International Journal of Pharmaceutics 415 (2011) 196–205

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A combined technique based on prilling and microwave assisted treatments for the production of ketoprofen controlled release dosage forms

Giulia Auriemma, Pasquale Del Gaudio*, Anna Angela Barba, Matteo d'Amore, Rita P. Aquino

Department of Pharmaceutical and Biomedical Sciences, University of Salerno, I-84084 Fisciano (SA), Italy

ARTICLE INFO

Article history:
Received 4 March 2011
Received in revised form 26 May 2011
Accepted 28 May 2011
Available online 14 June 2011

Keywords:
Prilling
Microwave
Drug release
Drying rate
Ketoprofen
Alginate

ABSTRACT

In this study the feasibility of joining prilling and microwave (MW) assisted treatments as combined technique to produce controlled release alginate beads was tested. Beads were produced by prilling (laminar jet break-up) using different polymer concentrations and loaded with ketoprofen, a slightly soluble non-steroidal anti-inflammatory BCS class II drug characterized by low melting point. MW assisted treatments applied using different irradiating conditions were performed as drying/curing step. The effect of formulation conditions and process variables on drying kinetics, particle micromeritics, shape, surface and inner characteristics of the matrix as well as drug loading and drug release behaviour was studied (USP pH change method). The properties of MW dried particles were compared to those dehydrated by convective methods (room conditions and tray oven 105 °C).

Results showed that MW dried ketoprofen loaded beads were obtained in a very narrow dimensional range retaining shape and size distribution of the hydrates particles. Compared to the traditional drying methods, MW treatments were able to strongly increase drying rate of the hydrated beads achieving faster and controllable dehydration kinetics. Moreover, different regimes of irradiation affected structural properties of the particles such as matrix porosity as well as the solid state of the loaded drug. DSC, X-ray and FTIR analyses indicated complex chemical interactions between the drug and polymer matrix induced by MW, related with the regime of irradiation, that contributes to the differences in release profiles. In fact, MW treatments under different time and irradiating regimes are able to modulate drug release from alginate beads; high levels of irradiation led to beads suitable for immediate release oral dosage forms whereas the lowest regime of irradiation led to beads that achieved a prolonged/sustained release of the drug till 8 h in simulated intestinal medium. This study showed that prilling in combination with microwave treatments is a useful and simple tandem technique to prepare dextran-based dried beads.

1. Introduction

Prilling or laminar jet break-up is a mild microencapsulation technique based on the breaking apart of a laminar jet of polymer solution into a row of mono-sized drops by means of a vibrating nozzle device (Brandenberg and Widmer, 1998; Del Gaudio et al., 2005; Sakai et al., 1985; Schneider and Hendriks, 1964). When polymer solution containing either solubilized or suspended drug has gelling properties, the resultant polymeric droplets can be sprayed into a gelation solution in which they are solidified in beads. Particle-systems obtained by prilling can be used to achieve controlled drug release, taste masking or immobilization of different cell lines by using various polymers as core/shell material due to their ability to move from sol to gel state (Del Gaudio et al., 2009; Koch et al., 2003; Serp et al., 2000). The prilling technology produces hydrated beads that can be used as self-consistent dosage form or as building blocks in the production of controlled drug release platforms.

After the production stage, beads require to be dried to avoid chemical or microbiological degradation. The drying process may have an impact on the final properties of the dried beads (solute migration, polymorphism, damages by overheating, structural modifications). Thus, its selection has a crucial role to assure that no adverse effects compromise the quality of the final products. Most conventional drying methods used in pharmaceutical preparations promote simultaneous heat and mass transfer based on convective transport mechanisms. Hot air (or inert atmosphere) streams constitute the drying medium used in fluid bed, rotary, tray and pneumatic driers (Chee et al., 2005). In recent years, microwave (MW) assisted heating has gained great interest in many applications with special reference to food processing (Schubert and Regier, 2005). The reasons can be found in the occurrence of benefits such as shorter processing times, improved products' uniformity and yields, possibility to reduce manufacturing costs due

* Corresponding author. Tel.: +39 089969247; fax: +39 089969602.
E-mail address: pdelgaudio@unisa.it (P. Del Gaudio).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.05.077

to energy saving. In addition, MW treatments are able to confer unique microstructures and properties to the end materials. Recently, researches showed that MW heating technology can be useful in the design of controlled release pharmaceutical dosage forms, such as solid dispersion, granules and tablets (Bergese et al., 2003; Gainotti et al., 2006; Jamuna-Thevi et al., 2009; Moneghini et al., 2008). Moreover, in the case of dextran-based formulations (i.e. pectin, alginate and chitosan) MW irradiation could be applied to modify the release behaviour of loaded drug as related to the extent of polymer crosslinking and drug–excipient complexation (Nurjaya and Wong, 2005; Wong et al., 2005). The peculiarity of the MW heating is the energy transfer mechanism. In fact, energy is delivered directly to materials through molecular interactions with electromagnetic field via conversions of electrical field energy into thermal energy (Hegedus and Pintye-Hódi, 2007; Loh et al., 2008). Rate of the energy "generation" and distribution throughout the material depend on the dielectric, thermal and other physical properties such as shape, structure and content of liquid dissipative phases (solvent, moisture). In particular, the dissipative character is the ability of a material to interact with the electric field, and it is expressed by the complex permittivity (ε'dielectric constant, real part; ε'' loss factor, imaginary part). Thermal properties such as the thermal diffusivity drive the heat distribution within the material (Acierno et al., 2004). As a matter of fact, materials with high moisture content easily interact with microwaves because of the high values of the loss factor. Therefore, it is expected that MW heating is suitable to dry carbohydrate hydrated beads manufactured by prilling.

In this study, the feasibility of joining prilling and MW assisted treatments as combined technique to producing controlled release alginate beads was tested. Beads were loaded with ketoprofen, a non-steroidal anti-inflammatory drug (NSAID) used as model drug. Ketoprofen is a class II slightly soluble drug in Biopharmaceutical Classification System (BCS), characterized by low melting point. MW assisted drying treatments applied using different irradiating conditions were performed as drying/curing step. The effect of formulation conditions and process variables on drying kinetics, particle micromeritics, drug loading and drug release behaviour were studied. In particular, the properties of MW dried particles were compared to those dehydrated by convective methods, used as control.

