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Release behaviour of clozapine matrix pellets based on percolation theory

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ABSTRACT

The release behaviour of clozapine matrix pellets was studied in order to investigate if it is possible to explain it applying the concepts of percolation theory, previously used in the understanding of the release process of inert and hydrophilic matrix tablets. Thirteen batches of pellets with different proportions of clozapine/microcrystalline cellulose (MCC)/hydroxypropylmethyl cellulose (HPMC) and different clozapine particle size fractions were prepared by extrusion–spheronisation and the release profiles were studied. It has been observed that the distance to the excipient (HPMC) percolation threshold is important to control the release rate. Furthermore, the drug percolation threshold has a big influence in these systems. Batches very close to the drug percolation threshold, show a clear effect of the drug particle size in the release rate. However, this effect is much less evident when there is a bigger distance to the drug percolation threshold, so the release behaviour of clozapine matrix pellets is possible to be explained based on the percolation theory.

1. Introduction

The pelletization process involves the agglomeration of active pharmaceutical ingredients and excipients in spherical units of size comprised between 0.5 and 1.5 mm called pellets (Bataille et al., 1993).

These multiparticulate systems offer a wide range of therapeutic as well as technological advantages compared with monolithic systems: they disperse as individualized units in the gastrointestinal tract reducing high local drug concentration (minimising side effects like the irritation of the gastric mucosa), maximising drug absorption and reducing peak plasma fluctuation. Another additional therapeutic advantage is that these dosage forms eliminate the dependence of the drug effect on gastric emptying, thus reducing intra and interindividual variability of the drug plasma concentrations (Bechgaard and Hegermann Nielsen, 1978; Bodmeier, 1997).

Examples of technological advantages of the pellets are their spherical shape, their narrow particle size distribution and their low friability which provide them very good flow properties and make it easier to coat them or include them into hard gelatin capsules as well as compress them into tablets (Reynolds, 1970). Moreover, these dosage forms allow combining non-compatible drugs or different drug release profiles in the same formulation (Pinto et al., 2001; Quintavalle et al., 2008).

Pellets can be manufactured in different ways. Nevertheless, the most popular method of producing pellets is by an extrusion–spheronisation process which involves four different stages: preparation of the wet mass (granulation), extrusion of the granulated mass to obtain cylindrical extrudates, rounding of the extrudates into spheres (spheronisation) and finally, drying of the spheres (Reynolds, 1970; Vervaet et al., 1995). The excipient most used to produce pellets by this technique is the microcrystalline cellulose due to its favourable plastic properties (Chatlapalli and Rohera, 1998; Delalonde et al., 1997).

Clozapine is an atypical antipsychotic whose main indication is the treatment of resistant schizophrenia, although it has also demonstrated efficacy in the treatment of other conditions like schizoaffective disorders, bipolar depression and some neurological disorders. One important advantage of this drug is its low risk of producing side effects like extrapyramidal side effects, tardive diskynesia or elevated prolactine levels compared with other antipsychotics (Elizondo, 2008; Iqbal et al., 2003), which makes it one of the most used drugs in the treatment of schizophrenia.

To the best of our knowledge, no pellet formulation of clozapine has been investigated; we therefore undertook formulation studies in order to take advantage of these dosage forms for clozapine treatment.

The percolation theory was introduced into the pharmaceutical field by Leuenberger and co-workers (Blattner et al., 1990; Bonny

Abbreviations: MCC, microcrystalline cellulose; HPMC, hydroxypropylmethyl cellulose; cp, centipoise; CI, compresibility index; HR, Haussner ratio; SI, sphericity index.

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Table 1
Composition and spheronisation conditions of the different batches manufactured.

	1	2	3	4	5	6	7	8	9	A	B	C	D
Clozapine													
Mixture	20%												
Size fraction:													
$x < 125\ \mu m$		20%			30%					20%		30%	
$125 < x < 250\ \mu m$			20%			30%		40%	20%				
$x > 250\ \mu m$				20%			30%				20%		30%
HPMC 4000 cp	10%									2%	2%	2%	2%
HPMC 100000 cp		40%	40%	40%	40%	40%	40%	40%	40%				
MCC	70%	40%	40%	40%	30%	30%	30%	20%	38.5%	78%	78%	68%	68%
Mg stearate									1.5%				
Granulation liquid (ml)													
Ethanol/water:													
(90%/10%)		240	236	238	240	240	248	233	240				
(85%/15%)	140												
(80%/20%)													
Distilled water:										85	88	65	60
Spheronisation													
Time (min)	3	2 + 1[a]	2 + 1[a]	2	2 + 1.5[a]	2 + 1[a]	2 + 0.5[a]	2 + 1[a]	2 + 0.5[a]	2	2	2	2
Speed (rpm)	1040	1040 (1600)	1040 (1600)	1040	1040 (1600)	1040 (1600)	1040 (1600)	1040 (1460)	1040 (1460)	1040	1040	1040	1040

[a] Additional spheronisation time at the speed indicated in parenthesis.

and Leuenberger, 1991, 1993; Holman and Leuenberger, 1988; Leuenberger and Leu, 1992; Leuenberger et al., 1987), retrieving practical consequences from the theoretical principles of the percolation theory. These findings have been applied nowadays to the design and optimisation of an increasing number of pharmaceutical formulations.

In relation with this theory, a cluster is defined as a group of neighbouring particles of the same component and is considered infinite or percolating when it extends from one side to the other side of the system (Stauffer and Aharony, 1992). Percolation threshold corresponds to the concentration of one component for which there is a maximum probability of appearance of an infinite cluster of it. At this concentration point, it is expected that some properties of the system change suddenly.

