SEM image analysis as a tool for the surface characterization of pharmaceutical pellets obtained by extrusion-spheronization

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This work focuses on the textural analysis of gray-level SEM images of pharmaceutical pellets prepared by extrusion-spheronization in order to obtain the gray level non-uniformity (GLN), the fractal dimension of pellet surface and the pore size distribution at the surface. Examples of the interest of this approach to analyze microcrystalline cellulose-based pellets and to elucidate the influence of surface properties on drug release profiles are discussed.

Keywords SEM images; textural analysis; pellets; extrusion-spheronization

1. Introduction

The term pellet designates any spherical matrix or reservoir microstructure (0.5-1.5 mm) formed by a group of particles that are joint together as a consequence of a certain agglomeration process, such as extrusion-spheronization [1]. The advantageous morphological characteristics of pellets, namely their rounded form and edge-free homogeneous surface, provide them with excellent flow behavior and high packing degree. Thus, pellets are particularly suitable for further technological processing, such as tableting, coating with polymeric films or filling in capsules [2]. The surface texture and the inner structure of pellets are strongly dependent on the nature of the components, the content in drug, the volume of wetting liquid, and the processing (e.g., extrusion, spheronization) and the drying conditions. These surface features determine friability, flowability, humidability, adhesion to substrates and drug release rate [3].

Various techniques can be used to characterize the surface of pellets, such as contact or stylus profilometry, non-contact or laser profilometry, and SEM image analysis. SEM micrographs enable to extract quantitative information of fractal geometry and surface texture. Image texture informs about the structural order of the surfaces as a function of the intensity, position and/or orientation of the pixels. To do that, parameters derived from the gray level co-occurrence matrix (GLCM) and from the number of consecutive cluster pixels in a given direction (run-length) (GLN) are used [4]. Information about the inner structure of pellets is commonly obtained using mercury intrusion porosimetry (MIP), which enables the characterization of pore volume, mean pore size and pore size distribution [5].

Microcrystalline cellulose (MCC) is the most widely excipient used to obtain pellets by extrusion-spheronization [6]. MCC wet masses exhibit an unique plasticity and tackiness performance [6] that has been widely investigated and served to develop the “gel crystallite” [7] and the “sponge” model [8]. Under a wide range of conditions, MCC leads to pellets of adequate size, shape, roughness and mechanical resistance [9]. However, MCC-based pellets show a low porosity due to the strong volumetric contraction underwent during drying, and hardly disintegrate once in contact with the aqueous medium [10]. These features result in slow penetration of water inside the pellets and drug release, which can lead to incomplete delivery of hydrophobic drugs [11].

The aim of this work was to evaluate the surface texture and microstructure of pellets prepared with various MCC varieties, to analyze the relationship between the information provided by SEM image analysis and mercury intrusion porosimetry, and to elucidate the influence on the drug release profiles.

2. Materials and methods

2.1 Materials

MCC varieties evaluated were Avicel PH101 (FMC, Ireland), Emcocel 5114 (E. Mendell, Finland), Unimac MG100 (Unitika Rayon, Japan) and MCC from India (Steeley-Berk, UK).

2.2 Preparation of pellets

One hundred grams of MCC were wetted with 90 mL of water in a RZR50 mixer (Heidolph, Germany) of 1 L capacity. The wet mass was extruded through a 1 mm mesh size screen using a Model 10 extruder (Caleva Ltd., UK) at 60 rpm, and the extruded mass was spheronized (load 20 g) for 10 min at 1200 rpm in a Model 120 spheronizer (Caleva Ltd., UK). The pellets were dried in a hot-air oven (J. Bonals, Spain) at 40°C for 24 h. Once dried, the pellets were maintained with silicagel at 20°C until assay.
2.3 Characterization of pellets

2.3.1 Shape and size

Digital images of pellets were obtained using an Olympus SZ-CTV microscope (Japan) connected to a video camera (Olympus DP12, Japan) and analyzed using PC Image VGA 24 v.2.1 (Foster Findlay Ass., UK). Size was estimated as the mean Feret diameter measured from four different angles (0°, 45°, 90° and 135°) for a minimum of 600 pellets per formulation; in all cases, the size data were best fit by a normal distribution. Circularity was calculated as $4\pi S/p^2$, where $S$ is the projection area, and $p$ is the projection perimeter.

2.3.2 SEM analysis

Pellet samples were mounted on double-sided tape on aluminum stubs and sputter-coated with gold/palladium, and micrographs were taken at appropriate magnification (scale factor 0.676 mm pixel$^{-1}$) for a detailed visualization of the surface using a Leo VP-435 SEM (Leo Electron Microscopy, UK). Surface images were 600x600 pixels coded on 256 gray levels (black = 0, white = 255). The surface fractal dimension and $GLN$ (gray-level non-uniformity) parameter were estimated as follows to characterize the roughness and the distribution of pores and holes appearing at the surface.

