Powder Technology 233 (2013) 341–346

Contents lists available at SciVerse ScienceDirect

Powder Technology

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

Screening and evaluation of variables in the formation of antibody particles by spray drying

Vahid Ramezani [a,b], Alireza Vatanara [a], Abdolhossein Rouholamini Najafabadi [a,*], Kambiz Gilani [a], Mohsen Nabi-Meybodi [a,b]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmaceutics, Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

ARTICLE INFO

Article history:
Received 18 March 2012
Received in revised form 14 July 2012
Accepted 20 July 2012
Available online 27 July 2012

Keywords:
Spray drying
Antibody
Plackett–Burman
Screening

ABSTRACT

We aimed to investigate the stability and particle properties of antibody by means of spray drying process. A Plackett–Burman factorial design was applied to identify the main factors of formulation and process which significantly influenced the responses. The formulations were consisting of the antibody as well as different ratios of either mannitol or trehalose as stabilizer. The spray drying parameters were inlet temperature, air flow rate, solution concentration, liquid feed rate and aspiration percent. Spray drying yield, particle size, size distribution, moisture content and protein stability were evaluated as the responses. Size exclusion chromatography as well as Fourier transform infrared (FTIR) spectroscopy was used to investigate remained antibody monomer and secondary structure, respectively. Particle size distribution was between 2.48 and 10.2 μm by narrow distribution of 0.71 to 1.96. The results of this study indicate that atomization flow rate and type of sugar with various ratios could efficiently enhance particle properties.

1. Introduction

Recombinant proteins play an emerging role in the treatment of various diseases and among them, therapeutic antibodies have the fastest growing area in biopharmaceutical industries [1]. Similar to other proteins, instability is the major concern in formulation of antibodies in large scale. Compared to liquid formulations, development of solid formulation improves antibody stability and elongates the product's shelf life [1].

Spray drying is one of the promising methods in the formation of particulate systems for pharmaceutical applications [2,3]. This method provides the opportunity to increase the stability of aqueous non-stable drugs like peptides and proteins into more stable powders in the form of particles. Spray dried powders are commonly small enough to be suitable for inhalation and other applications [4].

Although several studies have investigated the spray drying of antibodies, most of them focused on the evaluation of formulation ingredients rather than the process parameters [5–10]. Whilst the process consists of several critical steps such as liquid atomization, drying with hot air and collection of dry powder in a chamber exposed the hot air. Every parameter should be optimized to increase the product yield and to improve physicochemical properties of the powder [11,12].

Design of experiments (DOE) is a structured, organized method that is used to determine the relationship between the different variables affecting a process. Plackett–Burman factorial design is very useful for the identification and characterization of main factor from a large number of suspected contributor parameters for a desired response. This design can be implicated in preliminary studies to identify the rank order, magnitude and the sign of variable effect on specific responses [13].

In this study we have utilized this design for screening of the effects of various parameters in spray drying of a model antibody. The effects of different process variables including atomizing flow rate, inlet temperature, concentration of drug solution, drug solution flow rate, and aspiration rate on particle characteristics were investigated using a 12 blocks design.

2. Materials

Trehalose dehydrate, D(−)Mannitol, potassium phosphate dibasic and disodium phosphate were purchased from Merck, Germany. Human IgG (with molecular weight of about 150 kDa) and the pH of about 6.5 was purchased from Kedrion, Italy. In order to remove low molecular weight additives from concentrated antibody (50 mg/ml), it was dialyzed by the dialysis bag (cut-off 15 kDa) in deionized water. Dialyzed antibody was immediately used because of low stability.

* Corresponding author. Tel.: +98 21 6959057; fax: +98 21 6461178.
E-mail address: roholami@tums.ac.ir (A. Rouholamini Najafabadi).

0032-5910/$ – see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.powtec.2012.07.038

3. Methods

3.1. Processing of formulations by spray drying

Spray drying was performed using a lab scale spray dryer (Buchi, Switzerland) in conditions indicated by the experimental design. Antibody solutions were prepared by dissolving dialyzed antibody with the appropriate amount of additives to achieve final concentration of about 0.5 to 2 mg/mL and were immediately spray dried. The jacket of the spray nozzle was cooled by circulation of ambient temperature water. The cyclone as well as the collecting chamber was cooled with cold jacket in order to minimize the protein degradation.

3.2. Plackett–Burman screening design

In order to evaluate the effects of different spray drying variables on the characteristics of processed powders, a Plackett–Burman screening design was employed. The design and statistical analysis were performed by Design-Expert® V6 Software for design of experiments (DOE). Range of variable was determined according to previous studies [5,6,8,9,11,14]. Seven variables were evaluated for their effect on both antibody and particle properties. Independent variables were sugar type (A), sugar ratio (B), inlet temperature (C), air flow rate (D), solution concentration (E), liquid feed rate (F) and aspiration percent (G). Ranges of the dependent variable are listed in Table 1. Changes in yield, particle size and span parameter, moisture content, and antibody stability were considered as dependent variables. Stability of antibody was assessed by means of determination of residual monomer and β sheet content in protein secondary structure. The Plackett–Burman design suggested various experiment conditions in 12 blocks which are presented in Table 2. All the experiments were evaluated in random order to eliminate the effect of undesirable variables and all measurements were done in triplicate.

