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Research paper

Microstructural and drug release properties of oven-dried and of slowly or fast frozen freeze-dried MCC-Carbopol® pellets

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Abstract

The influence of the procedure and conditions of drying (oven-drying and freeze-drying after slow or fast freezing) and of the $CaCl_2$ concentration in the wetting liquid on the physical characteristics and drug release behaviour of microcrystalline cellulose (MCC)-carbopol 40:60 pellets containing theophylline or ketoprofen has been evaluated. The microstructural, morphological and mechanical properties can be modulated, to a large extent, through the control of the drying step and the $CaCl_2$ proportion. The drying step determines the volumetric contraction of the pellets and, consequently, the porosity parameters. When freeze-drying is applied, the freezing conditions have a marked influence on total porosity and mean pore size of the pellets. Slowly frozen pellets present the lowest porosity but the pores are the greatest. Pore size appears as a critical factor for achieving controlled release; the greater the pores, the faster the entrance of water and, consequently, the drug release. Therefore, if freeze-drying is used to remove water from wet pellets, the control of the ice formation is essential to modulate the release profiles. The practical possibilities of such modulation are especially clear for a slightly-water soluble drug, such as ketoprofen.

Keywords: Extrusion-spheronization; Freeze-drying; Pellets; Modified release; Mercury porosimetry

1. Introduction

Matrix pellets obtained by extrusion-spheronization are receiving increasing attention as modified-release drug delivery systems. A way to obtain the control of the drug release consists of including hydrophobic excipients, pH-dependent solubility polymers or less frequently, hydrophilic gel-forming polymers [1–4] in the pellet structure. Cross-linked poly(acrylic acid) derivatives, commercialized as Carbopol®, can be mixed at low proportions with microcrystalline cellulose (MCC) to obtain bioadhesive pellets of adequate morphological and mechanical properties [5,6]. The use of salts in the wetting liquid prevents the stickiness of the wet masses and enables the increase in the carbopol content, which is required to obtain an efficient controlled release [7–10]. The effect of the proportion of carbopol, the volume of wetting liquid, and the spheronization time and speed, on drug release has been evaluated in detail [7–10]. However, further research is required to enhance the performance of carbopol pellets as controlled release systems. In particular, the optimization of their microstructure may open new possibilities.

The rate and mechanism of drug release from pellets strongly depend on their microstructure [11,12]. In the case of matrix pellets containing Eudragit® and polyvinylpyrrolidone (PVP), drug loading, water required for granulation, and spheronization time were found to have a profound effect on porosity parameters. A good correlation

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between the total porosity or the mean pore diameter and drug release rate was found [11]. Changes in mean pore diameter can also be caused by the addition of small amounts of co-excipients to a specific formulation [4,13]. The influence of the composition of the granulation liquid on the total porosity of cellulosic pellets has been recently highlighted [14,15]. The disparity in final porosity of MCC pellets when prepared with water or with water/ethanol mixture has been mainly attributed to a different densification course during drying [16]. The contraction is driven by a compressive force due to capillary and osmotic forces in the liquid inside the pores, which causes the particles to be pulled together and the granule to shrink [16–18]. Since the pellets densify further during the drying phase, the procedure for water removal may be of crucial importance to their microstructural properties [18]. The discussion on the incidence of drying on the properties of pellets is usually only based on the speed at which the removal of water occurs, i.e. quickly (microwave-drying or freeze-drying) or slowly (oven-drying or desiccation on silica gel) [19]. Comparative studies on the contraction of cellulosic pellets as a function of the drying technique indicate that the reduction in the volume ranks in the order: oven $\geqslant$ fluidized bed $\gg$ freeze-drying [20]. It has been reported that freeze-dried pellets are more porous and have more pores open towards the surface, than those desiccated on silica-gel in which closed pores are predominant [21]. Although this is a general tendency, significant discrepancies in the literature about the effect of a drying technique on pellet characteristics and performance can be found [21–25]. These discrepancies may be related to an insufficient control of some critical variables of each drying process. For example, the freezing step conditions of the freeze-drying should exert a great influence on porosity. Additionally, it is not uncommon to use aqueous solutions of non-ionic or ionic substances that may alter water movement and its freezing and evaporation processes [26–28]. Therefore, a deeper study of the influence of these variables on the micro- (porosity and pore size distribution) and macrostructure (shape and surface roughness) and performance (compaction behaviour and drug release) of the pellets is required.

The aim of this work is to gain an insight into the influence of the incorporation of $CaCl_2$ to the wetting liquid and of the conditions under which the drying process is carried out on total porosity and porosity parameters and the drug release behaviour of MCC-carbopol 40:60 pellets containing theophylline (fairly-soluble drug) or ketoprofen (pH-dependent soluble drug).

2. Materials and methods

2.1. Materials

Theophylline anhydrous (Batch 2740443, Sigma–Aldrich, Spain), ketoprofen (Batch 007, Guinama, Spain), Carbopol® 974P (Batch AB17796, Noveon Inc., USA), microcrystalline cellulose Avicel® PH101 (MCC, Batch 6703C, FMC, Ireland), calcium chloride (Batch TA882282, Merck, Germany). Purified water was obtained by reverse osmosis (resistivity > 18.2 MOhm cm; MilliQ®, Millipore Spain). All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation and characterization of carbopol aqueous dispersions

2.2.1.1. Preparation. 0.5% Carbopol® 974P dispersions were prepared by a slow addition of 1 g of polymer (previously dried at 70 °C for 24 h) to 199 g of medium under mechanical stirring at 500 rpm (Jankel & Kunkel, Germany). The following media were used: distilled water, 0.145% and 0.190% $CaCl_2$ solution (equivalent to 0.29 and 0.38 salt:carbopol w/w ratio, respectively), MCC suspension (0.66 MCC:carbopol w/w ratio), theophylline solution (0.42 drug:carbopol w/w ratio) and ketoprofen suspension (1.1 drug:carbopol w/w ratio).

