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GRANULOMETRIC CHARACTERIZATION AND STUDY OF IBUPROFEN LYSINATE BY MEANS OF AN IMAGE PROCESSOR

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ABSTRACT

This article examines the possibility of using the microscope methods in conjunction with an **image processor** (Kontron IBAS 2000) to **granulometrically** characterise **ibuprofen lysinate**, a recently discovered soluble salt of ibuprofen (analgesic, anti-inflammatory and antipyretic). Likewise, the advantages this method has over conventional microscope methods and other existing procedures are analysed. The methodology applied is described and the findings reported. The crystal morphology of ibuprofen lysinate is acicular. All parameters determined follow a log-normal distribution pattern and not a Gauss distribution pattern. The Feret diameter distribution did not follow either of these patterns. As a result, the Feret diameters are the least representative parameters of this active agent's granulometry. On the other hand, the equivalent diameter is the most representative parameter. From a technological and pharmaceutical point of view, the drugs' appearance is that of a granulometrically homogenous powder. Finally, the article demonstrates that apart from being much easier and much more reliable, the method applied saves a considerable amount of time when compared with the conventional microscope method.

INTRODUCTION

The granulometric characteristics of a pharmaceutical powder can be determined using an optical microscope for particle sizes generally between 0.2 and 200μm using white light (down to 0.1μm if ultraviolet light is used and 0.01μm if an ultramicroscope is used), or using an electronic microscope for particle sizes between 0.001 and 0.2μm (although they have been used for particles of up to 10μm). Electronic microscopes, with a magnification of up to 200,000X and an approximate resolution of 25A, reveal details of the particles' surface morphology and provide the additional dimension of depth - seeing the same area from different angles - that may help to solve doubts as to the morphology of the particles in a sample (1). The resolution of the optical microscope used in this study was more than sufficient.

In our study, an image processor is connected to a TV camera placed above the optical microscope. The additional advantages of this method are numerous, and it also helps to counteract some of the disadvantages inherent in optical microscope techniques.

1) If we enter an initial calibration into the computer programme to convert pixels into units of length (μm, for example), the programme stores it and therefore does away with the need to physically measure each particle individually, as in the conventional microscope method.

2) The image processing programme allows the parameters for each particle to be measured automatically (the observer does not have to take any measurements). These parameters can be stored for processing at a later stage. Thus, as long as the particles are well isolated and contrasted, all the observer has to do to measure an individual particle is place the cursor over it, and the programme automatically performs the analysis. This clearly saves a tremendous amount of time.

3) One very important advantage this method has over other methods is the possibility of automatically obtaining a wide variety of parameters, using direct viewing and measurement of the particles. For example the area, shape, (using the so-called "shape factor"), several diameters like maximum diameter, minimum diameter, Feret diameters, equivalent diameter, the perimeter, etc., can be measured.

4) The image processor allows the quality of the images viewed under the microscope to be improved. Of particular interest here is the possibility of improving the particle-background contrast (obviously reducing measurement errors) and the possibility of eliminating

visual "background noise", using the simple subtraction, also automatic, of a "white image".

5) Although initially a certain amount of time is required to select the processing procedure that should be applied to the image to improve it (especially regarding contrast and clear definition of the edges of the particles), this method can be successively and automatically applied to other images, although different processes can be applied depending on the image type. In any event, only processing that substantially improves the image and does not falsify the parameters it measures on the basis of that image, can be used.

6) The measurements are far more accurate for the same magnifying level than the conventional microscope method. For example, magnifying 200X, a resolution of around 0.29μm is achieved. This can be increased by using greater magnification.

7) Instant images of each area studied and processed can be obtained without having to take a photograph and get it developed. This can be done simply by using a printer or a film printer, which provides a resolution level similar to photographic paper. In fact, the method has proved itself to be so practical that it has been used in routine quality control processes like, for example, the detection of particles in parenteral solutions (2,3). In some studies it has also been used to determine the particle shape and size distribution of active agents (4,5).

MATERIALS AND METHOD

Image Processing System

The device used for the experiment consists of a Nikon Apophot optical photomicroscope connected - via an adaptor - to an RC A TV camera and a Kontron IBAS 2000 image processing system. Basically, the system consists of a Z80 processor, a matrix processor and a 4MB image memory that can store up to 16 512 x 512 pixel images at the same time (6).

The image produced by the microscope is captured directly by the TV camera and stored as a 512 x 512 pixel matrix, with 256 grey-levels per pixel in the computer. In this study, a 200X magnifying lens was used, obtaining a spatial resolution of 0.29μm per pixel. The measurement routines were written in macro form so that they could be used automatically.

