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Research paper

Microscopic image analysis techniques for the morphological characterization of pharmaceutical particles: Influence of the software, and the factor algorithms used in the shape factor estimation

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Abstract

The present report highlights the difficulties of particle shape characterizations of multiparticulate systems obtained using different image analysis techniques. The report describes and discusses a number of shape factors that are widely used in pharmaceutical research. Using photographs of 16 pellets of different shapes, obtained by extrusion–spheronization, we investigated how shape factor estimates vary depending on method of calculation, and among different software packages. The results obtained indicate that the algorithms used (both for estimation of basic dimensions such as perimeter and maximum diameter, and for estimation of shape factors on the basis of these basic dimensions) have marked influences on the shape factor values obtained. These findings suggest that care is required when comparing results obtained using different image analysis programs.

Keywords: Image analysis; Granulation; Shape analysis; Extrusion; Spheronization; Morphology; Pellets

1. Introduction

Particle morphology is a key determinant of the behaviour of bulk solids and multiparticulate systems: many of the physical and chemical properties of such systems depend on particle shape and surface geometry [1]. Thus the morphological characterization of particles is of great importance in pharmaceutical technology. In the field of granulation and pelletization this characterization will be critical for some production steps like filling capsules, and specially the coating of pellets. Due to this great practical importance, we want to consider a new perspective of this problem like the lack of homogenization in formulae or the different programs of image analysis (IA) that can be used.

Early approaches for characterizing particle shape and size distribution were based on the combination of sieving and microscopy. Subsequently, techniques based on image analysis have come into wide use. In 1996, Barber [2] published an excellent review of technical aspects of particle morphology characterization by IA. Briefly, an IA system comprises at least the following components: some sort of microscope connected to a photographic or video camera, connected in turn to a computer processing system which (a) converts the analogue image into digital form (i.e. a pixel matrix) in accordance with user-specified criteria for definition of the colour or grey-shade of each pixel, and (b) applies other algorithms to characterize the size and shape of the particles identified in the image. Particle perimeter is estimated by any of various procedures. Particle area is typically estimated simply by counting the number of pixels within each particle. Particle diameter is determined: [2] (a) on the basis of the coordinates of each pixel forming part of the particle boundary, (b) on the basis of a series of radial chords drawn from the particle centre to the boundary, or (c) on the basis of measurement of a

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series of diameters drawn as the particle image is rotated (callipering), which permits a greater number of measurements than radial chords.

As noted by Barret [3] basically the morphological characterization of a particle requires three different aspects to be taken into account (Fig. 1). First, the particle projection must be assigned to that geometric shape category (e.g. circle) by which it is best described. Second, the morphological analysis should include assessment of the particle's roundness, and of the sharpness/roundedness of the particle's vertices, edges and sides. Third, surface texture must be evaluated, understood as minor roughness and irregularities affecting the particle surface at local level. Although these authors fail to further address surface texture. Anyway we think this is an important point of view in pharmaceutical field because this characteristic will affect properties like flow particles, or important manufacturing steps like coating. So, in Pharmaceutical Technology there are different articles [4–6] where the surface texture is studied. Morphological analysis that considers these three aspects, in conjunction with determination of particle size, will provide an approximate but useful idea of the particle's real morphology.

Up to this point most authors would agree; disagreement arises regarding the selection and means of calculation of the geometric parameters defining these three morphological aspects. This problem is not exclusive of pharmaceutical technology, but it is a common problem in others areas that need a exhaustive morphological characterization of particles and granules (Table 1). For this purpose different authors have proposed diverse shape factors, but considerable confusion exists as regards precise definitions and terminology. A general view of the Table 1 contents is sufficient to emphasize the difficulty to establish standardized criteria in the field of the evaluation of particle morphology. In this sense there are two fundamental questions: which is the more accurate shape factor for describing the shape of pharmaceutical particles? and once we choose the shape factor, which is the definition that must be used by us? The first question was treated in our previous articles [7,8]. For the second question we want to show some difficulties that are associated with this high number of variations in the equations for determinating the same shape factor, or going further, we want to show the limits of different AI programs, because they can limit the definition of the shape factor selected for our analysis.

In addition, the problems are complicated if we bear in mind that one common feature of most shape factors used to date is their close dependence on the method used by each particular IA program to estimate the basic dimensions of the particle (notably diameter and perimeter). This problem was noted by Barber [2], who states that "each size measure satisfies the concept of size as stated classically, illustrating the need to precisely state each concept for a clear operational definition". In addition, the great majority of shape factors have been proposed with the aim of comparing the shape of a particle with a perfect sphere. Without entering into the utility of this approach, most shape factors are poorly suited for identifying or characterizing particles of non-spherical shape.

In view of these considerations, the aim of the present study was to compare pellet morphology characterizations obtained with three widely used image analysis programs, and to argue that there is a need for standardization shape factor algorithms and terminology, to enable meaningful comparison of results between different studies and different laboratories.

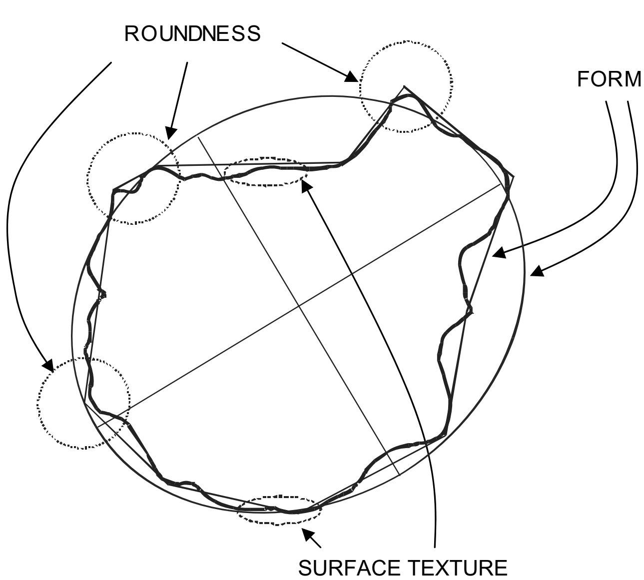

Fig. 1. Factors to be taken into account in the morphological characterization of a particle from its two-dimensional projection [3].

