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# MORPHOLOGICAL AND FRACTAL-BASED METHODS DESCRIBING INSULIN ZINC CRYSTAL HABITS

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## ABSTRACT

Computerized image analysis was used to obtain Fourier contour parameters and the fractal dimension of insulin zinc crystals (IZC). These methods are capable of characterizing and identifying small changes in the morphological and surface parameters of IZC with different crystal habits during dissolution. The Fourier contour analysis and the fractal dimension measurement technique described in this report could be useful tools to control batch-to-batch variations and to predict differences in the dissolution behaviour of IZC with different crystal habits.

## INTRODUCTION

Crystal habits may arise from differences in the relative growth rate of crystal faces. Work on crystal engineering in the past 10 years has

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demonstrated that crystal habit can affect important physical and biopharmaceutical properties of drug crystals. In crystallization from solution, the relative growth rate of individual surfaces can be varied by controlling such conditions as the agitation rate, presence or absence of small concentrations of additives[1-3] or by varying initial supersaturation[4]. Different dissolution rates have been linked to crystal habit variation. For example, faster rates have been observed with bipyramidal and acicular crystals than with platy crystals of nickel sulphate α-hexahydrate[5,6]. Similarly, cubic-shaped habits of phenytoin dissolved about 1.5 times faster than their needle-shaped counterparts[7]. Recently, crystal shape determined by the length-to-width ratio was employed to account for habit-related variations in the intrinsic dissolution rate[8].

Insulin zinc crystals (IZC) used for the preparation of long-acting suspensions are obtained from animal origin or manufactured by genetic engineering. It is important to maintain consistent crystal size, size distribution and crystal habit in insulin suspensions to assure similar kinetic profile and bioequivalency. In a previous study, it was demonstrated that IZC of different origins exhibit variations of their morphic features. Such variations can be quantitatively described by computerized image analysis of crystal contours[9].

The purpose of this study is to show that Fourier contour analysis and the fractal approach can rapidly characterize small changes in the crystal habit of IZC and can be valuable in predicting potential alterations in the dissolution kinetics of these crystals.

## MATERIALS AND METHODS

### Materials

Two commercial IZC$^S$ were used: 1. Ultralente Insulin Suspension (100 U/ml) of beef-pork origin (Connaught Novo lot # 979009 - Product

U1); and 2. Ultralente Iletin I Suspension (100 U/ml) also of beef-pork origin (Eli Lilly lot # 4PM94A - Product U2).

**Methods**

Dissolution tests were carried out in a cell containing 100 ml 0.2 M tris(hydroxymethyl)amino-methane buffer at pH 7.5. The buffer was maintained at 37 ± 0.5°C, using a water jacket and a circulating water bath. While stirring at a fixed rate, 1 ml of insulin zinc suspension was added to the buffer. Two ml aliquots were withdrawn at 1 min and 5 min dissolution times. The samples were then filtered (Millipore, HA 0.45 μm), and the crystals were transferred to a microscope slide for image analysis. The dissolution test was repeated at two different agitation speeds: 50 and 150 rpm, which were maintained by synchronized motors.

Fourier contour analysis was undertaken with the aid of computer program to characterize profiles by generating Fourier coefficients[10,11], which were employed to calculate shape descriptors[12,13]. For this purpose, images of the crystals were digitized, using an image analysis system reported previously in this journal[14].

Scanning electron microscopy was performed before dissolution and at different stages of dissolution (Jeol Model JSM-820, Tokyo, Japan).

Perimeter fractal dimension was assessed according to the walk-around step described elsewhere[15]. At least 100 perfectly grown crystals from randomly selected fields were analyzed for each parameter.

