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Small- and wide-angle X-ray scattering (SWAXS) for quantification of aspirin content in a binary powder mixture

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ABSTRACT

This article presents a novel application of small and wide angle X-ray scattering (SWAXS) in the assessment of aspirin and lactose content in a binary pharmaceutical powder formulation. It is shown that the content correlates with the intensity of the SAXS signal and the intensity of polymorph fingerprints in the WAXS spectra that are collected from the same samples. Because the polymorph WAXS fingerprints and the SAXS signal are two independent characteristics of the same sample, simultaneous SWAXS analysis provides the basis for a dual independent assessment of the same contents.

1. Introduction

Most pharmaceutical active ingredients (APIs), regardless of whether they are "small" organic molecules or large polypeptide and proteins, may have more than one crystalline form (Brittain, 1999). Typically, the therapeutic value of an organic small molecule (Bauer et al., 2001) or a protein (Merkle and Jen, 2002) is associated with its specific crystalline form or polymorph (Tedesco et al., 2002), since different polymorphs have different stability, solubility, dissolution rates, bioavailability, and thus, different therapeutic effects. Clearly, stability and, more importantly, bioavailability of a polymorph dictates the design of the dosage form and its manufacturing process (Singhal and Curatolo, 2004; Morissette et al., 2004). As such, efforts have been made to develop API crystallization processes that yield a specific and desired polymorph (Dunitz and Bernstein, 1995; Fujiwara et al., 2005). The issue of API polymorphism is not only limited to the crystallization stage but concerns the entire drug manufacturing process, as APIs may show process-induced polymorphic transformations (Morris et al., 2001). High-energy milling is a typical example of a process with the potential to alter the crystalline state of an API. Also excipients may undergo polymorphic transformations that ultimately affect the mechanical properties and disintegration of tablets (Mizumoto et al., 2005).

The effect of API polymorphism on drug bioequivalence is considered within the regulatory framework for the manufacturing of generic products (Raw et al., 2004) and quality control of solid pharmaceutical products is assessed by dissolution methods (Yu, 2008). These methods only evaluate the API concentration in solution and, when a drug product is dissolved the information regarding the crystalline structure of API and excipients is lost. Dissolution methods assess the contents of all polymorphs in a pharmaceutical product, regardless of their structure in the solid state. Changes in the dissolution rate and bioavailability may indicate that a different polymorph is present in the batch under consideration (Rasenack and Müller, 2002; Bauer et al., 2001). However, without an additional characterization technique for the API and the final product, the root cause of an out-of-specification dissolution rate may not be established, and the necessary corrective measures may not be taken. Hence, a technique that provides information both about the API content as well as its polymorph is required and X-ray techniques are ideal for this purpose. This problem has been recently addressed using transmission Raman spectroscopy (Aina et al., 2010; McGoverin et al., 2011). The transmission mode of Raman has proved advantageous over the backscattering mode in several situations (i.e., high sample fluorescence) and useful to assess API concentration in pharmaceutical products (Matousek and Parker, 2006; Buckley and Matousek, 2011).

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This paper shows how wide angle X-ray scattering (WAXS), an X-ray transmission and scattering technique, can be used to assess API and excipient content based on the intensity of their polymorph fingerprints (Suda et al., 2008; Chung, 1974; Fiala, 1980; Hodzic et al., 2008). It also shows that small angle X-ray scattering (SAXS) can be used to carry out the same content quantification. A SWAXS equipment has two independent detectors that collect the scattered X-ray spectra from a single sample at different angles, namely small (SAXS) and wide (WAXS). In this paper we show for the first time that these two spectra, which are originated by completely different properties of the sample, can be used to simultaneously quantify the content of components in a solid powder blend. The signal in the SAXS spectra is due to the electron density differences within the sample (Laggner et al., 2005) and a larger electron density differences leads to an increase in the intensity of X-ray scattering (Glatter and Kratky, 1982). Electron density differences exist, for example, between a solid surface and air. As we increased the concentration of the component with smaller particle size in a binary blend, the surface area (solid/air) and the intensity of SAXS scattering for powder samples increased.