2. Materials and methods

2.1. Materials

Sodium alginate European Pharmacopoeia X (MW ≈ 240 kDa) (Carlo Erba, Milan, I) employed as matrix in the preparation of gel-beads was used as purchased, without further purification. Water content (5% w/w) was determined by Karl Fischer titration (Tritomatic KF, Crison Instruments, SA, Barcelona, SP).

$CaCl_2$ anhydrous, granular (Sigma–Aldrich, Milan, I) was used, in aqueous solution, as cross-linking agent.

Ketoprofen was kindly donated by Dompè (Dompès.p.a. l'Aquila, I).

All other chemicals and reagents were obtained from Sigma–Aldrich (Milan, I) and used as supplied.

2.2. Drug loaded hydrated bead preparation

An appropriate amount of sodium alginate was dissolved in distilled water at room temperature under gentle stirring for 18 h in order to obtain 100 ml of polymer solution with concentrations ranging between 1.50% and 2.00% (w/w). Different amounts of solid ketoprofen (mean diameter 20.08 μm, span 38.44) were suspended into the polymer solution and stirred for 2 h in order to obtain different drug/polymer ratios (between 0.10 and 0.33). Beads were manufactured by a vibrating nozzle device (NiscoEncapsulator Unit, Var D; NiscoEngineering Inc., Zurich, CH), equipped with a syringe pump (Model 200 Series, Kd Scientific Inc. Boston, MA, USA), pumping the drug/polymer solution through a nozzle 400 μm in diameter. The experiments were performed at various volumetric flow rate, between 10 and 18 ml/min. The vibration frequency used to break up the laminar liquid jet was set between 250 and 300 Hz, amplitude of vibration 100%. The distance between the vibrating nozzle and the gelling bath was fixed at 25 cm. A stroboscopic lamp was set at the same amplitude as the frequency, in order to visualize the falling droplets. Drug/polymer droplets fell into an aqueous solution 0.3 M $CaCl_2$ where they were gelified under gentle stirring. The beads were held into the gelling solution for 10 min at room temperature then recovered and thoroughly rinsed with distilled water.

2.3. Bead drying

A commercial multimode microwave cavity (MW480 7-Days, De Longhi, Treviso, I) was used as MW drying apparatus. The MW source had a nominal power of 700 W (continuous regime) and a built-in duty cycle to simulate different power levels of 490 W, 350 W, 245 W (discontinuous regime) achieved through on/off cycles and hereafter reported as levels IV–I, respectively.

The irradiation time was set using an integrated timer. Desired power and duration of irradiation were selected using a touch-control panel. The MW oven was equipped with a Pyrex plate on which an appropriate sample-support was placed and rotated to achieve a uniform irradiation of the beads.

In brief, weighed amount of beads (about 2 g) was subjected to microwave treatment at various combinations of irradiation in time. The weight variation of beads was determined before and after the bead irradiation by using a four decimal place electronic balance (Crystal 200 CAL, Gibertini, Milan, I).

Residual moisture content, assayed by gravimetric method, was expressed as the moisture ratio, MR, i.e. ratio between actual and initial moisture.

Beads were also dried by convective conventional methods using a tray oven (ISCO mod. 9000, Milan, I) at 105 °C, and using room conditions (22 °C; 67% RH). All the drying runs were stopped when a constant weight was achieved (moisture ratio was evaluated as previously reported). Beads dried by convective methods were used as control.

2.4. Bead size and morphology

Hydrated and dried bead size distributions were measured by both an optical microscope (Citoval 2, Alessandrini, Milan, I) equipped with a camera and laser light scattering spectroscopy (Coulter LS 13320, Beckman Coulter, Inc., Brea, CA, USA) equipped with a 12 ml micro liquid module. The LS 13320 uses a 5 mW laser diode with a wavelength of 750 nm, reverse Fourier optics and binocular lens systems. During preliminary studies, ethanol was chosen as suspending medium. Beads were suspended into the small-volume cell filled with ethanol to obtain an obscuration between 10% and 12%. The particle size distribution was calculated by the instrument software, using the Fraunhofer model. The analyses were carried out in triplicate for each sample.

Scanning electron microscopy (SEM) was performed using a Carl Zeiss EVO MA 10 microscope with a secondary electron detector (Carl Zeiss SMT Ltd, Cambridge, UK) equipped with a LEICA EMSCD005 metallizator producing a deposition of a 200–400 Å thick gold layer. Analysis was conducted at 20 keV.

Projection diameter was obtained by image analysis (Image J software, Wayne Rasband, National Institute of Health, Bethesda, MD, USA). At least one hundred bead images were analysed for each preparation in order to calculate length-number mean and relative standard deviation for at least three different prilling processes. Perimeter and projection surface area obtained by image analysis were used to calculate a Sphericity Coefficient (SC) by the following equation (Almeida-Prieto et al., 2004, 2006):

$$SC = \frac{4\pi A}{P^2}$$

where A is the bead surface area and P its perimeter.

Surface roughness (SR) was calculated using fractal descriptors obtained by grey level distribution analysis measured on the SEM images of the beads. The images were taken at the same magnification. A scanned area of the SEM image, a box of 200 μm width and 200 μm length, was analysed by Image J software using the algorithm known as the "box counting method" (Chappard et al., 2003). The analysis was carried out on 5 different area sections for each bead image considered and a mean fractal descriptor was obtained. The analysis was repeated on at least 10 beads for each batch.

2.5. Calorimetric analysis

Beads' thermal characteristics were determined by differential scanning calorimetry (DSC) (Mettler Toledo DSC 822e module controlled by Mettler Star E software, Columbus, OH, USA), and compared with those obtained using both blank beads and drug as raw material. An appropriate amount of dried beads was crimped in a standard aluminium pan that was pierced and heated from 25 to 350 °C at a scanning rate of 10 °C/min. The characteristic peaks were recorded and the specific heat of the melting endotherm was evaluated. At least duplicates were carried out for each batch of sample, and the results averaged.