2.2.1.2. Rheological characterization. The rheological behaviour of the dispersions was analysed, in triplicate at 25 °C, in a Rheolyst AR1000N rheometer (TA Instruments, UK) fitted with thermostated concentric cylinders (4.5 cm diameter, recessed ends) and an AR2500 data analyzer, applying:

(a) Rotational viscometry (shear strength 0–10 Pa). The viscosity data were fitted to the Ostwald equation:

$$\eta = m \cdot \dot{\gamma}^{n-1}$$

in which η is the apparent viscosity, $\dot{\gamma}$ is the shear rate, and m and n are the consistency and fluidity indexes, respectively.

(b) Creep (0.1 Pa for 5 min) and recovery (5 min).

2.2.2. Preparation and characterization of pellets

2.2.2.1. Preparation. Wet masses of Carbopol® 974P (60 g), MCC (40 g) and, when needed, theophylline (25 g) or ketoprofen (66.6 g) were prepared by adding 160 ml of $CaCl_2$ solution of enough concentration to provide a 0.29 or 0.38 salt:carbopol w/w ratio, and mixing the components for 1 h at 125 rpm in a RZR50 mixer (Heidolph, Germany) of 98 mm inner diameter and 1 L capacity. The wet mass was extruded through a 1-mm mesh size screen using a Caleva Model 10 extruder (Caleva Ltd, UK) at 60 rpm, and the extruded mass was spheronized (load 20 g) for 30 min at 575 rpm in a Caleva Model 120 spheronizer (Caleva Ltd, UK). Portions of the pellets were dried under various conditions as follows: (a) hot-air oven (J. Bonals, Spain) at 40 °C for 24 h; (b) slow freezing at −30 °C in a freezer for 24 h, followed by freeze-drying under vacuum at −30 °C for 48 h (Labconco Corp., USA) or (c) fast freezing by immersion in liquid nitrogen (−196 °C) followed by

freeze-drying under vacuum at −30 °C for 48 h (Labconco Corp., USA). The identification codes of the resultant pellets are shown in Table 1. Once dried, the pellets were maintained with silica gel at 20 °C until assay.

2.2.2.2. Characterization. Shape and size. Pellet shape and size were characterized through the analysis (PC Imagen VGA 24 v.2.1, Foster Findlay Ass., UK) of the digital images obtained with an Olympus SZ-CTV microscope (Japan) connected to a video camera (Olympus DP12, Japan). 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 fitted by a normal distribution. Circularity was calculated as $4 \times \pi \times A/p^2$, where A is the projection area and p the projection perimeter.

Friability. Pellets were sieved on a 250-μm mesh and samples of those collected on the sieve (5 g) were assayed for 30 min at 20 rpm in a PTF 10ER (PharmaTest, Germany) previously loaded with 100 spherical glasses (4 mm diameter). After, the pellets were removed, sieved and weighed again, and the percentage of loss in weight was calculated.

Porosity. Total porosity and pore size distribution were determined in triplicate by mercury-intrusion porosimetry with a Micromeritics 9305 pore sizer (Norcross GA, USA), using a 3-ml powder penetrometer. Working pressures were around the range of 0.004–172.4 MPa.

Drug content. Five hundred milligrams of pellets nominally containing 100 mg theophylline or 200 mg ketoprofen was stirred in 900 ml of water (theophylline) or a pH 6.6 phosphate buffer (ketoprofen) medium for 24 h and the drug concentration was spectrophotometrically (Shimadzu UV-240, Japan) determined at 271 nm (theophylline) or 260 nm (ketoprofen). The experiments were carried out in triplicate.

Theophylline physical stability. X-ray spectra of pellets were recorded on a Phillips PW 1710 (Netherlands) diffractometer using CuKα radiation and scanning from 4 to 60 °2θ at a rate of 1.5 °2θ min^{-1}.

Drug release tests. Drug release profiles were obtained (500 mg of pellets, six replicates) using a USP 24 type II (Turu Grau, Spain) apparatus at 50 rpm and 37 °C, in 900 ml of water (theophylline) or pH 4.5 and 6.6 phosphate buffers (ketoprofen). The concentration of the drug in periodically withdrawn samples was determined spectrophotometrically (HP Agilent, Germany) at 271 nm (theophylline) or 260 nm (ketoprofen). In order to characterize the drug release profiles, the following modified Weibull equation [29]:

$$M = \frac{1}{q}(1 - e^{-a \cdot t^b}) \quad (1)$$

where M represents the fraction of drug dissolved, q is the dose/solubility ratio ($M_0/V \times Cs$), and a and b are constants, was fitted to the release data (up to the time in which M was just below 1) by non-linear regression (GraphPad Prism v.3.02, GraphPad Software Inc., San Diego, CA). M_0, V, and Cs represent the amount of drug in the pellets, the volume of medium and the solubility coefficient, respectively.

