Supercritical carbon dioxide spray drying for the production of stable dried protein formulations

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CHAPTER 1

General introduction

1. Protein dehydration

Therapeutic proteins and vaccines prepared in a liquid dosage form may be sensitive to stress, often undergoing physical and chemical degradation, such as deamidation, oxidation and aggregation in response to pH, temperature, agitation and surface adsorption [1, 2]. This instability of liquid protein formulations may negatively impact the safety and efficacy upon administration. In contrast, a dried protein is generally more stable because of the lack of water for reactant mobilization [2]. Thus, protein dehydration is a means for developing stable protein/vaccine formulations with prolonged shelf-life, as compared to liquid protein formulations.

The most commonly used protein drying methods are lyophilization and spray-drying [3, 4]. For lyophilization, protein formulations are frozen and the water is removed by sublimation at low pressure [4]. In the case of conventional spray-drying, a protein solution is atomized via a nozzle, and dried using heated air in a spraying tower [5]. In order to maintain the desired stability, and correspondingly the protein activity, the residual water content in dried protein formulations should generally be no more than 3% (w/w). Lyophilization and spray-drying methods are used to prepare commercially available pharmaceuticals and biologics, such as peptides [6], hormones [7-9], enzymes [10], blood plasma [11], DNA [12], inactivated viruses in vaccines [13, 14] and proteins [15-17]. However, both processes are not without their limitations; lyophilization is costly and time consuming, and the freezing and drying steps can lead to the denaturation and aggregation of proteins [18], while the combination or individual effect of heat and the air/water interface in conventional hot air spray-drying method may destabilize proteins [19-21].

An alternative to these drying methods is to use supercritical carbon dioxide ($scCO_2$), where high pressure CO_2 is used as the drying medium. CO_2 is non-toxic, non-flammable, inexpensive, readily available and recyclable [22], and is a supercritical fluid above 75.8 bar and 31.5 °C (Fig. 1). It is possible to tune the density, and thus the solvent power, of the $scCO_2$ by changing pressure and temperature [23]. It has previously been shown that $scCO_2$ spray drying can be used to prepare proteins powders from formulations containing immunoglobulin G, insulin, lysozyme, or myoglobin [24, 25]. One of the major benefits of drying processes with $scCO_2$ is that the dehydration can be carried out at ambient temperature, thereby avoiding the thermal denaturation of protein. In this thesis, spraying a protein formulation into $scCO_2$ has been investigated as a protein dehydration process.

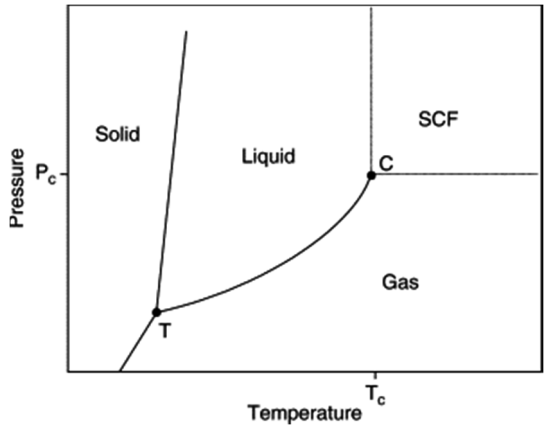

Fig. 1 Phase diagram, as a function of pressure (P) and temperature (T), of a substance that can occur as a supercritical fluid (SCF). Supercritical CO_2 occurs above the critical point (C), i.e., where pressure and temperature are ≥ 75.8 bar and ≥ 31.5 °C. The picture is taken from Nalawade et al. [38].

2. Supercritical carbon dioxide spray drying methods for proteins

With the supercritical CO_2 spray drying process, a protein formulation filled in a high pressure syringe pump is atomized by the CO_2 via a nozzle at a constant flow rate into a drying vessel filled with $scCO_2$ [26] (as shown in Fig. 2). The water removal from the atomized droplets is carried out in the vessel by mass transfer between water and CO_2 phases at a constant operating pressure and temperature. The atomization process influences the rate of water evaporation upon drying and the particle size of the resulting protein powder. For a batch process, the dried protein products are collected on a filter after the depressurization of the vessel. Powdered products of model proteins such as lysozyme [27], myoglobin [28] and immunoglobulin G [28] have already been prepared in order to evaluate the dehydration method by the $scCO_2$ spray drying process. However, in some cases, the $scCO_2$ spray drying could destabilize proteins or induce the formation of aggregates.