The WAXS spectra show fingerprints due to the crystalline structure of the individual components. In addition to assessing the content of components, a SWAXS instrument has a potential application in studying the effects of process variables on granular products. For example, the intensity of the SAXS spectra may be used to study how these variables affect the extent of interfaces with electron density differences (i.e., particle surfaces). Likewise, the intensity of the fingerprints in the WAXS spectra may be used to study their effect on the crystallinity of components. Therefore, SWAXS is a useful tool for evaluating and developing processes that affect API crystallinity (i.e., crystallization, drying, milling) (Morris et al., 1998) and for selecting adequate unit operations for the manufacturing of the final drug product (granulation, blending, tableting, etc.). However, this will be the subject of future studies.

In the current work, SAXS and WAXS were used to assess the aspirin and lactose content of a binary powder blend. The advantage is that these two independent methods are concurrently used to evaluate the content of the same samples.

2. Materials and methods

2.1. Sample preparation

The materials studied are two pure components, acid acetyl salicylic (ASA) and lactose monohydrate (LM), and four of their mixtures with different ASA content (20%, 40%, 60% and 80%). The mixtures were prepared in a 1-l Turbula blender (Turbula System Schatz: Willy A. Bachofen AG, Switzerland) operated at the rotation speed of 50 Hz for 10 min. Three samples of each material are analyzed using SWAXS.

2.2. SWAXS camera

A SWAXS compact Kratky camera with line-focus optics (HECUS X-ray Systems, Graz, Austria) mounted on a sealed-tube X-ray generator (Seifert, Ahrensburg, Germany) operating at 2 kW was used. The CuKα radiation (λ = 1.542 Å) was selected with a Ni-filter in combination with a pulse height discriminator. The SAXS spectra were recorded with a linear position-sensitive detector (PSD-50 M, HECUS X-ray Systems, Graz, Austria) in the angular range of $0.06^\circ < 2\theta < 8^\circ$. The WAXS spectra were recorded with an independent detector in the angular range of $17^\circ < 2\theta < 27^\circ$. SAXS and WAXS spectra are collected simultaneously.

Powder samples were placed into a glass capillary with an inner diameter of 2 mm, which was later sealed with wax and placed into the capillary rotation unit. The measurements were performed at room temperature with the X-ray exposure time range of 2000 s. The SAXS spectra were obtained by subtracting the background (empty capillary) from the scattering spectra of the capillary containing a powder sample. The background and the sample spectra were collected using the same instrument setup.

2.3. SAXS parameters

Powder SAXS characterization is done using the correlation between the angular dependence of the inner surface and the scattering intensity I (Glatter and Kratky, 1982) in Eq. (1):

$$\lim_{q\to\infty} I(q) = \frac{k}{q^3} \tag{1}$$

where q is the modulus of the scattering vector, $I(q)$ is the scattering intensity at q and k is the so called tail-end constant (Porod, 1982) that is a function of the interfacial area between the two phases (here solid and air). The q-scale, which is related to the scattering angle 2θ by $q = 4\pi \sin\theta/\lambda$, represents the magnitude of the scattering vector, and can be interpreted as the resolution. Calibration of the q-scale, i.e., calibration of the detector to the scattering angle, is performed using silver stearate which has a defined lamellar spacing (d-spacing of 48.68 Å). The intensity I of X-ray scattering is the Fourier transform of the spatial autocorrelation function and it is here normalized by setting the spectra with the highest scattering intensity to 100%. A parameter derived from the intensity of scattering is the so-called invariant Q (Eq. (2)),

$$Q = \int_0^\infty I(q)q^2 dq \tag{2}$$

Q is the second moment of the SAXS scattering curve from angle 0 to infinity (Eq. (2)), where scattering at angle 0 and infinity are derived via extrapolations, i.e., the Guinier (1994) extrapolation toward 0 angle and the Porod (1982) extrapolation toward infinity. This procedure is supported by well-established scattering theories (Glatter and Kratky, 1982; Porod, 1982). Q is sensitive to crystalline structural changes in the sample, as well as to the volume fraction of the phases present and the electron density differences between them (Glatter and Kratky, 1982; Porod, 1982).

