**COMPARATIVE DIGITAL ANALYSIS OF DIFFERENT PHENYTOIN CRYSTAL HABITS**

**ANALISIS DIGITAL COMPARATIVO DE DIFERENTES HÁBITOS CRISTALINOS DE FENITOINA**

**ABSTRACT**

The purpose of this work was to evaluate the information obtained using some digital techniques for measuring the external form of pharmaceutical solids, employing a combination of several contour descriptors by means of classical and mathematics descriptors and a modified method of texture analysis technique based on Gray levels co-occurrence matrix. In this case, phenytoin was used as a model, because changing some conditions in the recrystallization process different crystalline habits were obtained. The evaluations of the measurements were performed using techniques of principal component analysis and hierarchical cluster analysis. Final analysis indicated that applying the texture technique Gray levels co-occurrence matrix measurement, the results were similar to those obtained by combining contour measurement with classical and mathematics descriptors techniques, therefore, as the texture method is faster and easier to implement, could be a good alternative for use in quality control of multisource pharmaceutical solid.

**Keywords:** Descriptors, texture, crystallization, phenytoin.

**RESUMEN**

El propósito de este artículo fue evaluar la información obtenida empleando algunas técnicas digitales de medición de la forma externa de sólidos farmacéuticos, utilizando conjuntamente varios de descriptores de contorno, mediante descriptores clásicos y matemáticos, además de un método modificado de análisis de textura basado en la técnica de Matriz de Co-ocurrencia de Niveles de Grises. En este caso, se utilizó la fenitoína como modelo, ya que cambiando algunas condiciones del proceso de recristalización, se producen diferentes hábitos cristalinos. La evaluación de las mediciones se realizó con ayuda de las técnicas de análisis de componentes principales y el análisis de conglomerados jerarquizados. Los datos finales indicaron que con la técnica de Matriz de Co-ocurrencia de Niveles de Grises de medición de textura, se obtuvieron resultados similares a los obtenidos combinando los descriptores de medición de silueta clásicos y matemáticos, por lo tanto, por ser la técnica de textura más rápida y sencilla de aplicar, podría ser una buena alternativa para utilizarse en el control de calidad de las sustancias sólidas multiorigen de uso farmacéutico.

**Palabras clave:** Descriptores, textura, cristalización, fenitoína.

**INTRODUCTION**

External form characterization of pharmaceutical solids is important because it affects not only the properties of manufacturability, but also can have effect on the bioavailability properties. In this sense, phenytoin, an active multisource ingredient, used as an anticonvulsant and that presents significant changes in morphology depending on crystallization conditions and therefore, the crystal habit can vary depending on the crystallization procedure (1- 3).

The techniques of digital image analysis allow reliably in real time and faster particle morphology measurements than using manual method, however, management and routine processing are still difficult and less accessible (4, 5).

The common way to visual inspection of crystal habit is to compare the particle shape with reference shapes, as they are simple and easy to measure digitally as opposed to the actual particles that are more complex, if are used routine analytical techniques of high performance (6). These descriptors based on the particle geometrical shape, are called classical descriptors (CD).

On the other hand, there are others techniques, named mathematical descriptors (MD) which includes a image contour description, founded on the measurement of spectral transform, like applying the Fourier transform, or those based on fractal analysis, or eigenshape analysis, or coordinates analysis, or methods based on measurements made on specific points of a figure and finally, it is also possible to find hybrid methods of the above techniques (7 - 9). Most of MD have variants to describe either contour or surface of particles, because an image is presented not only as a silhouette, if not containing regions with surfaces.

Particle surface evaluation has the advantage of describing an entire region, doing procedures of measure more robust and provided better information that only contour measures, although more complex interpretation. Texture analysis is a method which describes merely the surface and is a good alternative to the difficulties encountered in the descriptions using as a reference geometric shapes (10-12).

**Descriptions based on the particle contour**.

Classical descriptors (CD) use different formulas depending on the software employed, therefore, these measures should be analyzed based on the equation applied instead of the descriptor name, to avoid confusion (13, 14). Simplest CD evaluate size and shape, they evaluate the size and shape, rather than the particle length and make a statistical comparison with the diameter of a circle that has the same geometric properties (equivalent diameter). The most important measurement used is Féret diameter (DFeret), although there are other measures (13, 15).

The so-called *form factors* provide more information, because they compare the diameter measured of particle contour, in relation to the area or perimeter of a circle (2D) or sphere (3D).

