Light induced fluorescence spectroscopy – a potential PAT tool for monitoring lactose based inhalation blending processes

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Summary

Light Induced Fluorescence (LIF) was evaluated as a potential Process Analytical Technology (PAT) tool for the assessment of blend uniformity in lactose-based dry powder inhaler (DPI) blends containing low active pharmaceutical ingredient (API) concentrations (<1% w/w). This technique has the potential to overcome the limitations of near infrared spectroscopy in terms of sensitivity for low drug concentrations.

Selectivity for the tested API was assured. The sample size measured by LIF was estimated to be approximately 2 mg, and density increases seem to decrease the LIF signal intensity.

A good calibration model was obtained for blends containing API contents from 0.08% to 0.16% w/w. Linear regression was used to generate a calibration model, due to the simplicity of the spectral features of API and lactose, allowing the quantitative characterization of blending processes.

The assessment of the blend uniformity depends on the understanding of blending mechanisms and kinetics. Convective blending takes place in the first minutes with rapid decrease of the sample RSD to acceptable levels. Diffusive mixing will occur during longer blending times. Density may also influence the signal intensity. The definition of the blending end time is dependent on the understanding of the multiple events taking place through time in the powder bed, and on the use of appropriate mathematical tools, coupled with aerosol performance testing.

LIF may be a suitable sensitive PAT method for blend uniformity monitoring of low-dose lactose-based DPI formulations, with simple analysis, allowing both qualitative and quantitative assessments of the blend uniformity.

Introduction

Powder mixing is one of the key unit operations involved in the manufacture of lactose-based DPIs, where the homogeneity of the final blend is crucial for a consistent delivery of the active pharmaceutical ingredient (API) to patients using a certain inhaled medicine. The homogeneity of the final blend is dependent on several parameters, such as the mixing principle, the API concentration, the material addition sequence, the blending container fill level, the blending speed and time and the scale of the blender [1]. Blending can be readily achieved during the development phase of an inhaled drug product where such blending parameters are scientifically evaluated.

The sampling thief is still the state of the art approach to sample powder bed blends for the assessment of uniformity of many blending processes, but it is known that this approach may not always provide representative samples of the powder blend, as it is very sensitive to sampling errors and depends on the physical and chemical properties of the formulation [2].

Process analytical technologies (PAT) are increasing being used to improve process understanding, process control and proactive manufacturing [3]. Nevertheless, on-line monitoring of blending requires fast, accurate, reliable, precise and sensitive techniques that require no sample preparation [1], which can be challenging for diluted blends. Near infrared (NIR) spectroscopy has been widely used as a preferential PAT solution for the assessment of blend uniformity, but the technique lacks sensitivity for low-concentration blends. Light-induced fluorescence (LIF) spectroscopy is a technology with the potential to achieve low detection and quantitation limits for certain pharmaceutical molecules, as fluorescence has the advantage of using two wavelengths, an excitation and emission wavelength, which increases the specificity of the method [1,3].

The present study describes an evaluation of the feasibility of LIF for the assessment of blend uniformity in DPI lactose-based formulations.

Experimental methods

The blends used in this assessment contained a micronized API and lactose monohydrate as excipient. Blends with increasing API loads (% w/w in blend) in the range of 0.08% to 0.16% were used for calibration. Blending was carried out at a 1 kg scale, using a Bohle LM40 bin blender (LB Bohle Maschinen Verfahren GmbH, Ennigerloh, Germany).

The LIF equipment used was Prozess 801 (Prozess Technologie, St. Louis, MO, USA) equipped with multiple, selectable narrow-band LEDs (240-400 nm), allowing the survey of the optimal wavelength for excitation, and a photomultiplier tube detector. The instrument consists of a wireless, blender-mounted base unit, and a measurement head which was attached to the blender via a sapphire window. Data analysis was performed with Microsoft Excel (Microsoft Corporation, Richmond, VA, USA) and NovaMath (SpectrAlliance Inc., St. Louis, MO, USA) applications. The most suitable excitation wavelength was selected by screening API and lactose using the different LEDs present in the LIF equipment. On-line blending assessment was performed out using an integration time of 100 ms and by averaging 10 points.