Methodology

The methodology required for this type of study includes initial viewing of different samples to determine what the morphology of the active agent's

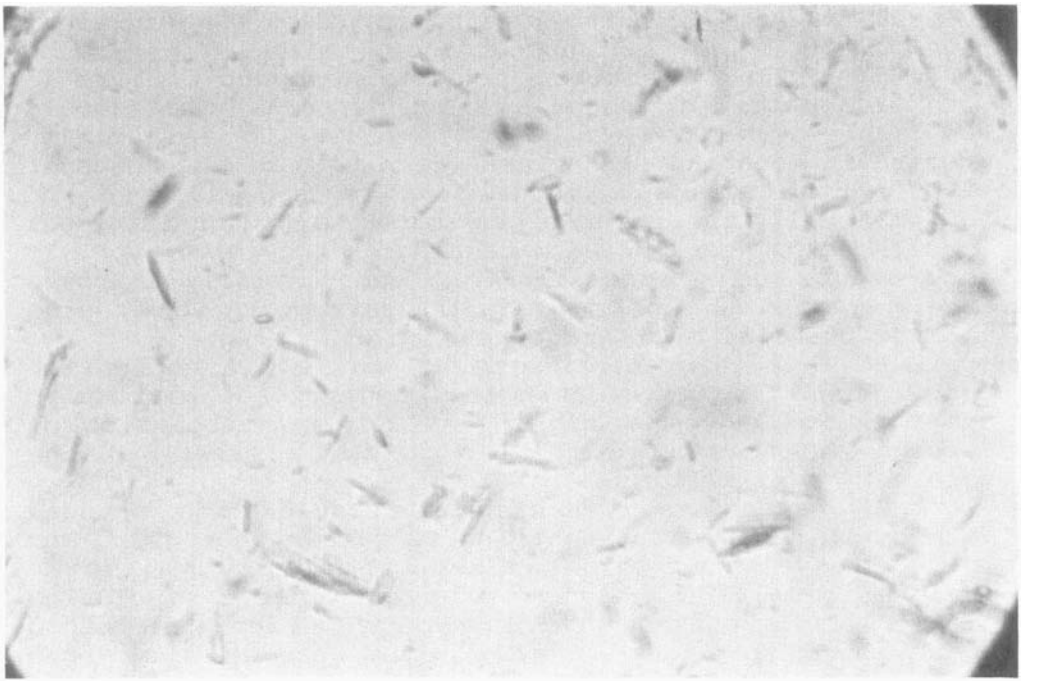

FIGURE 1
130x optical microscope photograph that shows the acicular morphology of ibuprofen lysinate's crystals.

particles is and if the shape is regular or irregular. Then, the processing to be applied to the images to improve them is decided. The parameters to be determined are also defined at this stage. Selection of these parameters is based on the prior study of the morphology of the active agent's particles. Then, a drop of the active agent's suspension is placed on vaseline on the slide (previously cleaned). The same procedure is used on subsequent samples to carry out the counts. Positioning the slide cover requires utmost care to avoid drawing the smaller particles towards the edges. In any event, it is always important to look at all the areas of the preparation.

A regular particle morphology was found for ibuprofen lysinate: the particles are acicular crystals (previously checked using X-ray diffraction). In other words, the crystals are long or needle-shaped (Figure 1).

With this shape of particle, an essential factor to determine is the maximum diameter (and the minimum diameter, although it is not as representative in this instance) of the particle, which will correspond to the length of the "needle". Similarly, as the particles are not spherical, it is always worth determining the equivalent diameter. Representation of the distribution of these parameters allows us to deduce what distribution pattern they match and gives us an idea of the homogeneity of the drug as far as particle size is concerned. However, as a wide range of parameters can be obtained automatically at the same time, other data can also be determined.

Applied Image Processing

Determination of the active agent's morphology is based on the standard grey-level binarisation of an image. The model of the image employed comprises a background with objects superimposed on it. The basic idea is to select thresholds of grey-level intervals that characterise the objects and construct an image with just two levels (usually black and white), one for the background and the other for the objects. The thresholds correspond to the troughs between the object phase peak in the grey-level histogram, on which the measurements are taken. This method is based on the supposition that the different objects to be measured have a fairly uniform grey-level which can be distinguished from the background. An image often needs to be pre-processed to make such suppositions valid.

The most appropriate image processing method, bearing in mind the above requirements, is a multi-stage one. These stages are:

1) Calibration

Firstly, the scale factor needs to be entered to obtain real length units for any geometric measurement. The factor can be entered directly, or the image measured interactively when the size of an object is known (micrometer).

2) Image Capture

Once the computer is connected to the TV signal output, the image of a zone of the sample can be displayed on the monitor. By scanning the sample and focusing on objects of interest, the optimum image for measurement is obtained and stored.

3) Pre-processing

All pre-processing steps are aimed at optimising the images for measurement purposes. Depending on the sample type, it may not be necessary to use all of them. A more detailed description of each operation can be found in reference material (7,8).

Firstly, the background image is captured (visual "background noise") and is subtracted from the original image to eliminate all the "background noise" that may be caused by the optics. To increase the contrast, the image is scaled up, gradually increasing its dynamic range to the maximum (0 to 255 grey-levels). Then, a contour delineation filter is applied. This specially designed filter emphasizes the contours (edges) between various phases and makes the influence of the halo effect negligible. Thus, the contrast between the phases that represent the crystals and the background is improved.

4) Segmentation

The next step is to binarise the image or, in other words, to convert the grey-level image into just two levels: white for the particles and black for the background. This is done interactively by selecting the range of greys that are to become white (or vice-versa).

Once the binary image is obtained, objects smaller than a specified size (area) are eliminated, whilst a "scrap" is applied to eliminate only points of distortion and not isolated particles that should be counted, and the edges of the "median-type" objects are softened. On some occasions, it may be necessary to fill in holes within the objects. This can be done automatically using the "afill" function. It is important to note that the image that is being processed can be compared at all times with the original. This avoids producing false results based on the processed image and is closer to reality.