However, none of these CD can specify precisely when a particle is exactly circular, or have vertices, or is rough, in same way they can confuse regular or irregular shapes when having similar ratio between length and width. In what appears to be consensus is on use of several CD simultaneously instead of only one to achieve an acceptable degree of external form definition (16).

Among the measurement parameters more explored are find: circularity (or its inverse, elongation), the presence of vertices and the roughness (surface roughness), homogeneity, solidity or image compaction, understood in a topological sense (17).

Although the CD are still widely used, the MD such as, fractal dimension and calculations by Fourier Transforms, are very likely to be promoted more comprehensive techniques with which can be measure both the edges as the particle surface, however, its interpretation remains complex, besides have different calculation approaches, thus making comparisons should be used with reference to previously constructed databases (18).

**Descriptions based on image texture.**

Unlike the images without texture, which can be processed exclusively based on contour features, texture is an inherent surfaces property (19). The texture characterization by means digital image is information from the statistical distribution of pixels differences in an image area, which can be coded by binary arrays and can be read as patterns changes or levels of gray intensity, which represent changes in surface elevations (20).

The main difficulty in texture characterization lies in the differentiation of tonality, since both (texture and tone) are complementary in an image, but is there a way to distinguish them empirically: small variations in gray indicates tone dominance; while large variations in small area is an indication of the texture influence (21).

**MATERIALS AND METHODS**

Benzoin, nitric acid, acetic acid, urea, sodium hydroxide pellets (Carlo Erba®), hydrochloric acid (HCL, Merck®), ethyl alcohol absolute anhydrous (J. T. Baker®), acetonitrile (Merck®), n-hexan (Merck®), all reactive grade.

Infrared Spectroscopy Fourier Transform (IR-TF) by PerkinElmer Spectrum BX®, the solid samples are diluted in KBr; X-ray diffractometer, Rigaku Miniflex®, source of Cu, Ka1 (1.542A˚) radiation, with angle between 3° y 50° are used; Thin layer chromatography (TLC) plates were made in aluminum and silica gel, 60 F254, Merck®; Differential Scanning Calorimeter (DSC), Netszch fox-200, Al crucibles and Ni atmosphere; Optical light microscope (BOECO BM-180 T/SP); Digital Camera, Webcam Microsoft Lifecam Vx6000.

Phenytoin was synthesized by us according to the following synthesis method: Starting from benzoin, this is oxidized with nitric acid in acetic acid medium to obtain the benzyl diketone. The benzyl compound obtained is put under reflux with an alcoholic solution of urea alkalinized with NaOH (30%) and then acidified with HCl (2M), to obtained 5,5-diphenyl-2 ,4-imidazolidinedione (Phenytoin).

*Identification and chemical structure evaluation of substance*: IR spectrum (500-3000 cm-1) and Powder X-ray diffraction (PXRD) patterns, is performed by comparing experimental data with database references (3, 22); Melting point by DSC (298 °C), and purity by TLC (using a mixture of ethyl acetone - chloroform (1:9 v/v) as mobile phase) after purification by successive recrystallizations of starting product.

This work describes only crystallization procedures on which different samples were obtained. In all of them, phenytoin is dissolved in each solvent, in the boiling point of the solvent and still hot and supersaturated, the solution is filtrated: The substance dissolved in ethanol, immediately leads to a temperature of -5 °C, for the sample M4. Phenytoin dissolved in acetonitrile, is left to reach an approximate temperature at 25 °C and then allowed to cool (10 -15 °C) until the formation of crystals, this corresponds to M7. Phenytoin dissolved in acetonitrile and to which deionized water is added quickly at 10 °C (equivalent to twice the volume of acetonitrile) and with constant stirring and filtered under vacuum to obtained M8. Phenytoin dissolved in hexane and left at room temperature for 24 hrs, produces M18. The substance in acetonitrilo, with the rapid addition of hexane and cooled to 10-15 ° C, produces M22 sample. In the collection of samples M4, M7, M18 and M22 and after the formation of crystals, the solvent is allowed to evaporate completely.

By texture test, stainless steel sieves are used, with an opening of 149 and 125 microns. Crystalline habits microphotographs are taken utilizing a digital camera and an optical light microscope.