2.4. Particle size analysis (QICPIC)

QICPIC is an optical particle analyzer whose operation is based on dynamic image analysis. The device consists of several subunits, all manufactured by Sympatec GmbH (System-Partikel-Technik GmbH, Germany). Powder samples are placed in a funnel, which is connected to a vibrating chute (VIBRI-module) that feeds particles to the image analysis unit at a constant rate. Two dimensional images of falling particles are taken by a high speed camera. The size of particles in these images is calculated using the equivalent projected circle (EQPC) diameter.

3. Results and discussion

3.1. Particle size analysis of pure components

The two raw materials used were ASA and LM. Their particle size distribution was established via QICPIC analysis. For LM, the mean particle size (volume-based) was 222 μm, while for ASA it was 459 μm. ASA had a slightly wider particle size distribution than LM (D_{10} = 252 μm and D_{90} = 706 μm versus D_{10} = 76 μm and D_{90} = 422 μm). Figs. 1 and 2 show the density distribution of particle sizes (right axis) and the cumulative distribution (left axis) for LM and ASA.

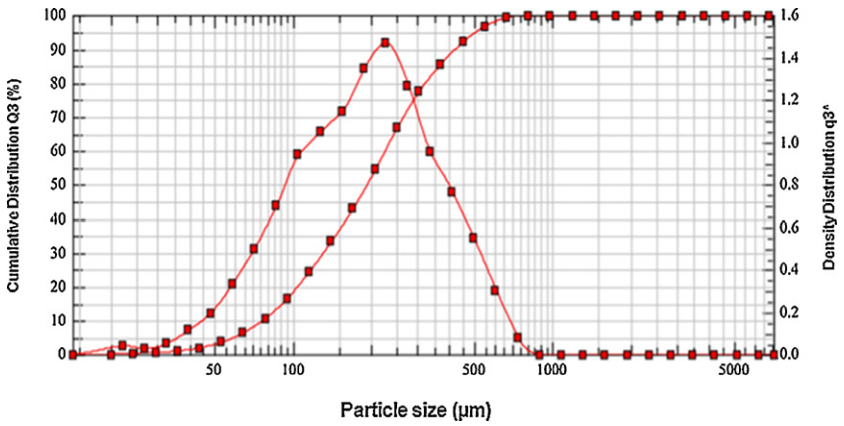

Fig. 1. Particle size distribution of lactose monohydrate (LM).

3.2. Quantifying the content of components in a blend using the intensity of their SAXS signal

Fig. 3-a shows the SAXS spectra of six powder mixtures with different ASA and LM content. The spectrum for pure ASA is in the front plane (Spectrum 1), followed by the spectra of samples with decreasing contents of ASA. The spectrum in the back plane (Spectrum 6) corresponds to a sample of pure LM (or 0% ASA). These spectra show the intensity of scattering (y-axis) versus the q-scale (x-axis), which is related to the scattering angle 2θ by $q = 4\pi \sin\theta/\lambda$. Electron density differences, such as those that occur at the interface between solid and air, are the reason for small-angle X-ray scattering (Glatter and Kratky, 1982; Porod, 1982). However, Bragg peaks of large crystal lattices also appear in the SAXS region. In fact, the SAXS spectra of our samples (Fig. 3) shows a Bragg peak due to the crystalline structure of ASA ($q > 0.45$). The signal due to electron density differences between the materials (solid A/solid B, solid-air) is more prominent at small q values ($q < 0.15$). Fig. 3-b shows these spectra in logarithmic scale to make the details of the Bragg peak and the SAXS baseline more evident. The intensity of the signal generated by electron density differences between the materials increases as the content of LM particles, which are finer and they have a substantially larger surface area than ASA particles, increases from 0% to 100%. In contrast, the Bragg peaks decrease with increasing LM contents.

The first step to develop a quantitative analytical technique is to use the signal due to different electron densities in phases to evaluate the parameter Q (Eq. (2)) for the six blends. The value of Q is dictated by the surface area of the particles in the sample (which depends on the proportions of fine LM and coarse ASA) and by the capacity of both materials for X-ray adsorption. The latter is very similar for organic pharmaceutical powders (Glatter and Kratky, 1982). In general, the parameter Q shows high reproducibility (three different samples were analyzed for each blend) for the blends studied, as we observe in the error bars of Fig. 4. Only the samples with pure ASA and 60% and 80% LM showed some variability. There are several sources for variability in the homogeneity assessment (not necessarily in this ranking): (1) sample size (Li et al., 2003; Mort and Riman, 1995). Generally, as the sample size becomes smaller, variability will increase, (2) sampling methodology (Muzzio et al., 1997) and (3) the error of the analytical technique itself (i.e., SAXS or WAXS). However, for the blends studied here, the error bars for Q were always within an acceptable range. The same arguments about the error in the homogeneity assessment apply to the WAXS-based technique described in Section 3.3.