On other occasions, if objects have been broken, a shape function can be applied. This "close" function consists of octagonal expansion and subsequent erosion with the same structural element several times. Groups of loosely joined objects processed in this way become properly joined together, whilst the rest are unaffected.

Finally, the use of a manual editing pen has been applied in some granulometric studies (to join, separate or fill in objects). However, this option should only be used for substances of a cohesive nature when the count is more complex (5). In the case in hand, the preparation provides perfectly isolated particle images, making the pen option unnecessary.

5) Measurements

Once the binary image is obtained, the objects of interest are interactively identified and the desired parameters are selected and measured automatically by the computer. The following parameters were selected to characterise ibuprofen lysinate:

- Area (A)
- Maximum and minimum diameters (Dmax -maximum length- and Dmin -minimum width-)

- Diameter of the equivalent area circle (DCIRCL)

$$DCIRCL = 2\sqrt{\frac{A}{\pi}}$$

- Feret diameters; defined as the difference existing between two tangents traced on the projection surface of the particle, both of which are perpendicular or parallel (dFerx or dFery)

- Shape factor (CIRSF); this factor represents a measurement of the shape of particles that deviate from the sphere (f=1)

$$CIRSF = \frac{4\pi A}{P^2} \qquad \text{(P - perimeter)}$$

Available literature reveals certain differences of opinion as to the number of particles that need to be counted if reliable conclusions are to be drawn. Although some authors consider that between 500 and 1,000 particles (9) should be counted, it is generally accepted that results are reliable on the basis of 200 particles onwards. In some studies, like those carried out by Marshall and York (5), just 100 particles are counted using an image processor. In this study, 208 particles were measured. This amount was considered sufficient to obtain a significant data population to make a statistical estimate of the measurement probability distribution and to characterise the drug correctly.

DISCUSSION AND RESULTS

The distribution of the maximum, minimum, equivalent and Feret diameters, the area and the shape factor were studied. Then, each distribution was looked at to see if it matched any of the most frequent distribution patterns: normal or log-normal. The statistical programme employed creates frequency histograms of the distribution of the values obtained from the image processing system (frequency compared to the value ranges for each parameter). The normal distribution or log-normal distribution pattern that matches the results best is represented on the histogram. Thus, in Figures 2 and 3, the best possible match of the equivalent diameter value distribution is represented by normal distribution and log-normal distribution, respectively. Note that the log-normal match is considerably better. This can be checked using the statistical programme through the Chi-square and Kolmogorov-Smirnov tests, and these also allow us to decide whether or not the match can be considered correct. In this event, given that the number of determinations for each parameter is high (over 200), the

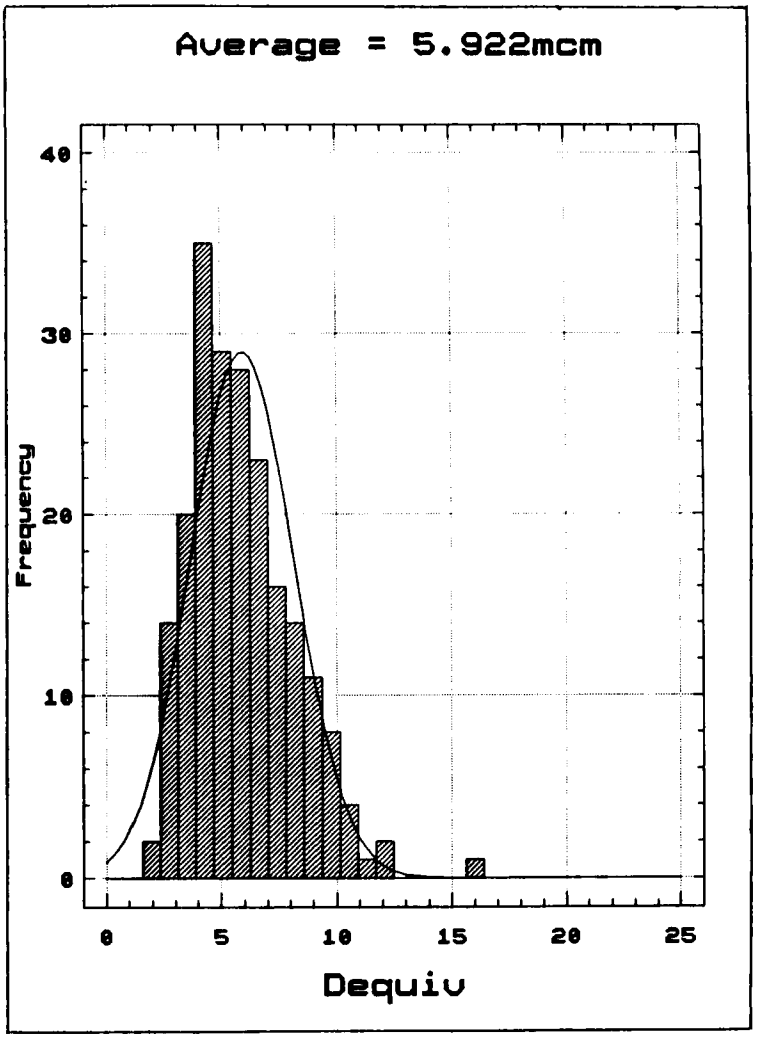

FIGURE 2
Equivalent diameter value distribution that best matches the normal distribution pattern.