**Assessment particles contour.**

*Shape Calibration*. As benchmarks in measurements with the software ImageJ (Rasband, W. National Institute of Mental Health, Bethesda, Maryland, USA), was carried out a preliminary assessment by measuring the figures of a circle, a rough circle, a square, a rough square, a rectangle, a columnar shape, a symmetrical star and a elongated star, see figure 1, in order to assess the relationship with common geometric shapes and the variation caused by the angularity and roughness.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| C:\Users\Oscar\Desktop\Desktop\1. Descriptores\Calibración de descriptores\circulo.tif | C:\Users\Oscar\Desktop\Desktop\1. Descriptores\Calibración de descriptores\circuloRug.tif | C:\Users\Oscar\Desktop\Desktop\1. Descriptores\Calibración de descriptores\Cuadrado.tif | C:\Users\Oscar\Desktop\Desktop\1. Descriptores\Calibración de descriptores\RectánguloRugoso.tif | C:\Users\Oscar\Desktop\Desktop\1. Descriptores\Calibración de descriptores\Rectángulo.tif | C:\Users\Oscar\Desktop\Desktop\1. Descriptores\Calibración de descriptores\Columnar.tif | EstrellaSimetrica2 | EstrellaLarga |
| a | b | c | d | e | f | g | h |

**Figure 1**. Geometric shapes in binary colors: a, circle; b, rugged circle; c, square; d, rugged square; e, rectangle; f, columnar shape; g, symmetric star; h, elongated star.

**Measure particles contour.**

From several microphotographs of each sample, particles are selected representing the external form, are isolated and converted into binary pixels using ImageJ software and then make the measurements:

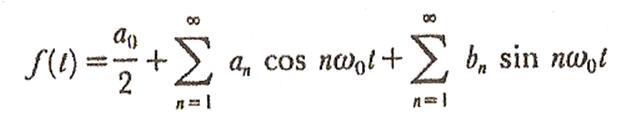
*Classical descriptors*:Four representative descriptors are selected, based on the results of the measurements and they are invariant with respect to particle size, besides they can be an approximation to measures of circularity, roughness, angularity and compaction. Also ImageJ is used, with plugins like particles8 and ShapeDescriptor1p. The following descriptors are selected and it showed equations with which are make the measurements: Aspect Ratio (AR): DFeret/amplitude; Roundness: 4Area/(Π\*(Larger diameter)2; Solidity: Area/Convex area; Regularity: Area/**(**Perimeter)2.

*Mathematics descriptors*: This study use radial Fourier descriptors (RFD) and the fractal dimension (DFractal), to make only the particle contours description.

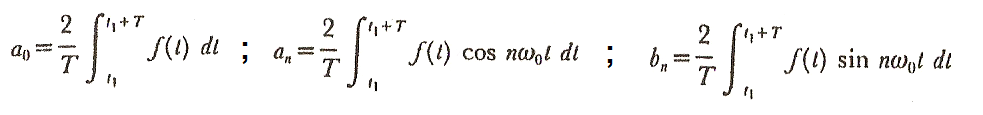
RFD analysis consists to take the edge of the original image and from a point inside the bounded area by this edge, the radials distance are measured to generate the function:

R = f(t) **Equation 1.**

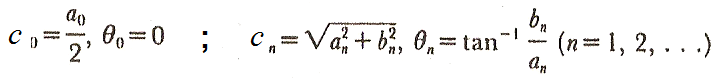
and thus obtained the coefficients of Fourier trigonometric series

 **Equation 2.**

where,



Taking coefficients *an* and *bn*, it can be calculating another, *cn*, which relates them as follows:



For identification and recognition of images through RFD, *an* and *bn* coefficients or *cn* coefficients can be used, according to the specific application, in this case *cn* (c0, c1, ..., c14 in this article) it used, by mean of the Matlab calculation package tools (Image Processing Toolbox. The mathworks, Inc. 1984-2007) (23).

Contour measurement using fractal dimension (DFractal), employs the box counting method, by which it can be calculate the figure perimeter length, through dividing the logarithm of self-similar pieces (N (m)) by the magnification factor "or size" (1 / m), according to the equation 3:

***DFractal = log (N(m))/log (1/m)* Equation 3.**

In practice, it can be plot the log (N (m)) vs log (1 / m), where DFractal is the slope.

The DFractal, quantitatively estimated the figure edge complexity with values between 1 and 2 for surface measurements, such as the edges of the figures and is invariant to the scale of measurement (24, 25).

