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International Journal of Pharmaceutics 283 (2004) 63–69
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Precision and detection limit of quality test for amorphous drug in powder X-ray diffractometry

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Received 20 February 2004; received in revised form 18 June 2004; accepted 18 June 2004
Available online 17 August 2004

Abstract

This report puts forward a method of powder X-ray diffractometry to estimate the precision and detection limit of the crystalline component in an amorphous drug. Cefditoren pivoxil (CP) was employed as a model drug. The major error source of the measurement at low crystal contents is shown to be the random noise in a diffraction pattern (halo pattern) of the amorphous material. For the analysis of the noise, the obstructive halo pattern should be eliminated from the observed pattern. The subtraction of the observed halo pattern from another one derived from the same material, extracts the random noise alone, although the noise is amplified by $\sqrt{2}$ times. The noise in the powder X-ray diffractometry was identified as the white noise. On the basis of the stochastic properties of the extracted noise and signal parameters (peak area) of CP, the relative standard deviations (R.S.D.) of the area measurements of the crystalline diffraction peaks were estimated over a wide range of crystal contents without repeated experiments. The detection limit was determined such that the crystal content at detection limit produced 30% R.S.D. of the measurements. The R.S.D. and detection limit obtained from FUMI theory were in good agreement with the results from the repeated measurements.

Keywords: Powder X-ray diffraction; Detection limit; Precision; FUMI theory

1. Introduction

The amorphous form is often utilized to improve the intestinal absorption of a drug due to its higher solubility and release rate compared with the crystalline form (Byrn et al., 1995; Hancock and Zografi, 1997). However, the amorphous form may be converted to the crystalline form during the inappropriate manufacturing process of drug products or storage in stress condition. Therefore, it is important to identify the existence of the crystalline forms in the amorphous drugs.

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doi:10.1016/j.ijpharm.2004.06.024

To analyze the amorphous drugs, polarized light microscopy, powder X-ray diffractometry, thermal analysis, IR spectrometry, solid-state NMR spectrometry, microcalorimetry, etc. are available (Saleki-Gerhardt et al., 1994; Yonemochi et al., 1997; Gao, 1998; Gustafsson et al., 1998; Ohta et al., 1999, 2000). The powder X-ray diffractometry has been frequently utilized, thanks to the simplicity of its operation, but the analytical performance, especially precision, has not been studied extensively.

The aim of this report is to propose a method of powder X-ray diffractometry to estimate the precision and detection limit of the crystalline component in an amorphous drug. The precision is usually expressed as the standard deviation (S.D.) and/or relative standard deviation (R.S.D.) of the measurements, and they are usually calculated through repeated experiments. However, statistics show that the high reliability of the S.D. estimate cannot be attained without many repeated experiments. For example, whereas 95% confidence intervals of S.D. estimates at $n = 40$ are about $\pm 25\%$ of the true value, they are $\pm 60\%$ at $n = 6$. This rule cannot be violated in any case. Thus, this report presents a new alternative method to repetition.

Cefditoren pivoxil (CP) has been developed as an oral antibiotic drug, and introduced to the worldwide market including USA, China, Korea and Japan. The low water solubility of the CP crystal is a critical problem in the development of oral drug products, since the low solubility leads to the low absorbability. To solve the problem, the CP crystal has been converted to its amorphous form in the manufacturing process. As for quality control, it is very important to evaluate the precision and detection limit of the crystalline component in amorphous CP. Thus, the CP is taken here as an example.

It is a natural assumption that the observed diffraction pattern at low crystal content is the linear sum of the 100% crystalline diffraction pattern (Fig. 1A) and 100% amorphous diffraction pattern (Fig. 1B) according to the existing ratio. Especially around the detection limit, the crystalline signal will appear slightly above the noisy amorphous diffraction pattern in the instrumental output (Fig. 1C). Therefore, the major error source in the measurement will be the random noise accompanied with the observation of the 100% amorphous material.