(a) Fractal dimension of the pellet surface. This was calculated by analyzing the Fourier amplitude spectrum (SPIP 4.6.0, Image Metrology A/S, Denmark); for different angles the Fourier profile was obtained and the logarithm of the frequency and amplitude coordinates calculated. The fractal dimension for each direction was then calculated as 2.0 minus the negative slope of the log–log curves (SPIP 4.6.0, Image Metrology A/S, Denmark).

(b) $GLN$. The 256 gray-level images were mapped to 16 gray levels. A collection of consecutive pixels with the same gray level in a given direction was a run [12]. For each direction, a two-dimensional matrix was computed (MATLAB R2007b, Mathworks Inc., Natick MA, USA); $g(i, j)$ represents the number of runs of length $j$ and gray level $i$. The run-length parameters were thus measured separately for the horizontal and vertical directions. $GLN$ values were estimated as [13]

$$GLN = \frac{\sum_{i=1}^{m-1} (2^i - 1) g(i)}{x}$$

where $x$ represents the number of run lengths on the whole image, $m$ is the number of gray levels, and $n$ represents the longest run length.

(c) Pore size distribution at the pellets surface. Grain analysis modulus was used (SPIP 4.6.0, Image Metrology A/S, Denmark). The segmentation of the image was carried out applying an automatic threshold. Log-normal distribution was fit to the pore surface frequency distributions, and the geometric means and the geometric standard deviations were estimated.

2.3.3 Porosity

Total porosity and pore size distribution were obtained in triplicate by mercury intrusion porosimetry with a Micromeritics 9305 pore sizer (Norcross GA, USA), using a 3-ml powder penetrometer. Working pressures were in the range 0.004–172.4 MPa. The following log-normal equation was used to analyze the bimodal pore size incremental distributions applying non-linear regression (GraphPad Prism v.4.02, GraphPad Software Inc., San Diego, CA)

$$V_p = \frac{V_1}{2\pi \ln \sigma_{g1}} e^{\frac{(\ln d - \ln M_1)^2}{2(\ln \sigma_{g1})^2}} + \frac{V_2}{2\pi \ln \sigma_{g2}} e^{\frac{(\ln d - \ln M_2)^2}{2(\ln \sigma_{g2})^2}}$$

where $V_p$ is the total incremental pore volume, $V_1$ and $V_2$ are the pore volumes of the two distributions integrated in the bimodal model, $d$ is the pore diameter, and $M_1$, $M_2$, $\sigma_{g1}$ and $\sigma_{g2}$ are the mean and the geometric standard deviation of the corresponding distributions. The distribution with the greatest mean value was assumed to correspond to intergranular empty spaces and to greatest pores at the pellet surface (distribution 1) and that with the lowest mean value to intragranular empty spaces (distribution 2). The fractal dimension of the pore surface of the pellets was estimated from the whole bimodal pore size distribution as follows [14]:

$$\ln \left( \frac{W_n}{r_n^3} \right) = D_s \left( \frac{V_n^{1/3}}{r_n^2} \right) + C$$

In this equation, $W_n$ represents the accumulated surface energy in the mercury intrusion process up to the $n$th stage ($W_n = \int_0^n \tau \cdot dV$), $C$ is the result of multiplying the surface tension by the cosine of the mercury contact angle, $r_n$ and $V_n$ are the pore radius and the pore volume at the $n$th stage of mercury intrusion process, respectively, $D_s$ is the fractal dimension of the pore surface, and $P$ is the applied pressure.
3. Results and discussion

The MCC varieties chosen for the study, i.e., Avicel PH101, Emcocel, Unimac and Indian MCC, remarkably differed in the mean particle size (47.3, 57.5, 39.9 and 22.4 µm, respectively) and content in lignine (0.66, 0.84, 0.35 and 0.95%, respectively) [15]. Pellets with a mean Feret diameter ranging between 650 and 750 µm were obtained (Table 1, Fig. 1a). The differences in size were due to the different volumetric contraction undergone by the extruded masses during drying [16] depending on the particle size of the MCC variety. In fact, a close relationship between these two parameters was observed (R²=0.906). The circularity values were similar for all pellet formulations and close to 0.87-0.924. Therefore, the roughness and the size of the pores were observed. Unimac and Emcocel pellets again a remarkable effect of the MCC source was observed. Emcocel and Emcocel pellets rendered low pore volume (0.04 cm³ g⁻¹) while MCC India pellets resulted in 6-fold greater pore volume (0.27 cm³ g⁻¹). Intermediate values were recorded for Avicel PH101 pellets (0.09 cm³ g⁻¹). Since these pore volumes comprise both inter and intrapellet empty spaces [19], the next step was to estimate, using eq. (2), the contribution of each component to the total pore volume as well as their pore size distributions (Table 2).