Thermo gravimetric analysis (TGA) was carried out in order to evaluate the water content in the different batches of dried beads (TG50 – Mettler Toledo, USA). Analyses were conducted at 10 °C/min heating rate between 25 and 200 °C.

Both DSC and TGA were performed in nitrogen atmosphere at a flow rate of 100 ml/min.

2.6. FTIR analysis

FTIR analysis was performed in a FTIR spectrophotometer (FT-IR Nexus, Thermo-Nicolet, West Palm Beach, FL, USA) equipped with a mercury–cadmium–telluride detector. The samples (ketoprofen, blank and ketoprofen loaded beads) were combined with small amount of potassium bromide and pressed to 3 tons in a manual press (OMCN s.p.a., Bergamo, I). The thin compacts produced were analysed using 256 scans with a 1 cm^{-1} resolution step. Each experiment was carried out in triplicate, and results averaged.

2.7. Powder X-ray diffraction studies (PXRD)

Dried samples were studied by means of X-ray diffraction measurement (XRD) with a Rigaku D/MAX-2000 diffractometer (Rigaku Corporation, Tokyo, J) using a Ni-filtered Cu-Kα radiation (40 kV, 20 A). 2θ range was set from 5 to 50°, step size 0.03°/2θ and 5 s counting time per step. A Rigaku imaging plate, mod. R-AXIX DSBC, was used for digitizing the diffraction patterns.

2.8. Drug content and encapsulation efficiency

Accurately weighed amounts of beads from each manufactured batch (about 50 mg each) were dissolved under vigorous stirring in PBS buffer (100 mM). Ketoprofen content was determined by UV spectroscopy at λ 254 nm (Lambda 25 UV/VIS Spectrometer, PerkinElmer, Waltham, MA, USA). Encapsulation efficiency was calculated as the ratio of actual to theoretical drug content. Each analysis was performed in triplicate; results were expressed in terms of mean ± standard deviation. Both drug content and encapsulation efficiency were calculated correcting the weight for the residual water and calcium chloride contained into the beads, as previously determined by Karl–Fischer titration (Tritomatic KF, Crison Instruments, SA, Barcelona, SP) and EDTA titration, respectively.

2.9. Kinetics of drug release

In vitro dissolution/release tests were conducted in sink conditions on given amounts of beads containing about 20 mg of drug using a USP 27 dissolution apparatus II: paddle, 100 rpm, 37 °C (Sotax AT7 Smart – Sotax, Allschwil, CH) on line with a UV spectrophotometer (Lambda 25 UV/VIS Spectrometer, Perkin Elmer, Waltham, MA, USA). Briefly, dried beads were added to the dissolution medium, 750 ml 0.1 M HCl for 2 h, then 250 ml of 0.20 M Na_3PO_4 was added and pH adjusted to 6.8 as described in the USP 27/NF monograph "Drug release from delayed-release articles". Data were analysed spectrophotometrically at λ 254 nm. Dissolution tests were conducted on six different batches of particles; mean values and standard deviation were reported.

3. Results and discussion

3.1. Hydrated ketoprofen loaded beads

Hydrated beads loaded with ketoprofen were manufactured by a NiscoEncapsulatorVar D unit. As described in a previous work alginate solution feeds ranging between 1.50% and 2.00% (w/w) were used and other process variables were accorded with Cross model (Del Gaudio et al., 2009; Soong and Shen, 1981) in order to obtain beads in a narrow dimensional range (diameters around 2 mm). Moreover, frequency of vibration of the encapsulation unit was kept as low as possible to avoid formation of satellite droplets during the prilling process (Sakai and Hoshino, 1980).

Ketoprofen hydrated beads were obtained with diameters ranging between 1993 and 2016 μm with a relative standard deviation lower than 3% (see Table 1). These beads show an almost spherical shape (SC 0.98 ± 0.02, where 1 corresponds to a sphere) with smooth and regular surface and some spots of solid ketoprofen recognizable inside the bead structure (data not shown).

3.2. Dried ketoprofen loaded beads

Homogenous amounts of hydrated beads were dried using microwave assisted heating under different radiation conditions (MW, power level I-IV), air-bulk heating (tray oven) at 105 °C and air-bulk room conditions.

As expected, drug content and encapsulation efficiency were not influenced by the drying method chosen. Drug content increased from 8% to 25% in accordance with the increasing of drug/polymer ratio (0.10–0.33). Encapsulation efficiency was remarkably high (over 93%) for all ketoprofen loaded beads without any variation due to the drying process as shown in Table 2.

3.2.1. Drying kinetics

In drying operation, two processes simultaneously occur: the energy transfer from the surrounding environment to let the surface moisture evaporate (mostly by convective phenomena in conventional methods) and the internal moisture transfer to the solid surface with its subsequent evaporation due to the first pro-

Table 1
Feed solution and mean diameter in both hydrated and dried state of beads manufactured by prilling. Each value represents the mean diameter ± S.D. ($n = 3$).

Alginate solution (% w/w)	Ketoprofen polymer ratio	Hydrated beads (μm)	Air-dried (μm)	Oven dried (μm)	MW level IV (μm)	MW level III (μm)	MW level II (μm)	MW level I (μm)
1.50	1:10	1995 ± 53	1002 ± 30	1216 ± 41	1428 ± 36	1346 ± 47	1305 ± 46	1252 ± 33
	1:5	2002 ± 46	995 ± 23	1221 ± 42	1412 ± 46	1353 ± 48	1318 ± 42	1244 ± 38
	1:3	2016 ± 41	998 ± 26	1198 ± 35	1424 ± 38	1352 ± 39	1299 ± 38	1249 ± 43
1.75	1:10	1993 ± 39	1003 ± 29	1218 ± 29	1418 ± 43	1366 ± 48	1308 ± 41	1246 ± 36
	1:5	1998 ± 41	998 ± 27	1225 ± 28	1426 ± 39	1353 ± 41	1315 ± 39	1253 ± 34
	1:3	2009 ± 38	1004 ± 30	1214 ± 32	1421 ± 41	1359 ± 39	1315 ± 31	1262 ± 24
2.00	1:10	2012 ± 45	1006 ± 31	1219 ± 21	1425 ± 42	1344 ± 46	1316 ± 42	1249 ± 39
	1:5	1996 ± 42	997 ± 21	1218 ± 26	1416 ± 37	1349 ± 39	1318 ± 39	1248 ± 37
	1:3	2003 ± 36	1002 ± 33	1212 ± 31	1420 ± 39	1350 ± 49	1309 ± 43	1252 ± 48

Air-dried: beads dried at room conditions.
Oven dried: beads dried at 105 °C.
MW: beads dried by microwave exposure at different irradiation levels.

