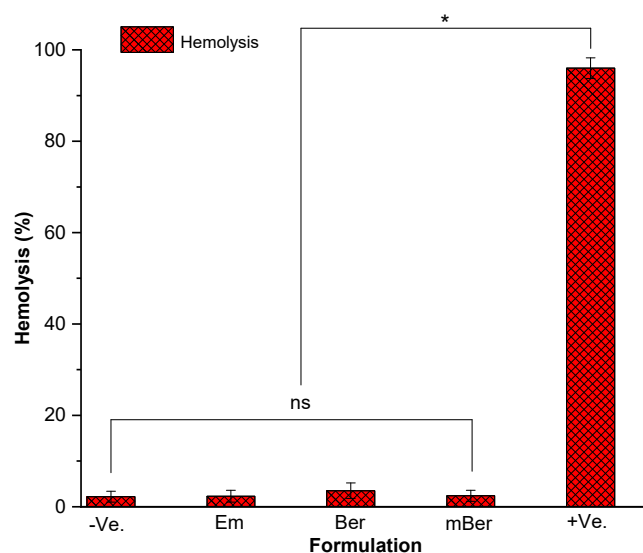
Ber had been released from the free stock solution, but about 65% of the Ber that had been incorporated into the micelles was still there. At a pH of 7.4 and after 24 h, the release of the Ber from the free solution was nearly complete, and the amount that was released reached over 98%, but only about half of the loaded amount of Ber in mBer was released.

As demonstrated by the release of Ber from mBer under *in vitro* assay conditions. Approximately one quart of the loaded Ber is released within the first 3 h after the micelles are introduced to the dialysis bag. After 24 h, just about half of the Ber loaded in mBer had been released from the micelles. On the basis of *in vitro* release tests, it would suggest that the micelles have the capacity to release Ber from the produced micelles in both burst-release and sustained-release behaviors. Ber incorporation close to the micellar surface or Ber molecules already existing in the microchannels of micelles are likely responsible for the initial burst release of Ber from the micelle particles [39]. The sustained-release behavior may result from the hydrophobic Ber being stably incorporated into the micelle core and slowly diffusing out of the micelles [40]. However, we predict

that increased blood volume dilution, dynamic clearance processes, and plasma protein binding will accelerate *in vivo* micelle dissociation. Previous research has demonstrated that the hydrophobic core and kinetic stability of F127 micelles may lengthen the circulating times of enclosed molecules [41]. Since *in vitro* release kinetics do not necessarily correspond to *in vivo* circulating times, it is reasonable to infer that mBer will circulate in the body for a longer duration than free Ber.

### Toxicity Evaluation Using *In Vitro* Hemolysis

A standard hemolysis assay was used to check the compatibility of a micelle formulation made for Ber with red blood cells. Free Ber in solution increased hemolysis slightly more than the negative control (cells were suspended in normal saline), an empty micelle vehicle, and Ber-loaded micelles, as shown in Fig. 8. Overall, compared to the positive control group (cells were incubated with distilled water), the Ber-loaded micelles did not cause any obvious hemolysis. The micelles produced were clearly very compatible with red blood cells, both on their own and when loaded with Ber.



**Fig 8.** Hemolysis tests after incubation of red blood cells with different samples include physiological saline as a negative control (-Ve.), empty micelles (EM.), free Ber (Ber), micellar-loaded berberine (mBer), and distilled water as a positive control (+Ve.). Data presented as mean  $\pm$  SD;  $n = 4$ . \* indicated a statistically significant difference ( $P < 0.05$ ). ns denotes no statistically significant difference

Hemolysis refers to the fast bursting of red blood cells. It can happen naturally under stressful circumstances. As such, it is one of the key indicators of both acute toxicity and compatibility issues with new foreign materials. The net surface charge of the particle and the chemical composition of the used materials are the main factors affecting the incidence of hemolysis [42]. Blood cells rapidly adsorb to some nanoparticles to produce a protein corona that affects their interaction with blood components [43]. Numerous studies have linked nanoparticle-induced coagulopathy to cardiovascular disease. Nanoformulation can disrupt the coagulation system and alter hemostatic equilibrium, causing life-threatening disorders such as deep vein thrombosis and intravascular coagulopathy [44]. Thus, researchers should carefully study the interactions between newly created nanoparticles and blood cells. This will improve nanoformulation hemocompatibility, simplify clinical studies, and speed up the market introduction of new nanoformulation-based products, both on their own and after being loaded with Ber, the micelles that were created as an outcome of this experiment showed a high level of compatibility with red blood cells. The results of this test suggest that the generated mBer can be safely administered via intravenous injection. The *in vitro* hemolysis assay showed the compatibility between the synthesized micelles and red blood cells. The non-toxicity of the unloaded Pluronic F127 polymeric nanocarrier has been demonstrated by the FDA. However, additional investigations pertaining to toxicity are imperative to furnish further substantiation concerning the toxicity characteristics of the micelles that have been loaded with Ber. A limitation of this study is its limited focus on the *in vitro* production and characterization of mBer. Future research needs to focus on testing the synthesized mBer *in vivo* models in order to further validate the safety and efficacy of this approach.

## ■ CONCLUSION

Using the thin-film hydration approach, we were able to create spherical Ber-loaded F127 micelles with high retention efficiency when the feeding Ber weight was

7 mg. The created micelles have an average hydrodynamic size of 82.2 nm, a polydispersity of 0.176, and a zeta potential of  $-47.4$  mV. Ber was loaded into Pluronic F127 in an amorphous state through physical interaction. Ber's *in vitro* release behavior from the micelles demonstrated both fast and sustained release properties. Ber-loaded micelles were shown to be relatively non-toxic and biocompatible via *in vitro* testing. This inventive method of Ber delivery has the potential to be utilized effectively for enhancing both Ber delivery and absorption.

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## ■ AUTHOR CONTRIBUTIONS

Noora Kadhim Hadi Alyasari designed and conducted the experiments. All authors analyzed the results, wrote the manuscript, and agreed to the final version of this manuscript.

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