The procedure used for measuring classical and mathematical descriptors is as follows: starting from microphotographs of recrystallized solid, independent particles are selected and binarized, then applied on the plugins of ImageJ, Particles8 and Shape descriptor1u, for classical descriptors selected; In the case of DFractal, is used FractalCount, also from imageJ and for the measurement of RFD, Matlab software is used.

**Assessment surface texture.**

The technique called gray level co-occurrence matrix (GLCM), is used to calculate second order images texture by "texture histograms" and measure the relationships or the likelihood of co-presence gray intensity between neighboring pixels *i* and *j*, which are at a distance *d* at an angle *θ*, getting a bidimensional array P*d, θ*(*i, j*) (26, 27). This matrix of co-occurrencia, evaluates in an image each one pixel neighbors, Mx × My (with x and y, binary numbers) and can be normalized by dividing each value obtained by the total pairs pixels of image.

In this case, it uses a modified procedure for the analysis of texture in the following way: The recrystallized solids are passed through a mesh of 149 microns with the help of a porcelain pestle; next, are take microphotographs of agglutinated particles without empty spaces, using an optical light microscope with a 10x objective. The images have a resolution of 640x480 pixels, which it must be converted to grayscale (8 bit) for analysis and are compared with the neighboring pixels that are at a 90° angle.

The entire image is used for this study and are selected, between 14 originally textural features proposed by Haralick et al., *Entropy* as a measure of ordering of the pixel values within a window and the *Inverse difference moment (IDM)*, representative of the contrast measurement (20, 28).

***Entropy****:* Randomized measure of intensity distribution. It provides information on "image regularity". Entropy is high when the elements Pd, θ (i, j) have similar values and is small when they are not.





*255*

*255*

[-ln *Pd,θ(i,j*)]

(*i,j*)

*Pd,θ*

**Equation 4.**

*j=0*

*i=0*

***IDM.*** It gives a homogeneity idea. The highest values are presented like as minor contrasts and largest elements are on the main diagonal line in the GLCM.

*j*

*i*

*P*

*i=0*

*j=0*

2

*d,θ*





1+(*i-j*)

)

,

(

Con *i ≠ j*  **Equation 5.**

**Statistical methods.**

Assessments are made both to the relationship between descriptors and silhouette calibrators, addition of the chosen descriptors and the different crystallines habits of phenytoin, the correlation technique by means of Principal Component Analysis (PCA) is used, which performs linear and independent combinations, of all initial variables which are orthogonal to each other to reduce the number to consider and establish new and smaller groups without significant loss of information. Then, taking these results as a criterion, the hierarchical cluster analysis (HCA) is used in all cases, through the square Euclidean method for nearest neighbors, to specify which variables are similar groups and collected the greater homogeneity among the samples analyzed.

In this case, PCA interpretation is based on plots of variance percentage vs. components or the graphical relationships of weights among the principal components; while for the HCA interpretation, are examined the graphical similarity representations by means of dendrograms which use cumulative percentage diagrams of samples. Those techniques are the most popular multivariate analysis methods (29).

**RESULTS AND DISCUSSION**

**Responses meaning by using these descriptors.**

Initially, it is necessary understand the obtained information by employing contour descriptors, applied in geometric calibration. Those are show in table 1.