2. Materials and methods

2.1. Image analysis

For the morphological characterization of particles we used three different IA programs: PCImage VGA 24 (Foster Finlay Ass.), ImagePro Plus v4.5.0.29 (Media Cybernetics Inc.), and SigmaScan Pro Image Analysis 5.0.0 (SPSS Inc.). In all cases, binary images were obtained from digitalized images using a relative grey-level threshold of 60% [4]. The pixel size selected was 4.26 μm, so we are under maximum pixel size as we defined in our previous papers [4,5].

2.2. Image obtention

Particle images were acquired with an Olympus SZ-CTN stereomicroscope equipped with a JVC TK-S350 video camera and an Olympus Europe Highlight 2000 cold light illuminating the sample perpendicularly from above against a black background [9] employing the optimal values established by Almeida and colleagues [7,8]. The images were digitalized with a Matrox Comet video card, using PCA Image VGA 24 software.

Table 1
Some geometrical shape factors that have been used in the characterization of particle morphology

Definitions commonly used	Modifications introduced in the previous definition	Name	Reference
$\frac{4\pi A}{P^2}$	A: area, P: perimeter	CIRCULARITY	[10,23]; PCImage user's guide
		SHAPE FACTOR	[18]
		FACTOR SPHERICITY	[24]
		SHAPE FACTOR	SigmaScapro and ImageProPlus user's guide
		APPEARANCE FACTOR	[25]
		ROUNDNESS	[26,27]
	×100	ROUNDNESS	[28]
	Inverse	SHAPE FACTOR	[29,30]
		SPHERICITY INDEX	[31]
		ROUNDNESS FACTOR	[24]
		SURFACE FACTOR	[32]
	$\frac{P^2}{4\pi A} \times 0.9399; \frac{P^2}{4\pi A} \times \frac{1}{1.064}; \frac{P^2}{4.256\pi A}$	ROUNDNESS	[13–17]
	$\frac{p^2}{A}$	COMPACNES	SigmaScanPro user's guide.
$\frac{D_{max}}{D_{min}}$	D_{max}: maximum Feret diameter	ELONGATION	[10]
	D_{min}: minimum Feret diameter	ASPECT RATIO	[33]
	D_{max}: maximum Feret diameter D_{min}: Feret diameter perpendicular to D_{max}	ASPECT RATIO	[34,35]
	R_1/R_2 — R_1 maximum radius or largest diameter	ROUNDNESS INDEX (E value)	[19–21]
	R_2 minimum radius or smallest diameter	ELONGATION	[36]
	l/b — l: length – maximum distance between two points on the perimeter b: breath – distance between the two points crossed by a line perpendicular to length at the long-axis midpoint	ASPECT RATIO	[13,37]
	b/l — l: length – maximum distance between two points on the perimeter b: breath – maximum distance between two perimeter points linked by a line perpendicular to length	ASPECT RATIO	[12,38]
	a/b — a, b side lengths of a rectangle drawn around of the projection of the particle	ELONGATION	[32]
$\frac{2\pi Re}{P}\sqrt{1-\left(\frac{b}{l}\right)^2}$	Re: mean distance between centre of mass and perimeter, measured every 1°	e_r	[9]
$\frac{2\pi Re}{Pf}\sqrt{1-\left(\frac{b}{l}\right)^2}$	Re: mean distance between centre of mass and perimeter, measured every 5° f: $1.008 - 0.231[1-(b/l)]$	e_r	[12]
$\frac{P}{cP} - \sqrt{1-\left(\frac{1}{AR}\right)^2}$	P: perimeter cP: convex perimeter AR: aspect ratio	e_r	[13]
$A/\left(\pi \times R_{max}^2\right)$		ROUNDNESS	[36]
P/cP		ROUGHNESS	[10,13]
$P/\pi D_{max}$	D_{max}: maximum diameter or 2 × max radius	PELLIPS	[10,36]
$\frac{A}{D_{min}D_{max}}$	D_{max}: maximum diameter and D_{min} minimum diameter Also −4 × max radius × min radius	RECTANG	[10,36]
$\frac{PD_{min}}{4A}$		MODELX	[10]
$A/(\pi(D_{max}/2))$		ROUNDNESS	[10]
$\sqrt{Area/cArea}$	cArea: convex area	FULLRATIO	[13]
$V_r = \frac{\sum_{i=1}^{n}\frac{\lvert r_i - r_m\rvert}{r_m} \times 100}{n}$	r_m: mean radius of each of the n vectors between the centre of mass and the perimeter coordinates	V_r	[8]
$V_p = \frac{\lvert 2\pi r_m - P\rvert}{2\pi r_m} \times 100$	r_m: mean radius, P: perimeter	V_p	[8]
$R = 1 - P_{smooth}/P_{rough}$	P_{smooth} is the perimeter measured with 72 points on the outline of the granule at 5° intervals and P_{rough} is measured with 360 points	Roughness factor R	[39]

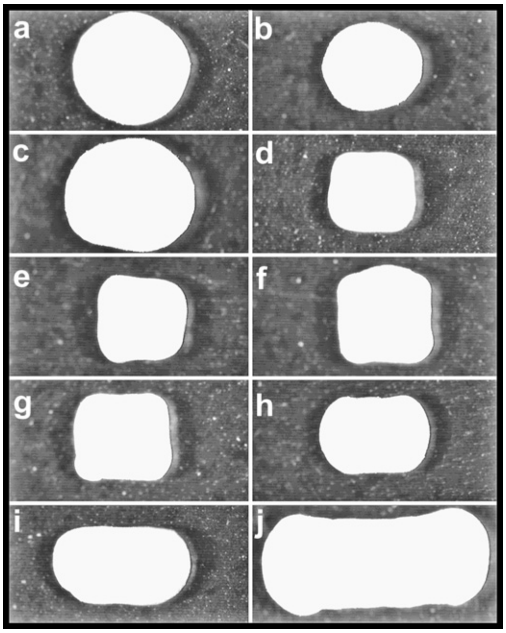

Fig. 2. Projections of 10 different particles of diverse shapes, obtained by extrusion–spheronization. Images were obtained by the image capture and digitalization procedure described in Section 2.

