FRACTAL CHARACTERIZATION OF DEXAMETHASONE SEM IMAGES

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Keywords: fractal, SEM, Hausdorff dimension, image processing, box-counting

Abstract: *Shape of most objects in nature is difficult to estimate using Euclidian geometry because their component elements look to be similar to the whole objects viewed at different scales, but not the same, as manmade objects are. Crystals are an exception. Analysis of the structure of these crystals was performed on a pure substance, dexamethasone. Qualitative and quantitative analyzes of digital images taken from an electronic microscope were performed at eight scales by using magnifiers between 600 and 80000. The quantitative analysis was based on the Hausdorff dimension evaluation for fractal objects. The simulated results show the range of fractal dimensions for each image scale according to the threshold at which the black and white images were transformed.*

1. INTRODUCTION

The parameters of manmade objects are given as a result of comparison with other entities called units. These objects are obtained by adding their component parts, and common term used in Euclidian geometry is algebra (from the Arabic: *jabara*) meaning to put together the parts. The topological dimensions are integer numbers (0 for a point, 1 for a line, 2 for a surface, 3 for a volume). From points, lines, areas and volumes is possible to get the basic shapes of traditional Euclidian geometry: triangles, squares, circles, cones, cubes, spheres, and so on. Each Euclidian dimension can be determined by a characteristic scale, and when these images are viewed at higher magnification, they do not reveal any new characteristics.

The physical objects in the real world are composed by a lot of “sub-units” depending on the scale which they are viewed. Actually, these “sub-units” are not the same; they have the same statistical properties as the whole object structure when they are examined at any scale. In order to understand and quantify the aspects regarding the pattern, shape and complexity of these objects, a new geometry was developed by Benoit Mandelbrot [1]. Actually, it was a purely mathematical concept using fractional dimension associated to the term *fractal* (coming from the Latin: *fractus*, derived from *frangere* which means to break into pieces), [2].

The fractal dimension describes the surface irregularity, the roughness, or how much the object surface occupies the space. The estimation of fractal dimension is based on measuring of the image characteristics where self-correlation there is. The fractal dimension is a parameter that can characterize the surface irregularities of solid materials at the supermolecular level from macroscopic to microscopic and nanoscopic scale. It does not uniquely describe patterns, and its information is not enough to reconstruct the real objects. Usually, the fractality is presented only over a limited range of scales, but intrinsic dimension has the ability to summarize the whole dataset in one value [3].

Biopharmaceutics and pfarmacokinetics use the concept of homogeneity for ideal drugs. The particle shape is assumed to be an ideal sphere, ellipsoid or parallelepiped having a smooth surface. But, in real cases, the situation is not so, and the shape cannot be easily described using deterministic models [4, 5]. The drug irregularities must be examined carefully because they are a very important clues regarding water solubility. More than, the irregularities of different sorts of drugs have to be compared not only qualitatively, but also quantitatively. The drug images are taken from different microscopes. From practically point of view, several problems have to be solved. The first aspect concerns to the influence of scale upon the fractal dimension value. Another one is the dependence of FD on the threshold at what the white and black image is processed, or the choosing of color which will be used (black, white or partial black and partial white).

The objectives of this paper are: the presentation of basic principles for fractal geometry evaluation, the giving an example of application for drug surface evaluation by electronic processing of the images taken from an electronic microscope, and the testing of fractal dimension dependence into a very large range SEM magnifiers.

2. MATERIALS AND METHODS

Dexamethasone is a very potent synthetic glucocorticoid used for treatment of inflammatory, autoimmune or oncologic conditions. Routes of administration of this active substance are limited by its poor water solubility. Between solubility and aggregate irregularities there is a certain relation [6]. The dexamethasone aggregates were investigated by a scanning electron microscope, SEM. The sample images are obtained from the detection of secondary electrons of the dexamethasone atoms excited by an electron beam. The SEM images have a very good resolution: 1024x1024 pixels, and were obtained from samples by multiplying between 600 to 80,000 times, corresponding to a linear resolution of 100 μm/div to 0.5 nm/div, divided into 8 scales.

Before fractal dimension calculation, the SEM image conversion from grey scale to black and white has to be done, based on an intensity threshold value. There are some criteria to choosing the optimum threshold, when pixels with intensity less than this threshold become white, and the other pixels become black.

In the next step, a grid of squares having the side equal to ***r*** is superimposed on the black and white image. We denote the number of squares from the resulting image that can be completely black, white, or partially white by the numbers $\mathbf{N_B}$, $\mathbf{N_W}$, and respectively $\mathbf{N_{PW}}$.