Table 2
Drug content, encapsulation efficiency (e.e.), water content, sphericity coefficient (SC) and surface roughness (SR) of the ketoprofen loaded beads dried by both convective methods and microwave irradiation.

	Sodium alginate solution (% w/w)	Drug:polymer (feed solution)	SC	SR	Drug content (% ± S.D.)	E.e. (% ± S.D.)	Water content (% ± S.D.)
Air-dried	1.50	1:10	0.93 ± 0.04	1.20 ± 0.04	8.02 ± 0.04	93.2 ± 0.3	12.3 ± 1.1
		1:5	0.92 ± 0.02	1.19 ± 0.04	16.04 ± 0.05	94.2 ± 0.4	10.6 ± 0.8
		1:3	0.92 ± 0.03	1.19 ± 0.05	24.92 ± 0.07	93.7 ± 0.5	10.4 ± 0.6
	1.75	1:10	0.94 ± 0.03	1.14 ± 0.03	8.04 ± 0.03	93.5 ± 0.3	11.4 ± 1.1
		1:5	0.93 ± 0.04	1.16 ± 0.04	16.03 ± 0.05	94.2 ± 0.4	11.1 ± 0.9
		1:3	0.93 ± 0.03	1.14 ± 0.03	25.02 ± 0.07	94.1 ± 0.5	10.6 ± 0.7
	2.00	1:10	0.94 ± 0.02	1.11 ± 0.02	8.04 ± 0.04	93.2 ± 0.3	10.4 ± 0.9
		1:5	0.94 ± 0.03	1.10 ± 0.03	16.08 ± 0.07	94.5 ± 0.5	9.6 ± 0.8
		1:3	0.93 ± 0.04	1.10 ± 0.05	25.07 ± 0.07	94.3 ± 0.5	9.7 ± 0.7
Oven-dried	1.50	1:10	0.91 ± 0.04	1.15 ± 0.05	8.06 ± 0.03	93.7 ± 0.3	10.2 ± 0.9
		1:5	0.91 ± 0.05	1.16 ± 0.02	16.08 ± 0.10	94.5 ± 0.7	10.2 ± 0.8
		1:3	0.92 ± 0.06	1.15 ± 0.04	24.98 ± 0.08	93.9 ± 0.6	10.4 ± 0.6
	1.75	1:3	0.92 ± 0.04	1.15 ± 0.04	8.03 ± 0.05	93.3 ± 0.2	10.2 ± 0.7
		1:2	0.92 ± 0.02	1.14 ± 0.05	16.06 ± 0.08	94.4 ± 0.6	9.6 ± 0.7
		1:1	0.91 ± 0.04	1.14 ± 0.04	24.94 ± 0.06	93.8 ± 0.4	9.5 ± 0.6
	2.00	1:10	0.92 ± 0.05	1.13 ± 0.03	8.05 ± 0.06	93.6 ± 0.4	9.9 ± 0.5
		1:5	0.93 ± 0.04	1.11 ± 0.02	16.10 ± 0.07	94.6 ± 0.5	9.5 ± 0.5
		1:3	0.93 ± 0.05	1.11 ± 0.04	25.12 ± 0.08	94.5 ± 0.6	9.5 ± 0.7
MW level I	1.50	1:10	0.92 ± 0.04	1.34 ± 0.04	8.06 ± 0.06	93.7 ± 0.4	5.7 ± 0.5
		1:5	0.91 ± 0.03	1.33 ± 0.05	16.11 ± 0.06	94.6 ± 0.4	5.6 ± 0.6
		1:3	0.90 ± 0.04	1.33 ± 0.05	25.16 ± 0.08	94.6 ± 0.6	5.6 ± 0.7
	1.75	1:10	0.91 ± 0.04	1.28 ± 0.03	8.04 ± 0.05	93.5 ± 0.4	5.6 ± 0.5
		1:5	0.92 ± 0.03	1.30 ± 0.05	16.06 ± 0.07	94.4 ± 0.4	5.6 ± 0.6
		1:3	0.90 ± 0.03	1.28 ± 0.05	25.09 ± 0.06	94.2 ± 0.4	5.4 ± 0.4
	2.00	1:10	0.91 ± 0.04	1.24 ± 0.04	8.05 ± 0.04	93.6 ± 0.3	5.5 ± 0.5
		1:5	0.92 ± 0.03	1.23 ± 0.03	16.09 ± 0.05	94.5 ± 0.3	5.4 ± 0.7
		1:3	0.92 ± 0.03	1.23 ± 0.02	25.04 ± 0.06	94.2 ± 0.4	5.4 ± 0.6
MW level II	1.50	1:10	0.90 ± 0.05	1.35 ± 0.04	8.08 ± 0.05	93.9 ± 0.4	5.5 ± 0.5
		1:5	0.91 ± 0.04	1.37 ± 0.05	16.08 ± 0.06	94.5 ± 0.4	5.4 ± 0.5
		1:3	0.91 ± 0.04	1.33 ± 0.06	25.08 ± 0.06	94.3 ± 0.5	5.4 ± 0.4
	1.75	1:10	0.90 ± 0.02	1.32 ± 0.05	8.05 ± 0.05	93.6 ± 0.4	5.4 ± 0.4
		1:5	0.91 ± 0.03	1.31 ± 0.04	16.06 ± 0.05	94.4 ± 0.4	5.4 ± 0.5
		1:3	0.91 ± 0.04	1.31 ± 0.05	25.16 ± 0.07	94.6 ± 0.5	5.5 ± 0.4
	2.00	1:10	0.91 ± 0.05	1.30 ± 0.03	8.06 ± 0.04	93.7 ± 0.3	5.3 ± 0.6
		1:5	0.91 ± 0.03	1.32 ± 0.03	16.05 ± 0.06	94.3 ± 0.4	5.4 ± 0.5
		1:3	0.92 ± 0.03	1.30 ± 0.04	25.11 ± 0.09	94.4 ± 0.6	5.2 ± 0.4
MW level III	1.50	1:10	0.91 ± 0.05	1.40 ± 0.06	8.06 ± 0.03	93.7 ± 0.2	5.4 ± 0.5
		1:5	0.90 ± 0.05	1.41 ± 0.07	16.10 ± 0.06	94.6 ± 0.5	5.3 ± 0.4
		1:3	0.90 ± 0.04	1.41 ± 0.06	25.06 ± 0.07	94.2 ± 0.5	5.3 ± 0.3
	1.75	1:10	0.91 ± 0.04	1.37 ± 0.07	8.04 ± 0.05	93.5 ± 0.4	5.3 ± 0.5
		1:5	0.91 ± 0.05	1.38 ± 0.06	15.99 ± 0.08	93.9 ± 0.6	5.4 ± 0.4
		1:3	0.92 ± 0.03	1.35 ± 0.05	24.98 ± 0.08	93.9 ± 0.6	5.2 ± 0.6
	2.00	1:10	0.92 ± 0.04	1.34 ± 0.05	8.07 ± 0.05	93.8 ± 0.4	5.4 ± 0.4
		1:5	0.91 ± 0.04	1.33 ± 0.06	16.05 ± 0.04	94.3 ± 0.2	5.2 ± 0.5
		1:3	0.90 ± 0.06	1.34 ± 0.07	25.13 ± 0.05	94.5 ± 0.3	5.2 ± 0.3
MW level IV	1.50	1:10	0.88 ± 0.04	1.45 ± 0.06	8.07 ± 0.04	93.8 ± 0.3	5.4 ± 0.6
		1:5	0.89 ± 0.05	1.48 ± 0.07	16.05 ± 0.05	94.3 ± 0.4	5.2 ± 0.3
		1:3	0.88 ± 0.04	1.44 ± 0.06	24.89 ± 0.11	93.6 ± 0.8	5.3 ± 0.3
	1.75	1:10	0.91 ± 0.04	1.41 ± 0.06	8.08 ± 0.04	93.9 ± 0.3	5.1 ± 0.4
		1:5	0.90 ± 0.05	1.43 ± 0.05	16.08 ± 0.07	94.5 ± 0.5	5.0 ± 0.5
		1:3	0.91 ± 0.03	1.42 ± 0.04	25.13 ± 0.08	94.5 ± 0.6	5.2 ± 0.4
	2.00	1:10	0.91 ± 0.03	1.33 ± 0.06	8.04 ± 0.07	93.5 ± 0.4	5.3 ± 0.6
		1:5	0.92 ± 0.04	1.32 ± 0.07	16.08 ± 0.04	94.5 ± 0.3	5.2 ± 0.4
		1:3	0.91 ± 0.03	1.33 ± 0.05	25.08 ± 0.06	94.3 ± 0.4	5.2 ± 0.5