When $q < 1$ and $b \neq 1$, mean dissolution time was estimated from the parameters of Eq. (1) as follows [29]:

$$\mathrm{MDT} = \frac{1}{bqa^{(1/b)}}\left[b(q-1)(-\ln\,(1-q))^{1/b} - \Gamma\left(\frac{1}{b}, -\ln\,(1-q)\right) + \Gamma\left(\frac{1}{b}\right)\right] \quad (2)$$

Table 1
Experimental design and properties of MCC:carbopol 40:60 pellets

Code	$CaCl_2$/Carbopol ratio	Drying process[a]	Feret diameter (μm)	Circularity	Porosity (%)
P1	0.29	OD	813.9 (191)	0.85 (0.08)	10.01 (0.69)
P2	0.38	OD	912.0 (251)	0.86 (0.10)	7.54 (0.32)
P3	0.29	FD fast	930.4 (197)	0.87 (0.04)	43.44 (0.81)
P4	0.38	FD fast	1097.3 (241)	0.89 (0.04)	41.62 (0.29)
P5	0.29	FD slow	1017.0 (206)	0.86 (0.08)	21.93 (0.37)
P6	0.38	FD slow	978.1 (202)	0.88 (0.08)	16.72 (2.87)
T1	0.29	OD	871.9 (281)	0.85 (0.09)	8.75 (0.27)
T2	0.38	OD	987.9 (238)	0.87 (0.08)	7.00 (0.03)
T3	0.29	FD fast	1002.7 (232)	0.91 (0.04)	46.01 (0.59)
T4	0.38	FD fast	1264.3 (369)	0.91 (0.04)	42.59 (0.36)
T5	0.29	FD slow	983.7 (213)	0.87 (0.08)	20.24 (1.53)
T6	0.38	FD slow	993.7 (253)	0.89 (0.08)	25.17 (1.58)
K1	0.29	OD	948.3 (240)	0.84 (0.09)	14.63 (0.64)
K2	0.38	OD	912.8 (202)	0.86 (0.08)	15.48 (0.12)
K3	0.29	FD fast	1076.3 (147)	0.87 (0.04)	37.09 (0.25)
K4	0.38	FD fast	957.5 (214)	0.88 (0.07)	33.46 (0.63)
K5	0.29	FD slow	966.5 (249)	0.85 (0.09)	27.38 (0.93)
K6	0.38	FD slow	1069.4 (215)	0.85 (0.08)	27.37 (0.48)

P, T, and K codes refer to placebo, theophylline, and ketoprofen pellets, respectively.
[a] OD, oven-drying; FD fast, freeze-drying after freezing by immersion in liquid nitrogen (−196 °C); FD slow, freeze-drying after freezing at −30 °C.

$\Gamma(\cdot)$ and $\Gamma(\cdot,\cdot)$ being the complete and incomplete gamma functions, respectively.

If $q > 1$ (i.e. the entire dose does not dissolve) and $b \neq 1$, the equation simplifies to:

$$\mathrm{MDT} = a^{-\frac{1}{b}} \cdot \Gamma\left(\frac{1}{b} + 1\right) \quad (3)$$

When the experimental data were not enough to provide a precise estimation of q, a and b, the value of b was assumed to be equal to 1, and Eq. (1) was simplified to the typical expression of a first-order kinetics:

$$M = \frac{1}{q}(1 - \mathrm{e}^{-a \cdot t}) \quad (4)$$

In this case, the mean dissolution time was estimated as the inverse of the dissolution rate constant, as follows:

$$\text{for } q < 1 : \mathrm{MDT} = \frac{q(q-1)\ln(1-q)}{aq} \quad (5)$$

and for $q > 1$, the mean saturation time is given by:

$$\mathrm{MDT_s} = \frac{1}{a} \quad (6)$$

Experimental design and statistical analysis. The pellets were prepared according to a factorial design of three factors: the $CaCl_2$/carbopol ratio (A) coded as (−1) and (+1) for the 0.29 and 0.38 ratios, respectively; the drug nature (B) coded as (1 0) for pellets without drug, (0 1) for pellets with theophylline, and (−1 −1) for pellets with ketoprofen; and the drying procedure (C) coded as (1 0) for oven-drying, (0 1) for freeze-drying after freezing in liquid nitrogen, and (−1 −1) for freeze-drying after freezing at −30 °C (Table 1). The response surface equations that quantify the effects of these variables on the different properties of the pellets were obtained by regression using Design Expert software (v. 6.06, Stat-Ease Inc., Minneapolis, 2002). A stepwise regression with backward elimination was used: the three factors and their double and triple interaction terms were initially considered and, then, those that provided a significant level above 0.05 were discarded. The analysis of the drug release data was carried out removing factor *B* from the design.

3. Results and discussion

3.1. Rheological characterization of carbopol dispersions

Carbopol pellet preparation required $CaCl_2$ in the wetting liquid to hinder the gelation process of the acrylic polymer [9,30]. Gelation can be also affected by the presence of other soluble components such as drugs [31]. To characterize the effect of $CaCl_2$, theophylline and ketoprofen on the gelation of Carbopol 974P, a rheological characterization of its aqueous dispersions was carried out. The 0.5% Carbopol 974P dispersion behaved as a pseudoplastic fluid at shear rates below 400 s^{-1} (Fig. 1 and Table 2). $CaCl_2$, at 0.29 or 0.38 w/w proportion referred to carbopol, caused a two order of magnitude decrease in m and a two-fold increase in n values. This is a consequence of a salting out effect caused by the ions on the carbopol microgels, and of a cross-linking effect of the calcium ions that act as bridges between two carbopol carboxylic acid groups. This causes the microgels to lose water and to shrink such as was previously found for other carbopol varieties [9,32]. As a consequence, at relatively high shear rates (above 400 s^{-1}) a shear-thickening behaviour was observed (Fig. 1a). As expected from the flow experiments, the compliance values recorded for dispersions containing $CaCl_2$ were significantly greater than those recorded for carbopol solutions.