Numerous studies applying the concepts of the percolation theory to the release behaviour of inert and hydrophilic matrix systems have been carried out. In inert matrix, the drug is released through both, the initial pores of the matrix and the pores formed when the drug has been dissolved (Caraballo et al., 1993). Therefore, the ideal formulation of an inert matrix, according to the percolation theory, would be above the drug percolation threshold since this fact assures the release of the total drug dose. The excipient must also be above its percolation threshold to avoid the disintegration of the matrix during the release process and to control the drug release (Bonny and Leuenberger, 1991, 1993; Caraballo et al., 1999; Melgoza et al., 1998). In the case of the hydrophilic matrix, the ideal formulation is above the excipient percolation threshold, since at that point a percolating cluster of the excipient is formed controlling the hydration and the release rate of the drug through the gel layer formed. Below the excipient percolation threshold the gel layer is less coherent resulting in a fast erosion of the matrix and conducting to a rapid diffusion of the drug (Caraballo, 2009).

The aim of this study is to investigate, for the first time, if the release behaviour of matrix pellets containing clozapine can be explained using the concepts of percolation theory, which have been previously used in the understanding of the release process of inert and hydrophilic matrix tablets.

2. Materials and methods

2.1. Materials

The following materials were used as matrix forming materials: hydroxypropylmethyl cellulose (Metolose 4000 and Metolose 100,000, Shin-Etsu Chemical Co. Ltd., Japan), microcrystalline cellulose (Vivapur 101, J. Rettenmaier&Söhne GmbH + Co. KG, Germany), magnesium stearate (Cooper, France) and clozapine (Ningbo Yuanfang Biochemicals Co. Ltd., China). Ethanol (Merck, Germany) and distilled water were used as granulation liquid. All reagents and solvents used were of analytical grade. Water was always used in demineralized quality.

2.2. Methods

2.2.1. Preparation of the pellets

Table 1 describes the studied formulations. Thirteen batches of 100 g of pellets with different proportions of clozapine/MCC/HPMC were prepared. The batches prepared contained 20%, 30% or 40% of clozapine. The drug particle size employed is indicated in Table 1. The content of HPMC was 2% for batches A–D, 10% for batch 1 and 40% for batches 2–9.

The powders were blended in a Turbula mixer (Willy A. Bachofen, Basle, Switzerland) for 5 min and the wetting was done in a planetary mixer (Kenwood Major, UK) during 20 min with ethanol–water mixtures in different proportions for batches 1–9 and distilled water for batches A–D.

Extrusion was performed on a single screw axial extruder (Pharmex 35T Gabler Machinenbau, Ettlingen, Germany) equipped with a 1 mm screen and operated at 50 rpm. Spheronisation was carried out on a Sphaeromat SPH 250 MA spheroniser (Gabler Machinenbau, Ettlingen, Germany) equipped with a serrated plate of 250 mm diameter and operated at different speeds depending on the batch. The spheronisation time also varied from 2 min to 3 min 30 s depending on the formula. An additional spheronisation time at a different speed was necessary for some batches (the speed of this additional spheronisation period is expressed in parentheses in Table 1). All the batches were dried in a ventilated oven (Binder FP400, Germany) at a temperature of 50 °C during 20 h.

2.2.2. Analytical methods

2.2.2.1. Rheological studies of the drug and excipients. Rheological studies for both, drug and excipients (clozapine, MCC, HPMC 4000 cp and HPMC 100000 cp) were carried out according to European Pharmacopoeia (2007).

The bulk and tapped densities were determined in triplicate for the excipients in a volumeter (type STAV II, J Engelmann AG, Germany). The bulk density (ρ_{bulk}) was determined by measur-

ing the bulk volume (V_{bulk}) occupied by 75 g of sample filled into a 250 ml graduated cylinder readable to 2 ml. The tapped density (ρ_{tapped}) was determined from the tapped volume (V_{tapped}) occupied by the powder after repeated taps until the difference between successive measurements was less than 2 ml. The values of densities were calculated from the measured volumes and its weight according to Eqs. (1) and (2):

$$\rho_{bulk} = \frac{m}{V_{bulk}} \quad (1)$$

$$\rho_{tapped} = \frac{m}{V_{tapped}} \quad (2)$$

Obtained values of densities were used for calculating the compressibility index (CI) as indicated in Eq. (3):

$$CI = \frac{100(V_0 - V_f)}{V_0} \quad (3)$$

The Hausner ratio (HR) was calculated according to Eq. (4):

$$HR = \frac{V_{bulk}}{V_{tapped}} \quad (4)$$

The flowability was performed also in triplicate in a granulate flow tester (Erweka GmbH type GTB, Heusenstamm, Germany) with an outflow opening diameter of 25 ± 0.01 mm, for both, clozapine and excipients.

The rest angle was also carried out in triplicate in a granulate flow tester (Erweka GmbH type GTB, Heusenstamm, Germany), with an outflow opening diameter of 25 ± 0.01 mm following the rule DIN 53916 and it was made only for clozapine.

2.2.2.2. Particle size analysis. The particle size analysis of the drug and excipients was carried out using laser diffraction in a Mastersizer X 2.18 version (Malvern Instruments Ltd., UK) equipped with MSX dry powder feeder. The results are expressed as percentiles 10, 50 and 90% of the volume diameter. Each sample was performed in duplicate and the results are expressed as the mean ± standard deviation.

2.2.2.3. Differential scanning calorimetry. Differential scanning calorimetry was performed in a Setaram 131, France DSC in order to investigate potential solid state interactions between drug and excipients. Standard aluminium sample pans (40 μl) were used and a heating rate of 10 °C/min was employed in the range of 25–400 °C.

2.2.2.4. Optical microscopy. Olympus SZ binoculars with a magnification of 10× interfaced via a digital camera Moticam 2300 to a computer with the software Motic Image v.2.0 and Leica S8APO binoculars with a magnification of 10× interfaced via a digital camera Leica DC 300 v.2.0 to a computer with the software Leica IM50 v.1.20 were used to perform the image analysis of the drug, excipients, and pellets.

2.2.2.5. Pellets size. Pellets produced were subjected to sieve analysis using a nest of standard sieves (Retsch, Germany). The fraction of pellets corresponding to the range 0.710–1 mm of the different batches was used for further characterisation. Mean particle diameter was determined using an image analyzer program (Image-Pro Plus v.6).