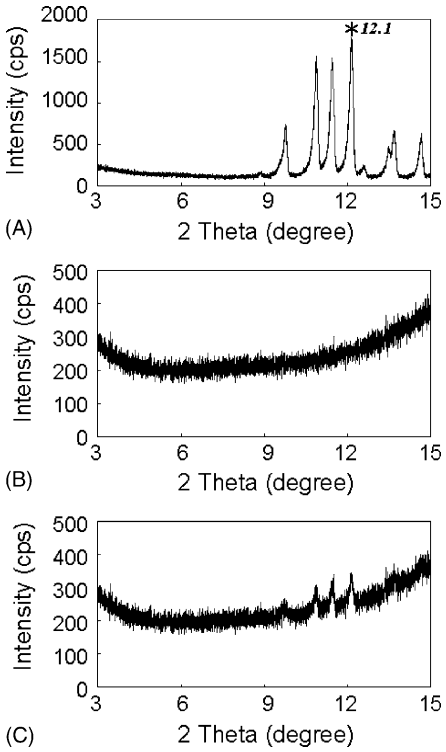

Fig. 1. Observed diffraction patterns of 100% crystalline CP (A) and 100% amorphous CP (B) and simulated diffraction pattern of CP at detection limit (C) in the powder X-ray diffractometry. The peak marked with 12.1 was used for the quantification. (C) The crystalline diffraction pattern at detection limit (4.8%) was added to the amorphous diffraction pattern (95.2%).

There exists a serious problem in attaining the purpose of this report. Some theories have been developed so far to estimate the precision in instrumental analyses such as atomic and molecular absorption spectrometry and HPLC (Ingle and Crouch, 1988; Boumans, 1994; Hayashi and Matsuda, 1994; Matsuda et al., 1998). For theoretical estimation, however, the background noise alone has to be extracted without the contamination of the signals or obstructive instrumental output. Unfortunately, the diffraction pattern of the amorphous material comprises both the background noise and halo pattern (Fig. 1B).

In this report, the noise alone is extracted from the mixture of the noise and halo pattern in the powder X-ray diffractometry. Since the extraction process changes the noise intensities, the stochastic properties of the original noise have to be assessed.

Finally, the R.S.D. of the measurements and detection limit for the determination of the crystal contents are calculated on the basis of the stochastic properties of the original noise and crystalline diffraction pattern.

2. Experiments

2.1. Material

Cefditoren pivoxil crystal (Meiji Seika Kaisha) was used as the 100% crystalline sample. The spray-dried sample was prepared by the following methods. Three hundred grams of the CP crystal was dissolved into 18 l of dichloromethane. The solution was spray-dried with a spray drier (L-8, Ohkawara) at inlet air temperature of 100 °C. The supply rate of the solution was 100 ml/min. The rotation speed of the atomizer was 15,000 rpm. After it was confirmed that the X-ray diffraction pattern of the spray-dried sample was a halo pattern, the spray-dried sample was used as the 100% amorphous one. Physical mixtures (PM) containing 5.0, 10 and 20% of crystalline component were prepared by mixing the 100% crystalline and 100% amorphous samples in the specified weight ratios in a vortex mixer (NS-8, As One Corp.) for 10 min.

2.2. Powder X-ray diffraction

The samples were analyzed in a powder X-ray diffractometer (RINT 2100, Rigaku) equipped with a monochromator (graphite) and Cu Kα beam at room temperature. The conditions were: tube voltage 40 kV, tube current 40 mA, scanning interval 0.002°, scanning speed 0.4°/min and scanning angle 2θ = 3–15°.

2.3. Estimation of precision

2.3.1. FUMI theory

If the noise in the diffraction pattern has the autocorrelation (i.e., not the white noise), the measurement R.S.D. will be difficult to estimate (Ingle and Crouch, 1988). This report adopts the function of mutual information (FUMI) theory, which enables the estimation by taking into account the noise made up of the white noise and Markov process (Hayashi and Matsuda, 1994; Matsuda et al., 1998).

The laboratory-made software, TOCO Version 2, or commercially available software, MAY2000 (Yazawa), was used for the calculation of the FUMI theory. The number of data points used for the analysis was 2048 sequential. The peak region over which the peak area was integrated involved 293 data points. The zero window, which was the region to determine the zero level of the *Y* axis by averaging the noise intensities, involved 40 data points.

2.3.2. Residual of a calibration curve

The relationship between the peak area and the content of the crystalline component was obtained by regression analysis using the X-ray diffraction patterns of seven measurements, each with 5.0, 10 and 20% PM samples. The square root of the residual variance thus obtained was taken as the estimated S.D. for the blank test.

2.3.3. Repetition with sample of low crystal contents

The S.D. of the peak area was calculated using the X-ray diffraction patterns of the seven measurements with 5.0% PM, and this was taken as the estimated S.D. for the blank test.