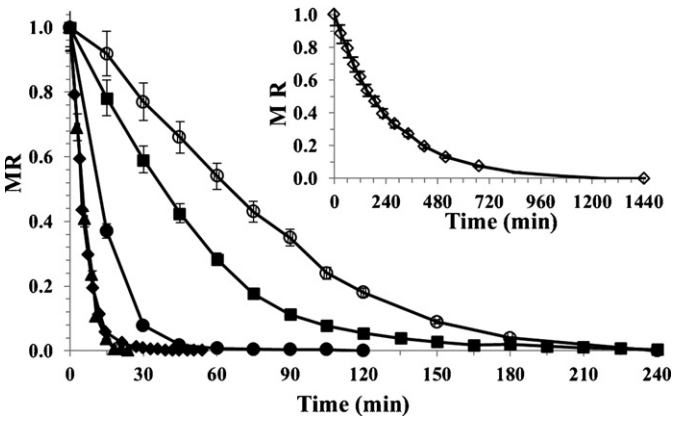

Fig. 1. Drying curves of ketoprofen loaded beads produced with 1.75% (w/w) alginate solution dried at different microwave irradiation levels: IV (-▲-); III (-♦-); II (-●-); I (-■-); tray oven drying at 105 °C (-○-) and room conditions drying (-◇-) are also reported. Mean ± S.D. (n = 6).

cess. The rate at which drying is accomplished is governed by the rate at which the two processes proceed (Mujumdar, 2006).

Our results showed that, as expected, drying rates were affected by the energy transfer mechanisms applied: dielectric (assisted by microwaves) and convective heating.

Hydrated beads are dissipative materials (fundamentally due to the water content), therefore are suitable to be processed by microwaves technique. The regime of MW irradiation had an important role on drying curves as shown in Fig. 1 for 1.75% alginate beads loaded with ketoprofen 8% (w/w). Drying rate varied with time for beads treated in continuous regime (level IV) or discontinuous regimes (MW irradiation levels I–III). Level IV and level III microwave treatments showed a drying rate almost superimposable in the first 15 min of the drying process. This might be due to the penetrative and volumetric heating nature of microwaves affecting the water migration from the inside out of the beads (Araszkiewicz et al., 2006). Diffusion of moisture and vapour inside the beads was easier when MW radiation was switched off for short period of time, as in level III, allowing the internal heat to diffuse to the external layer establishing an higher thermal gradient when water was predominant at the beginning of the beads drying process. As beads' water content decreased, level III of irradiation was not able to maintain a thermal gradient capable to match the continuous irradiation. Therefore, level III as well as weaker irradiations needed longer time to eliminate water from beads.

Convective heating methods, due to the high thermal capacity and the low thermal diffusivity of hydrated beads, require more prolonged process time (12–18 h at room conditions and 7 h for tray oven). In the drying at room conditions (Fig. 1), i.e. at mild but not really interesting conditions, the time of drying changed dramatically compared to MW irradiation at higher level where 20–50 min were need to obtain dried beads.