2.2.2.6. Sphericity index. The sphericity index (SI) of the fraction of pellets with size comprised between 0.710 and 1 mm was determined by image analysis employing the program Image-Pro plus v.6. This index was calculated as indicated in Eq. (5):

$$SI = \left(\frac{\overline{D_{min}}}{D_{max}}\right) \quad (5)$$

D_{min} and D_{max} are the smallest and largest diameter of a pellet (Galland et al., 2005).

2.2.2.7. Scanning electron microscopy. The morphology of the drug and excipients as well as the drug distribution within the pellets was studied by means of a scanning electron microscope (Philips XL-30, Eindhoven, Holland), after coating the samples with a thin layer of gold on a sputter coater (Edwards Pirani 501 Scan-Coat Six, Crawley, West Sussex, UK). Microphotographs were obtained at a magnification appropriate for particle size.

2.2.2.8. Particle density. The particle density was determined for both, drug and excipients, using a helium pycnometer (Micromeritics SA type Multivolume pycnometer 1305), employing a known mass of powder between 7.5 and 10.5 g. The sample volume is determined using Eq. (6):

$$V_s = V_c - \frac{V_r}{P_1/P_2 - 1} \quad (6)$$

where V_s is the sample volume, V_c is the cell volume, V_r is the reference volume, P_1 is the initial pressure and P_2 is the final pressure. The density of the powder mass, ρ, is given by Eq. (7):

$$\rho = \frac{m}{V_s} \quad (7)$$

The test was repeated two times for each product.

2.2.2.9. Study of the drug release. Hard gelatine capsules were filled with pellets of the different batches containing 25 mg of clozapine.

The dissolution studies for batches 1–9 were performed using the basket method (European pharmacopoeia, 2007) in a dissolution tester (Pharmatest DT70, Germany). 900 ml of pH 4 acetate buffer dissolution media (USP, 2009) was used for the assay. The temperature was fixed at 37 ± 0.5 °C and the stirring speed was 50 rpm. Samples were analyzed using a continuous flow-through system attached to an 8 cell UV/VIS (Specord 250, Germany) spectrophotometer. The wavelength was set to 260 nm. Readings of absorbance were taken every 10 min until 100% of the drug was released.

For batches A–D, the dissolution studies were carried out using the basket method (European pharmacopoeia, 2007) in a Sotax AT7 smart (Allschwil, Switzerland). 900 ml of pH 4 acetate buffer dissolution media (USP, 2009) was used for the assay. The temperature was fixed at 37 ± 0.5 °C and the stirring speed was 50 rpm. 5 ml samples were withdrawn at each time period during 7 h and measured in a UV/VIS Agilent 8453 (California, USA) at 260 nm. The clozapine content was calculated using a previously determined calibration curve. All the release data are expressed as the mean ± standard deviation (S.D.) of six samples.

2.2.2.10. Dissolution data analysis. Drug release data ($M_t/M_\infty \leq 0.9$) were analyzed according to Higuchi (1963) (8), Korsmeyer et al. (1983) (9), zero order (10), first order (11) and Peppas and Sahlin (1989) (12) equations:

$$\frac{M_t}{M_\infty} = bt^{1/2} \quad (8)$$

$$\frac{M_t}{M_\infty} = k_k t^n \quad (9)$$

$$\frac{M_t}{M_\infty} = k_0 t \quad (10)$$

$$\frac{M_t}{M_\infty} = M_0\, e^{k_1 t} \quad (11)$$

$$\frac{M_t}{M_\infty} = k_d t^m + k_r t^{2m} \quad (12)$$

Table 2
Densities of drug and excipients, compressibility index, Hausner ratio, rest angle and flowability.

Sample	Bulk density[a] (g/cm^3)	Tapped density[a] (g/cm^3)	Compressibility index[a]	Hausner ratio[a]	Rest angle[a] (°)	Flowability[a] (g/s)
MCC	0.335 ± 0.003	0.477 ± 0.002	29.65 ± 0.99	1.42 ± 0.02		0
HPMC 4000 cp	0.332 ± 0.002	0.502 ± 0.004	33.82 ± 0.74	1.51 ± 0.02		0
HPMC 100000 cp	0.310 ± 0.003	0.493 ± 0.006	37.28 ± 0.12	1.59 ± 0.00		0
Clozapine					36.67 ± 2.67	79.04 ± 2.11

[a] Mean value ± standard deviation (three replicates).

where M_t/M_∞ is the drug released fraction at time t (M_∞ corresponds to the drug amount released at infinite time), b is the Higuchi's release rate constant on Eq. (8), k_k is the korsmeyer's kinetic constant on Eq. (9), k_0 is the zero order release rate constant on Eq. (10) and k_1 is the first order release rate constant on Eq. (11). t is the release time, n is the korsmeyer's time exponent that depends on the release mechanism and the shape of the matrix tested (Ritger and Peppas, 1987), k_d is the diffusional rate constant and k_r the relaxational rate constant. m is the purely Fickian diffusion exponent (which depends on the geometrical shape of the releasing device through its aspect ratio).

The optimum values for the parameters present in each equation were determined by linear or non-linear least-squares fitting methods with SPSS version 17.0 software. The determination coefficient (r^2) was used to test the applicability of the release models.

3. Results and discussion

3.1. Preparation of the pellets

A homogeneity study of pellets was carried out on the first batch to validate the process. The content of clozapine was determined in five samples of the dry mix, granulated, extrudate and pellets, obtaining the following values for the corresponding coefficients of variation (3.22, 8.70, 4.34, and 1.99, respectively).

3.2. Rheological studies of the drug and excipients

Rest angle informs about the internal friction or cohesion between the particles (Vila Jato, 1997). Values below 40° suggest intermediate flow properties for clozapine. However, an adequate flow rate (79.04 ± 2.11 g/s) has been measured, using a funnel of 25 mm of diameter.

The data of the compressibility index of the MCC, 29.65 ± 0.99, as well as the Haussner ratio, 1.42 ± 0.02 suggest that this excipient has poor flow properties. Bigger differences between bulk and tapped densities have been found for HPMC 4000 cp and HPMC 100000 cp. Data of rheological studies are shown in Table 2.

3.3. Particle size analysis

The results obtained using the technique of laser diffraction in a Mastersizer can be observed in Table 3.

