Investigation of miscibility in freeze dried systems

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Abstract

The aim of this study is to investigate the feasibility of combining pair-wise distribution function (PDF) and principal component analysis (PCA) to detect miscibility in freeze dried amorphous-amorphous systems. Three formulations were freeze dried, analysed by X-ray powder diffraction (XRPD) and validated by differential scanning calorimetry. Results show that the combination of XRPD, PDF and PCA can be used as an alternative analytical tool to detect miscibility of binary amorphous formulations. This combined approach improves the clarity of the result presentation compared to XRPD-PDF analysis only.

Keywords

X-ray powder diffraction (XRPD), pair-wise distribution function (PDF), principal component analysis (PCA), phase separation, binary amorphous systems

Introduction

Freeze-drying is an increasingly important unit operation in the pharmaceutical field often required for stabilizing the growing number of proteins under development as drugs.[1,2] In order to stabilize lyophilized proteins, various additives such as sugars, polyols and polymers are often added to the formulation.[3-5] These excipients are often found to be in the amorphous state after freeze-drying, a characteristic reported to be essential for their stabilizing capability.[6]

Multicomponent amorphous systems such as freeze-dried protein formulations have the potential to phase separate during manufacturing and storage. Phase separation is of concern as it could result in a product where the protein is in a different phase than the stabilizer added to protect it from degradation.[7] Insight in this field is however limited, mostly due to the analytical challenges associated with detecting phase separation in lyophilized protein formulations. Methods typically used for identifying phase separation in amorphous systems, such as differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) are of limited use. The former technique is limited because of the vague glass transition temperature of proteins while the latter is limited due to subjectivity connected with evaluation of the pictures.[8]

Alternative analysis is the residual plot method. Here the difference between theoretical and measured PDF profiles is calculated. Two matching profiles will result in lower residuals, suggesting the sample is miscible; in phase separated samples, plot with lower fluctuation is expected and high fluctuation profile is considered miscible formulation.[9,10] Similarly, interpreting the residual plot is relatively subjective. Moreover, there is no fluctuation threshold guideline for this method. This aim of this study is to demonstrate the use of principal component analysis (PCA) in combination with XRPD-PDF for detecting phase separation in freeze-dried samples.

Materials and Methods

Polyvinylpyrrolidone 30k (PVP30), PVP 90k (PVP90), dextran 10k (DEX10), polyvinyl alcohol 3.7-7k (PVA) and trehalose dihydrate (TREH) were used as received. Formulations (A) PVP30-DEX10, (B) PVP30-PVA and (C) PVP90-TREH at various ratios from 1:9 to 9:1, including the pure solutions were freeze dried using a Martin Christ freeze dryer. Freeze