3. Theory

3.1. Noise extraction and correction

As mentioned above, the noise extraction and correction are essential to the accomplishment of the objective of this report. In the well-known measurement model, the observed halo pattern is assumed to be made up of the genuine halo pattern and random noise (Halo, Noise and Halo + Noise of Fig. 2). The two observed patterns, derived from the same material, differ only in the random noise. Therefore, the common halo pattern can be deleted by subtracting an observed pattern from the other. However, this noise extraction process enhances the noise (Enhanced Noise of Fig. 2).

The intensities of the random noises are implicitly assumed to be independent of each other (called the white noise). Under this assumption, it is easy to estimate the stochastic property of the original noise. Let the S.D. of the white noise be w. If two white noises are subtracted from each other, the resulting noise is known

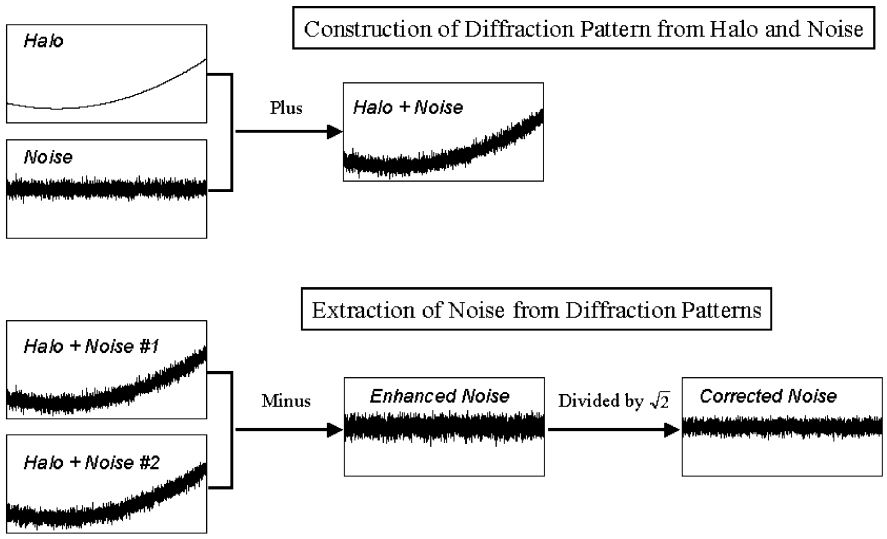

Fig. 2. Construction of diffraction pattern and extraction of noise.

to be white and the S.D. of the subtracted noise is $\sqrt{2}w$. This calculation is based on the error propagation rule (Ingle and Crouch, 1988; Day and Underwood, 1991). Consequently, we can obtain the S.D. of the original noise by dividing the S.D. of the enhanced noise by $\sqrt{2}$.

3.2. Types of noise

Many random variations in nature can be characterized by a fluctuation called 1/*f* noise (Hayashi and Matsuda, 1994). The 1/*f* noise is named after its power spectrum which is inversely proportional to frequency, *f*. However, there is a mathematical difficulty in treating the 1/*f* noise. In the FUMI theory, the 1/*f* noise is approximated by the mixture of the white noise and the Markov process. The power spectrum of the white noise is a horizontal line and that of the Markov process is downward sloping to the right. The power spectrum, *P*(*f*), of the mixed random processes takes the form (Hayashi and Matsuda, 1994):

$$P(f) = \frac{m^2}{1-r^2} \times \frac{2\alpha}{\alpha^2 + 4\pi^2 f^2} + w^2 \quad (1)$$

$$\alpha = \frac{(1-r)}{\Delta t} \quad (2)$$

where f is frequency, Δt is the data-sampling interval, m is the S.D. for the Markov process, r is the auto-correlation coefficient of the Markov process and w is the S.D. for the white noise. The first term of Eq. (1) represents the Markov process and the second term the white noise. The shape of power spectra is available for assessing the stochastic properties of real noises.

3.3. Precision profiles

The plot of the measurement R.S.D. against the concentration is called precision profile (Dudley et al., 1985). Fig. 3 illustrates the process for the R.S.D. estimation using the FUMI theory. The input of the FUMI theory is the actual crystal pattern (Signal) and noise (Corrected Noise), and the output is the precision profile and the crystal signal at the detection limit (Hayashi and Matsuda, 1994). The detection limit signal can be simulated from the smooth crystalline peak and halo pattern with noise as shown in Fig. 3.