3.4. Differential scanning calorimetry

DSC assays have been performed for the five pure components as well as for the 1:1 binary physical mixtures of each component with the drug. As Fig. 1 shows, displacement of the clozapine melting peak (at 184 °C) has not been observed. Therefore, solid state interactions between drug and excipients are not expected.

3.5. Optical microscopy

Drug and excipient particles have been observed using optical microscopy. It was observed that clozapine crystallizes in the hexagonal system, whereas the cellulose derivatives (MCC, HPMC 4000 cp and 100000 cp) showed fibrous appearance.

Table 3
Malvern® particle size analysis data for clozapine, MCC, HPMC 4000 cp and HPMC 100000 cp powders.

Material	D (v,0.1)[a]	D (v,0.5)[b]	D (v,0.9)[c]
Clozapine	6.26 ± 0.30	58.28 ± 3.12	205.13 ± 3.75
MCC	18.96 ± 0.25	59.3 ± 0.37	135.7 ± 1.05
HPMC 4000 cp	22.36 ± 0.14	72.53 ± 0.25	181.6 ± 1.20
HPMC 100000 cp	23.89 ± 0.16	75.85 ± 0.20	179.76 ± 1.45

[a] Mean ± standard deviation (n = 2) of the percentile 10 of the volume diameter of particles.
[b] Mean ± standard deviation (n = 2) of the percentile 50 of the volume diameter of particles.
[c] Mean ± standard deviation (n = 2) of the percentile 90 of the volume diameter of particles.

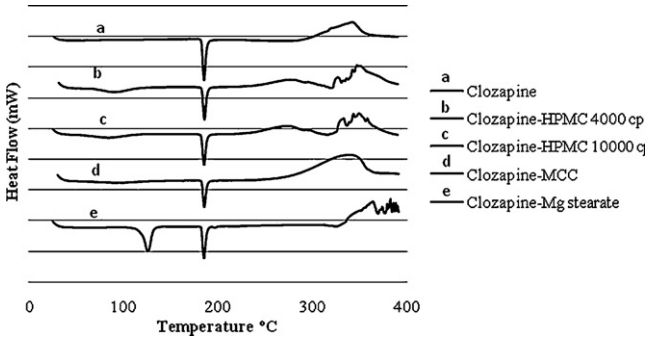

Fig. 1. DSC thermograms of (from top to bottom): (a) pure clozapine, (b) 1:1 blend of clozapine with HPMC 4000 cp, (c) 1:1 blend clozapine–HPMC 100000 cp, (d) 1:1 blend clozapine–MCC and (e) 1:1 blend clozapine-Mg stearate.

3.6. Pellets size

As indicated in Section 2, the selected size fraction of pellets was examined using image analysis. The obtained results are shown in Table 4.

The high values obtained can be attributed to the fact that using this technique, the particle size is estimated based on the measure of two dimensions, being one dimension (vertical) not considered.

Taking into account that there is a higher probability for the lower dimension of the particle being hidden, this would result in an overestimation of the mean diameter using this technique.

3.7. Sphericity index

As can be appreciated in Table 4, pellets formulations containing high concentrations of HPMC (batches 1–9) show a sphericity index relatively far from 1. Some difficulties were experienced concerning the management of high concentrations of HPMC as matrix former. On the other hand, batches containing 2% of HPMC show sphericity values of approximately 0.8, which can be considered adequate values (Galland et al., 2005).

Table 4
Pellets mean diameter (mm) and sphericity index.

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9	Batch A	Batch B	Batch C	Batch D
Pellet mean diameter (mm)													
Mean	1.159	0.966	1.003	0.898	0.911	0.941	0.943	0.989	1.009	0.979	0.991	1.042	0.992
S.D.	0.200	0.144	0.173	0.123	0.121	0.104	0.147	0.139	0.123	0.122	0.118	0.093	0.107
Pellet sphericity index[a]													
Mean	0.698	0.668	0.651	0.699	0.673	0.717	0.656	0.628	0.613	0.771	0.792	0.812	0.801
S.D.	0.133	0.108	0.134	0.118	0.115	0.102	0.129	0.119	0.116	0.096	0.097	0.078	0.081

[a] Calculated as the mean ratio between the smallest and the largest diameter of 75 pellets.

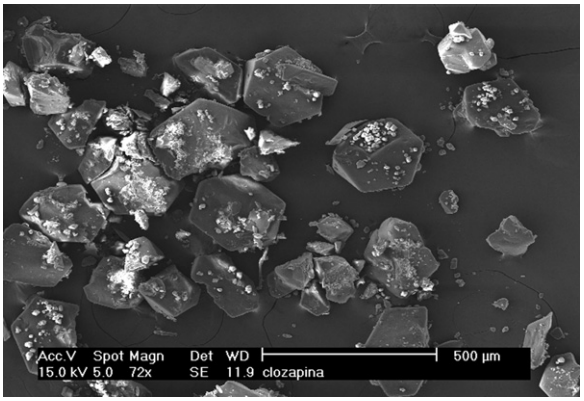

Fig. 2. Microphotograph corresponding to clozapine particles.

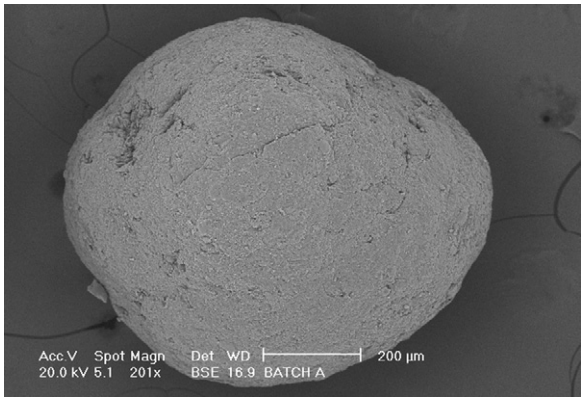

Fig. 4. Microphotograph corresponding to an uncut pellet from batch A (20% of clozapine (particle size smaller than 125 μm)/2% HPMC 4000 cp/78% MCC).