Drying kinetics was found to be independent on both ketoprofen loading and alginate solution concentration. Small differences in drying rate were recognized only for 2.00% alginate beads dried at level II and I irradiation where about 20 min more were needed to complete the drying (data not shown).

3.2.2. Bead morphology and micromeritics

Optical microscopy and laser light scattering (LLS) showed that reduction in mean diameter was different when MW treatments or other drying process were used.

In beads dried at room conditions (Table 1) the mean diameter was reduced to about 1 mm (relative standard deviation lower than 3%) because of the shrinking of volume due to the loss of water. Dimensional distribution ($p < 0.005$), as well as shape and sphericity coefficient of the beads, was not significantly affected by drying at room conditions (Fig. 2a). On the contrary, sphericity coefficient was found to be dependent on the alginate concentration used to formulate the beads, from 2.00% to 1.50% (w/w), with SC values decreasing from 0.94 ± 0.02 to 0.92 ± 0.02 (Table 2).

Tray oven dried beads (Table 1) exhibited a reduction in mean diameter of about 40% and SC values between 0.93 ± 0.04 and 0.91 ± 0.05 depending on alginate concentration. Little differences in surface roughness (from 1.16 ± 0.02 to 1.11 ± 0.02) were observed with the increasing of the sodium alginate concentration, while larger differences (between 1.20 ± 0.04 and 1.10 ± 0.03) were recognized for air-dried beads (Table 2).

Main differences in particle mean diameters were observed for beads dried at different regimes of MW irradiation (Table 1). In fact, beads dried in MW continuous regime (level IV) exhibited the highest mean diameter (1430 ± 39 μm) while beads exposed to the weakest irradiation (level I) presented the lowest mean diameter (1262 ± 24 μm). Moreover, no significant influence of drug content on diameter reduction was recognized.

Surface roughness as well as the number of cracks and craters on beads surface increased when level IV MW regime was used as shown in Table 2 and by SEM analysis (Fig. 2b and c). This phenomenon might be due to the higher temperature inside the beads that leads to the collapsing of the cross-linked alginate matrix structure when continuous irradiation was used (Araszkiewicz et al., 2006).

Interestingly, beads dried by MW at level IV were also characterized by pores detected both on the surface and in inner structure of the beads, in a narrow size distribution ranging from 150 to 250 nm (Fig. 3a and b). This wide interconnected nanoporous matrix may be related to the very fast elimination of water, peculiar of the continuous MW irradiation (Jamuna-Thevi et al., 2009). Surface porosity

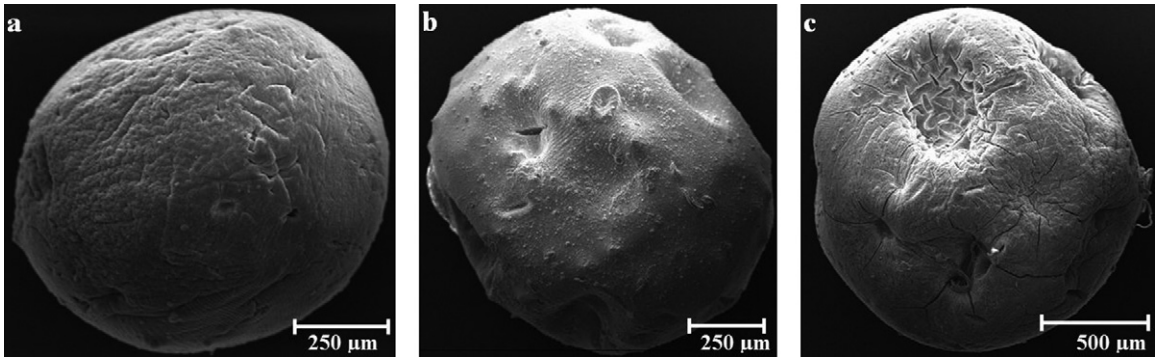

Fig. 2. SEM microphotographs of ketoprofen loaded alginate beads dried in different conditions: at room conditions (a), level I and, level IV microwave irradiation (b) and (c), respectively.

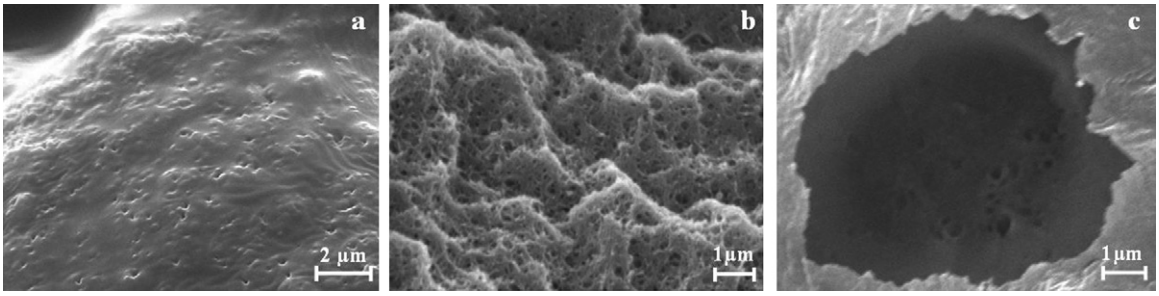

Fig. 3. SEM microphotographs of nanoporous ketoprofen loaded beads: porous surface (a) and porous inner matrix (b) of beads dried at level IV microwave irradiation; porous inner matrix of level I microwave treated beads (c).