Blend samples were taken and analysed by high pressure liquid chromatography (HPLC) for API assay determination.

Results and discussion

Figure 1 illustrates the raw spectra of the API and lactose monohydrate at the selected excitation wavelength (385 nm). A clear peak signal is visible for the API, whereas lactose shows minimal fluorescence at the selected wavelength. The penetration depth for the excitation laser was estimated to be 3 mm, equivalent to the measurement of a 2 mg sample of blend per revolution. The effect of the blend density was also assessed. Figure 2 shows that an increase in the blend density seems to decrease the observed LIF signal, indicating that density may have an influence in the outcome of LIF measurements.

For the API blend concentration in the range of 0.08% to 0.16%, a good calibration model ($R^2 > 0.88$, $p<0.05$) could be developed using the relationship between the LIF signal and the API assay by HPLC (Figure 3). Due to the simple spectral features of LIF, a linear regression was sufficient to generate the calibration model, indicating the suitability of LIF for the quantitative assessment of lactose-based blends in with API in the tested concentration range.

LIF was then used to determine the blend uniformity during the on-line monitoring of the blending of the API and lactose monohydrate. Figure 4 shows that the RSD for the LIF signal variation with time decreases rapidly in the first 5 minutes of blending to values lower than 5% (commonly accepted as reference for an uniform blend), and this RSD remains stable with time. However, the LIF signal intensity increases constantly until a plateau is reached around 40 min of blending time. Therefore, in the assessment of the blend uniformity of inhalation lactose blends, some considerations should be made, when using LIF as a tool for uniformity determination.

It should be considered that, contrarily to the invasive thief sampling process where samples are collected at different levels of the powder bed, the non-invasive LIF testing measures the blend on its surface. Moreover, the sample size analysed on each revolution is typically more than 10 times smaller than the one tested when thief sampling is used. Furthermore, in common blending processes, it is expected that the API will initially disperse rapidly in the lactose via convective mixing. This may be observed in the first 5 minutes of the blending process, where the RSD of the signal change decreases rapidly. However, API particles need to cover the lactose surface *via* diffusive mixing, a slower and gradual process, which is observed in the remaining time of the blending. Also, it is known that during blending the volume occupied by the powder bed will increase, and, consequently, density will decrease [5].This would correspond to a slight increase in the LIF signal over time. Nevertheless, qualitative analysis of the LIF signal profile during blending can be made. The application of mathematical modelling to the generated data, as exemplified by the determination of the LIF signal variation in defined time intervals (Figure 5), may allow a more detailed understanding of the blending kinetics. In this example, it is observed that after 30 minutes of blending, the variation of the LIF signal is essentially constant, potentially indicating the blending end point.

Therefore, the assessment of the uniformity of a blend using LIF needs to be performed in conjunction with appropriate mathematical tools together with testing for aerosol performance and the uniformity of the dosage units, either capsules or blisters. Overall, this technology allows the generation of useful data which will increase and improve the blending process control. However, during any evaluations, the limitations of the LIF spectroscopy, such as poorly-fluorescing APIs and the time-related event of photo-bleaching at the window, related with the inherent fluorescence properties of the different compounds, should be considered.

Conclusion

LIF may be a suitable PAT tool for the blend uniformity monitoring of low-dose lactose-based DPI formulations, with the advantages of high sensitivity and simple analysis and quantification (*e.g.* simple linear regression), allowing both qualitative and quantitative assessments of the blend uniformity. This technique can also be a complimentary method to NIR spectroscopy, which usually has a poor sensitivity at concentrations below 1% w/w.