3.8. Scanning electron microscopy

Figs. 2–4 show microphotographs of clozapine and uncut pellets from batches 2 and A. Both batches were prepared using the smallest clozapine particle size fraction and have been selected as representative of pellets containing 40% of HPMC and 2% of HPMC, respectively. As Figs. 3 and 4 illustrate, a clearly smoother surface can be observed in pellets containing 2% of HPMC.

On the other hand, Figs. 5 and 6 correspond to microphotographs showing the cross section of cut pellets from batches A and D, containing the smallest and the biggest clozapine particle size fractions, respectively.

Technological conditions have been forced to observe the effect of the different particle size on the drug distribution within the pellet. A clear difference in the drug distribution can be observed comparing the finest and the coarsest clozapine particles.

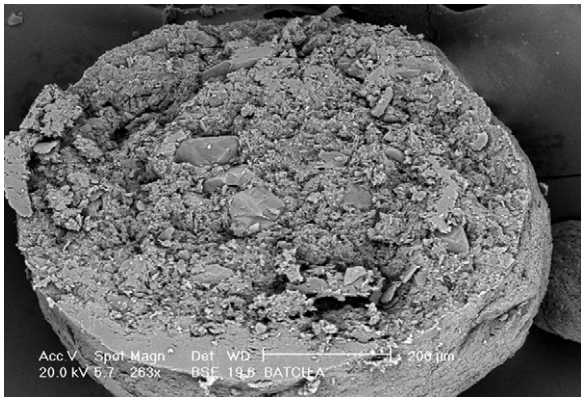

Fig. 5. Microphotograph corresponding to the cross section of a cut pellet from batch A (20% of clozapine (particle size smaller than 125 μm)/2% HPMC 4000 cp/78% MCC).

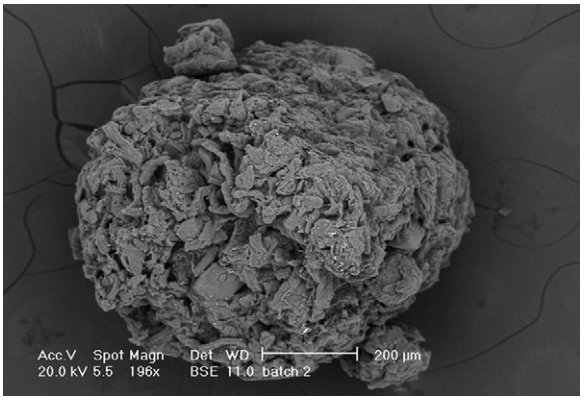

Fig. 3. Microphotograph corresponding to an uncut pellet from batch 2 (20% of clozapine (particle size smaller than 125 μm)/40% HPMC 100000 cp/40% MCC).

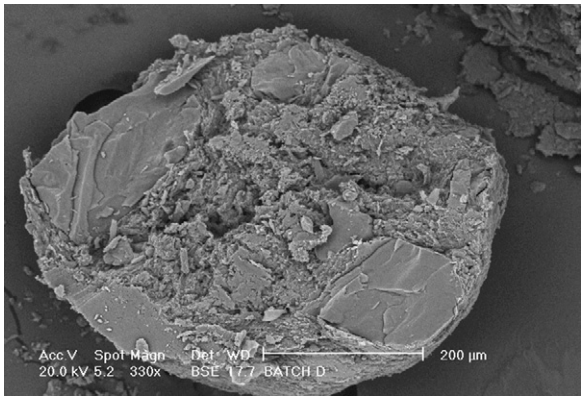

Fig. 6. Microphotograph corresponding to the cross section of a cut pellet from batch D (30% of clozapine (particle size bigger than 250 μm)/2% HPMC 4000 cp/68% MCC).

Table 5
Mathematical modelling and drug release kinetics from pellets of the different batches.

Batch	Higuchi		Korsmeyer			Zero order		First order		Peppas and Sahlin		
	b^a ($min^{-0.5}$)	r^{2b}	$k_k{}^c$(min^{-n})	n^d	r^{2b}	$k_0{}^e$(min^{-1})	r^{2b}	$k_1{}^f$(min^{-1})	r^{2b}	$k_d{}^g$($min^{-0.425}$)	$k_r{}^h$($min^{-0.850}$)	r^{2b}
1	0.135	0.948	0.089	0.614	0.806	0.021	0.905	0.028	0.772	0.098	0.017	0.953
2	0.075	0.873	0.127	0.422	0.847	0.005	0.682	0.006	0.573	0.190	−0.009	0.933
3	0.044	0.919	0.145	0.328	0.922	0.002	0.754	0.003	0.665	0.128	−0.004	0.985
4	0.062	0.855	0.176	0.333	0.838	0.004	0.652	0.004	0.574	0.184	−0.009	0.950
5	0.076	0.926	0.002	1.294	0.655	0.005	0.809	0.014	0.357	0.125	0.000	0.926
6	0.100	0.925	0.001	1.468	0.720	0.009	0.918	0.026	0.482	0.038	0.013	0.942
7	0.090	0.977	0.105	0.479	0.947	0.008	0.838	0.010	0.756	0.145	−0.002	0.986
8	0.099	0.981	0.035	0.730	0.984	0.009	0.954	0.018	0.838	0.058	0.012	0.991
9	0.106	0.941	0.011	1.019	0.904	0.009	0.901	0.021	0.665	0.069	0.011	0.945
A	0.141	0.958	0.050	0.790	0.893	0.022	0.949	0.035	0.808	0.058	0.027	0.975
B	0.102	0.985	0.069	0.597	0.969	0.010	0.874	0.015	0.771	0.115	0.004	0.982
C	0.127	0.970	0.057	0.712	0.920	0.017	0.934	0.027	0.814	0.080	0.017	0.977
D	0.116	0.988	0.083	0.588	0.963	0.014	0.906	0.019	0.837	0.115	0.008	0.987

[a] Higuchi's release rate constant.
[b] Determination coefficient.
[c] Korsmeyer's kinetic constant.
[d] Korsmeyer's time exponent.
[e] Zero order kinetic constant.
[f] First order kinetic constant.
[g] Peppas's diffusion kinetic constant.
[h] Peppas's relaxation kinetic constant.