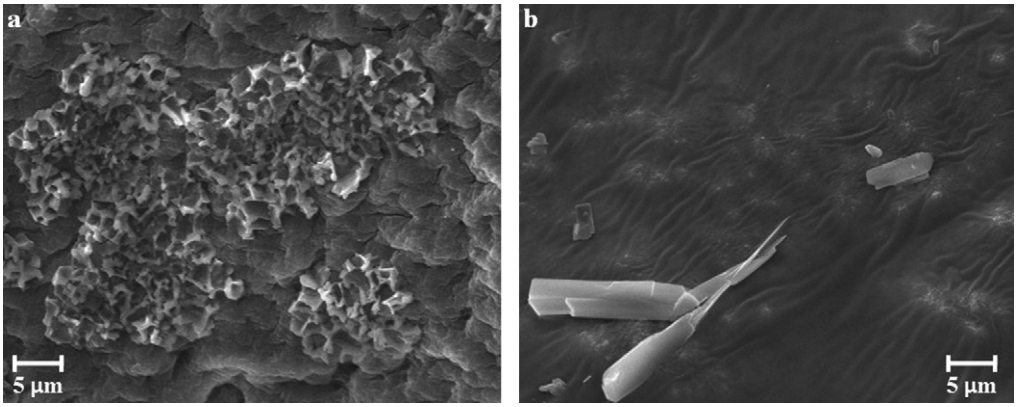

Fig. 4. SEM microphotographs of ketoprofen spots located on drug loaded beads surface after microwave treatment at different irradiation levels: IV (a) and III (b).

was not observed in any other batch of particles dried with, or without, microwaves. Pores with broader size distribution and larger diameter, ranging between 230 and 450 nm, were observed inside the matrix in the case of level I MW dried beads (Fig. 3c) due to the evaporation of water along preferential paths.

It is interesting to point out that small spots of ketoprofen in different solid states were observed on the bead surface when MW drying was performed at levels II, III and IV, whereas beads with unspotted surface were obtained when MW irradiation was carried out at level I. Ketoprofen was in amorphous state when drying was conducted at MW level IV (Fig. 4a) due to the harsh regime of irradiation (Cirri et al., 2009). Level III irradiation produced beads with ketoprofen in amorphous state accounting for almost 90% of the total drug located on beads (Fig. 5c) while level II led to beads with only few crystals of ketoprofen without any trace of amorphous drug on the particle surface. This observation was confirmed by XRD analysis (Fig. 5b) showing no peak related to the ketoprofen crystalline diffraction pattern for MW level IV irradiated beads. On the contrary, small peaks associated to ketoprofen in crystalline state were observed for MW level III irradiated beads.

FTIR spectroscopy analysis gave further information on the solid state of the encapsulated drug and on the interactions between ketoprofen and alginate in the beads matrix due to the microwave treatment. Fig. 6 reports the spectra of beads in the ν(C=O) stretching region.

As ketoprofen raw material, the sharp peak at 1697 cm^{-1} represents the stretching vibration of the carbonyl group in the dimeric carboxylic acid form of ketoprofen while the stretching

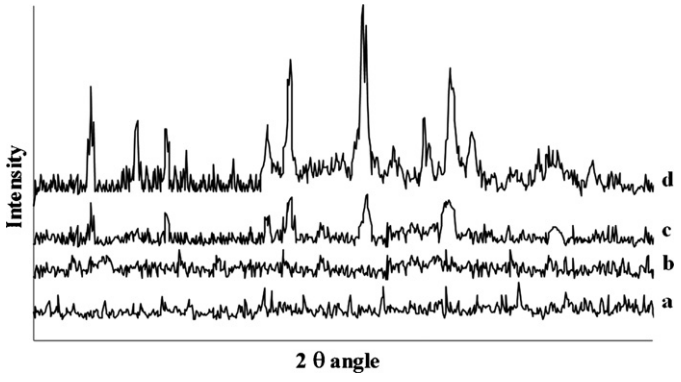

Fig. 5. X-ray diffraction patterns of blank alginate beads (a); ketoprofen loaded alginate bead treated at different microwave power levels: IV (b), III (c); and pure crystalline ketoprofen (d).

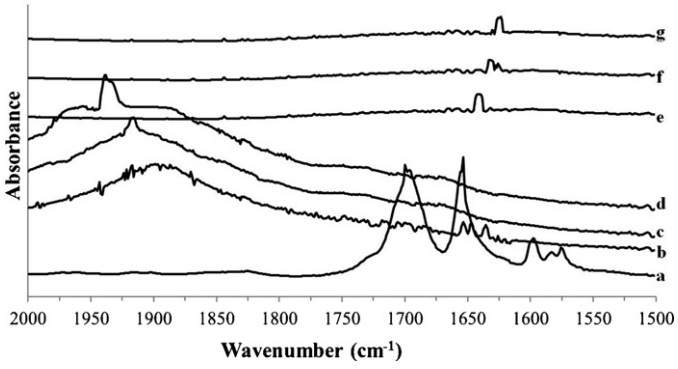

Fig. 6. FTIR spectra of ketoprofen and drug loaded alginate beads in the C=O stretching region: ketoprofen (a), ketoprofen loaded alginate beads dried at room conditions (b); ketoprofen loaded beads dried by microwave at level I (c) and at level IV (d); blank alginate beads dried at room conditions (e), blank microwave dried beads at level II (f) and level IV (g).

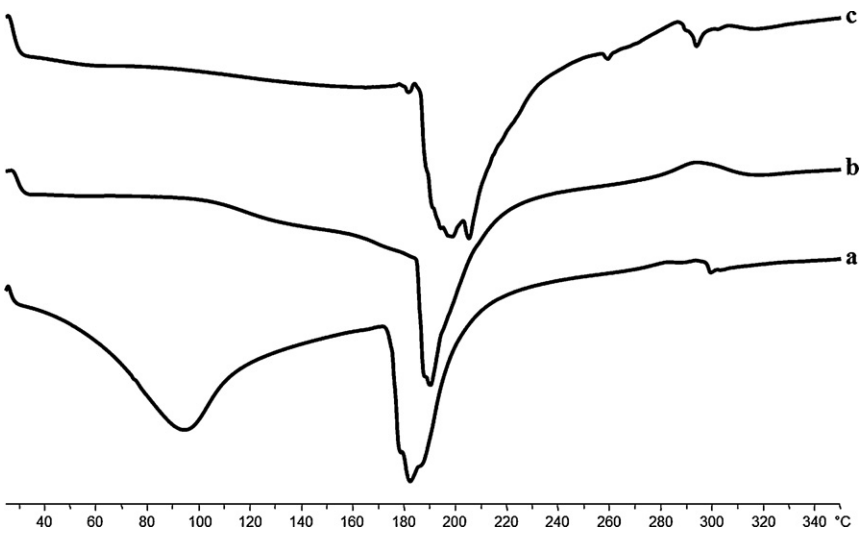

Fig. 7. Differential scanning calorimetry thermographs of blank alginate beads produced by prilling and dried at room conditions (a), level I (b) and level IV microwave irradiation (c).

