Evaluation of silk fibroin stabilization of doxorubicin

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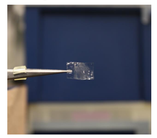
Introduction

Chemotherapy is typically administered systemically. Due to the systemic delivery route, significant and sometimes lethal side effects may occur. These side effects often limit the maximum allowable drug dose, reducing the drug's effectiveness. Local, sustained release drug delivery systems are a potential way to overcome this limitation. It is known that silk films can bind and slowly release doxorubicin, a common chemotherapeutic agent. However, it is not known if the sustained release formats stabilize the drug and reduce its solution degradation. This research will (1) identify potential degradation products of doxorubicin through forced degradation studies using liquid chromatography mass spectrometry, and (2) characterize the released molecules of doxorubicin-bound silk films to identify if silk materials support drug stabilization under aqueous conditions. We want to determine if the majority of the drug remains intact over the experimental time course.



Materials and methods

Three studies were conducted to identify degradation products and analyze the released solution from the silk-bound films. First, various concentrations of doxorubicin solutions were placed under stress degradation conditions to test for sensitivity: acid, base, temperature and oxidation. Over 28 days, degradation products of the acid-induced and temperature-induced solutions were identified by LC-MS analysis. The second study examined the released solutions of doxorubicin-bound silk films over 28 days at 37˚C in order to identify if any of the degradation products had appeared. In the third study, doxorubicin-bound films were placed under the same forced degradation conditions in order to confirm that silk could stabilize doxorubicin. All solutions were analyzed by liquid chromatography-mass spectrometry (LC-MS)—a machine that separates, detects, and identifies chemical substances by mass. Three replicates for each sample were analyzed, and the degradation to intact molecule ratio will be compared. Statistical difference between release sample and free-drug sample will be determined by t-test using Microsoft Excel. Significance will be defined as $p < 0.05$.

Results

A degradation product was identified in both acidic (HCl) and temperature (60˚C) conditions at a retention time of 4.6 minutes and a mass to charge ratio of 397-398. The degradation peak increased over time, but appeared at different time points depending on the condition. The increase of degradation product was confirmed by data analysis of the areas under the degradation peaks. Figures 1a and 1b detail the gradual increase of degradation product over time. Degradation products were not identified under oxidation or base-induced conditions because doxorubicin degradation occurred too quickly. Other degradation products were also identified but are not pictured below.

Figure 1a. HCl Degradation

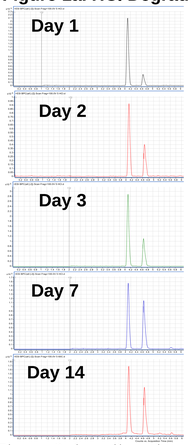


Figure 1b. 60˚C Degradation

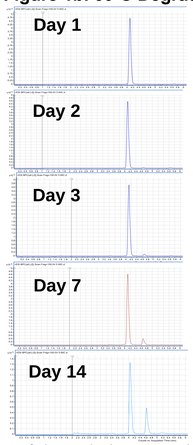


Figure 2. AUC vs. Time of Degradation in HCl and 60˚C

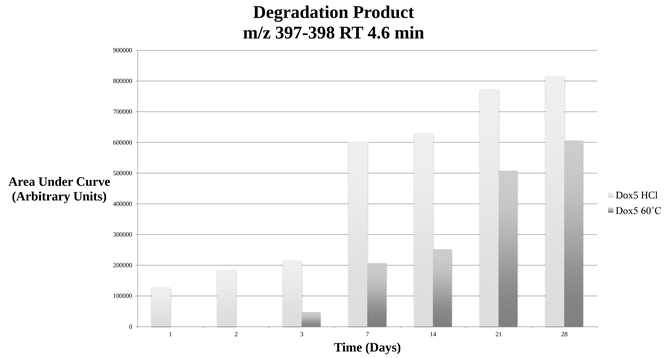
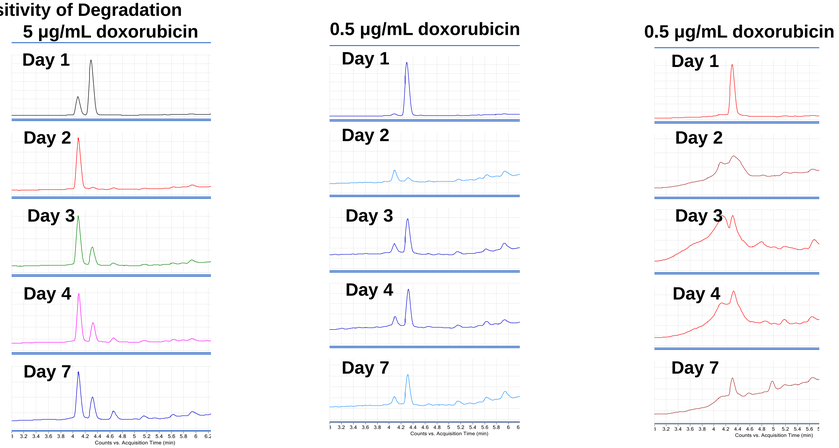


Figure 3 describes the sensitivity of degradation at various concentrations of doxorubicin. The higher concentration of 5 µg/mL doxorubicin showed accelerated degradation compared to the lower concentrations.

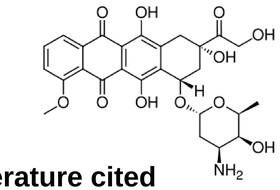
Figure 3. Sensitivity of Degradation



Data for film release and forced film studies have been collected and are still being analyzed to compare to the identified degradation products from the first study.

Conclusions

Many degradation products of doxorubicin have been successfully identified through the solution force degradation study. The next research goal would be to analyze the release study and film force study data for any signs of degradation. We hope to find that no such degradation exists under regular release conditions, which would support our hypothesis that immobilized drug remains intact over time. Although the research is still ongoing, local, sustained release drug delivery treatment is looking more and more feasible each day. In this way, we hope to one day overcome the challenges of the current systemic delivery route of chemotherapy for cancer patients.



Literature cited

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Acknowledgments

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