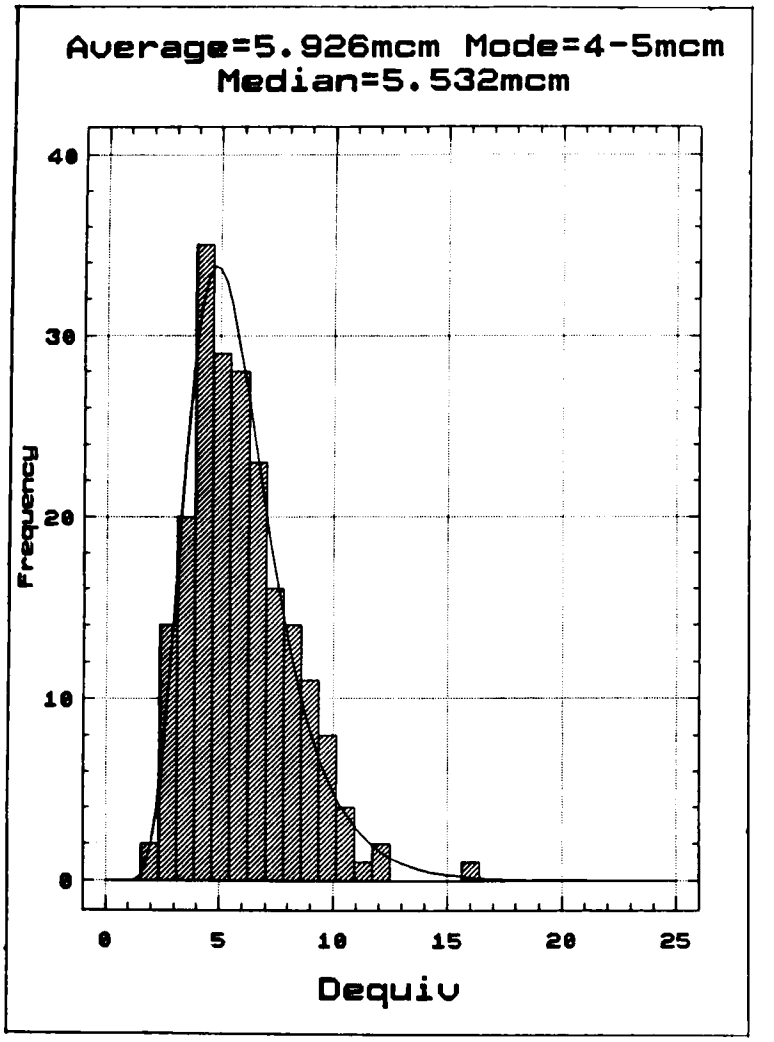

FIGURE 3
Equivalent diameter value distribution that best matches the log-normal distribution pattern.

TABLE 1
Levels of significance of the match between the parameters determined -and their logtransformations- and the normal or log-normal distribution pattern. The match is considered correct (significant) when the level of significance is above 10% and incorrect (not significant) when below.

	CHI-SQUARE TEST/LEVELS OF SIGNIFICANCE	
	NORMAL DISTRIBUTION PATTERN	*LOG-NORMAL DISTRIBUTION PATTERN*
Equivalent diameter	*$8.54.10^{-2}$% / INSIGNIFICANT*	*92.54% / SIGNIFICANT*
Log (Dequiv)	*32.35% / SIGNIFICANT*	
Maximum diameter	*$2.74.10^{-4}$% / INSIGNIFICANT*	*67.12% / SIGNIFICANT*
Log (Dmax)	*67.23% / SIGNIFICANT*	
Minimum diameter	*$2.08.10^{-3}$% / INSIGNIFICANT*	*20% / SIGNIFICANT*
Log (Dmin)	*19.37% / SIGNIFICANT*	
$Feret_x$ diameter	*$3.55.10^{-13}$% / INSIGNIFICANT*	*1.23% / INSIGNIFICANT*
Log ($DFeret_x$)	*0.801% / INSIGNIFICANT*	
Ferety diameter	*$1.67.10^{-8}$% / INSIGNIFICANT*	*9.46% / INSIGNIFICANT*
Log ($DFeret_y$)	*6.84% / INSIGNIFICANT*	
Area	*$5.55.10^{-14}$% / INSIGNIFICANT*	*75.98% / SIGNIFICANT*
Log (Area)	*28.81% / SIGNIFICANT*	
Shape factor	*0.087% / INSIGNIFICANT*	*70.11% / SIGNIFICANT*
Log (Factor F)	*60.47% / SIGNIFICANT*	

ji-squared test is applied to determine the level of significance with which the corresponding pattern matches the results. As a result, more reliable conclusions can be drawn; a level of significance above 10% means that the match can be considered correct. In this way, the distribution of the determined parameters, except for Feret diameters, is shown to match adequately the log-normal pattern and not the normal or Gauss pattern (Table 1).

As far as the Feret diameters are concerned, their distribution is not considered to match either of the two patterns. This can be easily understood because these diameters are not very representative; consider, for example, that the $Feret_x$ diameter may have different values for the same particle depending on the angle (orientation) of the particle to the horizontal plane.

The distribution of all other parameters studied best matches the log-normal pattern when superimposed (Figures 4 to 7). The Gauss distribution pattern is of no use in this particular study because no match can be considered to be correct in any of the cases.

