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Drug loading and release studies for milled silk particles of different sizes

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ABSTRACT

Milled silk particles with volume median particle size (d(0.5)) of 7 μm and 281 nm as well as silk snippets were used for loading of model drugs Orange G, Azophloxine, Rhodamine B, and Crystal Violet. Loading and release of these chemicals depended on the size of silk particles, pH, and the structure and properties of model drugs. Both types of silk particles reached equilibrium loading in less than 10 min due to high surface area whereas silk fibres needed more than 2–3 days to reach equilibrium, depending on the drug type. The uptake rate in fibres could be improved by increasing temperature. Both fibres and particles could slowly release the drugs over many days at 37 °C without a significant initial burst. As particle size decreased, the amount of model drug release also decreased. The release of drugs by the silk fibres was quicker than the silk particles.

1. Introduction

The slow release of drugs immobilised in a support material helps to provide a sustained dose at a therapeutic level, ensuring the optimum performance of that drug and helping achieve the desired physiological outcome with minimum side effects [1]. Improved drug efficiency, safety and the use of new therapies can be achieved by encapsulating pharmaceutical agents to various polymeric materials [2]. When developing materials for drug delivery, it is important to consider the material type and its physical nature such as size, as these can have a significant impact on the release profile of the drug being delivered. It is also important to choose a material that is degradable and biocompatible. Many studies have been carried out using polymeric micro and nanoparticles for drug delivery systems targeting diseased tissues [3–5]. Nanospheres (1–1000 nm) or microspheres (1–1000 μm) can be used for targeted drug delivery systems based on disease site and delivery path [6]. Nanospheres are usually used for short-acting drug delivery requirements via intramuscular, intravenous, subcutaneous, nasal, oral, transdermal, or ocular, either using solid powder carriers or via a liquid carrier [6–8]. Microspheres are generally used as depot drugs for long lasting delivery and usually administered subcutaneously or intramuscularly [6,7,9].

High strength, easy processing, biocompatibility, slow enzymatic breakdown, good oxygen permeability and ability to bind proteins and drugs make silk fibroin a suitable biomaterial [10,11]. In recent years, silk fibroin has been investigated for many biomedical applications such as drug delivery [12–17], tissue scaffolds [18–23], and wound healing [24–26]. Silk does not produce toxic products during decomposition, and has high cellular uptake properties which enhance its efficiency for drug delivery systems [27].

Studies conducted till date for drug delivery have used the bottom-up approach. In bottom-up methods [28–30], silk fibres are dissolved in aqueous solution of salts such as LiBr [31] or $CaCl_2$ along with ethanol [32] from which regenerated silk materials are fabricated [33]. Kosmotropic salts such as potassium phosphate [14] or alcohols (usually methanol) [12] are used during preparation of regenerated silk from silk solution to form β-sheet crystallites to make them water insoluble. Such regenerated silk such as films [12,16,34] or particles [6,11,14,35,36] have been used for drug delivery applications. In such bottom-up approaches, there are risks of protein degradation during dissolution of silk.

In contrast to bottom-up approaches, we have shown that native crystalline structure of silk fibres can be retained if top-down approach of milling is used for particle production [37]. In milling methods, fibres are processed through different types of milling systems to achieve a desirable particle size [38]. We have reported the production of micron and submicron silk particles using different milling approaches [39,40]. It was observed that submicron silk particles had almost similar β structure, thermal properties, and crystallinity to that of silk fibres [37]. Particles produced from the solution method and the milling method were also compared [41]. Particles from the solution methods formed aggregates as large as 40 μm while ultrafine particles (d(0.5) ~300 nm) produced by milling did not form aggregates.

In this work, we investigated the ability of these ultrafine particles to bind positively and negatively charged model drugs and compared the

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binding and release kinetics of these particles with fibre snippets. To our knowledge this is the first study on drug delivery properties of milled silk particles.

2. Experimental

2.1. Materials

All chemicals were of laboratory grade. Model drugs (Fig. 1) Orange G, Azophloxine, Rhodamine B, Crystal Violet, and PBS were purchased from Sigma-Aldrich (Australia). Deionised water was used in all experiments. Eri silk (from the silkworm *Samia cynthia ricini*) was used to produce silk powder and sourced from northeast India.

2.2. Silk particle fabrication by milling

The particle size distribution, and images of fibres and particles used in these experiments are shown in Fig. 2.