3.9. Particle density

The values of particle density determined by helium pycnometry for clozapine, MCC, HPMC 4000 cp and HPMC 100000 cp are the following: 1.307 ± 0.001, 1.534 ± 0.001, 1.305 ± 0.002 and 1.315 ± 0.001. Big differences in particle density have not been found, so this factor is not expected to cause significant segregation of the components within the formulation.

3.10. Study of the drug release

The low solubility of clozapine does not allow performing a standard release assay for this drug. Especially in this work, where the influence of formulation factors on the release kinetics will be studied, we must be sure that the low solubility of the drug will not mask the influence of the formulation. Therefore, as it has been indicated in Section 2, a pH 4 buffer dissolution medium (USP, 2009) has been employed in the release assay in order to increase drastically the solubility of clozapine.

This method has the disadvantage that the obtained release profiles will be faster than the in vivo expected drug release rate. Nevertheless it will allow keeping sink conditions, which are essential to study the influence of the formulation factors.

As it can be seen in Table 5, the majority of the batches show values of the Korsmeyer's time exponent "*n*" between 0.3 and 0.7, which can be attributed to diffusion kinetics. It can be observed in Fig. 7 that batch 1 shows the fastest drug release with almost 100% of drug released at the first hour, in contrast with the other batches, which release between 60 and 80% of drug in the first hour. This can be explained because batch 1 contains 10% of HPMC. Therefore, this polymer would be under its percolation threshold (Castellanos Gil et al., 2009; Contreras et al., 2010; Miranda et al., 2006). Below the HPMC percolation threshold, this excipient does not percolate the system and the drug release is not controlled by the gel layer. In this situation, the gel layer initially formed shows important "holes" which allow a fast water uptake, leading to a quicker release process, similar to conventional dosage forms. By contrast, above the excipient percolation threshold, a percolating cluster of this component exists and a coherent gel layer is formed from the first moment, which is able to control the hydration and drug release rate (Caraballo, 2009). The observed influence of the percolation threshold of the hydrophilic polymer on the release rate is a typical behaviour of hydrophilic matrix systems.

Batches 2–4 (20% clozapine (w/w)) show a marked bimodal release behaviour (see Fig. 7). These formulations show a burst effect, releasing the drug faster during the first 30 min and after

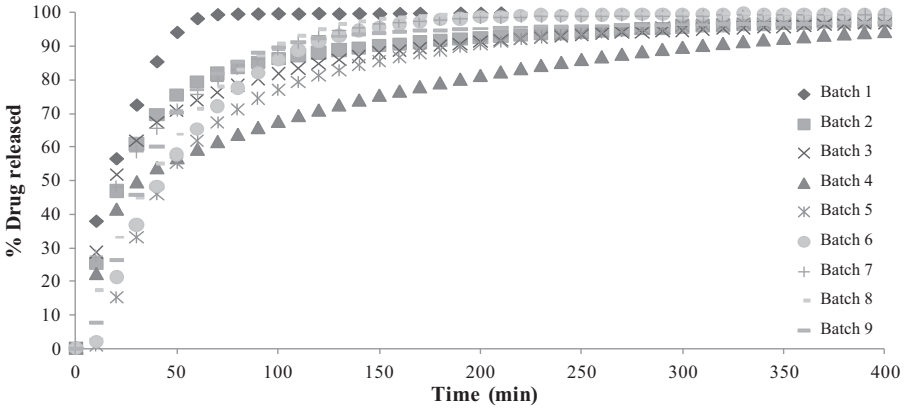

Fig. 7. Percentage of clozapine released versus time of batches 1–9.

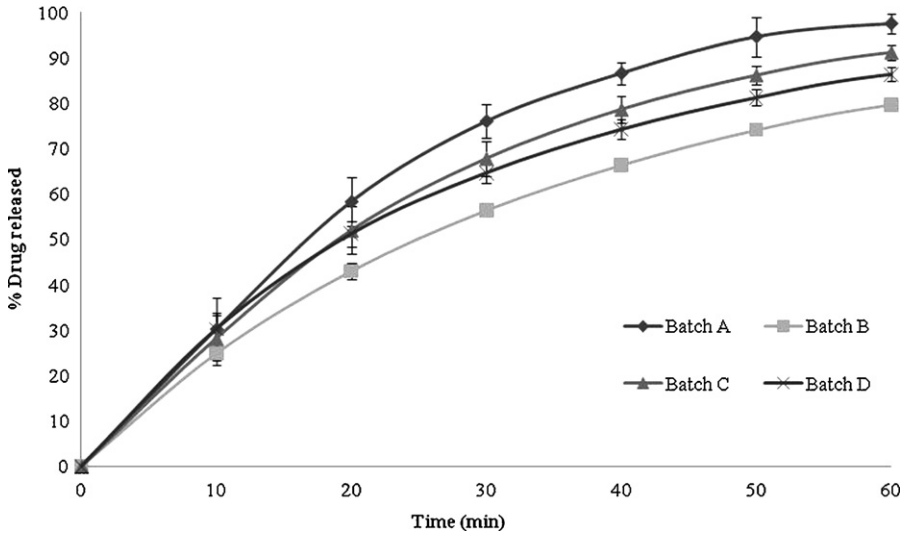

Fig. 8. Percentage of clozapine released versus time of batches A–D, having batches A and B 20% of drug and batches C an D 30% of clozapine. Batches B and D contain the coarsest particles of clozapine.

that, there is a change in the slope which can be due to the run out of the clozapine clusters connected with the pellet's surface. This behaviour is typical of systems below or very close to the drug percolation threshold because in these systems the clusters of drug particles in contact with the surface of the pellet are not connected with a percolating cluster of drug or, in case that they are connected, this cluster is an incipient cluster with a lot of bottlenecks which slowdown the drug diffusion (Caraballo et al., 1993), as corresponds to a percolating cluster very close to the percolation threshold.

Fig. 7 shows this effect affecting especially batch 4, which, after the initial phase, is releasing the drug clearly slower than batches 2 and 3, and furthermore slower than all the other batches plotted in Fig. 7.