Average, median and mode values are also different for this type of distribution, although they would coincide in a normal distribution.

A logotransformation was carried out on each variable determined (see Table 1) and it was found that a normal distribution pattern match is indeed correct in this case, as expected.

Cumulative histograms can also be obtained. These show the value below which all particles would be included or, in other words, the maximum value that the parameter can take for the set of particles determined. The superimposed curve is the so-called additive distribution curve. Figure 8 shows the cumulative histogram for the equivalent diameter values and their corresponding additive distribution curve.

Finally, Table 2 shows the values of the main statistical distribution parameters for a log-normal pattern. They do not match those that would be obtained for a Gauss distribution pattern for the reasons set out above.

As mentioned previously, the Feret diameters are the determined parameters that least match the log-normal distribution pattern. Therefore, they would be the least representative as far as attempting to draw conclusions from the study is concerned. An increase in the number of observations would supose an increase in the representativity of the parameters obtainable from the histograms, as the angle -orientation- of the particles (variable that affects them) is random. If we consider that in a granulometric study using a conventional microscope just one of the Feret diameters is determined

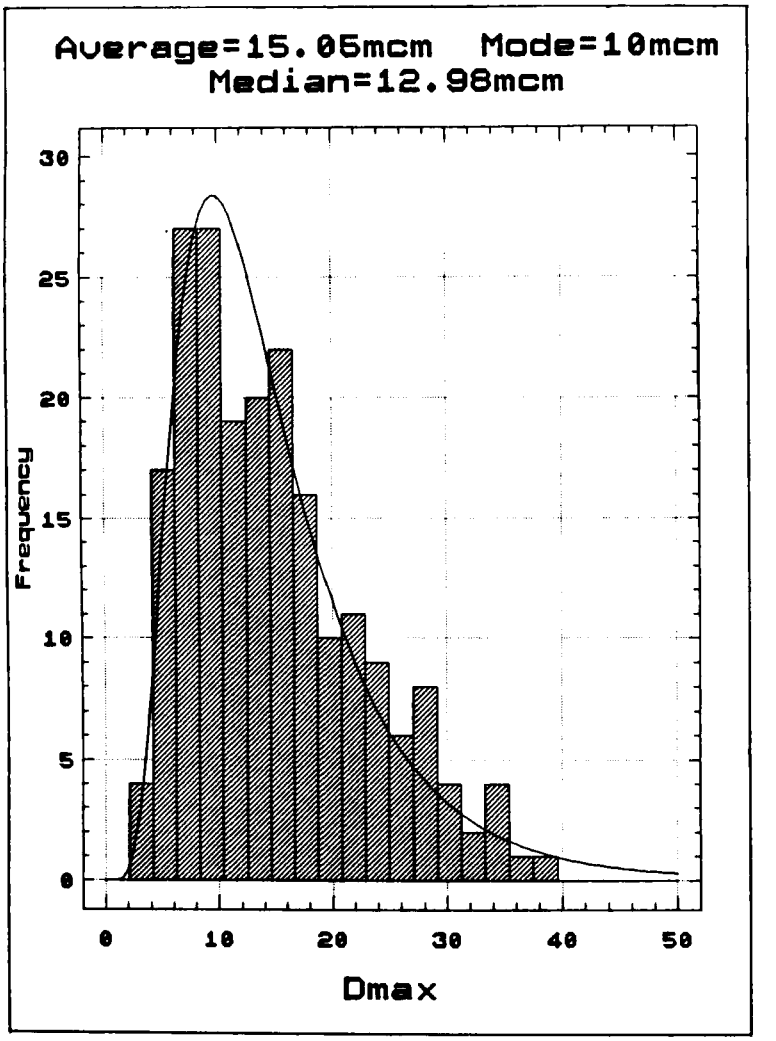

FIGURE 4
Maximum diameter value distribution that best matches the log-normal distribution pattern.

