European Journal of Pharmaceutics and Biopharmaceutics 88 (2014) 492–501

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Density dependent mechanical properties and structures of a freeze dried biopharmaceutical excipient – Sucrose

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ARTICLE INFO

Article history:
Received 18 February 2014
Accepted in revised form 26 June 2014
Available online 11 July 2014

Keywords:
Compression
Freeze-drying
Biopharmaceutical
Elastic modulus
Crushing strength
Structure
Cake
Mechanics model
Sucrose

ABSTRACT

Knowledge of the mechanical behaviour of freeze dried biopharmaceutical products is essential for designing of products with physical robustness that will not to crack, crumble or collapse during processing or transportation. The compressive mechanical deformation behaviour for freeze-dried sucrose cakes has been experimentally studied from a relative density (ρ_f/ρ_s) of 0.01–0.30 using a novel in-vial indentation test. Cakes exhibited more open like structures at lower densities and more closed structures at higher densities with some faces being present at all densities, as confirmed by SEM. The reduced elastic modulus $E_f/E_s = 0.0044(\rho_f/\rho_s)^1$ for all cake densities, indicating that face stretching was the dominant deformation mode assuming Gibson and Ashby's closed cell model. This linear scaling for the reduced elastic modulus is in line with various theoretical treatments based on tetrakaidecahedral cells and other experimental studies. Consistently, the wall thickness to cell diameter ratio scaled ρ_f/ρ_s with a power constant of 1.05. The maximum crushing stress was given by $\sigma_{max} = 3800(\rho_f/\rho_s)^{1.48}$ which agrees with a strut bending failure stress, assuming Gibson and Ashby's open cell model. Overall, the freeze dried cakes behaved as neither classic closed cell nor open cell materials, with their compressive elastic moduli reflecting a closed cell elastic response whilst their failure stresses reflecting an open cell failure mode. It was concluded that the mechanical response of freeze dried cellular materials depends upon their complex cellular structures and morphologies, and they cannot be rationalised using simple limiting case models of open or closed cell solids.

1. Introduction

Freeze drying is becoming an increasing popular way for the manufacture of biopharmaceutical products where it is the main method for the manufacture of commercial products. Commercial freeze drying is production of a large number (>100,000) of a single dose glass vials with a volume of typically 2–30 ml containing the active biopharmaceutical formulation. These vials are then distributed for ultimate parenteral delivery into the patient. However, the freeze-dried cakes are generally fragile and they tend to crack, crumble or collapse within the vial during unloading from the freeze-driers, shipping, packing and handling, or simply during storage. Though the loss of mechanical integrity of the cake is not thought to impact on product bioactivity, it is nevertheless a significant reason for concern, often resulting in product rejection by the client. Therefore, knowledge of the mechanical behaviour of freeze dried biopharmaceutical products will enable the formulation and manufacture of freeze dried products with predictable physical robustness.

Cellular solids are made up of an assembly of cells which have faces and solid edges. Major uses for cellular materials include thermal insulations, packaging, structural use e.g. bone replacement therapies, light weight structures with desired strength for specific applications, buoyancy [1]. Gibson and Ashby [2] have considered them to be a system of solid struts or plates or a combination of both which form unit cells. Cellular materials can be manufactured using various techniques [3]. Freeze drying has recently gained popularity as a process for producing novel porous materials such as 3D extra cellular collagen foams [4,5].

Numerous publications on simulating and modelling the mechanical behaviour of brittle cellular materials can be found in the literature and are tabulated in this paper. However, real world cellular solids are dimensional heterogeneous and most of the models used to describe them are based on simplified open or closed pore structures with uniform repeating volume units [6].

Freeze-drying, also regularly referred to as lyophilisation, is a commonly used drying process during which the water is removed

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http://dx.doi.org/10.1016/j.ejpb.2014.06.017
0939-6411/

from a frozen solution by vacuum sublimation. It is the drying technique of choice for many classes of products, especially for the manufacture of biopharmaceutical products [7,8]. The freeze-drying process is carried out in three steps: freezing, primary drying, and secondary drying. Ice is used as a porogen to create a network of pores resulting in a cellular structure after the ice is sublimed during the primary drying. The shape [9], density and hence mechanical strength of the final freeze-dried scaffold is therefore highly dependents on the ice characteristics. The size and shape of the ice crystals depends on the freezing rate [1,10,11], freezing temperature [12], freeze-drying pressure [13], direction of freezing [14] and temperature of freezing [12].

The structure of freeze-dried cakes varies as a function of density and differs in the continuity of the cell walls [15] and the cake mechanical strength [16]. Cellular graphene monoliths, produced by freeze-drying in densities from 5.10 to 0.56 g/cm^3, had different microstructures as observed by SEM showing more fibrous structures as the density decreased [15]. The solute concentration of the freeze-drying solution affects the pore size and hence the tensile strength. Freeze-dried collagen–glycosaminoglycan cakes at low densities relative to the solid wall material (0.0062) had low Young's modulus of 32 kPa compared to higher elastic modulus 127 kPa for cakes with 4 times higher relative density and 30% smaller pore sizes [16]. The mean pore size of freeze-dried chitosan–gelatin decreased from 375 μm to 75 μm with increasing solid concentration from 1 w/w% solution to 7 w/w% whilst the tensile strength increased from 0.47 MPa to 1.15 MPa respectively [17]. The pore structure in freeze-dried cakes is also affected by the morphology of components. Crystallisation of hydrogarnet in cement paste caused cracks and an alteration to the pore structure of freeze-dried cement scaffold [18].

