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Considerations on the quantitative analysis of apparent amorphicity of milled lactose by Raman spectroscopy

Samaneh Pazesh[a,*], Lucia Lazorova[a], Jonas Berggren[b], Göran Alderborn[a], Johan Gråsjö[a]

[a] Department of Pharmacy, Uppsala University, Uppsala, Sweden
[b] Recipharm Pharmaceutical Development AB, Solna, Sweden

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ABSTRACT

The main purpose of the study was to evaluate various pre-processing and quantification approaches of Raman spectrum to quantify low level of amorphous content in milled lactose powder. To improve the quantification analysis, several spectral pre-processing methods were used to adjust background effects. The effects of spectral noise on the variation of determined amorphous content were also investigated theoretically by propagation of error analysis and were compared to the experimentally obtained values. Additionally, the applicability of calibration method with crystalline or amorphous domains in the estimation of amorphous content in milled lactose powder was discussed.

Two straight baseline pre-processing methods gave the best and almost equal performance. By the succeeding quantification methods, PCA performed best, although the classical least square analysis (CLS) gave comparable results, while peak parameter analysis displayed to be inferior.

The standard deviations of experimental determined percentage amorphous content were 0.94% and 0.25% for pure crystalline and pure amorphous samples respectively, which was very close to the standard deviation values from propagated spectral noise.

The reasonable conformity between the milled samples spectra and synthesized spectra indicated representativeness of physical mixtures with crystalline or amorphous domains in the estimation of apparent amorphous content in milled lactose.

1. Introduction

The interest in the quantitative analysis of amorphous content of pharmaceutical solids has increased considerably the last years. This stems from the facts that firstly, amorphous solids may be used to solve certain formulation problems, such as low solubility of a drug, and, secondly, the processing of crystalline particles may result in, often undesired, formation of an amorphous phase (sometimes referred to as process induced disordering) that may still have an impact on physical and chemical properties of the materials and thus the final pharmaceutical product performance. Processing operations such as size reduction (Caron et al., 2011; Chamarthy and Pinal, 2008; Otte et al., 2012), compression (Kaneniwa et al., 1985) and dry mixing (Pazesh et al., 2013) has been shown to induce the formation of thermodynamically unstable amorphous regions of predominately crystalline particles. Therefore, the quantification of low levels of amorphous materials in powders has become an important part of the development of pharmaceutical preparations and this paper has been written in the context of this aspect.

Many analytical techniques may be used to quantify modest to high levels of amorphous content in a solid, including X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), solution calorimetry, isothermal microcalorimetry and dynamic vapour sorption. It is reported that the quantification limit of XRPD and DSC are greater than 5% (Shah et al., 2006), whereas solution calorimetry (Hogan and Buckton, 2000), isothermal calorimetry (Buckton et al., 1995) and dynamic vapour sorption (Mackin et al., 2002; Sheokand et al., 2014; Young et al., 2007) may enable the quantification of amorphous content of less than 1%.

Vibrational spectroscopy techniques, such as near- infrared spectroscopy (NIR) (Fix and Steffens, 2004; Hogan and Buckton, 2001; Savolainen et al., 2007), mid-infrared spectroscopy (MIR) (Agatonovic-Kustrin et al., 2001; Bartolomei et al., 1997) and Raman spectroscopy (Niemelä et al., 2005; Susi and Ard, 1974; Taylor and Zografi, 1998), have gained increasing interest in past

* Corresponding author at: Department of Pharmacy, Uppsala University, Uppsala Biomedical Center, P.O. Box 580, SE-751 23 Uppsala, Sweden.
E-mail address: samaneh.pazesh@farmaci.uu.se (S. Pazesh).

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decades for the analysis of chemical and physical properties of pharmaceutical solids. In a review, Strachan et al. (Strachan et al., 2007) give an overview of a number of such applications of the Raman technique, including the analysis of crystallinity of a solid. The limited water sensitivity of the Raman signal is a major advantage of this technique in the study of amorphous solids since water is frequently present as moisture in the amorphous phase.

A number of papers on the quantification of amorphous content of pharmaceutical substances by Raman spectroscopy have been reported in the literature. An early paper on the subject was authored by Taylor and Zografi (Taylor and Zografi, 1998) which was followed by other papers dealing with both quantification of amorphous content of drugs (e.g. Heinz et al. (Heinz et al., 2009), Mah et al. (Mah et al., 2015)) and excipients (Savolainen et al. (Savolainen et al., 2007) and Fix et al. (Fix and Steffens, 2004)).

According to literature, there are two ways of tackling the quantitative analyses of a Raman spectrum. The first group of methods is based on peak analysis, using peak variables such as amplitudes and areas or combinations thereof. In the second group of methods, the spectral values are used as they are, irrespectively of what spectral feature they may belong to, and subjected to multivariate analyses, such as principal component analysis (PCA) and classical least square analysis (CLS). Before the quantitative analysis, a raw Raman spectrum is often pre-processed in order to remove unwanted baseline and intensity variations in the Raman spectrum due to fluorescence and macroscopic variations in sample structure. The choice of pre-processing strategy may be crucial in order to obtain robust and accurate quantitative information from Raman spectra. Nevertheless, there are only few papers discussing the quantification of amorphous content of a pharmaceutical substance that also reports on the effect of the pre-processing procedure of raw Raman spectra for the out-come of the analysis. Hence, this is an aspect of the spectrum analysis that deserves more attention.