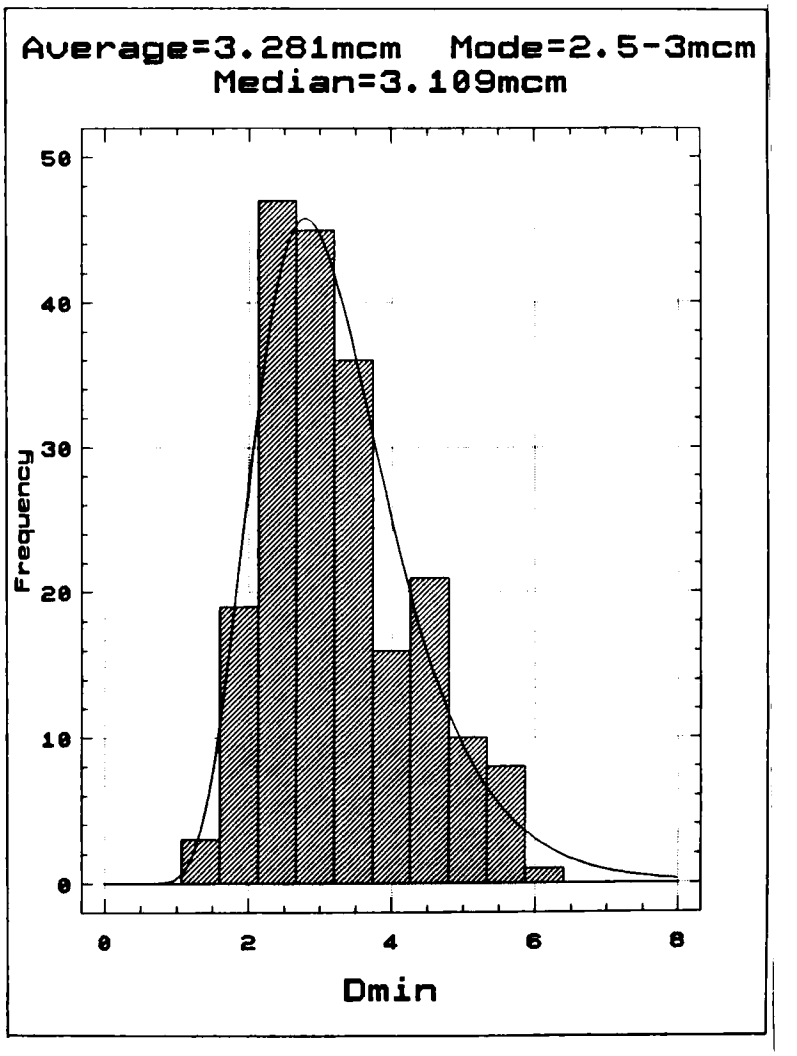

FIGURE 5
Minimum diameter value distribution that best matches the log-normal distribution pattern.

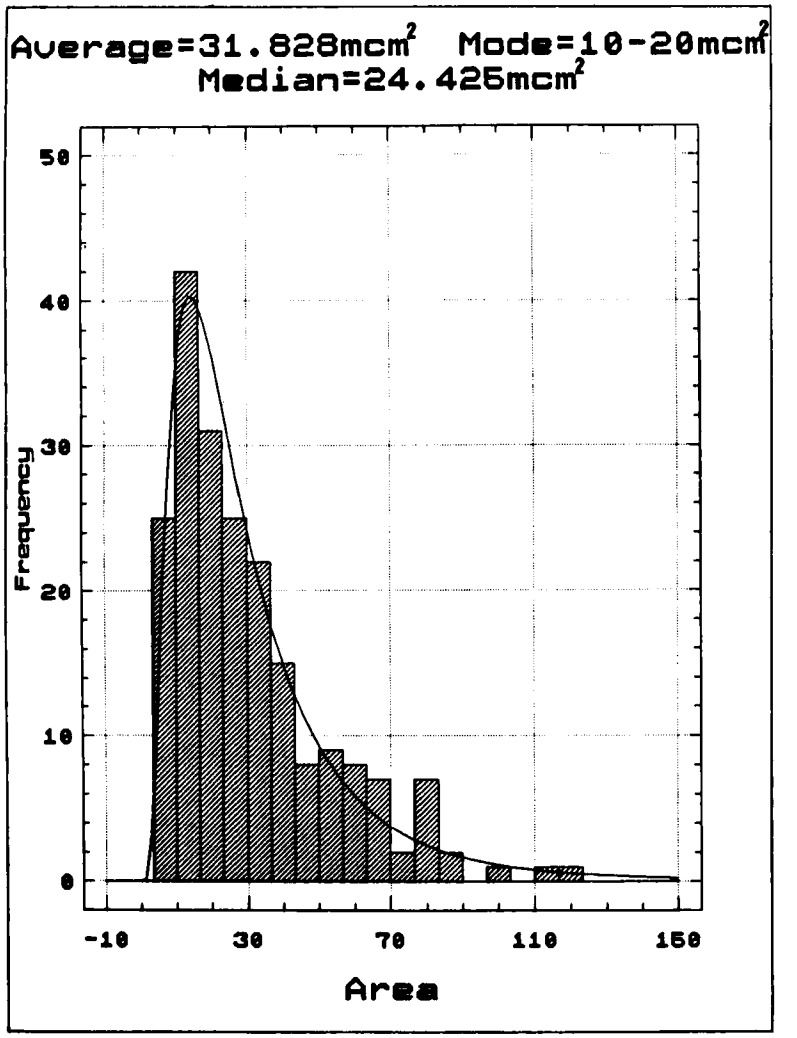

FIGURE 6
Area value distribution that best matches the log-normal distribution pattern.