The fractality will be evaluated by using Hausdorff dimension (or self-similarity dimension). In an affine space, a set of objects S is self-similar if this set can be obtained by the union of $N(r)$ not overlapping copies of it scaled with ratio r. From mathematical point of view, fractal dimension, DF, is given by the following relation [7, 8]:

$$DF = \lim_{r \to 0} \left(\frac{log(N(r)}{log(1/r} \right) \qquad (1)$$

where $N(r)$ is for the number of squares from the grid image. The method described before is called box counting method, and it is the most used one for fractal analysis [9].

3. RESULTS AND DISCUSIONS

A set of 8 images were taken for dexamethasone from SEM. Each image is investigated by following algorithm:

a - a grid of squares is added to the black and white image, r=3-200;

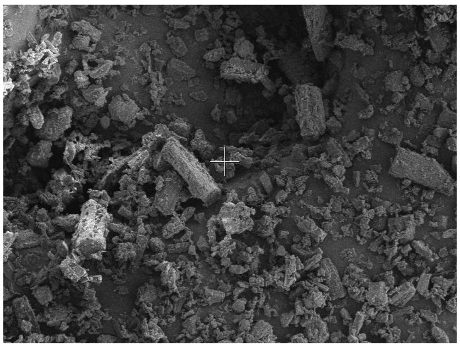
b - for each particular threshold, a black and white image is obtained;

c - an ascending value from 3 to 250 is set up for threshold;

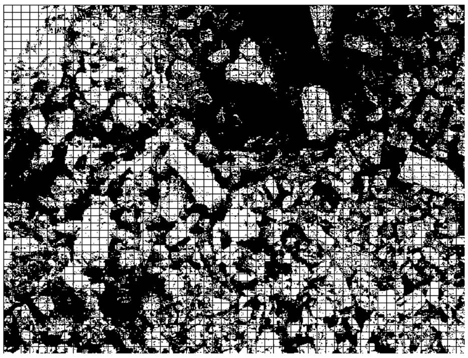
d - the number of black, white and partial white squares are counted;

e - the fractal dimension is evaluated from (1) for each pair of values, r-N.

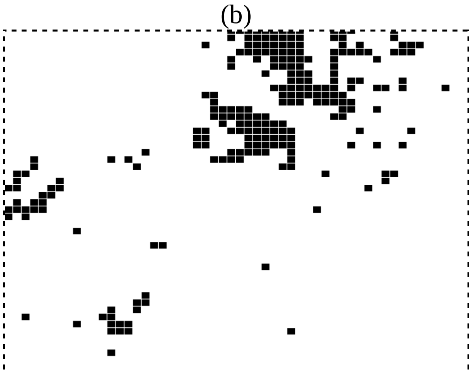
The first image, shown in Figure 1 (a), depicts dexamethasone SEM image multiplied by 600x, or 100 μm/div. Figure 1 (b) shows the black and white image and grid, for the side of squares of r=11 pixels.



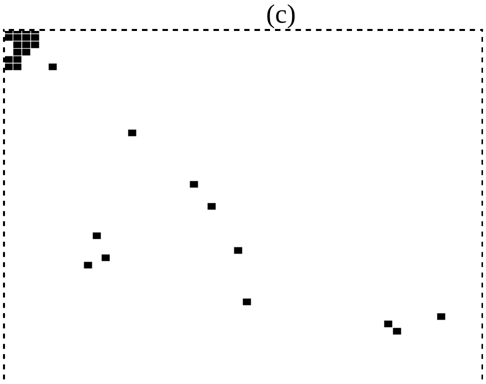
(a)



(b)



(c)

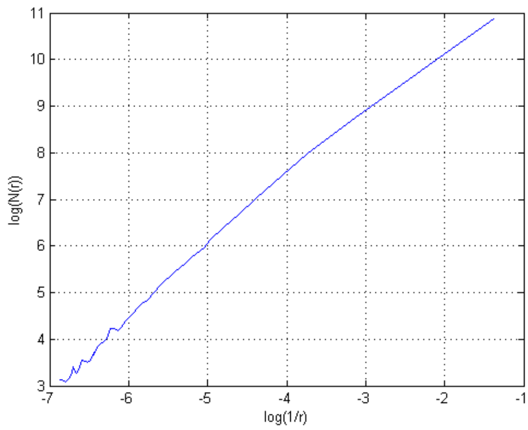


(d)