Details of the powdering processes were reported in our previous work [39]. Briefly, degummed Eri fibres were chopped with a cutter mill (Pulverisette 19 from Fritsch) until the snippets could pass through a 1 mm grid. Wet milling of snippets was carried out in an attritor (1S from Union Process, USA). Snippets (200 g) were mixed with 1500 mL of water. The slurry was treated in the attritor mill with 5 mm yttrium-doped zirconium oxide balls in water for 7 h. The slurry obtained from the attritor mill was further processed with a laboratory spray dryer (B-290 from Buchi) to produce dry powder. The d(0.5) of these particles was 7.05 μm. The spray-dried powder (2 g) was further milled in a bead mill (DYNO® Mill Research Lab) using 100 mL water at pH 10. The grinding medium was cerium-doped zirconium oxide (0.5–0.6 mm in diameter) having a volume of 60 mL. The milling speed was 2000 rpm. Processing time was around 3 h to obtain particle sizes with d(0.5) of 281 nm.

2.3. Drug loading

50 mL model drug solution with pH 2.1, 3.4, and 7 was added to reaction tubes containing 50 mg silk fibres, snippets, 7 μm, or 281 nm silk particles. To prepare 50 mL model drug solutions, the required amount of water was poured into the reaction tubes. The pH of the model drug solution was adjusted by adding 1 mL 0.5 N sulphuric acid for pH 2.1, acetic acid for pH 3.4, and potassium dihydrogen phosphate buffer (adjusted to pH 7 with 0.5 N sodium hydroxide) for pH 7. To neutralize the alkaline pH of 281 nm particles, an appropriate amount of 0.5 N sulphuric acid solution was used. Desired amounts of model drugs were added from stock solutions at a concentration of 1 g/L to attain 1.5–20% model drug concentration on the weight of silk materials. After pouring model drug solutions into the reaction tubes containing silk materials, the mixtures were vortexed for approximately 1 min. For experiments at ambient temperature, the reaction tubes were gently agitated with a rotating disk. For the experiments at higher temperatures, a laboratory dyeing machine (Ahiba Nuance Top Speed) was used. Temperatures of 40, 60, or 80 °C, for 1, 1.5, or 2 h were maintained in the dyeing machine. To determine model drug uptake by the silk materials, at set time intervals, 1.4 mL solutions containing model drug and particles were centrifuged at 25,000 rpm for 10 min and the aliquots were sampled. In the case of fibres, aliquots were sampled directly. The sample solutions were then analysed for their drug concentration using a UV–visible spectrophotometer (Carry 300) by measuring their absorption, after determining λ_{max} for each model drug. For standard curves, standard drug solutions were prepared, and linear equations were obtained to calculate the model drug concentrations in the tested solutions. Drug loading (% of drugs in silk, based on silk weight) and loading efficiency (% absorbed from the available drug in the solution) were calculated using the following equations:

$$\%\ Loading = A_0 - A_t$$

$$\%\ Loading\ \ efficiency\ = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 is the initial % of the model drug (based on silk weight) in the reaction solution and A_t is the % of model drug (based on silk weight) in the reaction solution at time t after exposure to the silk materials.

2.4. Drug release

Silk loaded with 5 mg of each model drug was placed in a vial containing 1 mL PBS solution (pH 7.4). In the case of fibres, separation of

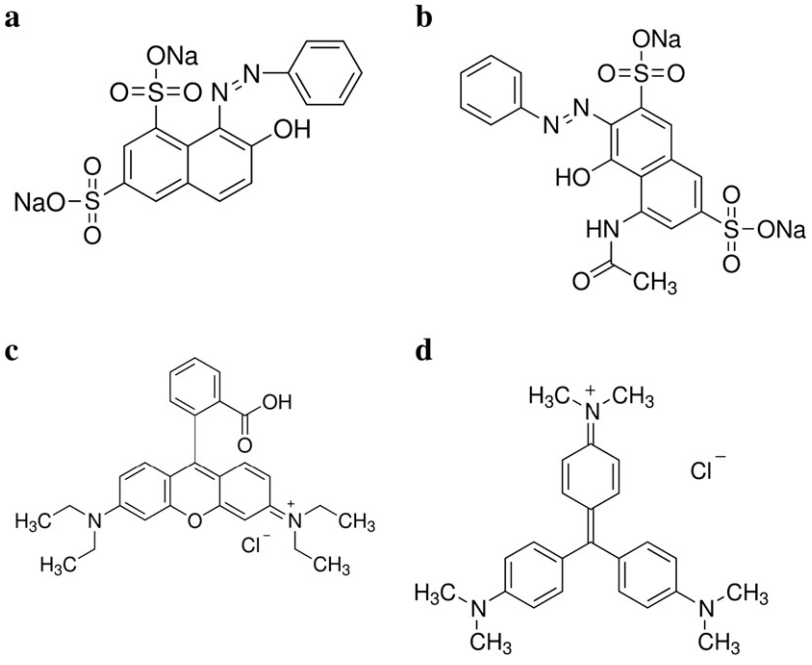

Fig. 1. Model drug formula a) Orange G, b) Azophloxine, c) Rhodamine B, and d) Crystal Violet (Source: www.sigmaaldrich.com).