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Newly isolated sporopollenin microcages from *Platanus orientalis* pollens as a vehicle for controlled drug delivery

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ABSTRACT

Sporopollenin microcages were produced from the pollens of *Platanus orientalis*. Paracetamol was loaded into the microcages. Pollen, sporopollenin, paracetamol and paracetamol-loaded sporopollenin microcages were characterized with FT-IR, TGA and SEM. The analytical analyses demonstrated that sporopollenin microcages were structurally intact, highly reticulated and thermally stable. The loading efficiency of the sporopollenin microcages was found to be 8.2% using the passive loading technique and 23.7% via evaporating loading technique. *In vitro* release and kinetics studies were performed to test the suitability of sporopollenin microcages for loading. These studies revealed that sporopollenin from *P. orientalis* can be suggested as a suitable carrier for drug loading and controlled release studies.

1. Introduction

Sporopollenin shells extracted from plant pollens have already emerged as promising micro-carriers for drug delivery [1]. Their thermal stability, non-toxicity, durability to chemical and biological agents, uniformity in size and porous surface morphology makes sporopollenin microcages suitable material for drug loading [2,3]. Recent studies have revealed the superiority of sporopollenin in drug delivery [4]. As known, in nature thousands of different pollens are produced by plants and each single pollen type can exhibit subtle variations in their morphology and physicochemical structure [5]. Sporopollenin from *Lycopodium clavatum* spores have been widely used in controlled release formulations but still many plant pollens remain unexploited [1,3,4,6]. In current study, sporopollenin from *P. orientalis* pollen grains were extracted for the first time and used for controlled release of paracetamol.

Platanus is a genus of large tree species native to northern hemisphere including North America, eastern Europe and Asia [7,8]. The species *Platanus orientalis* (London plane tree) is naturally distributed in regions covering South-Eastern Europe including Turkey and Southwestern Asia. Its natural habitats are valleys and wetlands; they are also commonly planted in parks and gardens in temperate regions. *P. orientalis* is a wind-pollinated tree and sheds large amounts of airborne pollens in spring [9]. *P. orientalis* pollen grains are tricolpate, medium-sized (mean polar axis: 19.8 μm and equatorial diameter: 15.8 μm based on SEM measurements) and reticulated. The reticulum consists of polygonal or rounded fine and coarse elements [8].

Paracetamol (*N*-acetyl-p-aminophenol) is the most widely used analgesic in the world. However, it is also known to be the top agent causing intentional or accidental poisoning [10]. The major indication of paracetamol poisoning is hepatotoxicity and in the worst condition of poisoning the kidneys and heart may also be affected. After 24–48 h of poisoning it causes fulminant hepatitis [11]. Normally during the metabolism of paracetamol P450 enzymes are responsible for just 5–10% metabolism, which results in the production of toxic metabolite called *N*-acetyl-p-benzoquinone imine (NAPQI). NAPQI then binds with glutathione and is released as non-toxic compound *via* urinary track [12]. But in case of over-dosage of paracetamol, the amount of glutathione becomes less to bind all the paracetamol by leading to production of toxic compounds in the liver. Considering the reports on paracetamol poisoning, it is needed to apply this drug not directly but in a controlled manner. Paracetamol-loaded sporopollenin microcages can facilitate release of the drug in more controlled manner due to its highly reticulated and micro porous nature. Reticulated surface can provide necessary cavities for drug particles. Compared to the burst release of free drug, which is responsible for hepatotoxicity, entrapped drug within the cavities of the sporopollenin microcages can alleviate side effects of the drug.

This study aimed to produce sporopollenin microcages from pollens of *P. orientalis*, to load paracetamol into sporopollenin microcages and to study release behaviour of loaded paracetamol from the reticulate sporopollenin microcages. The pristine pollen grains, sporopollenin microcages, paracetamol and paracetamol-loaded microcages were characterized by FT-IR, TGA and SEM analyses.

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2. Material and method

2.1. Pollen collection

P. orientalis pollen grains were collected in Kastamonu, Turkey (on 24.04.2013). After drying at room temperature for three days, the inflorescences were shaken and then pollen grains were sieved first with 10 μm sieve and then with 50 μm sieve. The identity of the pollen grains was confirmed by using light microscopy. Pollen grains were kept at −20 °C in sealed polyethylene tubes.

P. orientalis pollen grain is spheroidal in shape and has radial symmetry. The pollen has tricolpate apertures and fine to coarse reticulate ornamentation [8]. Mean values of polar axis and equatorial diameter of the pollen grains were measured as 18–23 μm and 17–24 μm. The thickness of the exine and intine were recorded around 1.4 μm and 0.7 μm, respectively.

2.2. Chemicals

Paracetamol was obtained from Sandoz Syntek Active Pharmaceutical Ingredients, Istanbul, Turkey. Na_2HPO_4, KH_2PO_4, NaOH, NaCl, KCl, CH_3OH, HCl and $CHCl_3$ were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Two buffer solutions were used in the experiments; phosphate buffer saline (PBS) (prepared from Na_2HPO_4, KH_2PO_4, NaCl and KCl) at pH 7.4 and hydrochloric acid solution (prepared from HCl and KCl) at pH 1.2. Distilled water was used in the experiments.