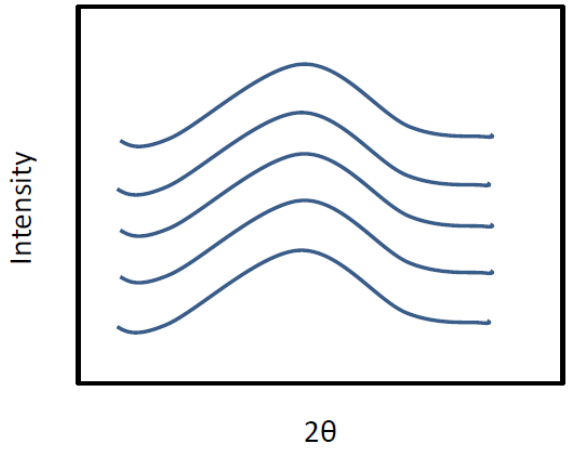
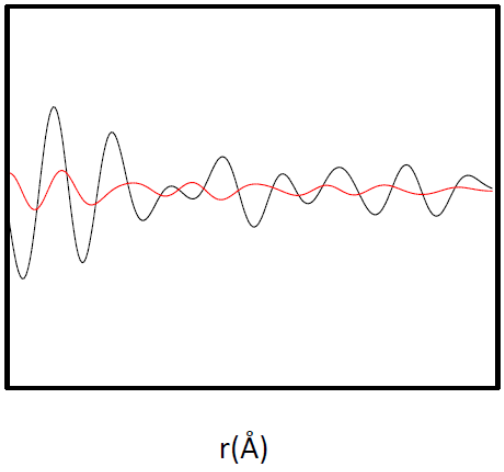
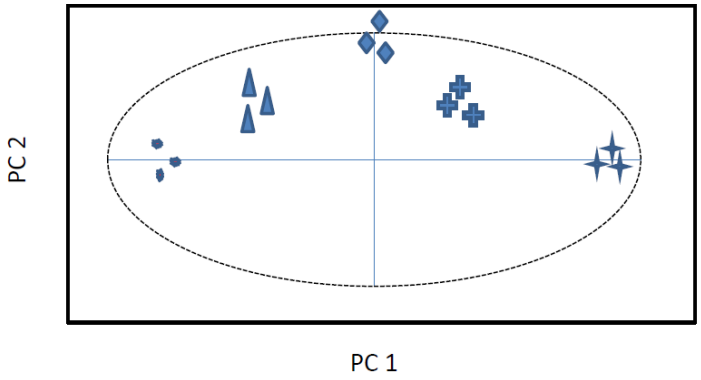
dried samples were stored in desiccators with silica gel until analysis. The samples were characterised and analysed using XRPD, PDF, PCA and validated by differential scanning calorimetry. During XPRD measurement, samples were exposed to ambient temperature and humidity. It was assumed that the exposure has minimal impact on the freeze dried cake due to relatively short timescale of the XRPD analysis (~4 hours).

Results and Discussion

Previously it has been reported that PVP30-DEX10[9] is a phase separated system, while PVP30-PVA[11] and PVP90-TREH[12] are miscible systems. Based on our DSC validation results (thermograms not shown) the findings were in agreement with literature results. In our PDF plots, subtle characteristic changes could be observed between theoretical and measured data at all formulation ratios that were studied. Owing to the small characteristic changes, interpreting PDF changes visually can be quite subjective, limited to one-to-one comparison and is highly dependent on the analysts' perspective. The reliability of qualitative comparison will also decrease with an increase in the number of PDF data compared simultaneously (i.e. replication data), not to mention it can potentially be quite overwhelming.

When XRPD-PDF analysis is combined with PCA, comparison of multiple PDF profiles with superior clarity on the subtle changes was achieved. The general workflow analysis of this research is summarised in Scheme 1. While step 1 and 2 are the 'common' procedures that have been reported, applying the third procedure is the crucial step in facilitating simultaneous analysis of multiple PDF data with the superior clarity. In our PCA plots, the PDF data of phase separated formulation (i.e. PVP30-DEX10) appears to be evenly distributed among the theoretical PDF suggesting no variation in theoretical versus measured PDF profiles (i.e. low fluctuation) and therefore the system is immiscible. To confirm this finding, the same experiment was repeated and similar results were obtained. In contrast, a skewed observation (i.e. high fluctuation) was found in PVP30-PVA and PVP90-TREH (Model results as shown in Scheme 1, step 3). This suggests that two latter systems are miscible. Overall, PCA plots were able to compare multiple samples simultaneously and proficiently distinguished systems that were immiscible or miscible.

Scheme 1: General workflow for XRPD-PDF combined with PCA analysis to investigate the miscibility of freeze dried systems.

Analysis procedure	Model results
Step 1: Acquiring XRPD data	
Step 2: Transformation of XPRD data to PDF plot	
Step 3: PCA analysis of XPRD-PDF data (each symbol represents a XRPD data or PDF plot)	

Conclusions

The XRPD-PDF-PCA results obtained in this study were comparable to the conventional DSC analysis. The combination analysis can be used as an alternative tool to screen and detect miscibility of binary amorphous freeze-dried formulations.

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