Finally, Fig. 4 shows that there is a linear correlation between the value of Q and the LM concentration in blends. Therefore, the basis for developing a quantitative SAXS-based method was established.

3.3. Quantifying the content of components in a blend using the intensity of their WAXS fingerprints

Section 3.2 described how the parameter Q, whose value is given by the intensity of SAXS signals, correlates with the contents

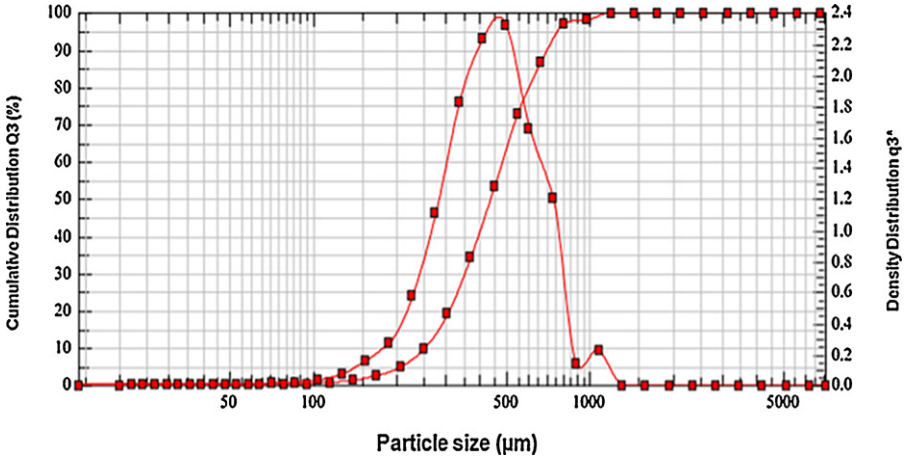

Fig. 2. Particle size distribution of aspirin (ASA).