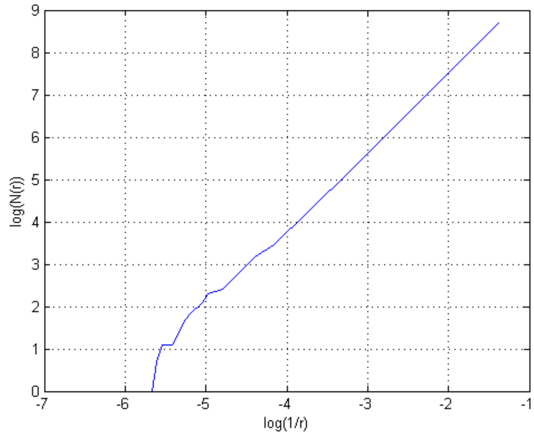
Fig. 1 The dexamethasone SEM image with linear resolution of 100 μm/div (a), a grid of r=11 (b), the images of black squares (c) and white squares (d).

The threshold was set up to 50. The following two figures, 1(c) and 1(d), show the squares corresponding to two situations of pixels inside of them: completely black, N_B, and white, N_W, respectively.

A Matlab program was written to add all N_B and N_W squares when the square size, r, was changes between 3-200. Fig. 2 (a) and (b) show this dependence on a log-log scale. According to (1), the following values were obtained for fractal dimensions: FD_{NB}=1.3717 and FD_{NW}=1.6291. These values correspond to the slopes of the straight lines from Fig. 2 (a) and (b). When it was taken into consideration the irregularities of white squares, the roughness was higher than for black squares as a result of the effect of secondary electrons in SEM.



(a)



(b)

Fig. 2 Dependence of black (a) and white (b) number of cells versus square dimension

In the second step, the threshold changed to cover the whole range pixel intensity. The algorithm described before was repeated, and the results were shown in Fig. 3 for black squares. It is easy to see that the fractal dimension is about the same for all threshold values.

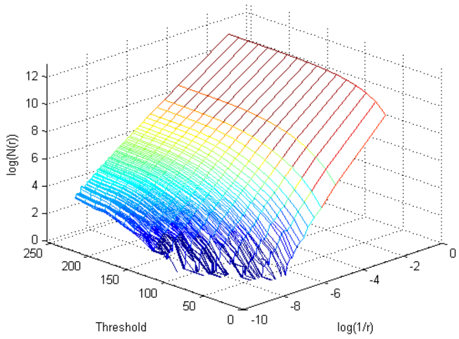
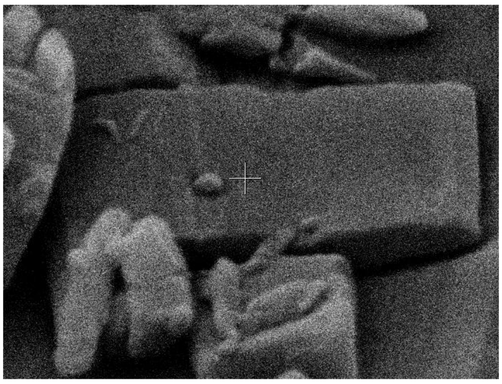
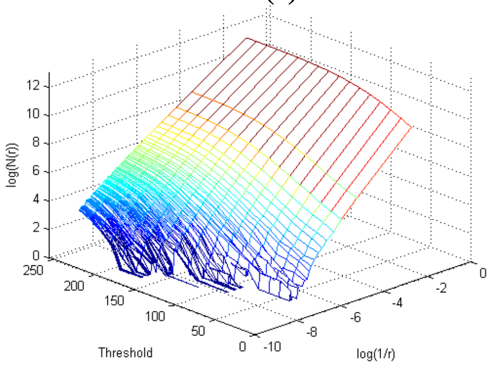


Fig. 3 3D representation of fractal dimension



(a)



(b)

Fig. 4 SEM image and 3D representation of fractal dimension for the highest SEM magnification

The SEM dexamethasone image for the highest resolution, 0.5 µm/div, corresponding to a magnify of 80.000x, is shown in Fig. 4 (a). The results presented in Fig. 4 (b) show that the fractal dimension does not depend too much on threshold value for black squares. In this scale, by applying (1), the following fractal dimensions were obtained: FD_{NB}=1.4193 and FD_{NW}=1.8659.

4. CONCLUSIONS

The dexamethasone drug irregularities were evaluated from SEM images having resolutions between 100 µm/div-0.5 µm/div by using fractal dimension. The influence of choosing different threshold values was also analyzed. Fractal dimension is about constant on a certain scale, and for all threshold levels. FD can be used to specify the drug morphology by microscopic investigations.

5. REFERENCES

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