2.3. Instruments

Infra-red spectra of untreated pollen grains of *P. orientalis*, sporopollenin microcages, paracetamol and paracetamol-loaded microcages were recorded using a Perkin Elmer 100 FT-IR Spectrometer 2.5 over the range of 4000–650 cm^{-1}. Scanning electron micrographs (SEM) of the samples were recorded on a QUANTA FEG 250. Thermograms were recorded on a thermogravimetric analysis system (EXSTAR S11 7300) under nitrogen atmosphere. Paracetamol concentration in the solutions was determined quantitatively on a UV–vis spectrophotometer (Shimadzu, Model 1601; Tokyo, Japan) by monitoring the absorbance readings at 243 nm. Briefly, we followed UV standard curve method to obtain the relationship between UV absorbance readings and the concentration of drug in the buffer systems (infra vide section of 2.6 for more details). An evaporator (Heidolph Hei-VAP Advantage) was used in evaporating loading technique.

2.4. Extraction of sporopollenin microcages

P. orientalis pollen grains (10 g) were treated with 40 mL of 4 M HCl solution at 50 °C for 1 h, recovered by vacuum filtration and extensively rinsed with water. Acid-treated pollen grains were then transferred into alkali solution (40 mL of 4 M NaOH solution) and kept at 90 °C for 12 h. Following the vacuum filtration, the sample was rinsed with water. This acid and base treatment was repeated under the same conditions. Then, acid- and base-treated pollen grains were kept in chloroform-methanol solution (1:1, v:v) at room temperature for 1 h. Finally, sporopollenin grains were washed extensively with water and air-dried at room temperature. The light microscope images of the intact pollen grains and sporopollenin microcages after treatment clearly confirmed the elimination of the genetic material from the pollen grains (Fig. 1).

2.5. Loading of paracetamol into sporopollenin microcages

2.5.1. Passive loading technique

The passive loading technique reported elsewhere [3] was used with some modifications for loading of paracetamol into the sporopollenin microcages. The passive loading technique was preferred due to the following reasons *i.e.*, a) it is eco-friendly (we avoid the use of harsh chemical methods), b) time saving and c) economical. Briefly, 100 mg of paracetamol was dissolved in 4 mL of ethanol. 200 mg of sporopollenin microcages were added into the solution and the suspension was vortexed for 10 min. Sporopollenin-paracetamol suspension was incubated at 4 °C for 4 h on a thermoshaker (350 rpm). Paracetamol-loaded microcages were filtered with 110 μm pore size filter membrane, washed with 3 mL of distilled water twice and incubated in a freezer at −80 °C for 30 min. Finally, paracetamol-loaded microcages were air-dried at room temperature for 24 h and then stored at −18 °C.

2.5.2. Evaporating loading technique

A new method called as evaporating loading technique was developed by our research group and used for the enhancement of the loading efficiency. A hundred mg of paracetamol was dissolved in 2 mL ethanol and suspended with 200 mg of sporopollenin. The suspension was vortexed for 10 min. Then, the sample was placed in an evaporator at 22 °C (ethanol did not evaporate at this temperature) and rotated for 4 h. Drug-loaded microcages were filtered through 110 μm pore size filter membrane. The paracetamol-loaded microcages were incubated in a freezer at −80 °C for 30 min and air-dried at room temperature for 24 h.

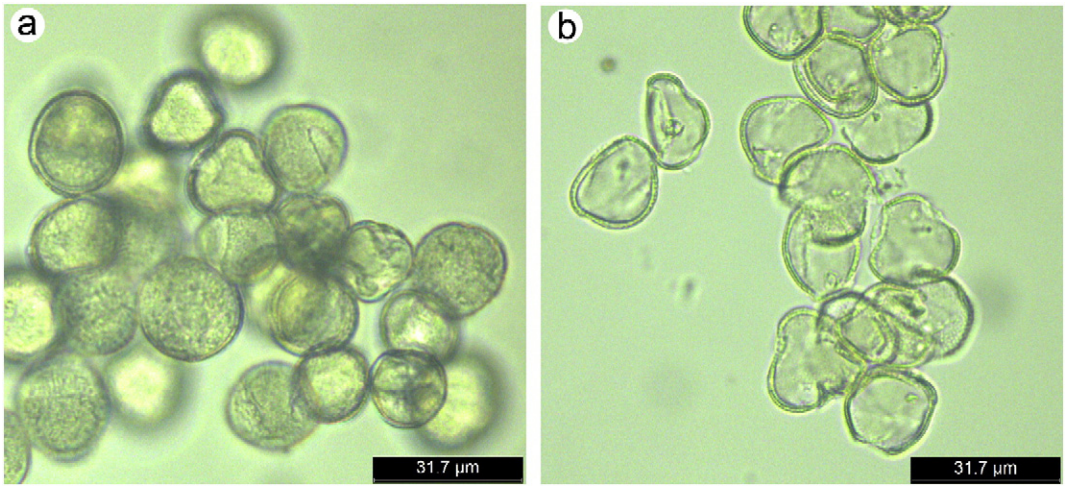

Fig. 1. Light microscopy images of a) pollen of *Platanus orientalis* and b) sporopollenin.