**Table 1**. Values obtained by applying classicals and mathematical descriptors and geometric shapes selected as calibrators.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Circle** | **Rough**  **circle** | **Square** | **Rectangle** | **Rough**  **square** | **Columnar** | **Simet. star** | **Long star** |
| **Solidity** | 1 | 0.903 | 1 | 1 | 0.869 | 1 | 0.78 | 0.694 |
| **Round** | 0.96 | 0.97 | 1 | 0.58 | 0.91 | 0.19 | 1 | 0.19 |
| **RA** | 1.040 | 1.030 | 1.000 | 1.720 | 1.100 | 5.390 | 1.000 | 5.330 |
| **Regulari** | 0.6206 | 0.4459 | 0.6206 | 0.6206 | 0.3492 | 0.3242 | 0.2493 | 0.1001 |
| **Dfractal** | 1.9132 | 1.9106 | 1.823 | 1.8383 | 1.8201 | 1.8139 | 1.7437 | 1.5839 |
| **c0** | 0.9707 | 0.9019 | 0.8073 | 0.7303 | 0.5627 | 0.4274 | 0.8817 | 0.208 |
| **c1** | 0.0022 | 0.0031 | 0.0046 | 0.0086 | 0.0161 | 0.0046 | 0.0045 | 0.0174 |
| **c2** | 0.0097 | 0.008 | 0.0018 | 0.1189 | 0.0094 | 0.166 | 0.0011 | 0.0907 |
| **c3** | 0.0002 | 0.0136 | 0.0056 | 0.0029 | 0.0079 | 0.0084 | 0.0016 | 0.0138 |
| **c4** | 0.0016 | 0.0059 | 0.0542 | 0.0105 | 0.0287 | 0.0848 | 0.0013 | 0.0501 |
| **c5** | 0.0007 | 0.0075 | 0.0032 | 0.0049 | 0.011 | 0.0071 | 0.0009 | 0.013 |
| **c6** | 0.0003 | 0.0011 | 0.0018 | 0.0301 | 0.0139 | 0.0412 | 0.0023 | 0.0319 |
| **c7** | 0.0004 | 0.0081 | 0.0046 | 0.0046 | 0.0121 | 0.0049 | 0.0039 | 0.0137 |
| **c8** | 0.0014 | 0.0038 | 0.0164 | 0.0135 | 0.0188 | 0.0159 | 0.0033 | 0.0249 |
| **c9** | 0.0001 | 0.0022 | 0.0016 | 0.0006 | 0.0052 | 0.0023 | 0.0015 | 0.0148 |
| **c10** | 0.001 | 0.0056 | 0.001 | 0.0031 | 0.0022 | 0.0012 | 0.003 | 0.0213 |
| **c11** | 0.0005 | 0.0035 | 0.003 | 0.0031 | 0.0004 | 0.0003 | 0.0023 | 0.0153 |
| **c12** | 0.0007 | 0.0018 | 0.0065 | 0.0082 | 0.0035 | 0.0063 | 0.0043 | 0.016 |
| **c13** | 0.0003 | 0.0058 | 0.0011 | 0.0029 | 0.0091 | 0.0019 | 0.0046 | 0.0119 |
| **c14** | 0.0015 | 0.0064 | 0.0004 | 0.0041 | 0.005 | 0.0092 | 0.0035 | 0.0129 |
|  | | | | | | | | |

With the measures arising from these software, the solidity descriptor tends to evaluate the figure edge softness and therefore, the values are decreasing with the presence of roughness, mainly when the vertices are sharpen; with roundness measuring, values increase with homogeneity or compactness, without an apparent influence of irregularities; as expected, with the AR measurement, values increase with elongation, and continue to grow with the rough; with the regularity measure, it is detected the effect of the vertices and ridges, in addition, the figure elongation makes decrease the values. It should be noted that in the case of the circle used as test, it is drawing by pixels, the edges are not really round and then the value is not exactly as expected.

For mathematical descriptors, the Dfractal shows a propensity to differentiate parallelepiped and spherical shapes from other geometrical shapes and shows a decline of values depending on the irregularity.

Given the complexity of RFD interpretation, it is better to explain them by similarity with other descriptors, using multivariate techniques like PCA and HCA .

From this analysis three new components are obtained, see figures 2a and 2b, which may largely explain the relationships of all descriptors (making an 87.85% of total variance).

|  |
| --- |
| **a** |
| **b** |
| **Figure 2**. Cumulative percentage of variance vs. the number of initial components (**a**) and Eigenvalues vs. number of initial components (**b**). |

The first component represents the homogeneity and compactness in topological sense, and only positively related to RFD c0; the second one, can be grouped by the irregularity, mainly caused by elongation and participate positively RFD c2, c4, c6, c8, c12, c14; the third component and proportionally with less weight than previous ones, is grouped in order to measure the roughness, with positive effect from the RFD c1, c3, c4, c5, c7, c8 and c13, in the figures 3a, 3b and 3c. These new components are linearly classified in the dendrogram of the HCA, see figure 3d.

|  |
| --- |
| **a** |
| **b** |
| **c** |
| **d** |
| **Figure 3**. Two-dimensional plots relationships of the three main components weights. (a) The weight ratio of component 1 to 2. (b) The weight ratio of component 1 to component 3. (c) The weight ratio of component 2 to 3. (d) Hierarchical cluster analysis plot shows the linear relationship of the DRF with the other descriptors. |

***Grouping used descriptors meaning***

Combining various descriptors and applying ACP and ACJ techniques to evaluate calibrator geometric shapes, it is infered that these geometric forms can be concentrated in two groups (which account for 99.32% of the variance), and appear to be based on the homogenization degree, according to graphical results of PCA and HCA, in the figure 4.