## RESULTS AND DISCUSSION

Fourier contour analysis of the amplitude spectra of U1 and U2 before dissolution is reported in Figure 1. The amplitude of normalized Fourier coefficients versus the harmonic number represents the signature of crystal profile[14]. Differences in amplitude over a broad range of harmonics between U1 and U2 were clear. For example, the coefficient of

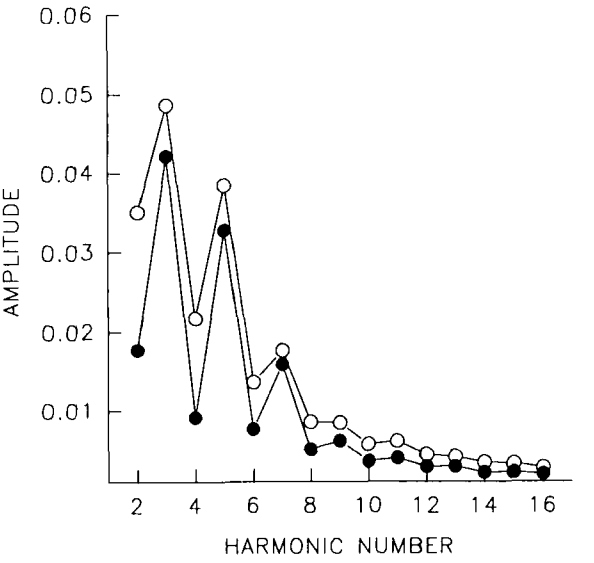


**Figure 1.** Amplitude spectra of U1 crystals (open circles) and U2 crystals (filled circles)

variation at harmonic number 4 was: $2.26 \times 10^{-2} \pm 4.2 \times 10^{-4}$ (variance) for U1 and $1.23 \times 10^{-2} \pm 1.7 \times 10^{-4}$ (variance) for U2. A slight change in operating conditions during IZC crystallization can alter crystal habit and bring about significant variations in particle signature (Figure 1).

Perimeter fractal dimension (PFD) was obtained from Richardson plots according to the walk-around step method[15]. The values obtained were PFD = 1.042 (r = 0.905) for U1 and PFD = 1.024 (r = 0.930) for U2, where r is the correlation coefficient of the fitted lines.

Linear plots of fractal dimension increments (FDI) of the particle populations were generated on a log-probability scale. Such plots demonstrated the range of fractals and FDI scatter of the two IZC$^S$ (Figure 2).

The variations observed in Fourier descriptors combined with PFD demonstrate that small changes in crystal habit can be easily detected and used to monitor batch-to-batch variations.

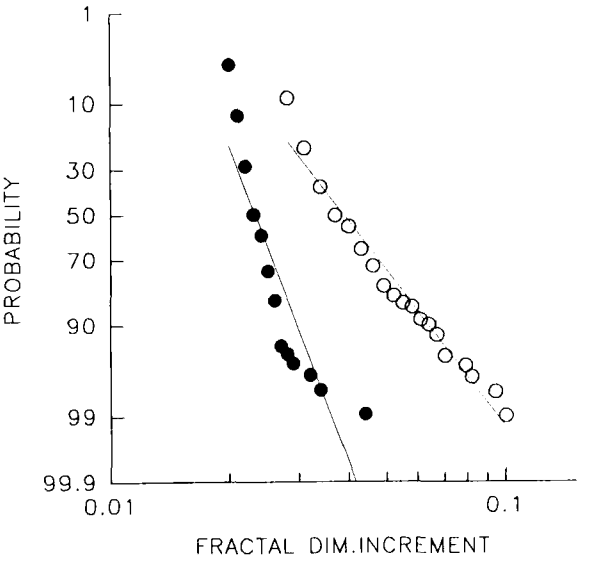


**Figure 2.** Log-probability plots of fractal dimension increment (FDI) of U1 crystals (open circles) and U2 crystals (filled circles)

Scanning electron micrographs of U1 and U2 taken before dissolution revealed these differences (Figure 3a and 3b).

**Effect of Dissolution on Shape and Surface Geometry**

To characterize the morphological changes of crystals during dissolution, Fourier descriptors were determined under different dissolution conditions. Quantitative differences in surface irregularity between the crystals are reflected by the degree of roundness and shape factor. Table 1 illustrates the changes in degree of roundness with varying agitation speeds. These values represent the average of at least 10 perfectly grown crystals and their corresponding standard deviations. It can be seen that unlike U1, U2 undergoes significant deformation during dissolution.