2.3. Pellets

Pellets with widely differing morphologies were obtained using various mixtures of excipient and wetting agent. Particles A, B, C in Fig. 2 were fabricated using a mixture of 75% of corn starch and 25% of white dextrin; particles D to H in Fig. 2 were obtained with a mixture of 85% of corn starch and 15% of waxy corn starch, and for pellets I and J, in Fig. 2, the mixture of powders used was 75% of corn starch and 25% of waxy corn starch. Powders were mixed in a Turbula T2C mixer for 15 min. Kneading was performed for 15 min with a Kenwood Chef Classic orbital mixer using distilled water as wetting agent. Extrusion was done with a Caleva model 10 extruder (extrusion sieve diameter 1 mm, 6 rpm). Spheronization (of 100 g of extrudate) was done with a Caleva model 120 spheronizer (2000 rpm, 15 min). Finally, the pellets were dried to constant weight in a Heraeus oven at 40 °C.

3. Results

3.1. Circularity estimation

Table 1 lists and defines some of the numerous IA-based particle shape factors used to date in pharmaceutical technology. At first sight these shape factors seem to be very diverse, in fact many are closely related variants. This wide variety of factors and names is evidently confusing, and makes it difficult to compare results from different laboratories. Thus for example one of the most widely used parameters, circularity $= 4\pi A/P^2$ ($A =$ area, $P =$ perimeter), appears in the literature under 6 or 10 different terms if we include identical variants such as $[100 \times 4\pi A/P^2]$ and the inverse $[P^2/4\pi A]$. Conversely, some terms are used with various meanings: thus for example the term "roundness" is defined by different authors in diverse ways: in some cases it means circularity (i.e. $4\pi A/P^2$ or variant thereof), in some cases it means aspect ratio (i.e. ratio of maximum diameter to minimum diameter), in some cases with entirely different meaning [7]. Similar usage inconsistencies were seen by us with other terms, including elongation and aspect ratio.

In view of these problems, the first important question in the use of image analysis techniques for morphological characterization of particles is the selection of the most useful parameter(s) in the given context. Thus for example our choice of parameter will differ depending on whether our aim is to assess proximity to perfect sphericity, or to assess the particle's basic shape (spherical or otherwise). Likewise, our choice of shape factor may be affected by the type of pellets to be characterized. Thus for example one study [11] shows that circularity is ineffective for discriminating different particle shapes, while [12] concluded that circularity is not effective for characterizing spherical particles because errors in image recognition can strongly influence the calculated value. Clearly, this type of error will likewise affect all factors based on the ratio of perimeter to area (see Table 1). For spherical particles, some authors [12] suggest the use of shape factors like AR (aspect relation) or e_r [9] (parameters defined in Table 1).

However, the selection of the most appropriate shape factor is only one among several problems. Once a shape factor has been selected, it is necessary to define the measured dimension used for its calculation, and the precise formula for this calculation. To illustrate the difficulties that may arise in this connection, we characterized the morphology of 10 individual extrusion–spheronization pellets, selected for their differences in shape, on the basis of dimensions measured with three different IA programs. The digitalized projections of each pellet (see Fig. 2) were obtained by the procedure described in the Section 2. Table 2 shows various dimensions of the 10 pellets (A–J) as measured by each program. These measured dimensions were then used to calculate various shape factors.

Estimates of circularity and related factors likewise vary markedly among different IA programs, despite the fact that these factors are calculated simply on the basis of perimeter and area. These among-program differences are basically attributable to differences in the method of estimation of perimeter, since area is determined in similar ways by the different programs. Specifically, area is determined from a black-and-white image, simply by counting all black pixels and multiplying by a scale factor; thus if the threshold value for conversion of the grey-scale image to a black-and-white image is the same (as in the present study), the area estimates obtained with different programs will be very similar (maximum variability of 0.1% for pellet J). This lack of variation among methods can be seen in the area estimates for our 10 pellet projections (Table 2). As

Table 2
Basic dimensions of the 10 particles (A–J) shown in Fig. 2, as obtained using the three image analysis programs

