Journal of Membrane Science 490 (2015) 256–265

Contents lists available at ScienceDirect

Journal of Membrane Science

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

A technique for drying and storing a protein as a soluble composite thin film on the surface of an ultrafiltration membrane

Raja Ghosh *, Rahul Sadavarte

Department of Chemical Engineering, McMaster University, 1280 Main Street West, Hamilton, Ontario, L8S 4L7 Canada

ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form
27 April 2015
Accepted 28 April 2015
Available online 8 May 2015

Keywords:
Protein drying
Ultrafiltration membrane
Thin film
Monoclonal antibody
Biopharmaceutical

ABSTRACT

We discuss a simple technique for drying and storing biopharmaceutical proteins such as monoclonal antibodies as soluble composite thin films on the surface of ultrafiltration membranes. This technique, which could also be used for drying other biopharmaceutical products such as vaccines, is being proposed as a simpler, faster and a significantly more cost-effective alternative to lyophilization. Protein and water are separated primarily by capillary-action based water absorption into an asymmetric ultrafiltration membrane, and selective retention of protein on the membrane surface by sieving. There is no requirement for sophisticated and expensive equipment as this technique can be carried out on the laboratory bench-top at ambient conditions, i.e. without either freezing or application of vacuum. Monoclonal antibody dried using this technique remained stable for one month at room temperature.

1. Introduction

Lyophilized or freeze dried forms of biopharmaceutical proteins such as monoclonal antibodies consist of molecular dispersion of the protein in an amorphous matrix made up of different excipients [1–4]. Dried proteins are more stable than proteins in solution due to the reduction in inter- and intra-molecular mobility resulting from water loss. Reduction in water content lowers protein degradation by oxidation and proteolysis and reduces undesirable effects such as toxicity and immunogenicity [5,6]. While protein solutions have to be kept frozen during storage, dried protein could be stored in a refrigerator or even at room temperature. Drying reduces both mass and bulk of a protein product, and this contributes towards ease in handling, transportation and storage [7]. The two main drawbacks of protein drying are increase in cost when using conventional drying methods such as lyophilization [7], and the need for reconstitution prior to use, which can sometimes be challenging [1,3].

Different stabilizers such as small molecules like sucrose and trehalose, or polymers such as dextran and polyethylene glycol are used during protein drying [3,8]. Such excipients are also used to stabilize other types of biopharmaceutical products such as DNA and vaccines during drying and subsequent storage [9,10]. There is lack of unanimity about the exact stabilization mechanism and two proposed mechanisms dominate the debate: one kinetic; and the other thermodynamic [5,6]. The kinetic mechanism is based on the suppression of global motions such as α-relaxation of the proteins within an amorphous matrix [11]. The thermodynamic mechanism is based on the substitution of hydrogen bonding [12]. Whatever the specific mechanism, the presence of stabilizers reduces the extent of protein aggregation both during drying and during reconstitution, and facilitates the retention of protein secondary structure during storage [13–15].

Lyophilization, which is widely used for drying biopharmaceutical proteins, consists of three distinct steps: freezing of protein solution; followed by dehydration of frozen solution by sublimation under vacuum; and finally secondary drying for more complete removal of water at a higher temperature, typically under vacuum [1–7]. It is a long drawn out process with the freezing step taking several hours, the sublimation step taking several days, and secondary drying taking several hours. Due to the requirement for refrigeration and high vacuum, lyophilization is expensive and requires highly specialized equipment. The freezing process is stochastic in nature and ice nucleation occurs at different times at different locations in material being frozen [16]. Many proteins are susceptible to stresses that develop due to the non-uniform nature of freezing [17]. Stabilizers and other excipients used during lyophilization could undergo differential migration during freezing, resulting in heterogeneity in morphology and composition of the freeze dried material [18]. Storage stability of proteins decreases with increase in product heterogeneity. Precipitation or crystallization of buffer salts during freezing could lead to pH shift, resulting in protein denaturation, and hydrolysis of excipients such as reducing sugars [19].

In this paper, we discuss a simple technique for drying and storing therapeutic proteins such as monoclonal antibodies in the

* Corresponding author. Tel.: +1 905 525 9140x27415.
E-mail address: rghosh@mcmaster.ca (R. Ghosh).

http://dx.doi.org/10.1016/j.memsci.2015.04.067
0376-7388/

form of soluble composite thin films on the surface of the macromolecule-retaining skin-side of asymmetric ultrafiltration membranes. The film consists of the protein dispersed in an excipient such as sucrose. In most currently used protein drying processes, dehydration takes place either by evaporation or sublimation directly from locations where the proteins are physically present. In the technique proposed in this paper, water is separated from the protein predominantly by convection, i.e. fluid flow, and translocated to a protein free location in the backing layer of the ultrafiltration membrane, from where it eventually evaporates. The technique is based on a recently reported micro-volume ultrafiltration method for protein purification [20]. In this paper, it was noted that if the retained protein remained on the membrane surface after ultrafiltration, it eventually formed a defined dry film-like deposit [20]. Also, the mildly hydrophobic surface of the polymeric ultrafiltration membrane used for micro-volume ultrafiltration minimized sample spreading, making it easy to reconstitute and recover retained material [20]. Concentration enrichment of protein solutions by removal of water across a membrane, followed by evaporation has also been previously described by other researchers [21]. The current work follows up on these earlier studies to develop an ultrafiltration-drying technique for drying and storing a recombinant monoclonal antibody in the form of a soluble film on the membrane surface. This technique could also potentially be used for drying and storing other types of biopharmaceutical products such as vaccines.