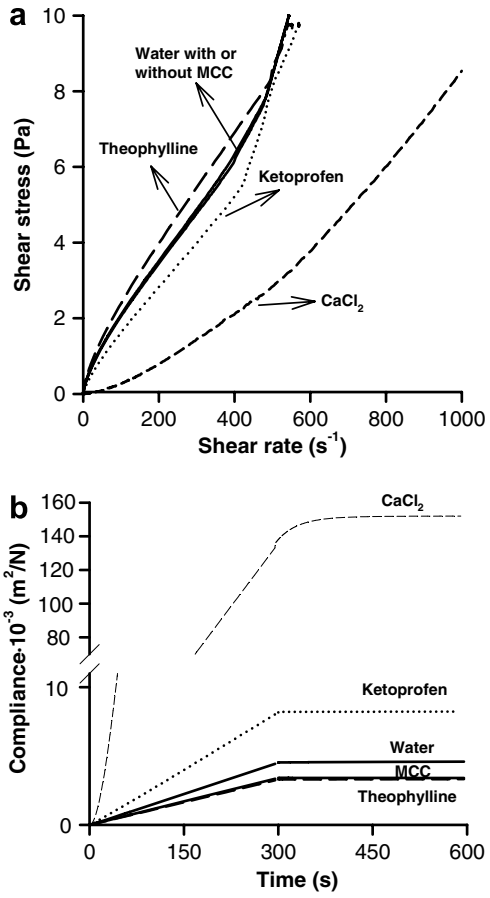

Fig. 1. Flow (a) and creep-recovery (b) profiles of 0.5% carbopol solutions prepared in different media.

The pH of 0.5% carbopol dispersion is 3.1. Theophylline has two pKa$_s$, 3.5 (weak base) and 8.6 (weak acid), and, although it may be partially ionized, no interaction with carbopol can be seen. Ketoprofen has a carboxylic acid group of pKa 4.25 [33] and most of the drug is not ionized at pH 3.1. Neither theophylline (0.21%) nor ketoprofen (0.55%) caused relevant changes on m and n values. Nevertheless, a slight increase in the compliance of carbopol dispersions in the presence of ketoprofen was observed when the creep-recovery profiles were recorded. The addition of ketoprofen caused an increase in compliance compared to the carbopol alone dispersion (Fig. 1b). The absence of

Table 2
Consistency (m) and fluidity (n) indexes and compliance of 0.5% Carbopol 974P dispersions, with and without $CaCl_2$, microcrystalline cellulose (MCC), theophylline or ketoprofen

Parameter	Dispersion medium					
	Water	$CaCl_2$/Carbopol ratio		MCC	Theophylline	Ketoprofen
		0.29	0.38			
m (Pa s^n)	5.13×10^{-2}	2.00×10^{-4}	2.01×10^{-4}	7.51×10^{-2}	6.73×10^{-2}	4.10×10^{-2}
n	0.8094	1.546	1.526	0.7808	0.7751	0.8004
r	0.9977	0.9849	0.9919	0.9992	0.9994	0.9996
Compliance (m^2 N^{-1})	4593.6	144,960	148,070	3834	3323	8225

an elastic component was confirmed for all systems. The addition of MCC did not significantly alter the rheological profile of carbopol solution (Fig. 1 and Table 2).

3.2. Preparation and characterization of pellets

The information provided by the rheological experiments confirmed that $CaCl_2$ is an additive able to significantly alter carbopol gelation, which can facilitate the preparation of carbopol:MCC pellets, whilst theophylline and ketoprofen do not exert any relevant effect. In fact, the wet masses were easily extruded and spheronized. Then, the wet pellets were dried following two main approaches: hot-air oven-drying and freeze-drying. For the last technique, special attention has been paid to the freezing step since freezing conditions may strongly determine the structure of water crystals and, consequently, of the pellets when water is removed. Specifically, a slow (in a freezer chamber at −30 °C) and a fast freezing (by immersion in liquid nitrogen at −196 °C) procedures were applied. Water can be found in the wet pellets as free water or bound to the solid components [34]. Free water is completely frozen at −30 °C, whilst freezing of bound water occurs at a much lower temperature [35]. The faster the freezing of free water, the more ice nuclei of smaller size are formed [26]. This should mainly condition the size of pores that are created as water sublimates. Despite the potential relevance of this fact, to the best of our knowledge no attention to the freezing rate and temperature conditions has been paid yet.

The properties of the dried pellets are summarized in Table 1 and the results of the statistical analysis are shown in Table 3. In all cases, the factors with a significant effect were identified. In the case of circularity, total porosity and mean dissolution time, equations with multiple-correlation coefficients (r^2) close to 1, indicative of a high predictive value, were obtained.