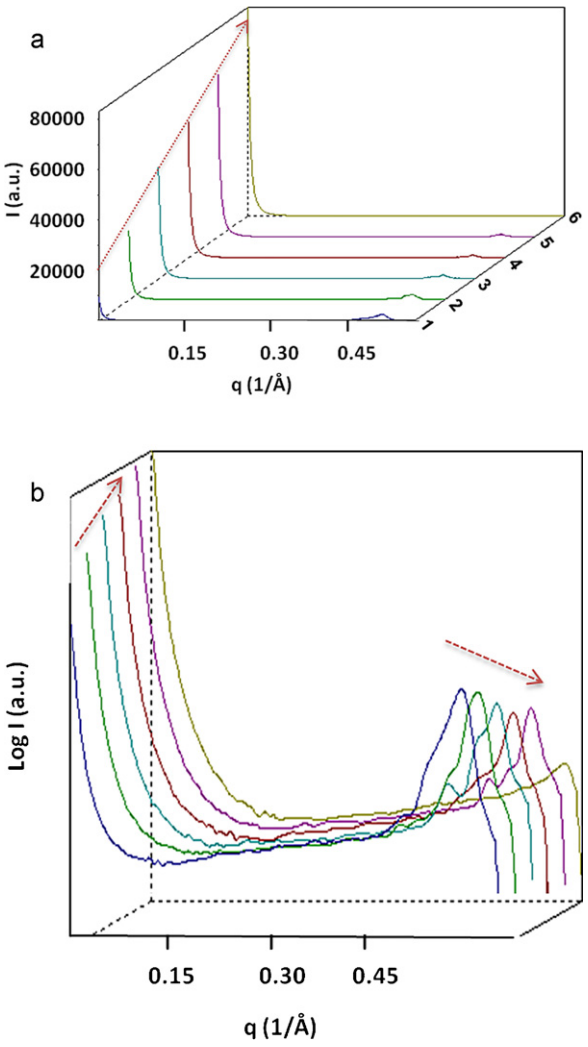

Fig. 3. (a) SAXS spectra (linear scale) of samples with (1) 100% ASA, (2) 80% ASA-20% lactose, (3) 60% ASA-40% lactose, (4) 40% ASA-60% lactose, (5) 20% ASA-80% lactose and (6) 100% lactose. (b) SAXS spectra (logarithmic scale) of the same samples. Here the Bragg peak of ASA and the baseline generated by electron density differences (solid-air, solid A/solid B) can be observed in more detail.

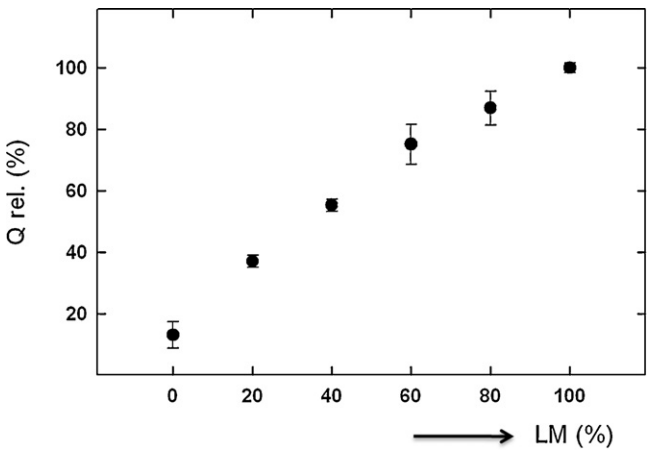

Fig. 4. Value of the invariant Q as a function of the LM content in samples.

of components in a binary blend. This section examines how the intensity of the fingerprints in the WAXS spectra of these same blends can also be used to quantify the content of components. The WAXS fingerprints are due to specific crystalline forms.

Fig. 5 shows the WAXS spectra of six powder mixtures with different ASA content. These WAXS spectra belong to the same samples as in the SAXS spectra (Fig. 3) and were in fact, concurrently collected. The WAXS spectra show the intensity of scattering (y-axis) versus the the scattering angle 2θ (x-axis). In those spectra, two LM fingerprints are identified at angles of diffraction $2\theta = 19.9^\circ$ and $2\theta = 23.6^\circ$ (Chen et al., 2001) and only one ASA fingerprint is identified at an angle of diffraction $2\theta = 26.7^\circ$ (Semalty et al., 2010). In order to assess the contents of ASA and LM in these samples, the area under these fingerprints is computed by integration. Fig. 6-a shows the relative area of the LM fingerprints (y-axis) at an angle of diffraction 2θ of 19.9 versus the LM content in the blends. There is a very strong linear correlation ($R^2 > 99\%$) between these variables. Fig. 6-b shows the relative area of the ASA fingerprint versus the LM content in the blends. This correlation is slightly weaker ($R^2 = 95\%$) because of the larger error in the estimation of the area of ASA fingerprints. The latter is attributed to the fact that these measurements are performed at the limit of this instrument detector. This instrument does not capture the scattering signal beyond