3.3. Size exclusion chromatography

Antibody stability and the percentage of soluble IgG aggregations were measured by size exclusion chromatography (SEC). The system was equipped with a pump (Waters, USA), a UV detector (Waters, USA) and a 300 mm TSK 3000 SWXL column (Tosoh Biosep, Germany). The isocratic mobile phase consisted of 0.1 M disodium hydrogen phosphate dehydrate and 0.1 M sodium sulfate, where, pH was adjusted to 6.8 with ortho phosphoric acid 85%. The flow rate was 0.5 ml/min and the injection volume was 20 μl with protein concentration of 5 mg/ml. Sample analysis was performed in triplicate.

3.4. Particle size analysis

Small amounts (about 10 mg) of each spray dried sample were dispersed in 5 ml acetonitrile with the aid of water bath sonication (Starsonic, Italy) for 2 min. The particle size of powders was measured by laser diffraction method (Malvern Instruments, UK) at obscuration between 0.15 and 0.20. Each sample was measured three times and size distribution is expressed as mean particle size. The distribution width expressed as span parameter is calculated as $(d_{90\%} - d_{10\%})/d_{50\%}$.

Table 1
Low and high levels in the Plackett–Burman design.

	Variables	Setting levels	
		Low (−1)	High (+1)
A	Sugar type	Trehalose	Mannitol
B	Sugar ratio	20%	80%
C	Inlet temperature	90	120
D	Atomizing flow rate	300	700
E	Solution concentration	0.5	2
F	Solution feed rate	10% (1.8 ml/min)	20% (2.7 ml/min)
G	Aspiration percent	80	100

3.5. Scanning electron microscopy (SEM)

The morphology of spray dried particles was examined using SEM (XL30, The Netherlands). Particles of representative samples were coated with gold at room temperature before the examination; and accelerator voltage for scanning was 25.0 kV.

3.6. Moisture analysis by Karl-Fischer titration

The moisture content of powders was determined by Karl-Fischer titration (Metrohm GmbH, Germany) using Hydranal TM composite as a titration reagent. About 10 mg of powder was dispersed in 1 ml of anhydrous methanol, and subsequently was injected to the titration cell.

3.7. Fourier transformation infra-red spectroscopy (FTIR)

Infra-red spectra were recorded on a Nicolet Magna spectrometer. Solid samples were produced by pressing 2 mg of protein powder with 200 mg KBr to make a clear window. The amide I region (1600–1700 cm^{-1}) of spectrum was analyzed in order to evaluate the changes in the secondary structure of antibody. Jasco Spectra Manager® software was used for the calculation of second derive of deconvoluted spectra of amid I band. This can help as peak position for curve fitting procedure using a mixed Gaussian/Lorentzian function. The percent of β sheet, α helix and turns were calculated with respect to β sheet absorption in 1640–1620 cm^{-1} as well as 1695–1690 cm^{-1}, α helix absorption in 1660–1650 cm^{-1} and turns from 1690 to 1665 cm^{-1} [15].

4. Result and discussion

Prediction of the effects of process parameters in spray drying of proteins is an essential requirement for development of this technology in bioprocesses. In this work, a Plackett–Burman design has been applied as screening method for the identification of important factors by reducing the number of experiments, although it is not suitable for the evaluation of interactions [16,17]. Therefore, these designs are extremely useful in preliminary studies where the aim is to identify variables that can be fixed or eliminated for further investigation.

Aqueous solutions containing antibody and excipients were spray dried according to a Plackett–Burman design in which the range of variables was selected according to the previous studies [5,6,8,9,11,14].

As shown in micrographs of Fig. 1, spray drying of pure antibody resulted in the formation of spherical microparticles with smooth surfaces in the sizes lower than 2 μm. All microparticles obtained from the experimental runs exhibited morphologies completely different from pure antibody. Formulations containing sugars demonstrated some wrinkled non-spherical shapes with narrow walls. Range of particle size was varied from 2.48 to 10.2 μm with the span ranges of 0.71 to 1.96 in different runs (Table 2).

Process variables influenced mean particle size and span parameters were affected by some process variables. By using the "Pareto chart of effects" (Fig. 2), main effects were rank ordered according to their significance. The main parameters that affected the particle size were sugar ratio ($p = 0.0462$), flow rate ($p = 0.0103$) and solution concentration ($p = 0.0465$). The ANOVA based analysis equation that described the magnitude and direction of main factors on this response is:

$$Y = +5.52 - 0.47A + 0.90B + 0.76C - 1.37D + 0.90E + 0.33F.$$