The procedure used for the drying was the only factor with a statistically significant effect on Feret diameter ($F_{2,15\,df} = 5.03$; $\alpha < 0.05$). The diameters ranked in the order: oven-dried (predicted value 907.8 μm) < freeze-dried after freezing at −30 °C (1001.4 μm) < freeze-dried after immersion in liquid nitrogen (1054.7 μm). Pellets subjected to conventional desiccation in an oven underwent an important volumetric contraction as previously reported for other formulations [20,36,37]. By contrast, freezing prevents such a contraction, especially when the process quickly evolved.

All pellets showed a spherical shape (Fig. 2). Although the differences among the formulations were relatively small (Table 1), significant effects of the three factors were found (Table 3). High levels of $CaCl_2$ in the wetting liquid led to the most spherical of pellets ($F_{1,12\,df} = 11.08$; $\alpha < 0.01$). This effect has been previously reported [7,8] and is explained by the improvement in the fluidity of the wet masses [9,30]. The addition of drug ($F_{2,12\,df} = 13.15$; $\alpha < 0.01$), as well as the drying conditions ($F_{2,12\,d.f.} = 23.77$; $\alpha < 0.01$) also affected the pellet morphology. The influence of drying is mainly related to the smaller volumetric contraction that the freeze-dried pellets underwent, which causes the pellets to be more spherical. A similar tendency in morphology was recently reported for MCC/propyl gallate pellets [19].

Mercury porosimetry is particularly suitable to characterize microstructure in terms of pore size distribution, mean pore diameter and pore volume [11,38]. The microstructure of the pellets (Table 1 and Fig. 3) was greatly dependent on the drying conditions (Table 3). The freeze-dried ones presented remarkably greater porosities than the oven dried pellets. When freeze-drying was applied, the overall porosity of fast frozen pellets (i.e. immersed in liquid nitrogen) was almost twofold that of those freeze-dried after slow freezing. As can be seen in Fig. 3, the procedure of freezing mainly determined the pore size distributions. In general, the slower the freezing, the greater the proportion of pores with a size above 1 μm. This can be attributed to the formation of few ice nuclei that progressively grow to form big ice crystals [26]. In contrast, despite fast frozen pellets having a greater total porosity, most of their pore volume is formed by pores with a diameter below 1 μm. To understand these findings two aspects should be considered. First, as mentioned above, the freezing of the pellets hinders the volumetric contraction during the drying. The faster the freezing, the smaller the contraction. This explains the differences in total porosity. Second, the structure of ice crystals depends on both the rate and the temperature of the freezing-step, and pellets frozen at −30 °C should have greater size crystals than those immersed in liquid nitrogen [26]. Therefore, after ice sublimation, pores with a greater section are formed.

Table 3
Dependence of the pellet properties on the $CaCl_2$/Carbopol ratio (A) coded as (−1) and (+1) for the 0.29 and 0.38 ratios, respectively; the drug nature (B) coded as (1 0) for pellets without drug, (0 1) for pellets with theophylline, and (−1 −1) for pellets with ketoprofen; and the drying procedure (C) coded as (1 0) for oven-drying, (0 1) for freeze-drying after freezing in liquid nitrogen, and (−1 −1) for freeze-drying after freezing at −30 °C

Property	Equation	F	r^2
Feret diameter (μm)	$D = 987.97 - 80.20\ C(1) + 66.77\ C(2)$	5.03[a,*]	0.40
Circularity	$C = 0.87 + 6.67 \times 10^{-3}\ A - 1.67 \times 10^{-3}\ B(1) + 1.30 \times 10^{-2}\ B(2) - 1.51 \times 10^{-2}\ C(1) + 1.80 \times 10^{-2}\ C(2)$	16.98[a,**]	0.88
Porosity (%)	$P = 24.80 - 14.23\ C(1) + 15.90\ C(2)$	78.46[a,**]	0.91
Mean dissolution time	$MDT_{Theophylline} = 0.074 + 0.028\ A + 0.019\ C(1) + 6.58 \times 10^{-3}\ C(2) - 6.07 \times 10^{-3}\ AC(1) + 0.016\ AC(2)$	732.14[b,**]	0.99
	$MDT_{Ketoprofen,\ pH\ 6.6} = 0.23 - 0.038\ A + 0.20\ C(1) - 7.33 \times 10^{-3}\ C(2) + 0.069\ AC(1) - 0.11\ AC(2)$	1435.49[b,**]	0.99
	$MDTs_{Ketoprofen,\ pH\ 4.5} = 2.22 - 0.31\ A + 1.68\ C(1) - 0.086\ C(2) - 0.18\ AC(1) - 0.32\ AC(2)$	589.77[b,**]	0.99

[a] 2,15 degrees of freedom.
[b] 5,30 degrees of freedom.
[*] $\alpha < 0.05$.
[**] $\alpha < 0.01$.