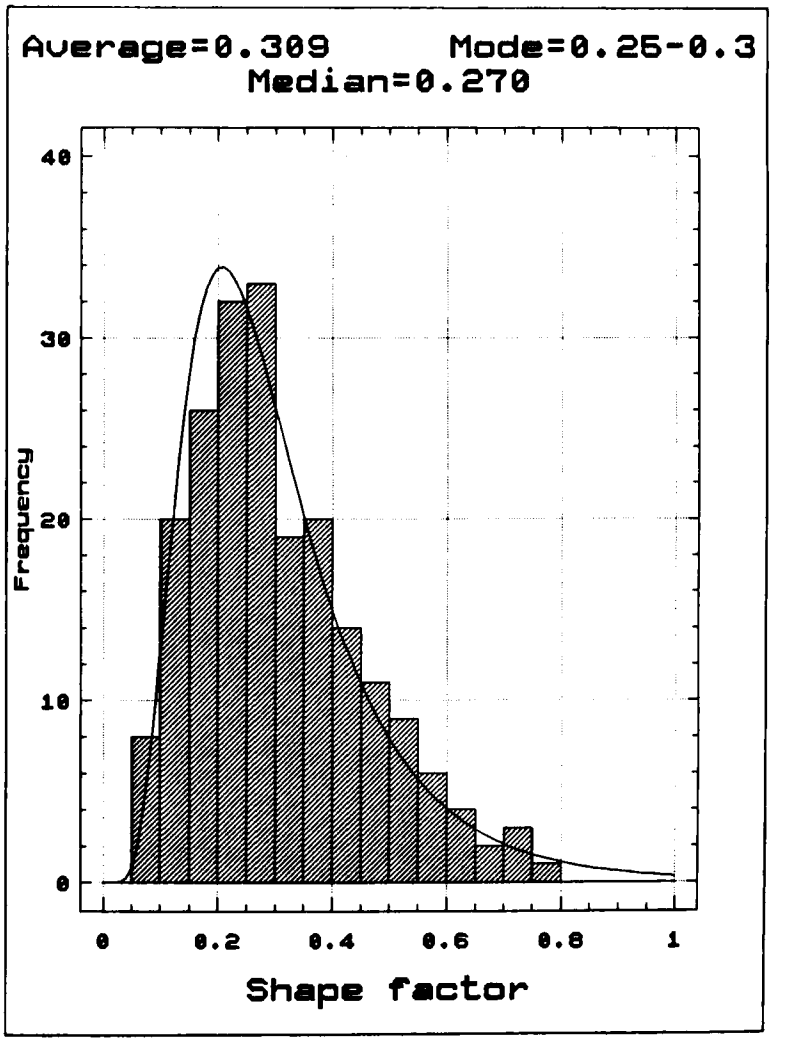

FIGURE 7
Shape factor value distribution that best matches the log-normal distribution pattern.

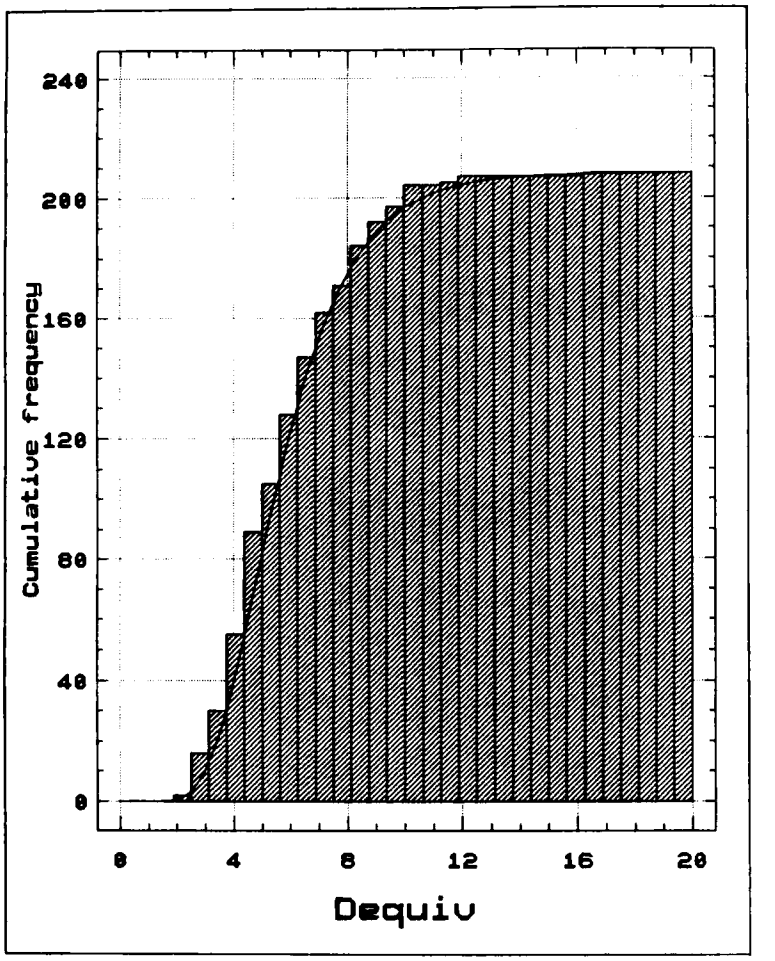

FIGURE 8
Cumulative histogram of equivalent diameter values.

(measurement of the particle projection over a micrometer), it allows us to deduce why it is necessary to determine a greater number of particles when using this technique. Therefore, it is important to state that the use of a Kontron IBAS 2000 image processor, apart from making determinations easier, allows us to reduce the number of determinations necessary to obtain reliable conclusions. This is thanks to the diversity of determinable parameters (more representative than Feret diameters and not obtainable through the conventional

TABLE 2
Values of the main statistical distribution parameters for the variables indicated for a log-normal distribution pattern.

	AVERAGE	MEDIAN	MODE	S	V.C.
Equivalent diameter	5.9257	5.5319	4-5	2.2751	38.39%
Maximum diameter	15.05	12.98	10	8.8327	58.68%
Minimum diameter	3.2808	3.109	2.5-3	1.1058	33.7%
Area	31.8282	24.4255	10-20	26.5913	83.54%
Shape factor	0.3087	0.2699	0.25-0.3	0.1714	55.52%

microscope technique) and the improvement that applied image processing brings to the quantification of those parameters.

