Application note

Examining the efficiency of silk fibroin biomaterial as a drug encapsulation particle

NanoDrop Eight Spectrophotometer

Silk fibroin derived from Bombyx mori silkworm cocoons has been utilized in sutures and bandages and is now showing promise as a drug delivery biomaterial.[1-3] The low immunogenicity and high biocompatibility and stability of silk fibroin make it an excellent pharmaceutical packaging vehicle.[3-4] The impressive durability and stability of silk fibroin biomaterials are driven by repeating Gly-Ala amino acids facilitating β-sheet structures through strong hydrogen bonding.[5-7] The silk fibroin particle formation involves self-assembly into micelles wherein the amphiphilic silk protein chains arrange in a favored thermodynamic structure, ultimately forming a structured matrix.[8-10] This quality of silk particles ensures robust drug encapsulation and enables protection against degradation mechanisms.[7] As outlined in Figure 1, to encapsulate drug molecules, the drug is mixed with silk fibers purified from Bombyx mori and the silk particles self-assemble to lock the drug within the matrix in the thermodynamically favored orientation.[8]

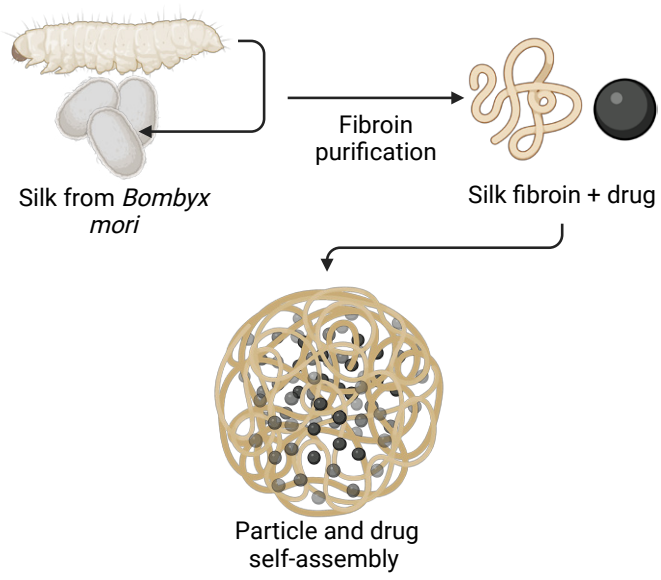

Figure 1. Self-assembly of silk fibroin purified from Bombyx mori silkworms and encapsulation of a drug product. Figure created with BioRender.com.

The encapsulation efficiency (EE%) is an important parameter to calculate when packaging drugs or other materials into particles. The equation for calculating EE% is listed below:[11]

$$EE\% = \left(\frac{\text{total drug added - residual drug}}{\text{total drug added}} \right) X\ 100$$

Equation 1. Encapsulation efficiency (EE%) calculation.

The EE% equation provides a value indicating how efficiently the particle system is packaging drugs by calculating the amount of drug packaged divided by the total drug added. The "residual drug" in Equation 1 refers to the amount of drug present in the supernatant after centrifuging the newly formed particles. The values used to calculate EE% are found via ultraviolet-visible (UV-Vis) spectrophotometry, an analytic technique that is useful for measuring small molecule pharmaceuticals that have an absorbance signature.

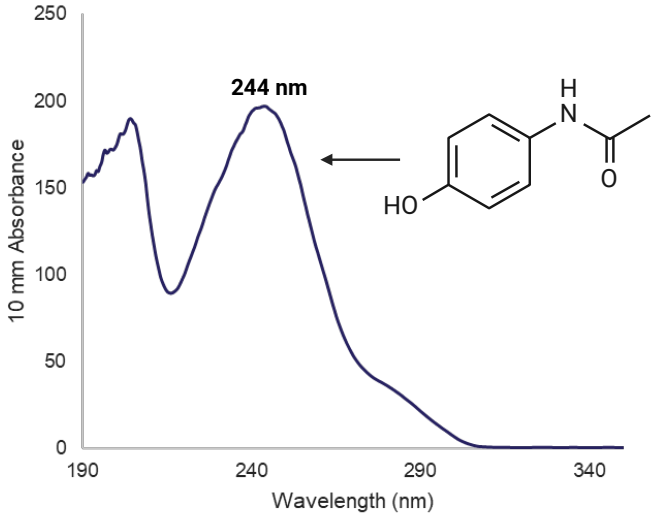

Figure 2. UV spectral signature (244 nm peak) and chemical structure of acetaminophen. Figure created with BioRender.com.