Parameters		Outlines									
		A	B	C	D	E	F	G	H	I	J
Area[a] (mm^2)	SigmaScan	1.106	0.755	1.576	0.676	0.724	0.899	0.847	0.804	1.006	2.131
	PCImage	1.106	0.756	1.578	0.673	0.725	0.900	0.848	0.805	0.997	2.127
	ImagePro	1.106	0.756	1.578	0.677	0.725	0.900	0.848	0.805	1.008	2.131
	Mean	1.106	0.756	1.577	0.675	0.725	0.900	0.848	0.805	1.004	2.130
	SD	0.000	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.006	0.002
	% variation	0.000	0.076	0.073	0.308	0.080	0.064	0.068	0.072	0.584	0.108
Perimeter (mm)	PCImage[b]	3.823	3.202	4.644	3.037	3.167	3.561	3.502	3.375	3.869	6.198
	SigmaScan[c]	4.013	3.377	4.907	3.164	3.295	3.718	3.665	3.543	4.091	6.439
	ImagePro										
	1[d]	3.723	3.103	2.975	3.101	3.489	3.419	3.301	3.788	6.056	3.301
	2[e]	4.001	3.363	3.155	3.283	3.696	3.646	3.536	4.037	6.427	3.536
	Mean	3.89	3.26	3.92	3.15	3.41	3.59	3.50	3.69	5.11	4.87
	SD	0.14	0.13	1.00	0.10	0.23	0.13	0.15	0.29	1.32	1.68
	% variation	3.63	4.05	25.41	3.33	6.78	3.58	4.30	7.84	25.78	34.51
Length (mm)	SigmaScan[h]	1.231	1.051	1.510	1.013	1.044	1.190	1.180	1.151	1.437	2.375
	PCImage[f]	1.227	1.047	1.518	1.010	1.043	1.189	1.176	1.147	1.428	2.373
	ImagePro[j]	1.203	1.044	1.491	0.910	0.933	1.141	1.011	1.141	1.430	2.364
	Mean	1.22	1.05	1.51	0.98	1.01	1.17	1.12	1.15	1.43	2.37
	SD	0.02	0.00	0.01	0.06	0.06	0.03	0.10	0.01	0.00	0.01
	% variation	1.24	0.34	0.92	6.00	6.34	2.39	8.59	0.44	0.33	0.25
Breath (mm)	SigmaScan[i]	1.184	0.921	1.317	0.988	1.045	1.173	1.147	0.847	0.852	1.201
	PCImage[g]	1.180	0.914	1.328	0.979	1.039	1.168	1.133	0.809	0.809	1.048
	ImagePro[k]	1.163	0.917	1.303	0.843	0.908	1.126	0.912	0.802	0.815	1.040
	Mean	1.18	0.92	1.32	0.94	1.00	1.16	1.06	0.82	0.83	1.10
	SD	0.01	0.00	0.01	0.08	0.08	0.03	0.13	0.02	0.02	0.09
	% variation	0.95	0.38	0.95	8.67	7.76	2.23	12.39	2.96	2.82	8.28
Mean Feret (mm)	SigmaScan	1.198	0.991	1.430	0.938	0.982	1.095	1.066	1.038	1.190	1.165
	PCImage	1.186	0.981	1.418	0.926	0.961	1.071	1.039	1.012	1.127	1.888
	ImagePro	1.185	0.984	1.426	0.945	0.986	1.108	1.084	1.047	1.204	1.918
	Mean	1.19	0.99	1.42	0.94	0.98	1.09	1.06	1.03	1.17	1.66
	SD	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.04	0.43
	% variation	0.61	0.52	0.43	1.03	1.38	1.72	2.13	1.76	3.49	25.73
R_{max} (mm)	PCImage[l]	0.624	0.526	0.766	0.507	0.532	0.598	0.594	0.581	0.721	1.190
	ImagePro[m]	0.614	0.526	0.765	0.507	0.534	0.602	0.596	0.575	0.719	1.192
R_{min} (mm)	PCImage[l]	0.564	0.442	0.610	0.407	0.417	0.464	0.429	0.378	0.380	0.430
	ImagePro[n]	0.564	0.447	0.613	0.410	0.417	0.472	0.434	0.388	0.386	0.439
D_{max} (mm)	ImagePro[o]	1.216	1.043	1.505	1.006	1.035	1.185	1.173	1.143	1.428	2.368
D_{min} (mm)	ImagePro[p]	1.151	0.904	1.280	0.824	0.840	0.962	0.88	0.782	0.791	0.915

[a] Defined as the total number of pixels included in the outline of the pellet, and multiplied by a scale factor as function of the magnification.
[b] PCImage estimates perimeter by applying a smoothing algorithm (not defined in the user's guide) to the pixel image, and calculating the length of the resulting smoothed outline (see Fig. 5b).
[c] SigmaScan estimates perimeter by summing distances along the outside of the projection, though "cutting the corners" (see Fig. 5c).
[d] ImagePro "perimeter 1" estimates summing the distances between the centres of those pixels constituting the particle outline (see Fig. 5a).
[e] ImagePro "perimeter 2" calculated by a similar procedure to that used by PCImage employing a smoothing algorithm (see Fig. 5b).
[f] PCImage calculates length as the maximum chord length of the particle (passing through the centre of gravity) regardless of orientation.
[g] PCImage calculates breadth as the maximum projection of the particle onto an axis orthogonal to the long axis (see Fig. 3b).
[h] SigmaScan calculates length as the maximum distance between two points on the perimeter.
[i] SigmaScan calculates breadth as the maximum distance between perimeter points linked by a line perpendicular to length (see Fig. 3a).
[j] ImagePro program calculates length as the Feret diameter along the major axis (see Fig. 3e).
[k] ImagePro program calculates breadth (in this program termed "width") as Feret diameter along the minor axis (see Fig. 3e).
[l] PCImage calculates min max and mean radius but these are not defined in the user's guide.
[m] ImagePro calculates R_{max} as the maximum distance between each object's centroid pixel position and its perimeter.
[n] ImagePro calculates R_{min} as the minimum distance between each object's centroid pixel position and its perimeter.
[o] ImagePro calculates D_{max} as the length of the longest line that can be drawn to pass through the centroid position and join two points on each object's perimeter.
[p] The ImagePro calculates D_{max} as the length of the shortest line that can be drawn to pass through the centroid position and join two points on each object's perimeter.

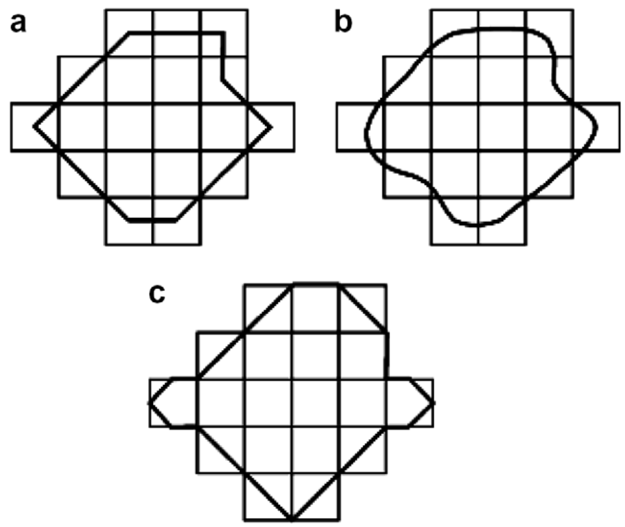

Fig. 3. Schematic representations of the procedures used for particle perimeter determination by the three image analysis programs: (a) ImagePro perimeter 1; (b) PCImage and ImagePro perimeter 2; (c) SigmaScan.