The first group stands out as it can be called compaction of the figures, where values are moving away as the figures are angular and rough, and shows no negative contributions; the second one, have acicular forms where elongation factor predominates, showing positive effects on the elongated star and columnar forms. The rectangle appears to have little contribution in either of the two components.

|  |
| --- |
| **a** |
| **b** |
| **c** |

**Figure 4**. Graphical representations of PCA and HCA to evaluate geometric shapes calibrators: (a) Total percentage of variance due to each component; (b) Weight ratio relationships between 2 principal components; and (c), conformation of two groups in a dendrogram.

***Phenytoin samples results*.**

For direct observation of the crystalline appearance of the sample in the figure 5, M4 solid looks like columns and needles; the M7 are presented as amorphous or irregular polyhedrons; the M8 habits appear as flat and rectangular plates well defined, with medium size; samples in M18 are also plates, but smaller, with irregular edges and show agglomeration; and M22, are rectangular blocks smaller than

the M4 and the smaller particles attached to the largest.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **M4** | **M7** | **M8** | **M18** | **M22** |
| M4 Aceit-1 10X | Photo002 | Photo012 | Photo015 | Photo008 |
| **M4-1** | **M7-1** | **M8-1** | **M18-1** | **M22-1** |
| **Photo024** | **Photo007** | **Photo006** | **Photo002** | **Photo022** |
| **M4-2** | **M7-2** | **M8-2** | **M18-2** | **M22-2** |
| Photo017 | Photo003 | Photo040 | Photo017 | Photo046 |
| **M4-3** | **M7-3** | **M8-3** | **M18-3** | **M22-3** |
| M4 -11 | M7-1 | M8-10 | M18-2 | M22-3 |
| **M4-4** | **M7-4** | **M8-4** | **M18-4** | **M22-4** |
| M4 -7 | M7-3 | M8-5 | M18-5 | M22-10 |
| **M4-5** | **M7-5** | **M8-5** | **M18-5** | **M22-5** |
| **M4 -8** | **M7-12** | **M8-14** | **M18-12** | **M22-12** |
| **M4-6** | **M7-6** | **M8-6** | **M18-6** | **M22-6** |

**Figure 5.** Mn-1, Mn-2, Mn-3, are representative microphotographs of different samples analyzed. Mn-4, Mn-5, Mn-6, are the forms of isolated and binarized particles by means ImageJ software.

Table 2 summarizes the results of measurements made to these micrographs, with the different descriptors and the definitions obtained in the calibration. It can be infered, according to the AR descriptor, M4 habits are essentially elongated and irregular; M22 and M8 are very similar and have average values with respect to other habits; the lack of circularity of all samples, and the irregularity of the sides and edges is evidenced by DFractal measurement; with solidity descriptor, M7 and M18 show more rough silhouettes; based on roundness descriptor results, M4 and M8 are the least compact in its relation length-width; measuring regularly, the samples M7 and M18 appear to be more rectangular and angular than M8 and M22, and even more than M4, which are individually more elongated.

**Table 2**. Data for all descriptors evaluated

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | M4 | M7 | M8 | M18 | M22 |
| **roundness** | 0.210 | 0.602 | 0.412 | 0.556 | 0.417 |
| **regularity** | 0.298 | 0.460 | 0.402 | 0.459 | 0.235 |
| **AR** | 6.455 | 1.736 | 2.946 | 1.889 | 2.820 |
| **solidity** | 0.905 | 0.877 | 0.894 | 0.871 | 0.894 |
| **Dfractal** | 1.6182 | 1.6751 | 1.6276 | 1.6125 | 1.6264 |
| **c0** | 0.3767 | 0.6059 | 0.5308 | 0.6065 | 0.5443 |
| **c1** | 0.0196 | 0.0314 | 0.0333 | 0.0482 | 0.0293 |
| **c2** | 0.1364 | 0.0791 | 0.1174 | 0.0952 | 0.1260 |
| **c3** | 0.0139 | 0.0319 | 0.0293 | 0.0310 | 0.0174 |
| **c4** | 0.0651 | 0.0217 | 0.0448 | 0.0287 | 0.0451 |
| **c5** | 0.0120 | 0.0127 | 0.0171 | 0.0149 | 0.0105 |
| **c6** | 0.0360 | 0.0115 | 0.0193 | 0.0170 | 0.0170 |
| **c7** | 0.0105 | 0.0120 | 0.0085 | 0.0125 | 0.0082 |
| **c8** | 0.0256 | 0.0088 | 0.0131 | 0.0101 | 0.0088 |
| **c9** | 0.0088 | 0.0089 | 0.0071 | 0.0061 | 0.0060 |
| **c10** | 0.0177 | 0.0069 | 0.0101 | 0.0069 | 0.0066 |
| **c11** | 0.0069 | 0.0075 | 0.0073 | 0.0057 | 0.0063 |
| **c12** | 0.0116 | 0.0048 | 0.0068 | 0.0045 | 0.0057 |
| **c13** | 0.0062 | 0.0061 | 0.0064 | 0.0036 | 0.0045 |
| **c14** | 0.0084 | 0.0045 | 0.0053 | 0.0048 | 0.0044 |