For example, acetaminophen has a signature peak at 244 nm in the UV range and the absorbance is easily assessed with a UV-Vis instrument (Figure 2). The amount of acetaminophen in a sample can be evaluated by creating a standard curve of known concentrations and determining the absorbance contribution from each sample with a UV-Vis spectrophotometer. In this way, unknown acetaminophen concentrations can be calculated from the known concentrations in the standard curve.

The Thermo Scientific™ NanoDrop™ Eight 8-channel Microvolume UV-Vis Spectrophotometer measures 8 samples simultaneously in less than 20 seconds, reducing time spent at the instrument. With the implementation of pedestals using auto-ranging pathlength technology, the maximum absorbance limit of the NanoDrop Eight instrument is 200A (1.0 cm equivalent), which equates to 3.0 mg/mL of acetaminophen or about a typical 325 mg dosage.[12]

This dosage of acetaminophen is highly absorbing in the UV range at 244 nm. For a typical cuvette-based spectrophotometer, error-prone serial dilutions would be required to ensure acetaminophen absorbance is within the instrument's detection limit, which is usually around 1.0A (1.0 cm equivalent). With the NanoDrop Eight instrument, the shorter pathlengths remove the requirement of performing dilutions, making a measurement of highly concentrated acetaminophen fast and accurate. This wide absorbance range also supports a standard curve that contains a wide range of concentrations, allowing flexibility for any percentage of drug encapsulation.

Experimental Procedures

In this experiment, silk fibroin particles encapsulating acetaminophen were prepared following the method from Lammel et al.[13] Four particle samples were created by mixing 5% silk solution (Advanced BioMatrix, 5154), 1.25 M potassium phosphate, and 6.0 mg/mL of acetaminophen (Sigma-Aldrich, A7085) to a 1:5:1 volumetric ratio, respectively. The samples were then stored at 4°C for two hours to permit self-assembly. The particles were then spun down in a centrifuge for 15 minutes at 2000 g and 1.0 mL of supernatant was reserved for determining residual acetaminophen concentration with the NanoDrop Eight instrument.

A Custom Method – Standard Curve program was created in the NanoDrop Eight software. The standard curve consisted of six standards ranging from 3.0 mg/mL to 0.09 mg/mL in 1:1 serial dilutions. The Analysis Wavelength was set to 244 nm, which was predetermined by measuring pure acetaminophen in the UV-Vis application and noting the absorbance peak. Automated Pathlength was turned on to ensure the optimal pathlength was utilized based on the absorbance intensity of the sample. Baseline Correction was also turned on to 750 nm to subtract background signal from the sample measurements. All standards and supernatant samples were measured using 2.0 µL volumes in replicates of five. The encapsulation efficiency was calculated using Equation 1 and the acetaminophen concentrations were calculated by the NanoDrop Eight software and the standard curve.

Results

The linear relationship depicted in Figure 3 between absorbance at 244 nm and acetaminophen concentration illustrates the Beer-Lambert Law's principle of direct proportionality. This is evidenced by a linear relationship with an R^2 value of 0.998, indicating a high degree of correlation.

Table 1 lists the average concentration, standard deviation, and EE% for each sample based on replicates of five. The average concentration of residual acetaminophen was 0.84 mg/mL and the standard deviation for each sample was ≤0.02 mg/mL. The low standard deviation illustrates the high degree of reproducibility in the NanoDrop Eight instrument's measurements.

