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Evaluation method for the drying performance of enzyme containing formulations

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Abstract

A method is presented for fast and cheap evaluation of the performance of enzyme containing formulations in terms of preserving the highest enzyme activity during spray drying. The method is based on modeling the kinetics of the thermal inactivation reaction which occurs during the drying process. Relevant kinetic parameters are determined from differential scanning calorimeter (DSC) experiments and the model is used to simulate the severity of the inactivation reaction for temperatures and moisture levels relevant for spray drying. After conducting experiments and subsequent simulations for a number of different formulations it may be deduced which formulation performs best. This is illustrated by a formulation design study where 4 different enzyme containing formulations are evaluated. The method is validated by comparison to pilot scale spray dryer experiments.

Keywords: Downstream processing; Enzyme deactivation; Enzyme production; Kinetic parameters; Formulation design; Differential scanning calorimetry

1. Introduction

Spray drying is a very important unit operation used for numerous industrial applications such as waste treatment, production of inorganic salts, pharmaceuticals and food stuffs. In spray drying, a solution or suspension is fed to an atomizer and the droplets formed are mixed with a hot gas. This causes the solvent of the droplets to evaporate, leaving a dry powder product. Industrial enzymes are often subject to spray drying because product handling is easier and the enzyme storage stability is better in a powder product than in a liquid formulation.

Enzymes are widely used biocatalysts and these complex proteins are highly dependent on their structure to perform the catalytic act [1]. However, the enzymes may unfold and thereby lose the native structure during the drying process due to the high temperatures prevailing in the spray dryer. This thermal inactivation is of primary concern to enzyme producers as any activity loss evidently deteriorates the final product value.

Obviously, measures are taken to avoid the thermal inactivation during spray drying. This includes altering process parameters such as feed rate, initial droplet size and drying chamber temperature (see, e.g. Ref. [2]). Also, the inactivation may be reduced by mixing the enzyme containing feed with different compounds (e.g. carbohydrates and surfactants [3,4]) prior to drying, i.e. design activity preserving formulations.

In this work, a method for evaluating the performance of activity preserving formulations is presented, allowing fast formulation screening.

2. Previous studies

The enzyme inactivation during spray drying occurs at a certain rate depending on the nature of the enzyme, formulation ingredients and process operating parameters. In the literature a number of studies are devoted to quantify the rate of the inactivation reaction and simulate the progress of the reaction during drying. In theory, such simulations allow for determination of the optimal operating conditions and the best formulation.

An approach used commonly in the literature to quantify the reaction rate is to assume that the inactivation occurs as an irreversible first order reaction

$$N \rightarrow D \tag{1}$$

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Nomenclature

A	preexponential factor (may be water concentration dependent) (s^{-1})
E_a	activation energy (J $mole^{-1}$)
k	rate constant (s^{-1})
k_0	preexponential factor (water concentration independent) (s^{-1})
k^*	preexponential factor (water concentration dependent) (s^{-1})
n	constant
P	point on baseline (mW)
Q	DSC output (mW)
R	gas constant (J $mole^{-1}$ K^{-1})
T	temperature (K)
T^*	reference temperature (K)
w	water mass fraction
X	enzyme activity loss

where N and D are the native and the inactivated enzyme, respectively.

The reaction rate is described by the following (or a similar) expression

$$\frac{dX}{dt} = k(1 - X) \tag{2}$$

where X is the degree of conversion of the reaction given by (1). That is, $X = 0$ corresponds to all enzyme being in the native state while $X = 1$ when all enzyme is inactivated. Thus, X represents the fraction of enzyme which has been degraded and is therefore simply referred to as *activity loss* throughout the text.

The rate constant k is determined by an Arrhenius type expression

$$k = A \exp\left(\frac{-E_a}{RT}\right) \tag{3}$$

The preexponential factor A as well as the activation energy E_a may, according to the studies, depend on the water concentration of the medium surrounding the enzyme. Specific values or water concentration dependent functions for A and E_a are usually determined experimentally.

A study following the outline above is among others undertaken in the series of papers by Daemen et al. [5–8] and by Liou [9], Meerdink [10] and Yamamoto et al. [11].

Except Yamamoto et al. [11] all of the authors mentioned use the same approach to measure the rate of inactivation as a function of sample moisture content and temperature. An enzyme powder sample moistened with a predetermined amount of water is filled into a small special designed air- and watertight aluminum box and kept for a specific period of time at a constant temperature in an oven. Carrying out multiple experiments changing either time interval, temperature or moisture content and subsequently measure the residual enzyme activity yields data to which water concentration dependent parameters (A and E_a) may be fitted.

The experiments conducted by Yamamoto et al. [11] are quite similar but a sample incubated in a constant temperature bath is used.

Generally, the parameters determined by either of the experimental methods are not well-defined because the data obtained are limited. The main problem with the experiments is that they are difficult and time consuming to conduct [10].

Nevertheless, applying the parameters found the equations for the rate of inactivation, Eqs. (2) and (3), may be used quantitatively in droplet drying simulations.

Simulations of this kind are conducted by calculating droplet moisture content and temperature during the course of drying using a drying kinetics model. The results obtained are readily combined with Eqs. (2) and (3) to estimate the residual enzyme activity subsequent to drying.

To evaluate the performance of the simulations the results are often compared to data obtained from various drying experiments. These include spray driers [8], single droplet suspended from a glass filament [9–11] or sampling of free falling droplets [10]—see references for detailed descriptions of experimental setups.

Qualitatively, simulations generally respond correctly to changes in, e.g. spray dryer outlet temperature [8] or formulation [11].

Additional to the work discussed above, a number of authors perform quite similar experiments or simulations [12–15]. It is, however, noted that most literature on this subject has been written in the period from the early 1980s to the middle 1990s. Later literature focuses on the challenges in the formulation design of pharmaceutical proteins. Although numerous publications exist in this area, they are of little relevance in terms of formulation screening because the methods used are quite time consuming as they focus on, e.g. structural alterations of a particular protein due to drying or the specific effects of a formulation change. Readers with interests in that area are encouraged to consult the very elaborate review paper of Wang [16].

3. Objectives

The objective of this paper is to present a fast method for comparing the drying performances of different enzyme containing formulations in terms of maintaining the highest residual enzyme activity. The method may help reduce the number of expensive pilot and full scale spray drying experiments when designing new or improving existing formulations.

3.1. Method outline

The basis of the method mentioned above is to find the kinetics of the inactivation reaction, as given by (1), for each formulation. This is used to calculate the enzyme activity loss in the formulation at temperatures and moisture contents relevant for spray drying. Finally, it may be deduced which formulation performs best by comparing the activity losses in all considered formulations.

The reaction kinetics are estimated from experiments using a differential scanning calorimeter (DSC). In DSC a sample of a