The standard procedure used in the literature to transform a Raman spectrum of a sample of milled particles into amount disordered content, is to use a standard curve obtained from spectra of reference samples. These reference samples are typically physical mixtures of completely crystalline and completely amorphous particles of known proportions, i.e. two-state systems with domains that are either crystalline or amorphous. The representativeness of such a two-state system for the physical structure of milled particles that are considered partially amorphous may however be questioned. Two conceptions are used in the literature (Chamarthy and Pinal, 2008; Luisi et al., 2012) to describe the physical nature of partially amorphous particles. Firstly, particles can be considered as a one-state system where the degree of disorder depends on the concentration of defects or the size and orientation of crystallites forming the particle. Secondly, particles can be considered as a two-state system where the degree of disorder depends on the proportion of amorphous and crystalline domains. The physical form of milled particles is thus an intricate issue as the exact physical nature of the disorder in milled samples is not obvious. Thus, a careful interpretation of the determined percentage amorphous content must be seen as an apparent measure and will at least partly be a relative measure of the degree of disorder rather than an absolute measure of amorphicity. The term apparent amorphicity or apparent amorphous content is used in this paper to acknowledge the fact that the quantitative analysis of a disordered solid results in a single percentage value although the exact nature of the solid is unknown and possibly complex.

The overall aim of this study is to address the question of the possibility to derive physically sound values of the apparent amorphous content for milled powders with a special reference to the importance of the combination of pre-processing and quantification methods for the determination of amorphous content in a powder. Lactose, one of the most common pharmaceutical excipients, is used as model material in the study. The overall aim was sub-divided into three sub-objectives. The first objective was to evaluate the effect of a comprehensive series of pre-processing methods combined with a series of quantification methods for the determination of fraction of apparent amorphous solid in a powder. The quantification methods could be divided into two main approaches; either based on peak parameter analysis or multivariate analysis (as illustrated in Figs. 1 and 2). In this part of the study, a series of physical mixtures of crystalline and amorphous lactose was used. The second objective of the study was to evaluate how spectral noise influenced the variation in determined fraction of amorphous content for the physical mixtures under investigation. This can be done by simulations, but, if possible, a derivation of an analytical expression will facilitate this evaluation. In this paper, an expression for propagation of errors for the combination of linear background and PCA analysis was derived. The third objective was to determine and compare the apparent amorphous content of milled powders of lactose, using the same pre-processing and quantification methods as were used in the assessment of amorphous content (Figs. 1 and 2), and to evaluate the representativeness of the calibration method in the estimation of apparent amorphous content.

2. Materials and methods

2.1. Materials and sample preparation

Crystalline α-lactose monohydrate Pharmatose® 200 M, (DFE Pharma, the Netherlands) was used as received. Amorphous lactose was prepared from a 10% (*w/w*) aqueous solution of α-lactose monohydrate, using a Büchi Mini Spray Dryer B-290 Advance (Büchi Labortechnik AG, Flawil, Switzerland). The aspirator rate was set to 100%, the spray gas flow to 40 L/min and the feed pump rate to 4.0 mL/min. The inlet and outlet temperatures were maintained at 155 °C and 98 °C respectively.

Ball milling of crystalline lactose was performed in a planetary ball mill (PM 100, Retsch, Germany) at 25 °C and 30 ± 3% relative humidity (RH) with rotation speed of 400 rpm. 1 g of lactose was milled in a stainless steel milling jar of a volume of 12 cm^3 with 50 stainless steel balls with a diameter of 5 mm corresponding to a ball to powder mass ratio of 25:1. In order to prepare lactose samples that could represent low, intermediate and high degree of apparent amorphous content of lactose by the ball mill used in this study, a series of pre-trials was conducted. Based on these pre-trials, milling times of 10, 300 and 1200 min were used for sample preparation. For the two longer milling times, a combination of milling periods and pause periods was applied to allow cooling of the jar and thus minimize heating of the sample, i.e. after each milling period of 20 min a pause period of 5 min was used.

For standard curves, physical mixtures with 0%, 5%, 10%, 15%, 30%, 50%, 70%, 85%, 90%, 95% and 100% (*w/w*) amorphous content with total weight of 2 g were prepared by combining spray dried lactose powder, representing 100% amorphous lactose, and Pharmatose powder, representing 100% crystalline lactose. Constituents of each mixture were added by geometrical dilution and mixed in a mortar with a pestle at 25 °C and 30 ± 3% RH.

2.2. Experimental methods

2.2.1. X-ray powder diffraction

Powder X-ray analysis were performed using a Bruker D8 Advance diffractometer equipped with a position sensitive detector (PSD), LynxEye (Bruker AXS, Inc., Madison, WI, USA)