Using the residual acetaminophen concentrations reported by the NanoDrop Eight software and the known input concentration of 6.0 mg/mL, EE% was calculated for each sample. The EE% for all four samples was on average 85.98%, indicating efficient drug packaging by silk fibroin. Acetaminophen is a neutral molecule at physiological pH, given its pKa of 9.38, which may be the reason for the maximum EE% of 86.54%.[14] With an isoelectric point of around 4.2, silk fibroin could be more attracted to a positively charged drug at physiological pH, which would ultimately increase the EE%.[8,13]

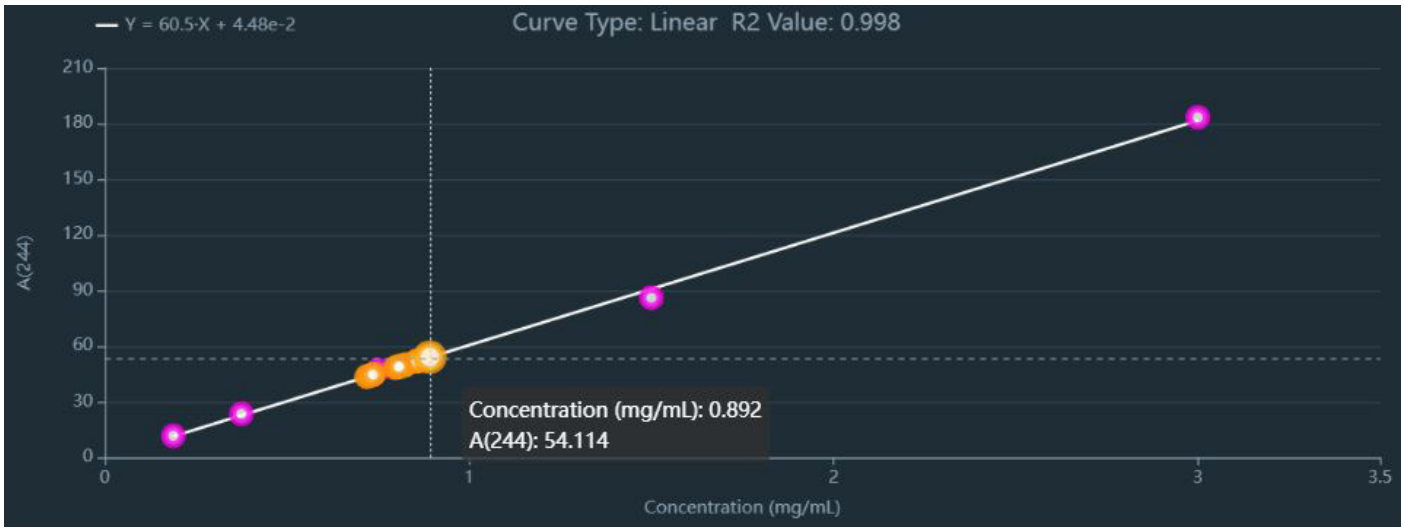

Figure 3. Acetaminophen standard curve with a sample highlighted showing the concentration and absorbance at 244 nm using the NanoDrop Eight software. Standards are shown in pink and unknowns are shown in orange.

Sample Name	Concentration (mg/mL)	Standard Deviation (mg/mL)	EE%
Sample 1	0.87	0.01	85.47
Sample 2	0.81	0.01	86.54
Sample 3	0.87	0.02	85.48
Sample 4	0.81	0.01	86.44

Table 1. Encapsulation efficiency (EE%) calculated based on the residual concentration of acetaminophen for four separate silk particle samples measured in replicates of five.

Conclusion

Calculating the EE% in researching and manufacturing drug particles is important to consider for new particle formulations, time-based stability testing, or batch-to-batch quality control. With UV-Vis spectrophotometry, the absorbance and concentration of small molecule pharmaceuticals are easily measured provided the spectral signature peak is within 190 – 850 nm. In this study, the NanoDrop Eight spectrophotometer has been shown to quantify non-encapsulated acetaminophen at 244 nm with a high degree of linearity and reproducibility through the use of a standard curve. The auto-ranging pedestal pathlength technology supports a wide range of acetaminophen concentrations without the need for dilutions, reducing sample preparation time and errors caused by dilutions for typical cuvette-based spectrophotometers.

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