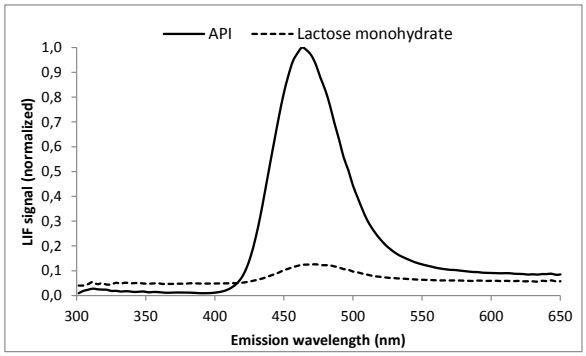

Figure 1 – Fluorescence spectra of API and lactose monohydrate (excitation wavelength: 385 nm)

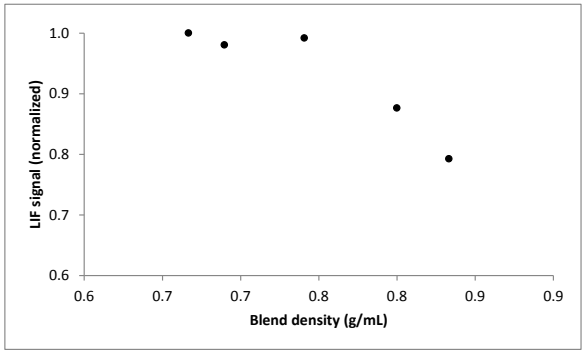

Figure 2 – Effect of blend bulk density on the LIF signal

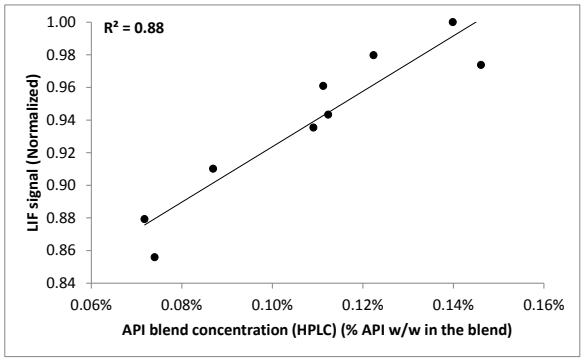

Figure 3 – Correlation between LIF signal (normalized) and concentration of API (% w/w) in the different blends, as measured by HPLC

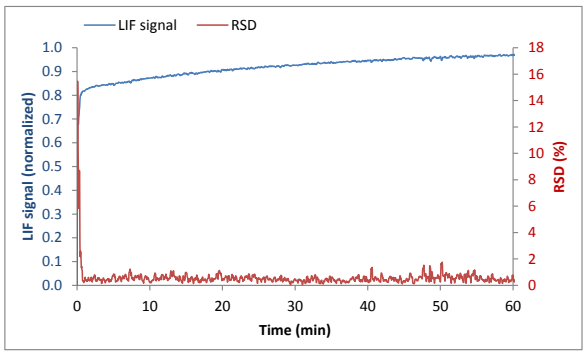

Figure 4 – LIF signal and respective coefficient of variation (RSD) change through time by on-line blending monitoring

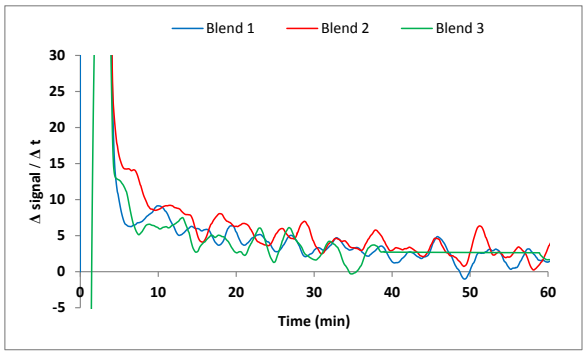

Figure 5 – LIF signal variation in defined time intervals through time by on-line blending monitoring

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