This behaviour of batch 4 can be explained on the basis of percolation theory, taking into account the influence of particle size on the percolation thresholds: batches 2–4 contain the same drug percentage (20%), the only difference being that batch 4 contains the coarsest drug particle size fraction, batch 2 the finest and batch 3 an intermediate size fraction. It has been demonstrated (Caraballo et al., 1996; Millán et al., 1998) that smaller relative particle size in a powder mixture is related with a lower percolation threshold of this component, indicative of higher percolation efficiency. By contrast, pellets containing the coarsest drug particles would show a higher drug percolation threshold for this component. In other words, we need to add a higher concentration of these big particles in order to have this component acting as an outer phase of the system.

Therefore, from the point of view of percolation theory, batches 2–4 have different percolation thresholds, with the highest threshold corresponding to batch 4. This way, even having the same concentration of drug, this component could be percolating the system for pellets corresponding to batches 2 and 3, but acting as an isolated component in batch 4, because the concentration of drug needed to percolate the system is higher in this formulation due to its higher relative (and absolute) particle size.

Taking into account the release behaviour of batch 4 and the difference between this batch and the other batches containing 40% HPMC (batches 2–9), it could be expected that batch 4 is below, or at least very close to the drug percolation threshold.

The drug release is faster for batch 2, containing the smallest drug particles, which is also in agreement with the exposed theory.

On the other hand, as it can be observed in Fig. 7, batches 5–7 show more uniform release profiles (less biphasic behaviour). As discussed previously, this can be indicative of a better connection of the drug clusters in contact with the surface with a percolating cluster inside the system, as corresponding to pellets containing a higher drug concentration (30% (w/w) clozapine). This could be the reason why the effect of the drug particle size in the release rate is not as marked as in batches 2–4. Batches 5–7 are all clearly above the drug percolation threshold, so the change in the percolation threshold due to the particle size is much less significant. We have to take into account that the critical properties depend exponentially on the distance to the percolation threshold, showing important variations close to this point and much more discreet changes for formulations situated far from the percolation threshold.

Batch 8 (40% (w/w) clozapine) is the less biphasic batch. In agreement with the previous explanation, this can be attributed to the fact that clozapine is far above its percolation threshold, so the release process does not significantly depend on the groups of particles connected with the surface of the pellet.

One formulation containing magnesium stearate has also been performed and assayed (batch 9). Important differences have not been found, its release profile being in between those of the other formulations.

With respect to batches A–D, a similar beginning (a little slower for batch B) is observed, but after approximately 20 min a difference between batches with clozapine particle size higher than 250 μm and clozapine particle size smaller than 125 μm is evident. As it was explained before, for a given drug concentration, the decrease of the percolation threshold caused by the lower particle size results in an increased distance to the percolation threshold. This means that the percolating cluster will be better connected (in terms of percolation theory, the strength of the infinite cluster, i.e., the number of lattice sites belonging to the infinite cluster will increase), leading to a faster release (Fig. 8).

On the contrary, a slower release is observed for pellets containing the largest drug particles. As it has been previously mentioned, this fact can be explained attending to the higher drug percolation threshold for these larger particles which reduces the distance to the percolation threshold and therefore the strength of the percolating cluster. As explained above for batches 1–9, after surface-connected clusters of drug are dissolved, the release rate decreases.

Also in agreement with the behaviour previously depicted for batches 1–9, the effect of particle size is more significant for batches

A and B, which contain a lower drug concentration. Batch B, containing the coarsest particles can be situated very close, or even below the percolation threshold, as a consequence of the increase in the percolation threshold for the larger particles. This would explain the important difference in the release profile with respect to batch A.

The observed influence of the drug percolation threshold on the release rate is typical of inert matrix systems, so it can be stated that the studied formulations show an intermediate behaviour between hydrophilic and inert matrix systems. This can be attributed to the fact that MCC and HPMC have been used as matrix former excipients. MCC is a polymer having a low swelling capacity, whereas HPMC shows an important ability to swell, forming a gel layer that is able to control the drug release.

4. Conclusions

It has been possible to explain the release behaviour of clozapine matrix pellets based on the percolation theory. The matrix pellets manufactured have an intermediate behaviour between hydrophilic and inert matrix systems, since MCC and HPMC has been used as matrix former excipients. Therefore, it has been appreciated that the distance to the excipient (HPMC) percolation threshold is important to control the release rate, but also the drug percolation threshold has a significant influence in the release process. Batches with 20% (w/w) clozapine, very close to the drug percolation threshold, show a clear effect of the drug particle size in the release rate. Smaller relative particle size is related with higher percolation efficiency, leading to a faster drug release as can be observed for batches 2 (20% (w/w) clozapine smaller than 125 μm) and A (20% (w/w) clozapine smaller than 125 μm) while bigger relative particle size is related with a slower release of the drug after the dissolution of the clozapine of the clusters connected with the pellet's surface. Far above the drug percolation threshold, the effect of the drug particle size is clearly less evident.

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List of symbols

ρ_{bulk}	bulk density
V_{bulk}	bulk volume
ρ_{tapped}	tapped density
V_{tapped}	tapped volume
V_0	initial volume of the poured powder
V_f	final volume of the tapped powder
D_{min}	the smallest diameter of a pellet
D_{max}	the largest diameter of a pellet
V_s	sample volume
V_c	cell volume
V_r	reference volume
P_1	initial pressure
P_2	final pressure
ρ	true density
M_t	drug amount released at time t
M_∞	drug amount released at infinite time
b	Higuchi's release rate constant
k_k	Korsmeyer's kinetic constant
k_0	zero order release rate constant
k_1	first order release rate constant
n	Korsmeyer's time exponent
k_d	diffusional rate constant
k_r	relaxational rate constant
m	purely Fickian diffusion exponent

References

Bataille, B., Ligarski, K., Jacob, M., Thomas, C., Duru, C., 1993. Study of the influence of spheronization and drying conditions on the physico-mechanical properties of neutral spheroids containing Avicel PH 101 and lactose. Drug Dev. Ind. Pharm. 19, 653–671.