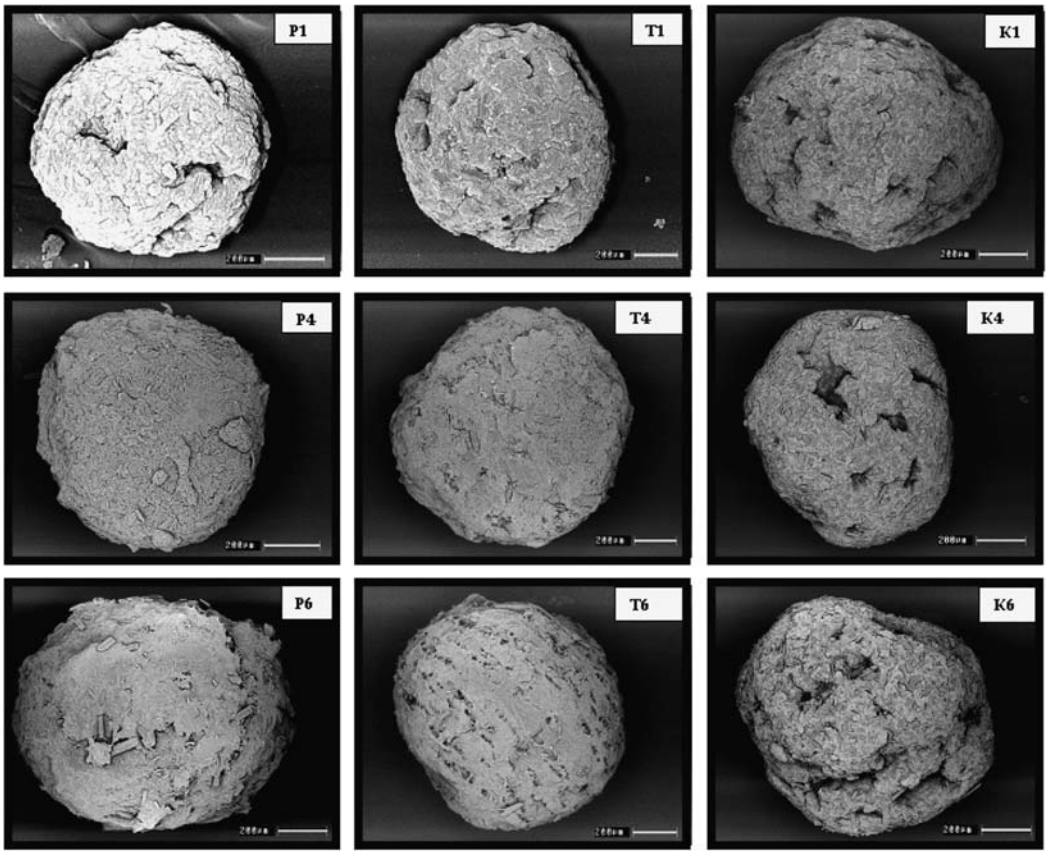

Fig. 2. TEM photographs of pellets of different formulations.

The microstructural differences had no repercussions on the mechanical properties (friability values close to zero), but remarkably affected drug release profiles from the pellets (Fig. 4). Although theophylline release quickly proceeded (100% released in 30 min), some differences in release rate could be noticed. These differences in the drug release rate cannot be attributed to a change in the hydration state of the drug, since theophylline remained in the anhydrous form (peaks at 7 and 12°2θ) and no monohydrate formation (peaks at 9 and 11°2θ) was detected in the X-ray spectra of the formulations [39]. Theophylline release profiles were well fitted to a first order kinetics (Table 4), which indicates that release rate mainly depends on the amount of drug that remains in the solid state [13]. The response surface equation for MDT (Table 3) indicates that the release rate mainly depends on the $CaCl_2$/carbopol ratio (A: $F_{1,30\,df} = 2252.19$; $\alpha < 0.01$), and to a less extent on the drying procedure (C: $F_{2,30\,df} = 512.23$; $\alpha < 0.01$) and on the interaction of both factors (A·C: $F_{2,30\,df} = 192.03$; $\alpha < 0.01$). From a practical point of view, these results highlight the main role of $CaCl_2$. An increase in its proportion caused a noticeable decrease in

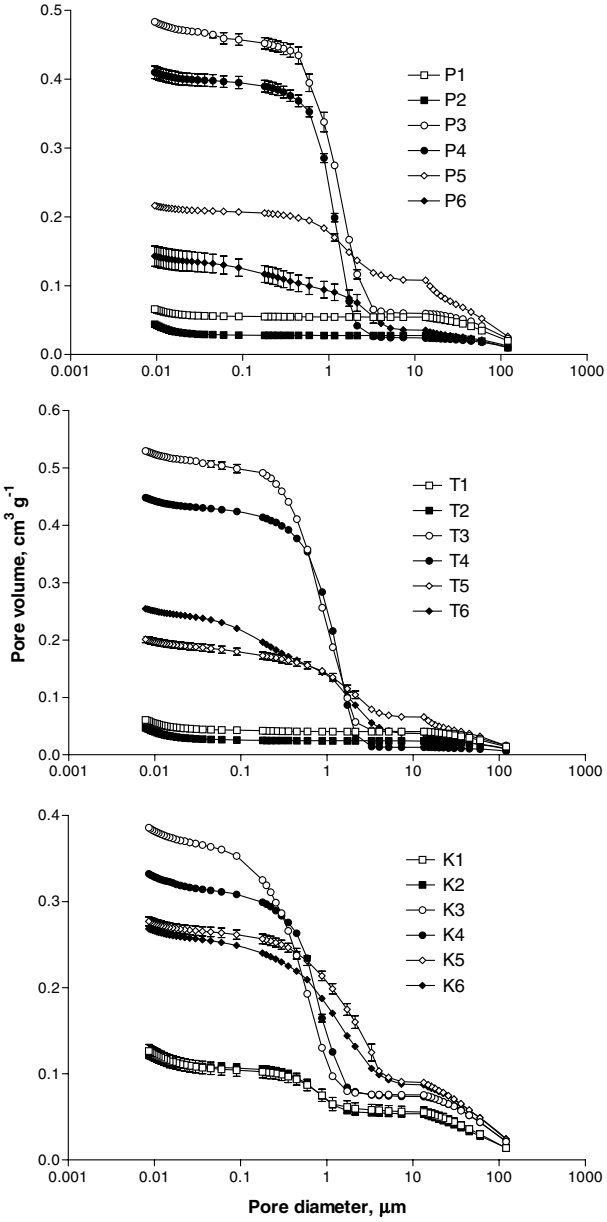

Fig. 3. Cumulative pore-diameter distribution plots of placebo, theophylline and ketoprofen pellet formulations prepared with different $CaCl_2$ proportions and dried using different procedures.