The mechanical characteristics of cellular materials are known to depend primarily on the elastic modulus and rupture strength of the material of construction, material porosity (which also affects density), and the cell geometry microstructure [19]. Overall cellular mechanical properties e.g. specifically elastic modulus and crushing stresses, and their dependence on relative foam density and solid cell wall properties, can be predicted using simple scaling analyses of the unit cell deformation processes as elegantly reported by Gibson and his co-workers [2,20]. Tabulated here are other models reported which may be used to describe the mechanical behaviour of brittle freeze-dried cellular structures.

It is currently acceptable to the freeze-drying community to limit the final product description to the cake's physical shape and colour, and occasionally the texture, with various subjective terms such as 'rough surface' 'grainy appearance' 'soft in nature', 'crumbly'. However, to eliminate this subjectivity, freeze-dried cakes can be better described by their cellular morphology, their mechanical behaviour and their composition. Many cellular mechanical behaviour models use a unit cell structure for predicting the materials stress–strain curves [22,28,30], or use composite material models to describe the elastic deformation of cellular materials [30] or use computer simulations based on the deformation behaviour of simple geometrical models representative of the physical structure of the cellular materials [22,27,29]. As the real structures of cellular materials can be quite complex, [26] suitable representations and accurate predictions of the actual mechanical behaviour can be difficult. Simpler models [31] have been used to predict the mechanical behaviour of complex cellular materials.

Processing conditions can also influence the mechanical behaviour of the cellular materials by controlling the molecular orientation and mobility, extent of crystallization and crystal orientation and any secondary bonding between molecules. In other words, it can be difficult to predict *a priori* the mechanical properties of a new phase being produced, especially in the cases here where is it typically a non-equilibrium glass i.e. amorphous. Therefore most models for mechanical performance rely on the relative density of the foam ρ_f/ρ_s which is an easily determined parameter for providing an empirical and theoretical relationship with E_f/E_s as is evident in Table 1. ρ_f/ρ_s significantly effects the mechanical properties of the cellular structures, so it is common to associate the relative density/volume fraction to the model unit cell structures/geometry [30,32,33].

Here the relationship between density, structure and compressive mechanical performance of freeze-dried cakes of sucrose, used widely for stabilizing and preserving biopharmaceutical formulations, is investigated in detail for the first time.

2. Method and materials

Sucrose (Sigma: S5016-1 kg, UK) was used to make 1, 2.5, 5, 20, 30 and 40 w/w% solutions with purified, de-ionised water. A fill volume of 2 ml in the borosilicate glass vials (VCDIN6R, Schott, Germany) was used for all solutions. Solutions were freeze-dried (FD) using a Virtis Advantage plus (SP scientific, New York, USA) freeze drier. The freezing rate was 0.3 °C/min, primary drying for 190 h at −40 °C and secondary drying at for 24 h at 20 °C. All the vials were sealed under a 50 mTorr vacuum at the end of the freeze-drying run and stored at 18 °C.

2.1. Mechanical test instrument

The indenter probe, a flat-faced 10 mm diameter punch made of poly methyl methacrylate, was directly attached to a 250 g load cell (FUTEK, UK). The load cell is connected to computer via a strain bridge amplifier (FYLDE, UK) and attached to a z direction motion stage. The load cell performance was validated using a series of calibrations weights. A stepper motor controller controls the motion of a linear motion stage with a 0.5 μm resolution. The system was controlled by Labview style software.

The sealed vials containing the cakes were equilibrated at 22 °C before testing. Immediately on removing the stopper from a vial, a compression indentation test was carried out on the cake within the glass vial using a loading velocity 10 μm/s, recording force data every 50 μm. The cakes were approximately 6.0 mm thick and 19.6 mm in diameter. The force measured (g) versus displacement (μm) was recorded during the compression into the central zone of the dried cakes. Time from opening the vial to the completion of the experiment typically took 2 min and was conducted at a nominal 22 °C and 40 %RH.

The global compressive strain ε was calculated using:

$$\varepsilon = \frac{h_0 - h}{h_0} \tag{1}$$

where h is the height of the sample after movement of the load cell/crosshead, and h_0 is the original cake height. As the cake sample is enclosed within the walls of the vial it was assumed that there is no lateral spreading of the cake; nominal compressive strains are representative of the overall global deformation.

The h_0 was measured by descending the indenter into an empty vial and determining the height at which the indenter touched the vial base; h_1. The indenter was retracted and a vial with sample is placed in the same position. The height at which the indenter makes contact with the cake is noted as h_2. The original height of cake h_0 is calculated by subtracting h_1 from h_2. The global compressive strain ε is limited to the range 0–1.

The compressive indentation stress (σ_i) was calculated using;

$$\sigma_i = \frac{F}{A_0} \tag{2}$$