Bechgaard, H., Hegermann Nielsen, G., 1978. Controlled-release multiple-units and single-unit doses. A literature review. Drug Dev. Ind. Pharm. 4, 53–67.

Blattner, D., Kolb, M., Leuenberger, H., 1990. Percolation theory and compactability of binary powder systems. Pharm. Res. 7, 113–117.

Bodmeier, R., 1997. Tableting of coated pellets. Eur. J. Pharm. Biopharm. 43, 1–8.

Bonny, J.D., Leuenberger, H., 1991. Matrix type controlled release systems: I. Effect of percolation on drug dissolution kinetics. Pharm. Acta Helv. 66, 160–164.

Bonny, J.D., Leuenberger, H., 1993. Matrix type controlled release systems II. Percolation effects in non-swellable matrices. Pharm. Acta Helv. 68, 25–33.

Caraballo, I., 2009. Critical points in the formulation of pharmaceutical swellable controlled release dosage forms—influence of particle size. Particuology 7, 421–425.

Caraballo, I., Fernández-Arévalo, M., Holgado, M.A., Rabasco, A.M., 1993. Percolation theory: application to the study of the release behaviour from inert matrix systems. Int. J. Pharm. 96, 175–181.

Caraballo, I., Melgoza, L.M., Alvarez-Fuentes, J., Soriano, M.C., Rabasco, A.M., 1999. Design of controlled release inert matrices of naltrexone hydrochloride based on percolation concepts. Int. J. Pharm. 181, 23–30.

Caraballo, I., Millán, M., Rabasco, A.M., 1996. Relationship between drug percolation threshold and particle size in matrix tablets. Pharm. Res. 13, 387–390.

Castellanos Gil, E., Iraizoz Colarte, A., Bataille, B., Brouillet, F., Caraballo, I., 2009. Estimation of the percolation thresholds in ternary lobenzarit disodium–dextran–HPMC hydrophilic matrices tablets: effects of initial porosity. Eur. J. Pharm. Sci. 38, 312–319.

Contreras, L., Melgoza, L.M., Villalobos, R., Caraballo, I., 2010. Study of the critical points of experimental HPMC–NaCMC hydrophilic matrices. Int. J. Pharm. 386, 52–60.

Chatlapalli, R., Rohera, B.D., 1998. Physical characterization of HPMC and HEC and investigation of their use as pelletization aids. Int. J. Pharm. 161, 179–193.

Delalonde, M., Bataille, B., Baylac, G., Maurice, J., Sabatier, R., 1997. Definition of indices for the mechanical design of wet powders: application to the study of a natural polymer, microcrystalline cellulose. Int. J. Pharm. 146, 159–165.

Elizondo, J.J., 2008. Clozapina: una visión histórica y papel actual en la esquizofrenia resistente al tratamiento. Ars Pharm. 49, 135–144.

European Pharmacopoeia, 2007. Dissolution test for solid dosage forms, sixth ed. European Directorate for the Quality of Medicines and Healthcare, pp. 266–275.

Galland, S., Ruiz, T., Delalonde, M., Krupa, A., Bataille, B., 2005. Texturing the spherical granular system influence of the spheronisation stage. Powder Technol. 157, 156–162.

Higuchi, T., 1963. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci. 52, 1145–1149.

Holman, L.E., Leuenberger, H., 1988. The relationship between solid fraction and mechanical properties of compacts—the percolation theory model approach. Int. J. Pharm. 46, 35–44.

Iqbal, M.M., Rahman, A., Husain, Z., Mahmud, S.Z., Ryan, W.G., Feldman, J.M., 2003. Clozapine: a clinical review of adverse effects and management. Ann. Clin. Psychiatr. 15, 33–48.

Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., Peppas, N.A., 1983. Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25–35.

Leuenberger, H., Leu, R., 1992. Formation of a tablet: a site and bond percolation phenomenon. J. Pharm. Sci. 81, 976–982.

Leuenberger, H., Rohera, B.D., Haas, C., 1987. Percolation theory—a novel approach to solid dosage form design. Int. J. Pharm. 38, 109–115.

Melgoza, L.M., Caraballo, I., Alvarez-Fuentes, J., Millán, M., Rabasco, A.M., 1998. Study of morphine hydrochloride percolation threshold in Eudragit® RS-PM matrices. Int. J. Pharm. 170, 169–177.

Millán, M., Caraballo, I., Rabasco, A.M., 1998. The role of the drug/excipient particle size ratio in the percolation model for tablets. Pharm. Res. 15, 216–220.

Miranda, A., Millán, M., Caraballo, I., 2006. Study of the critical points in lobenzarit disodium hydrophilic matrices for controlled drug delivery. Chem. Pharm. Bull. 54, 598–602.

Peppas, N.A., Sahlin, J.J., 1989. A simple equation for the description of solute release III. Coupling of diffusion and relaxation. Int. J. Pharm. 57, 169–172.

Pinto, J.F., Lameiro, M.H., Martins, P., 2001. Investigation on the co-extrudability and spheronization properties of wet masses. Int. J. Pharm. 227, 71–80.

Quintavalle, U., Voinovich, D., Perissutti, B., Serdoz, F., Grassi, G., Dal Col, A., Grassi, M., 2008. Preparation of sustained release co-extrudates by hot-melt extrusion and mathematical modelling of in vitro/in vivo drug release profiles. Eur. J. Pharm. Sci. 33, 282–293.

Reynolds, A.D., 1970. A new technique for the production f spherical particles. Manuf. Chem. Aerosol News 41, 40–43.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. J. Control. Release 5, 23–36.
Stauffer, D., Aharony, A., 1992. Introduction to Percolation Theory. Taylor&Francis, London.
USP 32, 2009. Clozapine Official Monograph, Rockville, pp. 2005–2007.
Vervaet, C., Baert, L., Remon, J.P., 1995. Extrusion–spheronisation. A literature review. Int. J. Pharm. 116, 131–146.
Vila Jato, J.L., 1997. Tecnología Farmacéutica 1: Aspectos fundamentales de los sistemas farmacéuticos y operaciones básicas. Síntesis, Madrid.