As it can be seen in figure 6a, from accumulated graph of components vs percentage of variance, obtained from the PCA technique and gathering calibration and geometric shapes of the different habits of phenytoin, it is founded that all information can be explained by only two new groups (explaining 99.5% of the variance). In the figure 6b, The component weight plot shows that all samples can be collected on compaction component (positively value); in the second component, which is the irregularity by elongation, the results are negatively influenced by the roughness, in the following order: M4> M8> M22> M18> M7.

In cluster analysis, HCA, two group dendrogram confirmed shape composition, see figure 6c.

|  |
| --- |
| **a** |
| **b** |
| **c** |

**Figure 6**. Graphical representations of PCA and HCA to evaluate contour of samples with the calibrators: (a) Total percentage of variance defined mainly 2 components; (b) Plot of components weight relationships; and (c), classification of 2 groups in dendrogram.

***Microtexture results*.**

Table 3 summarizes the results of texture analysis by GLCM technique of entropy and IDM, for the five samples previously ground and sieved. Microphotographs of these samples can be seen in figure 7. Leer fonéticamente

Diccionario - [Ver diccionario detallado](http://www.google.com.co/dictionary?source=translation&hl=es&q=micro&langpair=en|es)

1. **nombre** 
   1. microordenador
   2. It can observe that these two textural characterization results are equally related between the samples, but reversely; also, apply within the definition of regularity for entropy and reciprocally, with homogeneity for IDM.

**Table 3**. Values from GLCM texture analysis.

|  |  |  |
| --- | --- | --- |
| **Sample** | **Entropy** | **IDM** |
| M4 | 7.20 | 0.40 |
| M8 | 6.92 | 0.45 |
| M22 | 6.65 | 0.48 |
| M18 | 6.26 | 0.52 |
| M7 | 6.20 | 0.57 |

Results classification of entropy and IDM using principal component analysis (PCA) and HCA, shows good agreement with a sorting of the samples by "visual appearance" or by expert eye inspection. The order of lowest to highest is as follows: M4 seems to have the smoothest surface, followed by M8 after M22, M18 and assuming the most irregular M7.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **M4** | **M7** | **M8** | **M18** | **M22** |
| Photo001 | Photo001 | Photo012 | Photo004 | Photo002 |
| **M4-1** | **M7-1** | **M8-1** | **M18-1** | **M22-1** |
| Photo006 | Photo003 | Photo008 | Photo006 | Photo005 |
| **M4-2** | **M7-2** | **M8-2** | **M18-2** | **M22-2** |
| Photo009 | Photo008 | Photo010 | Photo011 | Photo011 |
| **M4-3** | **M7-3** | **M8-3** | **M18-3** | **M22-3** |
| **Figure 7.** Examples of samples comminuted, sieved and then binarized, for texture measurements. | | | | |

**CONCLUSIONS**

Although it is commonly stated that in order to characterize the particles by classical descriptors is necessary to measure circularity (or elongation), angularity and roughness; in this paper it confirms that it is better to use a fourth descriptor, which may be called compaction, in agreement with other articles (30-32).

If only classical descriptors are used to characterize particle crystal habit, it is gets subjective and inaccurate representations. Applying mathematical descriptors or texture GLCM method, numerical values have no physical meaning and its interpretation is still unclear and should be employed as parameters for comparison between samples or between samples and a reference.

With the GLCM modified texture analysis method, one can use a whole picture and not just part of it, which could be very useful in routine analysis processes.

The results reveal that phenytoin habits are irregular both in elongation, as rough edges.

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