The signal parameters necessary for the R.S.D. estimation are collected from the actual signal as shown in Fig. 1A. The noise parameters (m, r, w) are obtained by least squares fitting the theoretical power spectrum (Eq. (1)) to the observed one. Given a calibration curve, the measurement R.S.D. is described as a function of

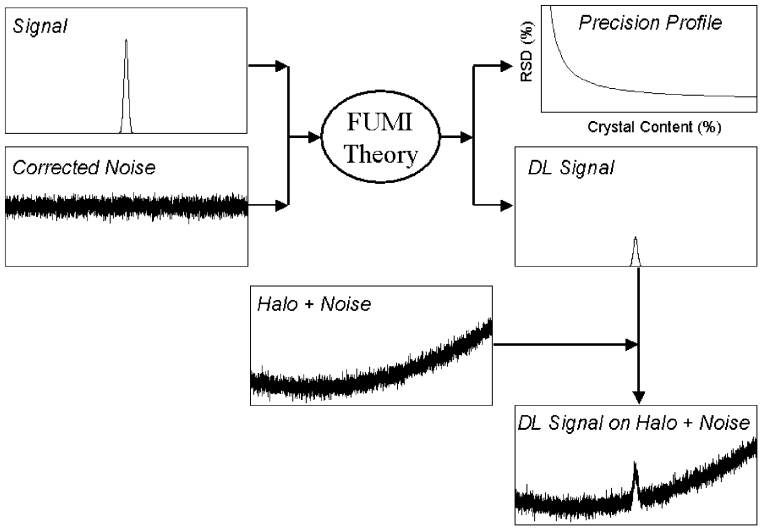

Fig. 3. Input and output of R.S.D. estimation theory (FUMI theory) and simulation of detection limit signal. DL: detection limit.

the concentration (here, crystal content) with the signal and noise parameters as constants.

We should note that the parameter that we can obtain by analyzing the noise power spectrum is the S.D. of the noise intensities, but our objective quantity is the S.D. or R.S.D. of peak measurements in the instrumental analysis. The measurement S.D. and R.S.D. can be calculated from the noise S.D. by the FUMI theory (Hayashi and Matsuda, 1994).

3.4. Detection limit

The definition of the detection limit is often given as:

$$\mathrm{DL} = 3.3 \times \frac{\sigma}{\mathrm{Slope}} \quad (3)$$

where DL is detection limit, "Slope" is the slope of the calibration curve and σ is the S.D. estimated for the blank samples or around the expected detection limit (ICH, 1996). By definition, the detection limit signal is equal to the signal which produces 30% R.S.D. of measurements (note that (σ/Slope)/DL = 1/3.3 = 30%, where σ/Slope means the S.D. of estimated crystal contents). In this report, the σ included in Eq. (3) is obtained by different methods: FUMI theory, residual of a calibration curve and repetition with samples of low crystal contents.

4. Results and discussion

4.1. Noise properties of power X-ray diffraction

Fig. 4A shows the subtracted pattern of amorphous CP, which looks like a random noise with no halo pat-

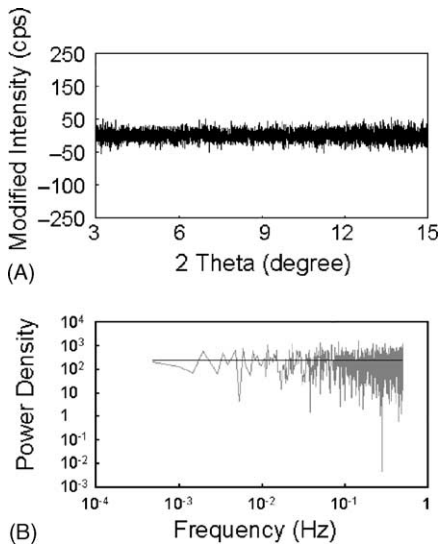

Fig. 4. The extracted noise pattern (A) and its power spectrum (B) of amorphous CP. (A) An observed diffraction pattern of 100% amorphous CP (Fig. 1B) was subtracted from another one derived from the same material. (B) Zigzag line: observed power spectrum; solid horizontal line: the least squares fitting.

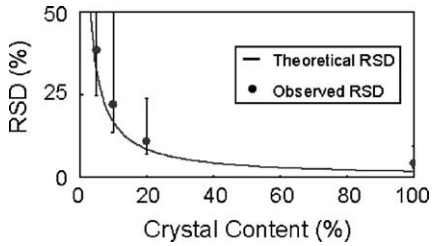

Fig. 5. Precision profile for determination of crystalline CP. Closed circle: the observed R.S.D. (n = 7); bar: 95% confidence intervals of observed R.S.D.; solid line: theoretical estimation by the software, TOCO or MAY2000 (FUMI theory).

tern. It corresponds to the enhanced noise in Fig. 2. In contrast to the halo pattern, the noise in the subtraction pattern is enhanced from the original one.