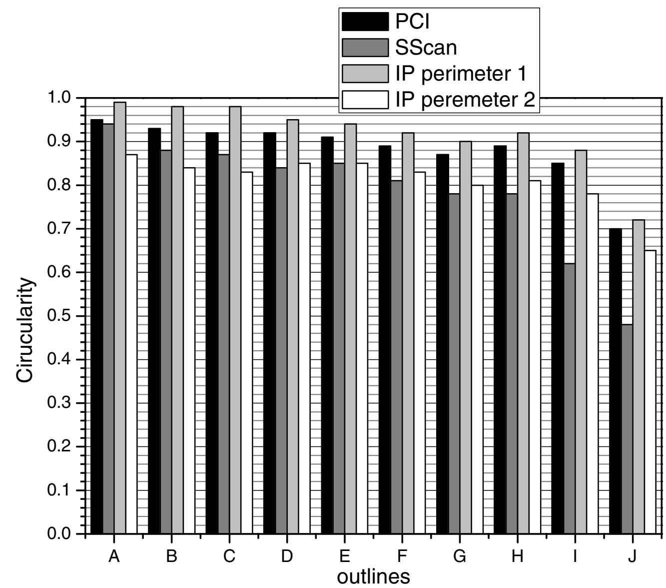

Fig. 4. Circularity values of the 10 particles (A–J) shown in Fig. 2, as calculated using perimeter measurements obtained with the three image analysis programs.

noted, however, perimeter estimates show greater variation (most than 25% for pellets C, I and 34.5% for J), reflecting the different procedures used by the different programs: indeed, the ImagePro program offers various perimeter estimates. Fig. 3 schematically illustrates the ways in which the three IA programs estimate perimeter. Briefly, ImagePro estimates perimeter ("perimeter 1") by summing the distances between the centres of those pixels constituting the particle outline (Fig. 3a); SigmaScan estimates perimeter by summing distances along the outside of the projection, though "cutting the corners" (Fig. 3c); PCImage estimates perimeter by applying a smoothing algorithm to the pixel image, and calculating the length of the resulting smoothed outline (Fig. 3b), probably applying a correction factor in the same way as other works [13–17]. ImagePro likewise offers a second perimeter estimate (perimeter 2), calculated by a similar procedure to that used by PCImage but using different smooth factor. SigmaScan perimeter tends to be larger than ImagePro perimeter 1, the magnitude of the difference increasing with increasing ratio of pixel to particle size.

The importance of these differences in the method of perimeter calculation is clear from Fig. 4, which shows that for a pellet with near-circular projection (pellet A), the circularity estimate varies from 0.87 to 0.99. Similar differences are observed for markedly non-circular projections: for example, circularity estimates for pellet I range from 0.62 to 0.88. Then we can affirm that differences observed in the circularity measurements are due to the next two reasons: (a) First of all, we have the discrimination problem of the pellet shape, because a shape factor like circularity identify as the same shape circular and square pellets with rounded edges (A and E in Fig. 2). But this was already discussed in a previous article [8]. (b) The second reason for the circularity values is basically attributable to differences in the method of estimation of perimeter with the IA program, independently of the definition used for the circularity. And this is a consideration that has never been studied before in the characterization of spherical pellets. So we want to emphasize its importance on the final results of the IA.

Concerning the limits for the circularity parameter, as noted, a critical analysis was carried out [12] about the limits of circularity values proposed by different authors as the criteria for classifying a pellet as being spherical; namely the value of 0.88 [10], the value of 0.93 [18], or the reciprocal circularity value of 1.2 (equivalent to a circularity value of 0.83) [14]. Podczeck et al. [12] concluded that most of the proposed threshold values are low restrictive. However, the values shown in Fig. 4 strongly suggest that much of the among-study variation in circularity values (and corresponding thresholds for consideration as spherical) must be attributable to variation in the method of estimation of perimeter by different IA programs, not to genuine disagreement about which pellets should be considered "spherical" and which not. Therefore, as demonstrated in this work, as significant differences among the available image analysis system algorithms exist that result in differing values for commonly calculated pellet characterization descriptors, at this time the focus should be directed to, first resolving these differences before beginning the debate on what should be the numerical limits of circularity that should serve as the criteria for considering a pellet as being spherical.

3.2. AR estimation

A shape factor for which there is a clear need for standardization of criteria is aspect ratio, or elongation, i.e. the ratio of maximum to minimum diameter. However, the value obtained is very sensitive to the way in which the IA program determines these diameters (Fig. 5). Fig. 6 shows AR values for the pellet projections shown in Fig. 2, as determined by each of the three IA programs, and Fig. 7 as determined by one of the programs using different values for maximum and minimum diameter. As can be seen, the results obtained are highly dependent on both pellet morphology and calculation method. We see that for

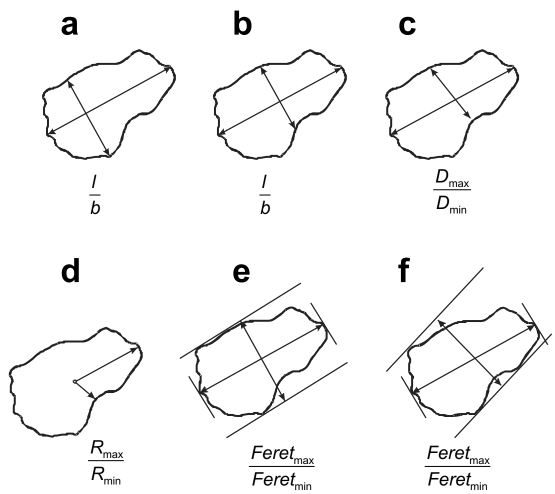

Fig. 5. Schematic representations of six different procedures for determination of the shape factor commonly denominated "aspect ratio" or "elongation": (a) length l is the maximum distance between two points on the perimeter, and breadth b the maximum distance between two perimeter points linked by a line perpendicular to the long axis (SigmaScan); (b) length l is the maximum distance between two points on the perimeter, and breadth b the distance between the two perimeter points linked by a line perpendicular to the long axis at its midpoint (PC Image); (c) D_{max} is the maximum distance between two points on the perimeter, and D_{min} the minimum distance between two points linked by a line crossing the centre of mass of the particle; (d) R_{max} is half D_{max} as defined for (c), and R_{min} the minimum distance between the midpoint of the long axis and the perimeter; (e) $Feret_{max}$ is maximum Feret diameter, and $Feret_{min}$ the Feret diameter determined perpendicular to the long axis at its midpoint (ImagePro); (f) $Feret_{max}$ is the maximum Feret diameter, and $Feret_{min}$ the minimum Feret diameter.