This is confirmed in Table 2 which shows significant shape factor variations. Shape factor, the ratio of perimeter L and area A of the closed boundary ($L^2/4\pi A$), represents a measure of contour complexity[11]. The

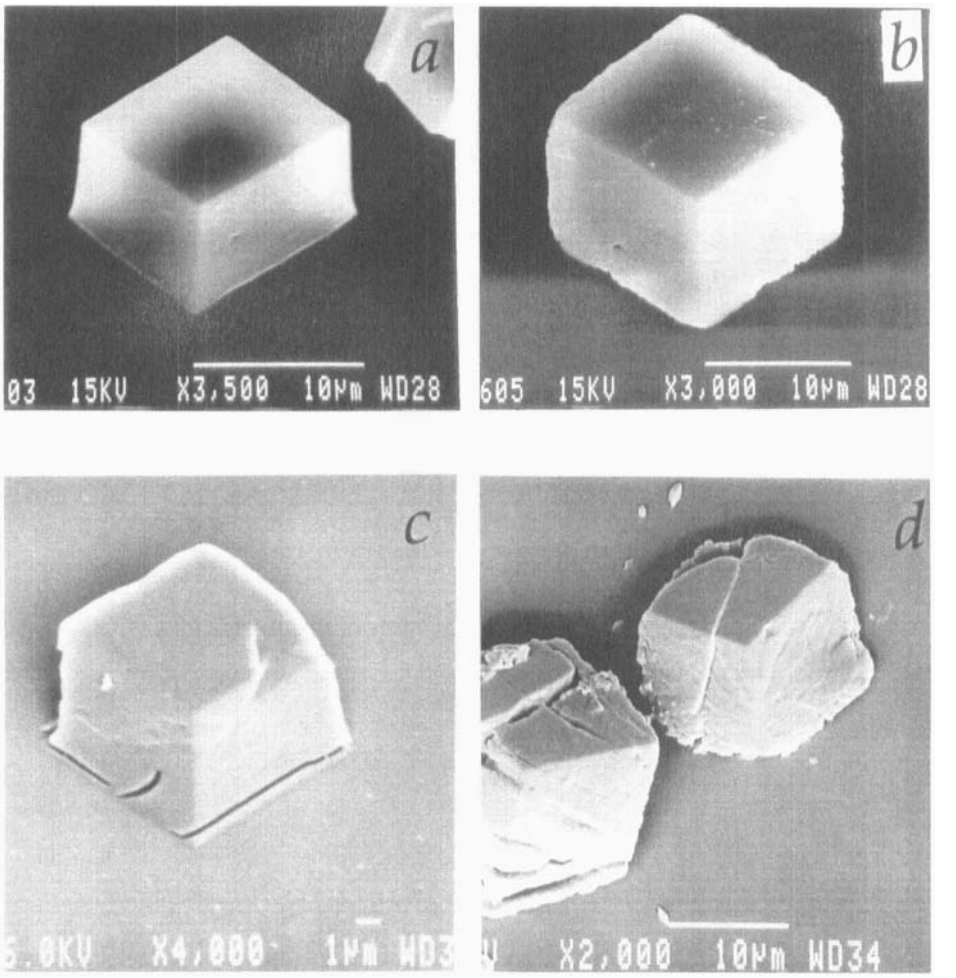


**Figure 3.** Scanning electron micrographs of insulin zinc crystals; a and b are U1 and U2 before dissolution and c and d are U1 and U2 after 5 min dissolution respectively.