The stochastic property of the random noise can be examined by the power spectrum. In Fig. 4B, the zigzag line is the actual power spectrum of the subtracted amorphous CP pattern and the solid horizontal line is the theoretical power spectrum (Eq. (1)) that was fitted by the simplex least squares. This parameterization numerically indicates that the S.D., m, of the Markov process is negligibly smaller compared to the S.D., w, of the white noise. Therefore, the subtraction pattern can be concluded to be the white noise.

The precision profile of Fig. 5 shows the usual pattern of uncertainty in instrumental analyses, i.e., downward sloping to the right (Hayashi and Matsuda, 1994; Matsuda et al., 1998). Among the peaks in the powder X-ray diffraction pattern of the CP (Fig. 1A), the highest peak at $2\theta = 12.1^\circ$ was used as a target peak for quantification. The area of the peak was measured with the zero window as mentioned above. The experimental R.S.D. values of area measurements (closed circle) were obtained from seven samples of 5.0, 10, 20 and 100% crystal contents. The theoretical R.S.D. (solid line) was estimated based on the S.D. of the original noise which should be $1/\sqrt{2}$ of the subtracted noise. The theoretical estimates agree well with the R.S.D. values of the repeated measurements.

4.2. Comparison between FUMI theory and the conventional methods of estimation of the detection limit

As for the detection limit, the results of the three methods were listed in Table 1. The difference among the three methods is only the ways of estimating the S.D. of measurements. The FUMI theory is based on the stochastic properties of noise and signal (peak area, peak height, etc.), the second method applies the residuals of the least squares fitting to a calibration curve and the third method directly estimates the S.D. from the experimental data (n = 7). The values of detection limits calculated by the three methods are comparable, indicating the successful applicability of the FUMI theory in the powder X-ray diffractometry.

Table 1
Detection limit (crystal content) determined by various methods

Method	Detection limit (%)
FUMI theory[a]	4.8
Residual[b]	6.3
Repetition[c]	5.5

[a] In FUMI theory, the S.D. of Eq. (3) was obtained from a single measurement, but a calibration curve prepared with 20% PM (n = 7) was used.

[b] In residual, the calibration curve was drawn from 5.0, 10 and 20% crystal contents (n = 7 for each content), and the residual was calculated for the S.D.

[c] In repetition, the responses of the 5.0% crystal content samples (n = 7) were used for the S.D.

The diffraction pattern of the CP at the detection limit (=4.8% crystal content) was superimposed over the 100% amorphous pattern (see Fig. 1C). From visual inspection, the signal-to-noise ratio (S/N) of the crystal peak at $2\theta = 12.1^\circ$ seems to be about three to one. This S/N corresponds to the widespread understanding of detection limit.

If the noise in the amorphous diffraction patterns (Fig. 1B) was characterized by an auto-correlated process such as Markov process and $1/f$ noise, the extraction and correction of the noise would be more difficult than proposed in this paper. Fortunately, the noise in the amorphous diffraction pattern of the CP is proved to be the white noise in the powder X-ray diffractometry used. Under the assumption of the white noise, the intensity at a data point is independent of the intensities at neighboring data points and this independency enables the correction (division by $\sqrt{2}$).

Similar experiments were carried out to identify the noise property of the amorphous lactose. Fig. 6 shows the power spectrum of the enhanced noise in these experiments. An equivalent result and the same conclusion were drawn. The method proposed here would be

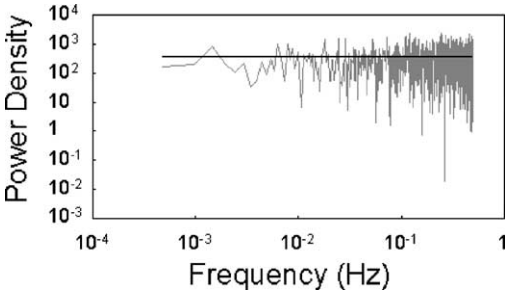

Fig. 6. The power spectrum obtained from the experiment using amorphous lactose.

applicable to many quality tests of amorphous drugs in power X-ray diffractometry.

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