As the solubility of water is low in $scCO_2$, modifiers (such as dimethyl sulfoxide (DMSO) [29], DMFA [28], ethanol [28], methanol [30], ethyl acetate [28], dichloromethane (DCM) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) [31]) have been added to the $scCO_2$ to enhance the drying kinetics. In a previous drying study, ethanol was used to enhance the solubility of water in $scCO_2$ when spray drying protein-trehalose

formulations. However, residual ethanol was found in the resultant CO_2-dried powder, which may lead to destabilization of the proteins, as detected by changes in protein structure and an increase in aggregate formation [32]. To eliminate the ethanol, a secondary drying step, such as vacuum drying, was required [33]. For these reasons, the use of organic solvents in CO_2 spray drying is not desired.

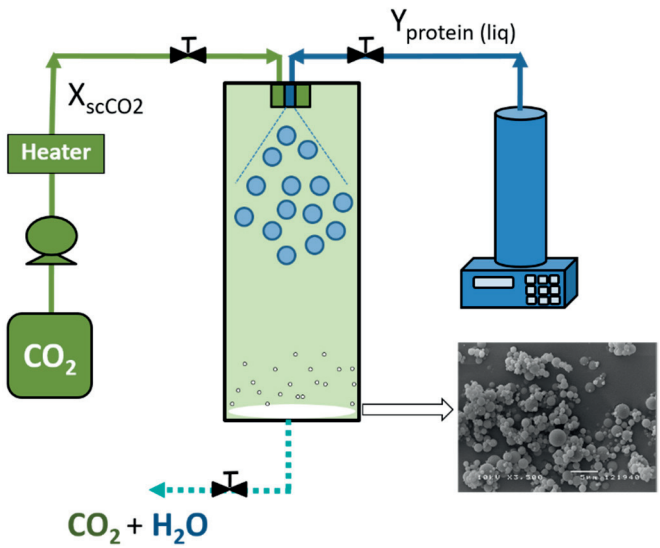

Fig. 2 Schematic set-up of the CO_2 spray dryers used in this study. P and T are the abbreviations of pressure and temperature, while X_{scCO2} and $Y_{protein(liq)}$ indicate the flows of $scCO_2$ and liquid protein formulation.

3. The challenge of supercritical drying methods and the choice of excipients on protein integrity

In contrast with numerous studies about harmful effects of lyophilization and conventional spray drying on protein structure [34-36], there is only limited information available in the public domain about the effects of $scCO_2$ drying methods on the stability of proteins, both during processing and storage. Since the drying process using $scCO_2$ fundamentally differs from freeze-drying and spray drying, the detrimental effects on protein integrity may also be different. The main factors of $scCO_2$ drying, such as pressure, temperature, atomization,

acidification, flow rates of carbon dioxide or protein solution, may affect the stability of proteins.

From previous studies, it has been observed that $scCO_2$ drying affects the structure and bioactivity of excipient-free protein formulations [32, 33]. Upon reconstitution of the dried products, the bioactivities of lysozyme [37] and lactate dehydrogenase [37] had decreased as compared to their original solutions. Moreover, changes in the protein structures were found, with one study showing a decrease in the α-helix content or an increase in the β-sheet structure of lysozyme [29]. For $scCO_2$ spray dried myoglobin (with or without a sugar excipient), no changes in the secondary structure were observed after the drying process, although there was a decrease in the heme-myoglobin interaction as well as the formation of insoluble myoglobin residues [26]. In this case, adding a sugar excipient helped stabilizing the protein [26].

