Monitoring The Reconstitution Time of Lyophilised Protein Formulations using UV Spectroscopy

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Introduction

Traditional methodology for quantifying the reconstitution time of lyophilized protein formulations involves the visual identification of the endpoint.

higher concentration lyophilised protein products (>50mg/ml) have come onto the market, it has become apparent that the product's long reconstitution periods and tendency to foam make the endpoint identification more difficult.

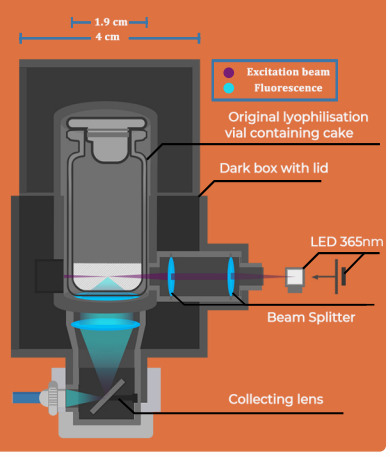
Due to its manual nature, visual identification of the reconstitution endpoint can be subjective and can result in variable and inconsistent values.

By monitoring the UV fluorescence of a lyophilised protein during reconstitution the endpoint can be observed. Analysing the data using principal component analysis allows the precise determination of the endpoint.

Methods

Vials containing the lyophilised formulations were placed in a 3D printed dark box to eliminate any stray light and enable the collection of UV signal through the vial. The signal was collected via 2 optical fibre cables. The acquisition time for all measurements was 500 msec.

Spectra were collected and analysed using principal component analysis. For each sample the first derivative of the first principal component was ploted over time, and using a semi-automated technique the endpoint of reconstitution was determined as seen in the fig. 3.



Results

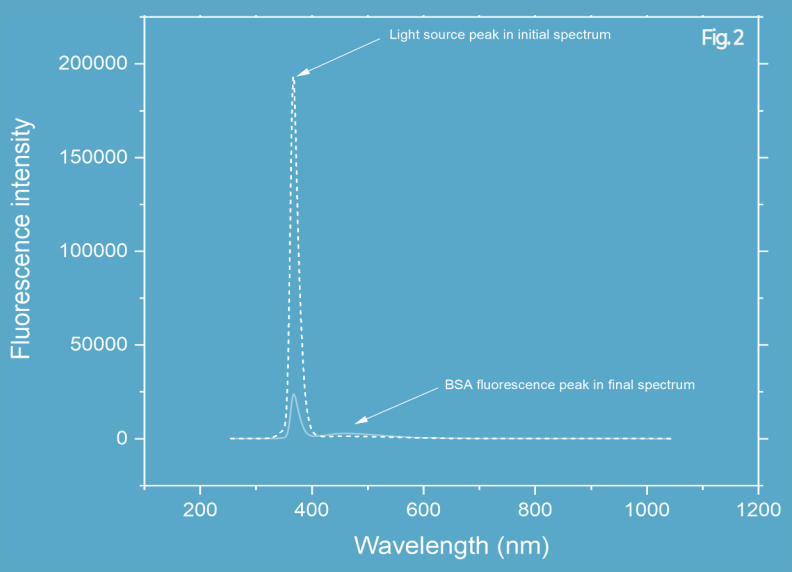
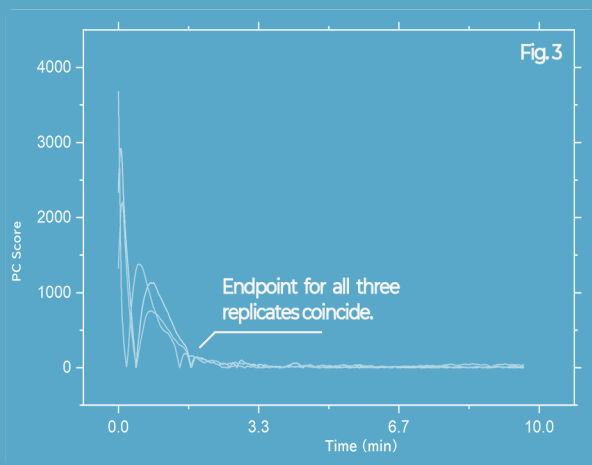


Fig. 2 First (dotted) and last (solid) spectra (of 1800) of an instrumentally observed reconstitution for a 50mg/ml lyophilised BSA formulation. Increasing opacity causes reduction of scattering and decreases peak intensity at 365nm (LED excitation peak). Increasing solubilisation of BSA increases peak intensity at 428nm (BSA fluorescence peak).

Due to the effects of differences in protein concentration on the spectra intensity, peak height changes are not directly comparable between different formulations. Consequently, preforming an analysis of the underlying patterns in the spectra using principal component analysis was performed to ensure that only significant changes are being represented in the results. The first derivative plot of the first principal component of the data captured during reconstitution is displayed over time in Fig. 3.



The spectra werecollected in sequence over time, each spectrum representing the same solution at a different point in time. The PC-1 eigen values ranged from 88% up to 96%. Therefore, it was concluded that the first principal component was capable of describing a large percentage the spectral variation with time due to physical changes in solution.
Plotting the first principal component scores over time reveals a reproducible pattern of changes in one formulation (F01) during reconstitution. Shown in Fig. 3, the PC-1 curves are derivatised to facilitate the determination of the reconstitution endpoint. Reconstitution endpoint was determined when the score value remained stabilised about the baseline value, for a continuous period (300 sec).

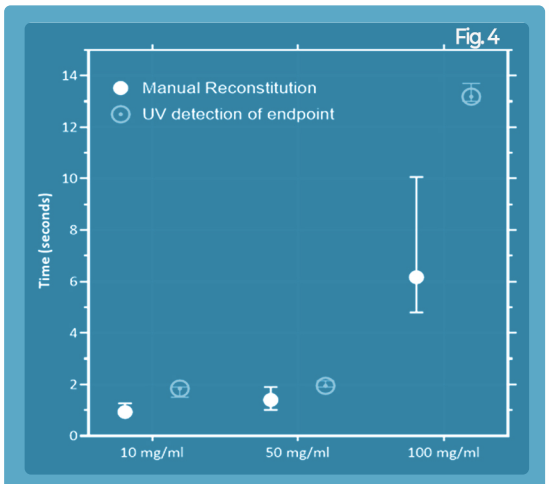


Fig. 4 Comparing between Reconstitution time measurements obtained visually versus those obtained by UV spectra PCA. For 10, 50, 100 mg/ml BSA lyophilised formulations (n = 6 for instrumental detection).

Lyophilised formulations below 50 mg/ml show reconstitution times (~2 min) that are independent of protein concentration.

High concentration formulation (100 mg/ml) takes significantly longer (~14 min) to reach complete reconstitution.

Visual observation produces times at an average of 7.18 min with a standard deviation of ±2.47 min at the highest concentration of protein

Instrumental method produced a much narrower range of reconstitution times at 13.3 ±0.37 min. The instrumentally measured reconstitution times are on average longer than those obtained by visual observation, which is attributed to the presence of subvisible particles and/or human error.

Conclusions

A method capable of easily and precisely identifying the reconstitution time presents many new opportunities in formulation design. The instrumental method is customisable for a wide variety of use-cases, affordable, easy to set-up, and easy to use. Furthermore, the results obtained using this method are easily cross compared, improving the usability of the reconstitution time of lyophilised product as a critical quality attribute.

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