**TABLE 1**

Roundness (Mean ± S.D.) at Different Agitation Speeds

| IZC | No Agitation | 50 RPM | 150 RPM | Level of Significance |
|---|---|---|---|---|
| U1 | 0.691 ± 0.039 | 0.690 ± 0.030 | 0.651 ± 0.035 | n.s. |
| U2 | 0.729 ± 0.062 | 0.673 ± 0.056 | 0.614 ± 0.033 | $p < 0.05$ |

**TABLE 2**

Shape Factor (Mean ± S.D.) at Different Agitation Speeds

| IZC | No Agitation | 50 RPM | 150 RPM | Level of Significance |
|---|---|---|---|---|
| U1 | 1.298±0.048 | 1.295±0.065 | 1.337±0.054 | n.s. |
| U2 | 1.234±0.090 | 1.318±0.075 | 1.472±0.183 | $p < 0.05$ |

increase in the shape factor of U2 was larger than that of U1, and contour irregularity of the U2 crystal increased with greater agitation.

The fact that anisotropic crystals undergo changes in surface geometry during dissolution can best be described by fractal analysis. The fractal dimension of crystals is a measure of surface irregularity[16-18]. Table 3 shows the PFD obtained before and after IZC dissolution. Linear plots of FDI were generated on a log-probability scale for U2 (Figure 4). For U1, FDI scatter was almost identical before and after dissolution. Scanning electron micrographs taken after 5 minutes of dissolution confirmed this observation (Figure 3c and 3d).

Recently it has been demonstrated that at least under some circumstances the fractal dimension of physically observed object reflects quantitatively the physical process that produced it[19,20]. This work indicates that variations in the surface geometry of IZC during dissolution are related to crystal habit. Such variations could lead to changes in the surface area accessible for dissolution and, consequently, affect overall dissolution rates. These observations could explain the findings of Graham and Pomeroy who reported wide differences in the dissolution rates of commercial insulin formulations[21]. Moreover our findings support the views of Farin and Avnir[22] that fractal dimension may be more important

**TABLE 3**

Changes in the Perimeter Fractal Dimension (PFD) of IZC after 5 Minutes of Dissolution

| | U1 Crystal | | U2 Crystal | |
|---|---|---|---|---|
| | PFD | r* | PFD | r* |
| Before Dissolution | 1.042 | 0.905 | 1.024 | 0.930 |
| After Dissolution | 1.047 | 0.906 | 1.033 | 0.922 |

* r is a correlation coefficient

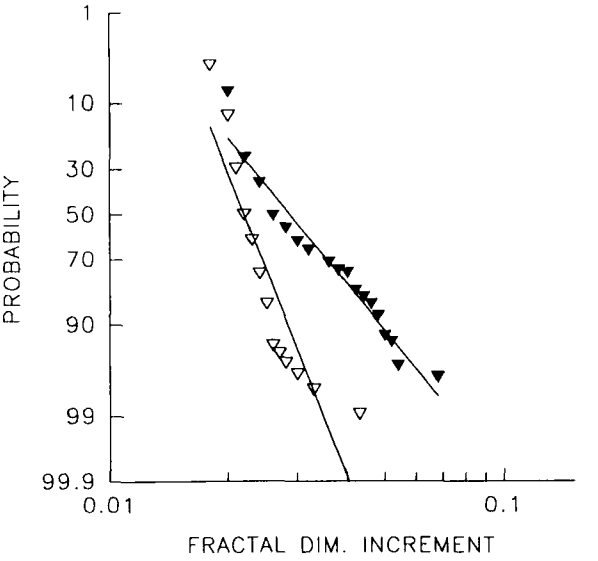


**Figure 4.** Log-probability plots of fractal dimension increment (FDI) of U2 before (open triangles) and after dissolution (filled triangles).

than previously thought in deriving more rigorous dissolution equations that can adequately describe the kinetics of powder dissolution.

## CONCLUSION

This study describes how particle signature and PFD can be used to characterize and differentiate between the crystal habits of IZC. The results obtained indicate that small changes in morphic features produced during crystallization and dissolution can be quantitatively assessed by these parameters. Their application could be helpful in controlling batch-to-batch variations and in predicting differences in the dissolution rates of IZC and other crystal habit modifications.

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