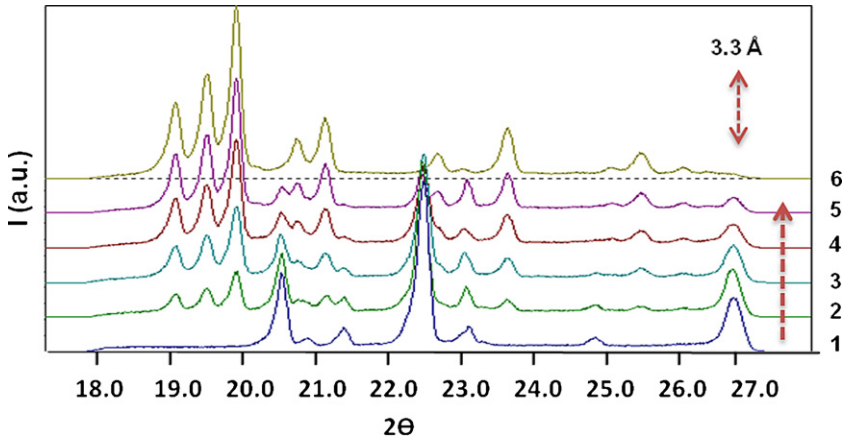

Fig. 5. WAXS spectra of powder samples. The spectra correspond to samples with (1) 100% ASA, (2) 80% ASA-20% lactose, (3) 60% ASA-40% lactose, (4) 40% ASA-60% lactose, (5) 20% ASA-80% lactose and (6) 100% lactose. The q values that correspond to the scattering angles ($q = 4\pi \sin\theta/\lambda$) indicated on the x-axis are displayed on top of the Bragg peaks.

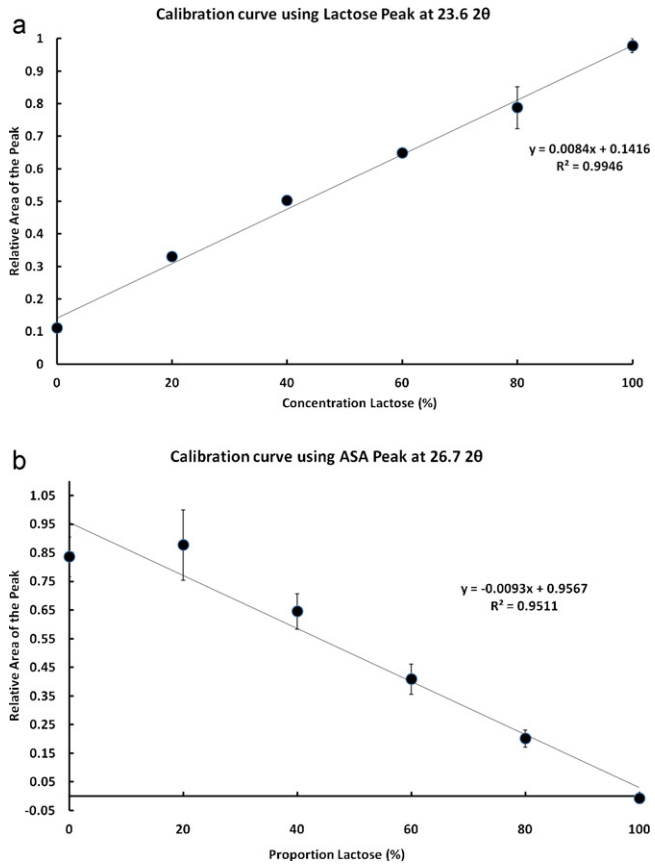

Fig. 6. (a) Correlation between the lactose (LM) content (*x*-axis) in blends and the relative area of the LM Bragg peak (*y*-axis) at an angle of diffraction $2\theta = 19.9$. (b) Correlation between the lactose (LM) content (*x*-axis) of blends and the relative area of the ASA Bragg peak (*y*-axis) at an angle of diffraction $2\theta = 26.7$.

an angle of diffraction $2\theta = 27^\circ$ and the ASA fingerprint is at an angle of diffraction $2\theta = 26.7^\circ$.

4. Conclusion

The results presented in this paper show that the SAXS and WAXS spectra simultaneously collected from a same sample provide the basis to develop a method for assessing the aspirin and lactose content in a powder blend. A SWAXS instrument has two independent detectors that collect the X-ray scattered spectra in two different ranges: small-angle (SAXS) and wide-angle (WAXS). While the signal in the SAXS spectra is attributed to electron density differences between the powder surface and the air or between phases of different chemical composition, the WAXS fingerprints are due to a specific crystalline structure. The intensity of the SAXS signal and WAXS fingerprints was successfully correlated with API and excipient content in the samples of a powder formulation. Hence, the SWAXS instrument can provide the basis for a dual independent assessment of the same contents.

One advantage of X-ray techniques lies in their capability to penetrate matter, which allows for an analysis of the internal structure of coated tablets, products with internal pores and products that are packaged. The SWAXS ability to simultaneously assess API content using two independent methods makes it very attractive for process development and quality control of products, particularly for pharmaceutical dosage forms whose compound bioavailability and therapeutic value is associated with crystalline structure.

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