pellets with circular, elliptical or even squarish projections, AR values obtained by the different programs scarcely differ. However, for pellets with more elongated and/or irregular shapes (i.e. pellets G–J), the different programs give markedly different AR values. These differences are largely attributable to differences in the way in which the different programs measure breadth (i.e. minimum diameter), since although the way of estimating length (i.e. maximum diameter) varies from program to program, the values obtained are very similar in the case of spherical pellets. The SigmaScan program calculates length as the maximum distance between two points on the perimeter, and breadth as the maximum distance between perimeter points linked by a line perpendicular to length (Fig. 5a); the ImagePro program calculates length as the Feret diameter along the major axis, and breadth (in this program termed "width") as Feret diameter along the minor axis (Fig. 5e); PCImage calculates length as the maximum chord length of the pellet (passing through the centre of gravity) regardless of orientation, and breadth as the maximum projection of the pellet onto an axis orthogonal to the long axis (Fig. 5b). If the pellet projection is of regular shape (with similar maximum and minimum Feret), these three programs will give similar measures of breadth, and thus similar estimates of AR, but if the pellet is more irregular, however, the measures of breadth (and thus the estimates of AR) will differ. The variation in AR increases with increasing pellet irregularity.

In fact, these calculation-method-dependent differences in the value of AR are even greater if we apply some of the other methods proposed in the literature. For checking these theoretical considerations we proceeded to measure the shape factor AR in mind the possibilities offered by the different programs cited before, and the literature studied. The results presented in Fig. 7 show the values obtained for this shape factor using such methods, on the basis of pellet dimensions determined with ImagePro. As can be seen, AR estimates thus obtained are similar for near-circular projections, but show considerable among-method variation in the case of non-circular pellets.

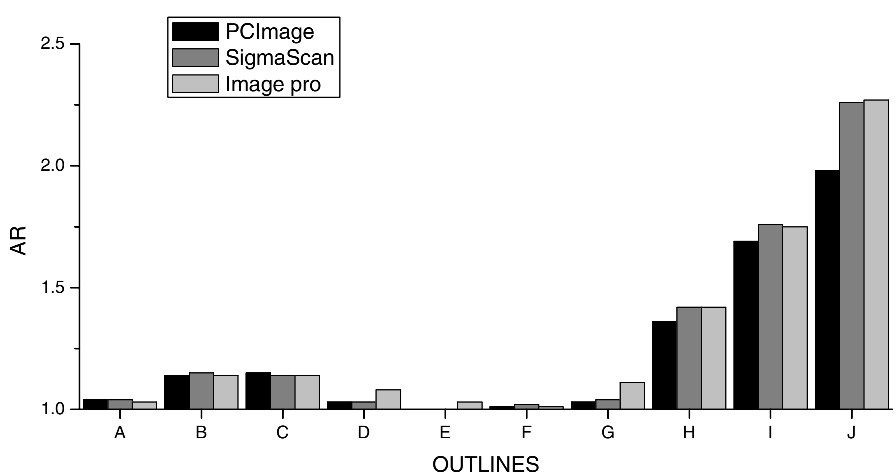

Fig. 6. Aspect ratios of the 10 particles (A–J) shown in Fig. 2, as calculated by the different procedures used by the three image analysis programs.

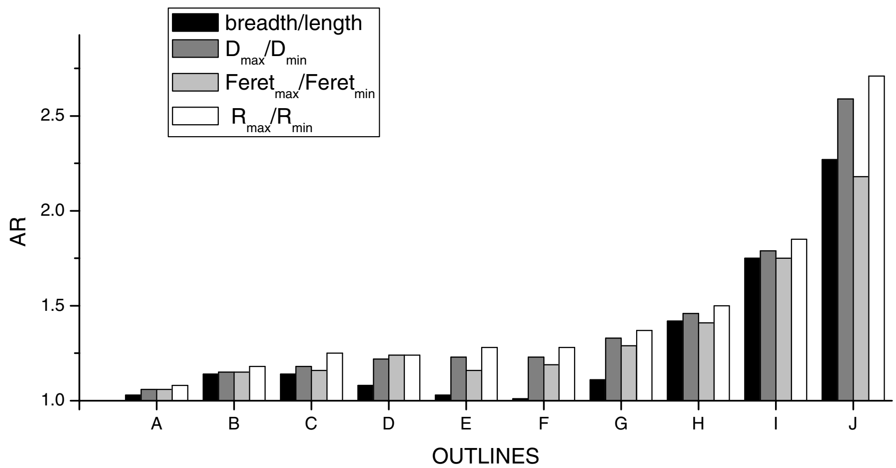

Fig. 7. Ratios of the different max/min pellet dimensions obtained for the 10 pellets (A–J) shown in Fig. 2, using ImagePro.

The marked differences in AR estimates provided by the different IA programs are clearly of sufficient magnitude to cause significant confusion. In this connection, the differences found in the AR and circularity estimated values may be the origin of the highly different threshold values proposed by different authors as the sphericity criterium. For example Podczeck et al. [12] suggest that the maximum AR value should be 1.1, in contrast with 1.2 as proposed by other authors [11,19–22] or 1.55 as proposed by [10]. All these authors use different formulae to calculate AR: thus some authors [11,19,20] calculated "roundness index" ("*E* value") as the ratio of maximum to minimum radius measured with a ruler from photographs, while other investigators [21,22] used the same terms to refer to the ratio of maximum to minimum diameter measured using a projection microscope and finally the AR was defined as the ratio of length to breadth (breadth = the longest distance perpendicular to the long axis) [9,12] basically as in SigmaScan. Clearly, then, AR estimates depend heavily on the procedure used: this needs to be taken into account when evaluating different studies, and is a strong argument in favour of procedural standardization.