The working principle of ultrafiltration-drying is shown in Fig. 1. Protein solution is applied on the skin-side of an asymmetric ultrafiltration membrane (Fig. 1A). An asymmetric membrane consists of a thin and tight nano-porous skin layer with very low surface porosity and pore size atop a much thicker and very open microporous layer with significantly greater porosity and pore size [22–24]. Water and other permeable species present in the solution are drawn into the membrane by capillary action while protein is retained on the membrane surface. The location of retained protein and direction of water transport within the membrane are indicated in a micrograph-cartoon figure prepared using a transmission electron micrograph of the asymmetric ultrafiltration membrane used in this study (see Fig. 1B). In ultrafiltration-drying, the dehydration of protein solution takes place primarily by water absorption into membrane while surface evaporation plays a secondary role. As shown in Fig. 1A and B, the liquid entering the membrane rapidly spreads out laterally due to pore interconnectivity within the macroporous backing layer of the membrane. This increases the air-liquid contact area and speeds up the rate of water evaporation from the membrane. When the protein retained on the membrane surface is partially dehydrated, a solution containing the stabilizing excipient is added (see Fig. 1A) to facilitate the retention of protein secondary structure, and to minimize protein aggregation. Eventually, a dried thin composite film consisting of the protein molecules dispersed within an amorphous matrix consisting of the stabilizing excipient is obtained on the membrane surface.

Asymmetric polyethersulfone (or PES) ultrafiltration membranes having different molecular weight cut-off (MWCO), i.e. 10 kDa, 30 kDa and 50 kDa were used for ultrafiltration-drying. Humanized IgG1 monoclonal antibody (hIgG1-CD4) was used as the model biopharmaceutical protein while sucrose was used as the stabilizing excipient. The kinetics of the ultrafiltration-drying was studied. Dried protein films obtained on the membrane surface were characterized by optical microscopy, transmission electron microscopy (TEM) and atomic force microscopy (AFM). The films were stored at room temperature and at 45 °C for one month and the stability of reconstituted monoclonal antibody was assessed using techniques such as circular dichroism (CD), hydrophobic interaction membrane chromatography (HIMC) and dynamic light scattering (DLS). Ultrafiltration-dried monoclonal antibody remained stable at room temperature for one month.

2. Experimental

2.1. Materials

Monoclonal antibody hIgG1-CD4 (Batch 12) was kindly donated by the Therapeutic Antibody Centre, Oxford, United Kingdom. FITC-albumin (bovine albumin fluorescein iso-thiocyanate, A-9771) was purchased from Sigma-Aldrich (St. Louis, MO). Sucrose (SUC600) was purchased from Bioshop Canada Inc. Canada. Purified water (18.2 MΩ cm) used in this study was obtained from a DiamondTM NANOpure (Barnstead, Dubuque, IA) water purification unit. Polyethersulfone (PES) ultrafiltration membrane sheets (10 kDa MWCO, OMEGA, part OT010SHEET; 30 kDa MWCO, OMEGA, Part OT130SHEET; 50 kDa

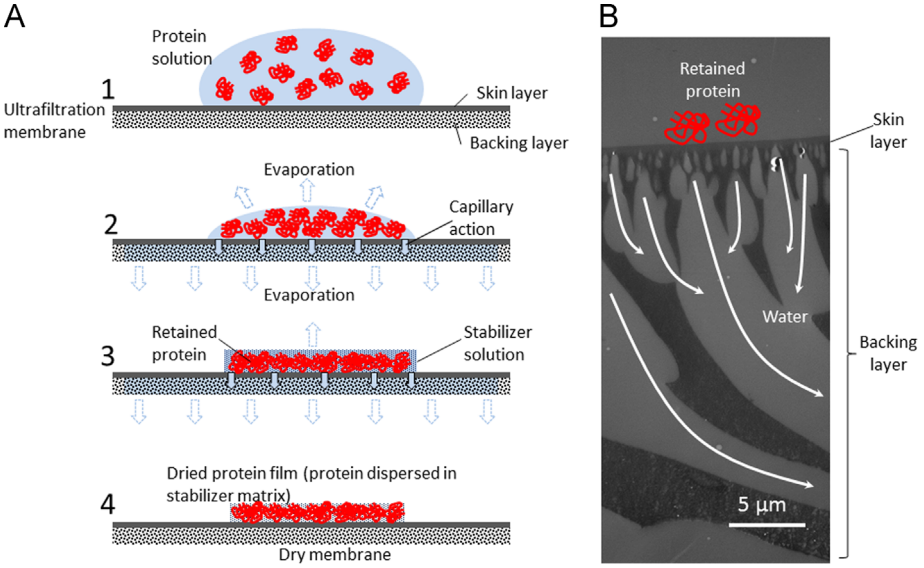

Fig. 1. (A) Working principle of ultrafiltration-drying (B) TEM micrograph-cartoon figure showing the location of retained protein and transport of water away from it during ultrafiltration-drying.