When freeze-drying proteins, formulation excipients (e.g., buffer, sugar and surfactant) are often used to stabilize the protein during drying [3]. For example, trehalose is an osmolyte that can form H-bonds with a protein molecule, helping to maintain the structure and functionality of the protein when it is subjected to chemical and/or thermal stress, dehydration and freeze damage from ice crystal formation during freeze-drying [3]. For $scCO_2$ spray drying, the processing conditions are very different compared to freeze drying, and may therefore require different excipients and formulations to achieve the same protein stability. To date, however, the efficacy of protein stabilizers during $scCO_2$ spray drying has yet to be fully elucidated. Moreover, the complexity of the pressurization system and the properties of $scCO_2$ (e.g., atomizing $scCO_2$ and $scCO_2$ drying medium) make it difficult to study the influences of $scCO_2$ spray drying parameters on protein integrity. However, through a systematic study of the processing parameters, it may be possible to better understand the $scCO_2$ spray drying mechanisms and the role of formulation excipients in maintaining the protein integrity, making it ultimately possible to tune the processing conditions in order to prepare stable dried protein formulations.

4. Aims of thesis

The main goals of this thesis are to understand the $scCO_2$ spray drying mechanisms and parameters that influence the stability of proteins, to evaluate the excipients to stabilize protein formulations during $scCO_2$ spray drying, and to study the scalability of the $scCO_2$ spray drying process. For this study, lysozyme and myoglobin were used as model proteins. More specifically, the detailed aims are as follows:

- To study the $scCO_2$ spray drying parameters (i.e., pressure, protein solution and CO_2 flow rate, feed volume) without the use of organic solvents, in order to produce dried protein formulations with minimal residual water content in a single drying step
- To evaluate the scalability of the $scCO_2$ spray drying process
- To gain fundamental insight into the effect of the CO_2 spray drying parameters at sub- and supercritical conditions (65-130 bar and 25-50°C) on the stability of myoglobin
- To understand the effect of the CO_2/water interface and pH shift on heme destabilization and aggregation in myoglobin solutions using a gas bubbling method at atmospheric conditions.
- To evaluate the influence of pharmaceutical excipients on the stability of myoglobin in terms of heme binding and aggregation during $scCO_2$ spray drying

5. Outline of the thesis

Particle characterization methods are important for evaluating the properties of drug-containing particles engineered by $scCO_2$ processes, in order to ensure that they exhibit the desired characteristics for drug delivery. **Chapter 2** is a review of the particle characterization techniques most commonly used in evaluating particles produced by $scCO_2$ technology.

As the use of organic solvents in $scCO_2$ was previously shown to affect protein integrity, a single-step organic solvent free $scCO_2$ spray drying process is investigated in **Chapter 3**, with the aim to produce a dried protein powder with a target residual water content of max. 3% (w/w). The residual water content of the powdered product is studied as a function of pressure, volume of protein feed solution, and the flow rates of both the CO_2 and the protein solution. The study is carried out by using lysozyme as a protein model. The best processing conditions are further used to prepare dried formulations of other model proteins, including a-lactalbumin, a-chymotrypsinogen A and a monoclonal antibody. In addition, the scalability of the $scCO_2$ spray drying process is evaluated by simply controlling the atomization by maintaining the same gas-to-liquid mass ratio on each scale.

Even when using the best conditions obtained in Chapter 3, $scCO_2$ spray drying without organic solvent still resulted in the destabilization of the model protein myoglobin, as was seen from the partial loss of heme and the formation of protein aggregates. The study of **Chapter 4** aims to reveal the influence of the critical parameters associated with the $scCO_2$ spray drying process, such as pressure, temperature, pressurized CO_2 and the combined spraying and drying steps, on myoglobin's structural integrity. In relation to this, **Chapter 5** shows the effect of acidification and the CO_2/water interface on myoglobin stability using a gas bubbling method under atmospheric conditions. The results are compared to those obtained with N_2 gas bubbling, which has been used as a control, to study the influence of gas/water interface on proteins without lowering the pH. Building upon the results from Chapters 4 and 5, **Chapter 6** demonstrates the effect of pharmaceutical excipients on myoglobin, for further development of stable dried myoglobin formulations prepared via $scCO_2$ spray drying. Finally, the overall results are summarized and the main conclusions and prospects are discussed in **Chapter 7**.

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