3.3. e_r, V_r and V_p estimation

We have also investigated among-program differences in the estimation of three other shape factors recently introduced, the factor e_r proposed by Podzeck et al. [12], the variant of e_r proposed by Junila et al. [13], and the factors V_r and V_p [8]. The formulae for the calculation of these shape factors are defined in Table 1.

Values of e_r, V_r and V_p for the pellet projections in Fig. 2 are shown in Figs. 8 and 9. For calculation of e_r values, we used three different measures of perimeter (ImagePro perimeter 1, ImagePro perimeter 2, SigmaScan perimeter, PCImage perimeter; see Fig. 3), and in each case the length and breadth measurements obtained with the program in question; mean radius was determined for the ImagePro and SigmaScan calculations as mean distance between perimeter pixels (every 5°) and the pellet's centre of mass, and for the PCImage calculations as the mean of 64 radial chord measurements each obtained after a pellet rotation. For calculation of V_r and V_p values, we used the coordinates of all pixels constituting the perimeter obtained using a SigmaScan constructed macro (with automatic edge tracking of multiple pellets), or the coordinates of the vertices on the object outlines if the outline option of the ImagePro software is used. The SigmaScan perimeter 2 was calculated starting from the sum of the existent distances between each one of the border pixels of the pellet's outline, recognized by the image analysis program. Evidently, PCImage could not be used for the calculation of V_r because this software is not able to provide each coordinate of the pellet perimeter. For this reason, it has not been included in the comparison of results of V_r in function of the program used in Table 1. As can be seen from Fig. 8, e_r values estimated using these different programs and procedures vary considerably, with significant practical implications. For example, if we consider the value of 0.6 proposed as the minimum value for sphericity [12], it can be seen that some pellets (e.g. E and H) will be classified as spherical or non-spherical depending on the IA program used (see Fig. 8). Furthermore, and as we have pointed out previously [8], e_r values are – independent of the calculation procedure used – very sensitive to differences in aspect ratio, so that values drop rapidly with increasing ellipticity. Conversely, e_r is insensitive to shape differences of pellets with AR close to one: indeed, it may show higher values for squarish projections (e.g. E and F) than for circular projections (e.g. A). The modified e_r calculation procedure [13] gives systematically higher values than Podczeck's e_r, though again the magnitude of the difference depends on the program used.

Values of V_r and V_p calculated are likewise shown in Fig. 9. As can be seen, V_r values estimated with ImagePro and SigmaScan were very similar for the most spherical pellets like A, B or C, but show important differences for highly irregular pellets such as J. It is necessary to keep

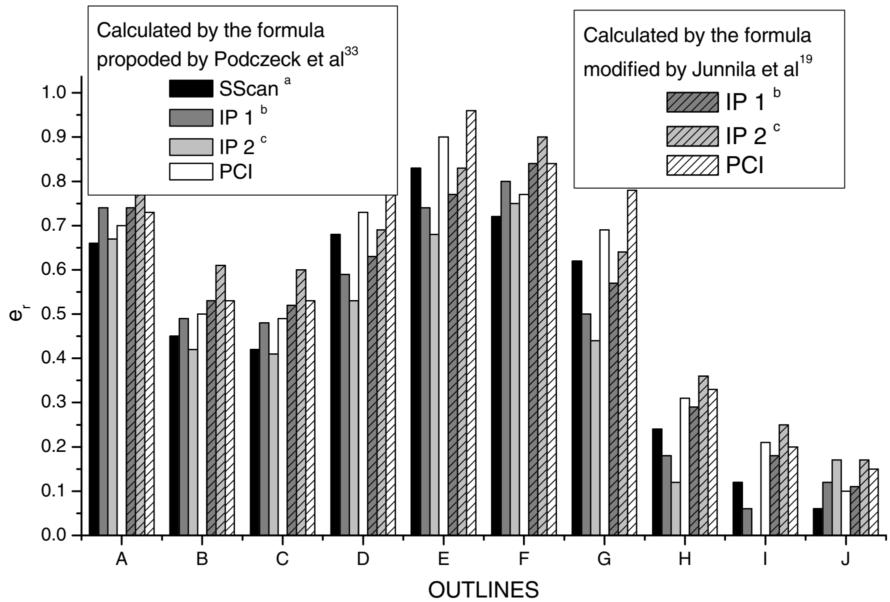

Fig. 8. Values of the shape factor e_r for the 10 particles (A–J) shown in Fig. 2, as calculated using basic particle dimensions obtained by the different image analysis programs. (a) Calculated using the SigmaScan perimeter estimate; (b) calculated using ImagePro perimeter 1; (c) calculated using ImagePro perimeter 2.