### Table 1 Mean Feret diameter, circularity, GLN, fractal dimension of the pellets surface (Df) and mean diameter of the pores at the pellets surface (M6).

<table>
<thead>
<tr>
<th>MCC</th>
<th>Feret diameter a, µm</th>
<th>Circularity b, GLN c</th>
<th>Df d</th>
<th>M6 e, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicel</td>
<td>696.6(110.0)</td>
<td>0.887(0.069)</td>
<td>3.32E4(2.26E2)</td>
<td>2.400(0.026)</td>
</tr>
<tr>
<td>Emcocel</td>
<td>654.9/150.3</td>
<td>0.895(0.067)</td>
<td>3.14E4(1.98E2)</td>
<td>2.511(0.040)</td>
</tr>
<tr>
<td>Unimac</td>
<td>723.4(98.2)</td>
<td>0.884(0.072)</td>
<td>3.28E4(4.53E2)</td>
<td>2.422(0.036)</td>
</tr>
<tr>
<td>MCC India</td>
<td>744.2(112.3)</td>
<td>0.878(0.073)</td>
<td>4.43E4(1.63E2)</td>
<td>2.276(0.019)</td>
</tr>
</tbody>
</table>

Fig. 1 Cumulative distribution of Feret diameter (a) and circularity (b) of pellets prepared using several MCC varieties.

SEM images of pellets were taken (Fig. 2) and the texture was characterized using two parameters: surface fractal dimension (Df) and GLN. Fractal methods used for image analysis correlate the gray level with the high of the pixel [17,18]. Thus, a 2D-image is considered a flat x–y map of pixels that can be transformed into a 3D image, with the gray level as the z dimension. This approach has great interest for analyzing heterogeneous or multiphase materials [13]. GLN values ranged between 31400 for the roughest pellets, which were prepared with Emcocel, to 44300 to the MCC India-pellets with the smoothest surface (table 1). Therefore, the source of MCC causes relevant differences in the surface roughness. The Df values (Table 1) were also affected by the MCC variety and Emcocel pellets exhibited greater Df values than those prepared with MCC India (2.511 vs. 2.276). Pore size distribution at the pellet surface was estimated from SEM images using the grain analysis option of the SPIP software. The pore sizes were fitted to a log-normal distribution (Fig. 3) in order to estimate the geometric mean (M6) and the geometric standard deviation (Table 1). The mean size of the pores at the surface of the roughest pellets was 2.04 µm, while the pore size at the surface of the smoothest pellets was 2-fold greater (4.39 µm). Furthermore, M6 values positively correlated with those of GLN (R²=0.977) while negatively correlated with those of Df (R²=0.924). Therefore, the roughness and the size of the pores at the surface of the pellets strongly depend on the particle size of the MCC.

Data obtained using mercury intrusion porosimetry are shown in Fig. 4a. Once again a remarkable effect of the MCC source was observed. Unimac and Emcocel pellets rendered low pore volume (0.04 cm³ g⁻¹) while MCC India pellets resulted in 6-fold greater pore volume (0.27 cm³ g⁻¹). Intermediate values were recorded for Avicel PH101 pellets (0.09 cm³ g⁻¹). Since these pore volumes comprise both inter and intrapellet empty spaces [19], the next step was to estimate, using eq. (2), the contribution of each component to the total pore volume as well as their pore size distributions (Table 2).
Fig. 2 SEM images of the surface of the pellets.

Avicel PH101

Emcocel 5114

Unimac MG100

MCC India

Fig. 3 Cumulative distribution of the size of pores at the surface of pellets prepared with different MCC varieties.

Table 2 Total pore volume ($V_p$), volume of the inter ($V_i$) and intragranular ($V_2$) empty spaces, mean diameter of the inter ($M_i$) and intragranular ($M_2$) pores and fractal dimension of the inter ($D_{s1}$) and intragranular ($D_{s2}$) empty spaces.