The equivalent diameter is considered by most authors to be the most representative parameter for irregular particles. This is also reflected in this study because this diameter has a 92.54% level of significance as far as matching the log-normal distribution pattern is concerned. This is the highest level of significance found in this study. Therefore, we can safely say that the number of observations made is more than enough - and could even be decreased - to draw reliable conclusions from the parameter. It also proves that it is possible to reduce the number of determinations - thanks to the method employed - when compared to the conventional microscope technique.

Although both the maximum and minimum diameters -the most representative parameters of the physical reality of the particles- correctly match the log normal distribution pattern, the maximum diameter does so substantialy better. This is understandable if we bear in mind that the maximum diameter is just one measurement (the length of the crystal) whereas the minimum diameter can be measured at different points along the acicular crystal, depending on its shape, even though the measurement corresponds to its minimum width. These points can be at the central zone, intermediate zones or the ends. Therefore, the fact that the minimum diameter matches the log-normal distribution pattern less is due, at least in part, to its dependence on the irregularity

of the particles along their length, something which does not affect the determination of the maximum diameter. Once again, an increase in the number of observations would lead to a better minimum diameter value distribution match when compared to a distribution pattern (although the value obtained is in fact correct).

The degree of dispersion shown by each parameter can be evaluated through the variation coefficient. The highest degree of dispersion was shown by the area, since this parameter was the one that varied most. Returning to the parameters that are most representative of the particles' physical nature, (Dmax and Dmin), the fact that the maximum diameter value dispersion is considerably higher is worthy of note, as this indicates that the ibuprofen lysinate crystals are acicular crystals with a more or less constant minimum diameter (consider the negligible amount of real variation that 33.7% represents in 2-4 micra particles) whilst their length is more variable. However, despite the greater degree of length variability (maximum diameter), the dispersion should not be considered from a solely statistical point of view. It should also be evaluated from a pharmaceutical point of view. Thus, even the maximum diameter could be considered to be a parameter that varies little because, although the variation coefficient gives a higher value (58.68%), all particles measure between 1 and 40 micra. A lesser degree of dispersion is found for the equivalent diameter values as 207 out of the 208 particles determined measure between 2 and 12 micra. Bearing in mind the sizes of the particles dealt with, we can deduce that ibuprofen lysinate is a granulometrically homogenous powder from a technological and pharmaceutical point of view.

The deviation of the drugs' acicular crystals from the sphere is reflected in the shape factor (average value 0.3087 and median value 0.2699).

CONCLUSIONS

1. Applying an image processor to the microscope method is shown to be an efficient way of studying the granulometry of pharmaceutical powders, and offers numerous advantages over other methods.

2. Processing applied to the image (to improve the determination of variables that are quantified on the basis of the image) and the diversity of the parameters that can be determined (not just measurement of the particles using a micrometer, as in the conventional microscope method), mean that reliable conclusions can be drawn from the results of a smaller number of observations. What is more, if we consider that the

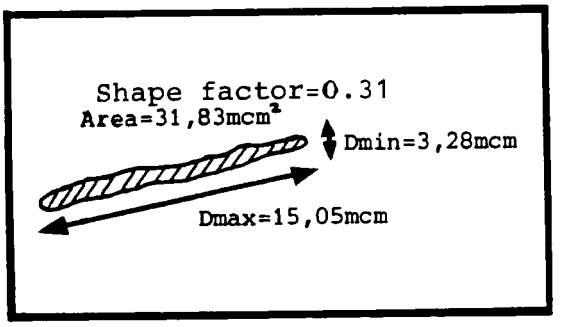

FIGURE 9
Representation of an ibuprofen lysinate particle type, based on the average values of the parameters that are the most characteristic of its physical nature.

Kontron IBAS 2000 image processor allows us to automate all our readings, we can conclude that the method employed saves a considerable amount of time.

3. The Feret diameters are seen to be the only parameter whose distribution does not match either of the two most frequently employed patterns (normal and log-normal). This is due to their close dependence on the angle (orientation) of the particles and helps us to understand why it is necessary to count more particles if reliable conclusions are to be drawn when using a conventional microscope method (just one of the Feret diameters is usually determined when this method is used).

4. The equivalent diameter is the parameter that best matches a log-normal distribution curve (92.54% level of significance). This confirms that it is the most representative non-spherical particle parameter.

5. The minimum diameter's dependence on the irregularity of ibuprofen lysinate's acicular crystals along their length is the reason why this parameter does not match the log-normal distribution pattern as well as the maximum diameter. However, it can be improved (even though it is already correct, according to the Chi-square test) by increasing the number of readings.

6. Ibuprofen lysinate is made up of acicular crystals whose average minimum diameter is equal to 3.28 micra (V.C. = 33.7%) and whose average maximum diameter is equal to 15.05 micra (V.C. = 58.68%).

7. All the ibuprofen lysinate particles studied have a maximum diameter of between 1 and 40 micra, and a minimum diameter of between 1 and 6.5 micra.

8. Ibuprofen lysinate can be considered a granulometrically homogenous drug based on technological and pharmaceutical criteria (and not just statistical values of the dispersion parameters). Its equivalent diameter is between 2 and 12 micra.

9. The deviation of the drugs' acicular crystals from the sphere is reflected by the shape factor.

10. For illustrative purposes, determination of the main statistical distribution parameters shown in Table 2 allows us to think of a particle type, as shown in Figure 9.

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