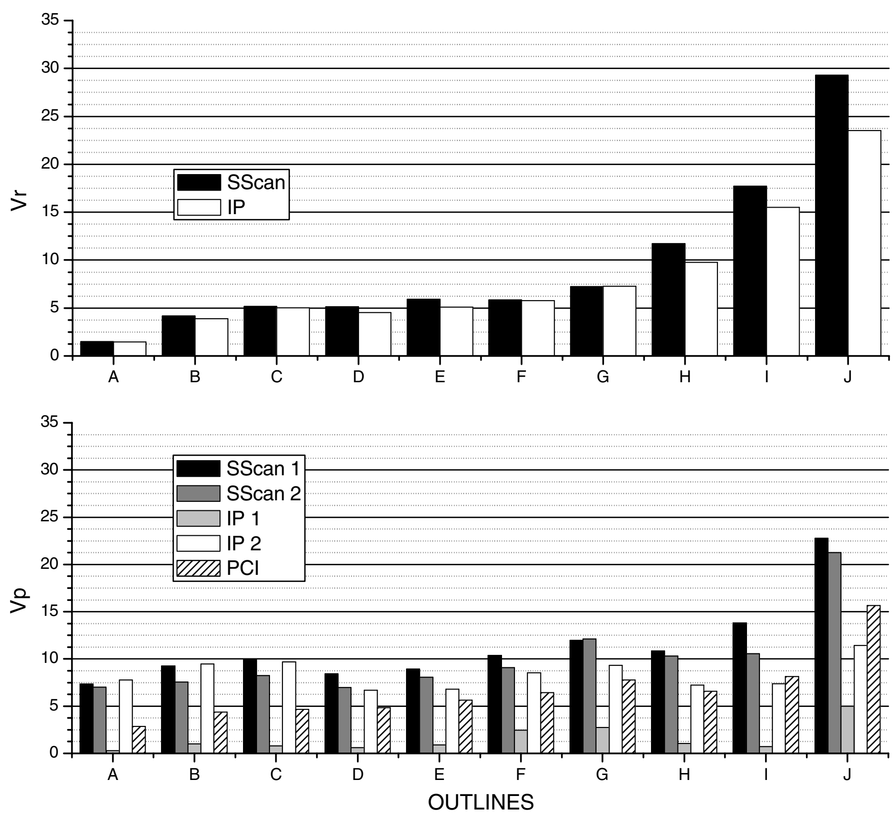

Fig. 9. Values of the shape factors, V_r and V_p, obtained for the 10 particles (A–J) shown in Fig. 2, as calculated using basic particle dimensions obtained by the different image analysis programs. Calculated using ImagePro perimeter 1 (IP 1), calculated using ImagePro perimeter 2 (IP 2), calculated using the SigmaScan perimeter estimate (SScan 1) and calculated from pixel centre to pixel centre, in the same way as ImagePro perimeter 1, but using the coordinates obtained with SigmaScan (SScan 2).

in mind that V_r is expressed as a percentage variation, so in theory it can adopt values between 0 and 100 [8]. In practice, the value of this parameter typically lies between 0 and 30. For this reason, even the biggest variations observed represent a reduced variability in the case of spherical pellets (as the pellet E). In both cases pellet radii were deter-

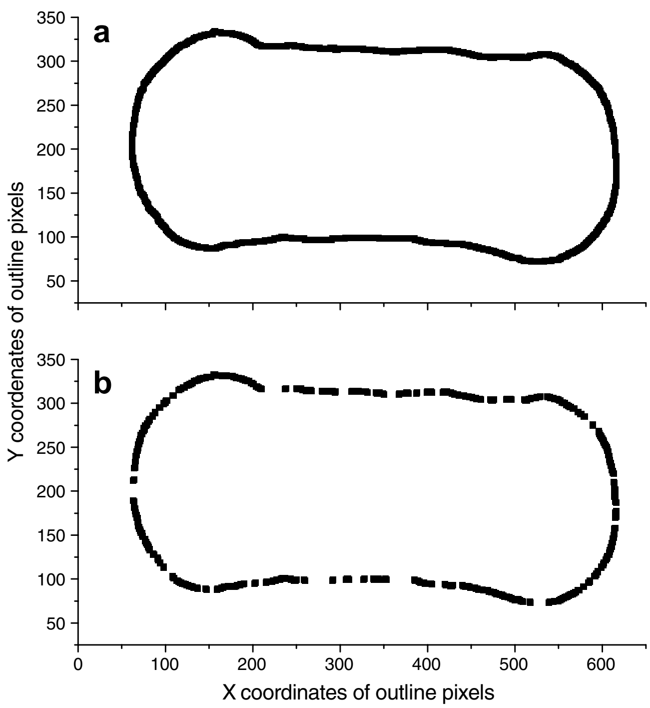

Fig. 10. Pixels used for estimation of the edge of particle J (a) by SigmaScan and (b) by ImagePro.

mined on the basis of the coordinates of all pixels constituting the perimeter: the accuracy of this approach depends above all on image quality and the grey-scale threshold values used, and in the present analysis the same image was used in both cases. The observed differences in the V_r estimates obtained using these two programs probably originate from the different number of coordinates used for the radius determinations: thus SigmaScan uses all pixels (about 1400 in the example shown in Fig. 10), while ImagePro excludes from its analysis all pixels judged to lie on a straight line between other pixels named as vertices (leaving about 500 pixels in the example shown in Fig. 10).

In contrast to V_r, V_p shows marked differences depending on the method used for calculation of the perimeter (Fig. 9), though strictly only two of the procedures used (SigmaScan method 2, and ImagePro using perimeter 2) follow the formula given in our original proposal of this shape factor [8]. However, it is interesting to see how this shape factor is affected when different measures of perimeter are used. As can be seen from Fig. 9, similar values of V_p were those obtained using SigmaScan and ImagePro perimeter 2, except in the case of highly irregular pellets with high aspect ratio. The minimal differences between V_p values determined with the two SigmaScan perimeters are attributable to the fact that we used high-resolution images (i.e. pixel size small with respect to pellet size), so there are little differences between pixel-to-pixel distances measured centre-to-centre or edge-to-edge. In the case of ImagePro, marked differences were observed between V_p values for irregular pellets, depending on the type of perimeter estimate used: this reflects the smaller number of coordinates used to determine pixel perimeter (while for more regular pellets this effect is not important). Finally, PCImage and ImagePro perimeter 1 give very different V_p values to the other procedures, reflecting the very different algorithm for perimeter determination.

4. Conclusions

The present study confirms the lack of standardization of shape factors in pharmaceutical particle analysis, with different image analysis programs supplying markedly different shape factor values. This is an important question if we bear in mind the limits founded in the bibliography for considering a particle as spherical. Clearly, this is potentially confusing and hinders meaningful comparison of results among studies and laboratories. Very interesting studies are currently being published on relationships between particle shape and diverse particle properties, but at present the non-standardization of shape factor estimation methods means that these studies cannot be evaluated and compared.

Furthermore, it is noteworthy that various studies have investigated important technical aspects of image analysis for particle characterization in pharmaceutical technology (image capture, light, and digitalization), but that there have been no serious attempts to develop standardized procedures for shape factor estimation.

So, we would suggest that there is a need for efforts to develop some sort of consensus standardization of shape factor algorithms and nomenclature, involving both pharmaceutical researchers and image analysis software designers, with the aim of facilitating research using this very useful tool. But it is highly improbable that IA system manufacturers will rewrite their code, then our fundamental recommendations could: always include adequate system information to allow interested researchers to study the acquisition of algorithm details.

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