<table>
<thead>
<tr>
<th>MCC</th>
<th>Pore volume $V_p$, cm$^3$g$^{-1}$</th>
<th>Volume of inter $V_i$, cm$^3$g$^{-1}$</th>
<th>Mean diameter of inter $M_i$, µm</th>
<th>Mean diameter of intragranular $M_2$, µm</th>
<th>Fractal dimension of inter $D_{s1}$</th>
<th>Fractal dimension of intragranular $D_{s2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicel</td>
<td>0.0908</td>
<td>0.0308</td>
<td>74.99(2.60)</td>
<td>0.34(2.04)</td>
<td>2.373(0.010)</td>
<td>2.527(0.024)</td>
</tr>
<tr>
<td>Eemcocel</td>
<td>0.0462</td>
<td>0.0260</td>
<td>72.11(2.61)</td>
<td>0.29(2.58)</td>
<td>2.406(0.009)</td>
<td>2.711(0.035)</td>
</tr>
<tr>
<td>Unimac</td>
<td>0.0360</td>
<td>0.0265</td>
<td>72.44(2.57)</td>
<td>0.22(3.53)</td>
<td>2.401(0.011)</td>
<td>2.766(0.028)</td>
</tr>
<tr>
<td>MCC India</td>
<td>0.2695</td>
<td>0.0695</td>
<td>87.50(2.87)</td>
<td>0.49(1.76)</td>
<td>2.285(0.001)</td>
<td>2.226(0.012)</td>
</tr>
</tbody>
</table>

* Geometric mean (geometric standard deviation) after fitting to log-normal bimodal distributions ($R^2>0.95$).

* Values estimated by linear regression (standard error, $R^2>0.99$).
The volume and the mean size of the intergranular empty spaces were quite similar for all pellets \( (V_i \approx 0.03 \, \text{cm}^3\text{g}^{-1} \) and \( M_i \approx 72-75 \, \text{μm} \) except for MCC India pellets (Table 2), which showed notable greater \( V_i \) and \( M_i \) values. Oppositely, the volume and the mean size of the intragranular empty spaces \( (V_2 \) and \( M_2 \) were clearly dependent on the MCC source. The extreme values were shown for Unimac and MCC India, while Emcocel and Avicel PH101 showed an intermediate behaviour. The fractal analysis of pore surface was carried out applying a model based on the thermodynamics of the intrusion process [20]. The results obtained are shown in Fig. 4.b. The double logarithmic plot deviated from the linearity when the whole pore sizes interval was considered, which indicates that the value of the fractal dimension is not constant but depends on the analysis scale as previously reported [21]. In the case of polydisperse pore size distributions, the quality of the fitting can be notably improved by analyzing each subpopulation separately [22]. The evaluated pellets had two pore size distributions, interpellet empty spaces and pores of high size and intrapellet empty spaces of small-to-medium size, and this could be the reason for the non-linear pattern. In fact, two linear regions can be distinguished in Fig. 4.b: one corresponding to interpellet spaces and the greatest pores at the surface of the pellets \( (D_{s1}) \), another corresponding to intra-pellet empty spaces \( (D_{s2}) \) with a transition region between them. The \( D_{s1} \) values were from 2.285 for MCC India pellets (the smoothest ones) to 2.406 for Emcocel pellets (the roughest ones). The \( D_{s2} \) values showed a similar trend, i.e., the roughest pellets have rough inner pores. The size of the pellets and the surface texture explain the differences found in \( D_{s1} \) values [3]. No statistically relevant correlation was found between \( D_{s1} \) and Feret diameter \( (R^2=0.502) \). However, a closer correlation was observed for \( D_{s1} \) and \( D_f \) and particular with GLN values (Fig. 5.a).

In sum, the surface texture and the inner structure of the MCC pellets prepared by extrusion-spheronization can be finely tuned by the features of the MCC variety. The possibility of obtaining highly porous pellets and with large pores at their surface enables the preparation of rapid release formulations. Oppositely, if a sustained delivery is wanted, pellets with small pores at the surface should be prepared. If the lignin content is above 60%, the particle size of MCC

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**Fig. 4** Cumulative pore size distributions obtained using mercury intrusion porosimetry (a) and fitting to eq. (3) in order to estimate the \( D_{s1} \) and \( D_{s2} \) values (b). With the aim of clarity, the values of \( Ln(W_j/r_n^2) \) were increased in \( 5^*, 10 \) or \( 15^\text{**} \) units.

**Fig. 5** Linear dependence of \( D_f \) or GLN on \( D_{s1} \) (a) and influence of the MCC particle size on \( D_{s1}, D_{s2} \) and GLN (b). Values found for Emcocel are framed (b).
determines the surface and the microstructure of the pellets. Emcocel deviates from this general rule since its lignin content is just 0.35%. SEM image analysis is shown as a useful tool able to provide complementary information to that provided by mercury intrusion porosimetry; e.g., SEM enables to characterize the pore size distribution at the pellet surface, with is hard to do using other techniques.

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