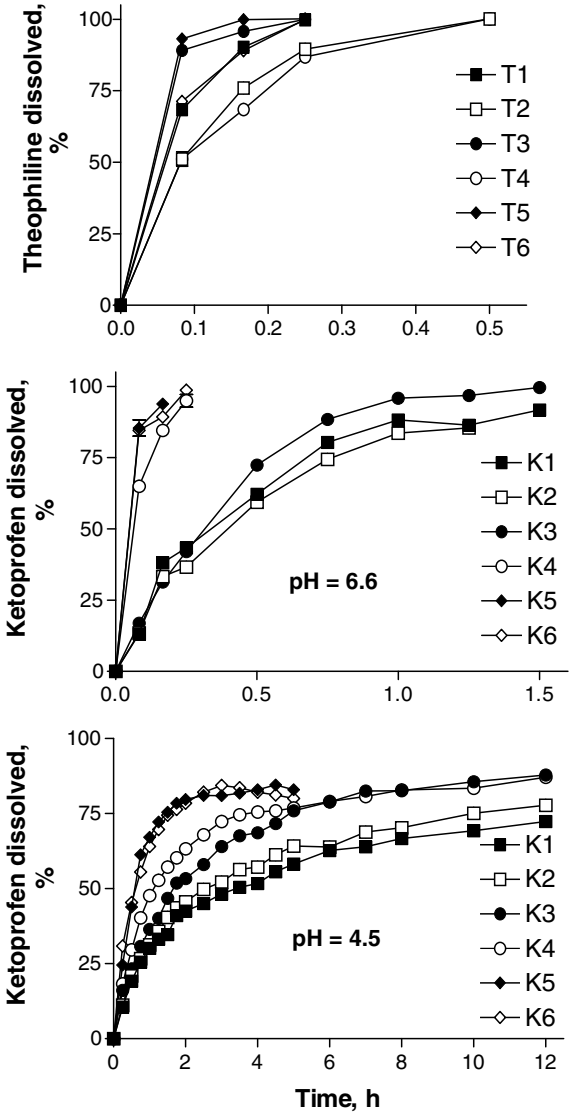

Fig. 4. Theophylline and ketoprofen release profiles from pellets prepared with different $CaCl_2$ proportions and dried using different procedures.

theophylline release rate, disregarding the drying procedure. The electrolyte may act as a cross-linker between the carboxylic acid groups, strengthening the polymer network and making the entrance of water in the pellet more difficult [40]. Neau et al. [8] also observed that an increase in $CaCl_2$ content of carbopol:MCC pellets slowed down clorfeniramine release. On the other hand, pore size seems to play a more relevant role than pore volume on theophylline release rate. The greater pore size of freeze-dried pellets that were slowly frozen facilitates the penetration of water and, consequently, a faster delivery.

The pH-dependent solubility of ketoprofen [41] prompted us into carrying out the release test in pH 6.6 and 4.5 media (Fig. 4). At pH 6.6, ketoprofen solubility is maximum since this drug is almost completely dissociated, and release rate was faster than at pH 4.5. Freeze-dried pellets that were slowly frozen or that contained the greater $CaCl_2$/carbopol ratio delivered the dose in ca. 15 min at pH 6.6. The release profiles were also well-fitted to a first order kinetics (Table 4) and the response surface of the MDT (Table 3) indicates that an adequate control of the $CaCl_2$ proportion and the drying conditions enables a precise modulation of the ketoprofen release rate through their direct effects ($F_{1,30\,df} = 308.09$ and $F_{2,30\,df} = 2815.04$, respectively) and their interaction ($F_{2,30\,df} = 619.65$; $\alpha < 0.01$).

Ketoprofen release at pH 4.5 was not complete owing to the low solubility of the drug (Fig. 4). Nevertheless, remarkable differences on release rate were observed (Table 4). The values of q (ca. 1.2) indicate that ketoprofen solubility at pH 4.5 is around 0.83 times the dose contained in the pellets (equivalent to 0.18 mg/ml). On the other

Table 4
Parameters of Weibull function fitted to theophylline and ketoprofen release profiles

Code	q	a	b	R^2	Sum of squares	df	MDT (h)
T1	0.995 (5×10^{-3})	13.72 (0.31)	–	0.9980	8.433×10^{-3}	28	0.071 (0.002)
T2	0.994 (7×10^{-3})	8.55 (0.17)	–	0.9981	6.987×10^{-3}	28	0.114 (0.005)
T3	1.006 (9×10^{-3})	27.02 (1.66)	–	0.9961	1.611×10^{-2}	22	0.037 (0.002)
T4	0.981 (1×10^{-2})	7.45 (0.32)	–	0.9859	6.320×10^{-2}	34	0.124 (0.007)
T5	0.988 (1×10^{-2})	30.36 (1.92)	–	0.9984	6.216×10^{-3}	16	0.031 (0.002)
T6	1.003 (2×10^{-2})	15.22 (1.09)	–	0.9895	3.821×10^{-2}	22	0.066 (0.004)
pH 6.6							
K1	1.074 (1×10^{-2})	2.52 (0.12)	–	0.9819	1.020×10^{-1}	52	0.397 (0.021)
K2	1.083 (2×10^{-2})	2.17 (0.09)	–	0.9891	4.824×10^{-2}	46	0.460 (0.014)
K3	0.952 (1×10^{-2})	2.25 (0.06)	–	0.9944	3.949×10^{-2}	52	0.363 (0.019)
K4	1.021 (2×10^{-2})	12.76 (0.89)	–	0.9901	3.254×10^{-2}	22	0.078 (0.002)
K5	1.055 (2×10^{-2})	27.73 (3.77)	–	0.9906	3.073×10^{-2}	16	0.036 (0.001)
K6	1.092 (9×10^{-3})	30.36 (2.43)	–	0.9952	1.736×10^{-2}	22	0.033 (0.003)
pH 4.5							
K1	1.269 (3×10^{-2})	0.46 (0.01)	0.67 (0.02)	0.9900	4.576×10^{-2}	117	4.23 (0.14)
K2	1.228 (2×10^{-2})	0.49 (0.01)	0.69 (0.01)	0.9937	3.336×10^{-2}	117	3.57 (0.21)
K3	1.105 (1×10^{-2})	0.52 (0.01)	0.76 (0.01)	0.9957	3.079×10^{-2}	117	2.77 (0.21)
K4	1.193 (8×10^{-3})	0.81 (0.01)	0.78 (0.01)	0.9909	5.849×10^{-2}	117	1.50 (0.18)
K5	1.213 (6×10^{-3})	1.69 (0.04)	1.10 (0.03)	0.9911	3.880×10^{-2}	72	0.60 (0.06)
K6	1.204 (9×10^{-3})	1.51 (0.04)	0.91 (0.03)	0.9843	7.685×10^{-2}	87	0.66 (0.05)

hand, the values of b show that, from a kinetic point of view, the behaviour of pellets that freeze-dried after slow freezing is remarkably different to that of the pellets that undergo other drying procedures. Since the b values of the slowly frozen pellets are close to 1, the modified Weibull function simplifies to the classical first order kinetics. Other formulations had lower b values. Consequently, the value of the rate constant of the first order kinetics changes as the release progresses. This may be a consequence of the structural alterations that the pellets undergo on their surface as water penetrates and carbopol gelates, which may alter ketoprofen diffusion.

When the entire dose could not be dissolved, a mean saturation time MDT was estimated [29]. The response surface for the MDTs (Table 3) shows that the $CaCl_2$/carbopol ratio ($F_{1,30\,df} = 146.39$; $\alpha < 0.01$), the drying procedure ($F_{2,30\,df} = 1346.03$; $\alpha < 0.01$) and their interaction ($F_{2,30\,df} = 55.20$; $\alpha < 0.01$) determine the release process; the drying procedure being the most relevant factor from a quantitative point of view.

Since theophylline and ketoprofen release profiles are strongly dependent on pellet microstructure, the pore size intervals that are critical for the release process were investigated. In the pore size interval of the mercury porosimetry assay, the correlation between MDT and the accumulated pore volume of pellets (up to different specific pore size limit values) was studied by linear regression. The values of the correlation coefficient of each linear regression are plotted against the pore size limit value in Fig. 5. As can be shown, the pore volume corresponding to the pores greater to 5 μm appears as determinant for theophylline release rate. In the case of ketoprofen, the critical pore size is around or above 1 μm. These results suggest that drug solubility conditions the pore size limit, below which the porosity does not significantly contribute to the release process.

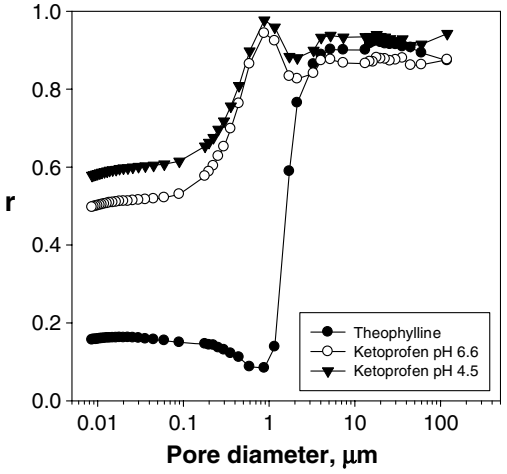

Fig. 5. Dependence of the correlation coefficients of the lineal regression of MDT vs. accumulated pore volume corresponding to pores of size equals to or above the value shown in the X-axis.

4. Conclusions

The size, shape, mechanical properties and release behaviour of MCC-carbopol pellets with a high proportion in carbopol can be tuned by changing the $CaCl_2$/carbopol ratio and the drying conditions, mainly because of the influence of these variables on pellet microstructure. Oven-drying causes the contraction of the wet masses and results in smaller pellets with a lower total porosity and mean pore size, compared to the freeze-dried ones. In the case of the freeze-drying, the frozen rate is a critical

factor; pore sizes negatively correlating with the rate of freezing. Pore size, which ranks in the order: slowly-frozen freeze-dried pellets > quickly-frozen freeze-dried pellets > oven-dried pellets, plays a main role in the drug release rate. This highlights the importance of taking into account the specific conditions of a given drying procedure in order to achieve adequate release profiles.

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