of the carbonyl group of the monomeric acid is recognizable at 1655 cm^{-1} (Fig. 6a) as previously reported in literature (Marini et al., 2009; Sánchez-Dasi et al., 1998). Crosslinking of alginate chains by Ca^{2+} in air-dried blank beads resulted in small peak at 1640 cm^{-1} related to the carbonyl group (Fig. 6e). MW treated blank alginate beads exhibited the peak corresponding to the carboxylic group at 1637, 1635, 1631 and 1625 cm^{-1} for levels I–IV of irradiation, respectively. Reduction in the wavenumber absorbance bands was found to be directly correlated with the intensity of the MW treatment (Fig. 6f and g) indicating an increasing in alginate interchain interactions via carboxylic group confirming previous observations (Wong et al., 2002).

Drug loaded beads dried by convective methods (tray oven at 105 °C and room temperature) (Fig. 6b) presented these peaks shifted to different wavenumbers (1635, 1646 and 1653 cm^{-1}) and reduced in intensity. Other absorbance bands were observed at 1887 cm^{-1}. Signal shifting indicated the breakage of the dimeric ketoprofen molecules, the formation of hydrogen bonds between alginate carbonyl group and ketoprofen hydroxyl group as well as interactions between ketoprofen hydroxyl group and Ca^{2+} embedded in the polymer matrix.

Microwave dried drug loaded beads (Fig. 6c and d) exhibited sharp peaks at 1938 and 1916 cm^{-1} for level I and level IV irradiation, respectively, that may suggest the formation of new strong

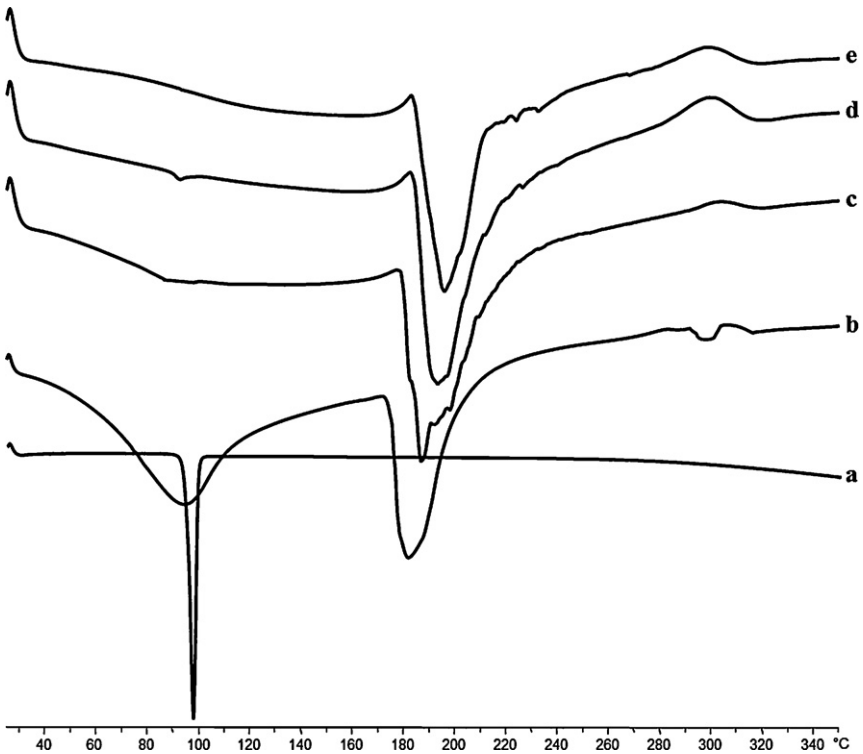

Fig. 8. Differential scanning calorimetry thermographs of pure ketoprofen (a) and ketoprofen loaded beads dried at room conditions (b) and by microwave irradiation at level I (c), level III (d) and level IV (e).

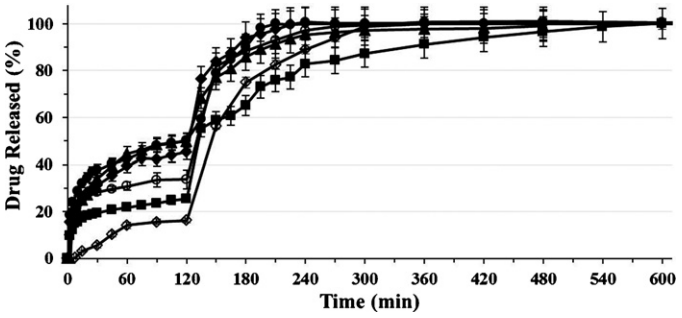

Fig. 9. Release profiles of dried beads formulated with 1.75% (w/w) alginate solution and loaded with ketoprofen 8% (w/w) dried at room temperature (-◊-); in tray oven at 105 °C (-○-) and by microwave treatments at: level I (-■-), level II (-●-), level III (-♦-) and level IV (-▲-). Mean ± S.D. (n = 6).

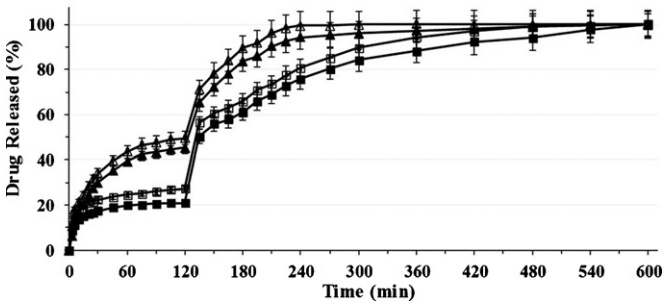

Fig. 10. Release profiles of beads loaded with 8% (w/w) ketoprofen formulated with alginate solution 1.50% (w/w): level I (-□-) and level IV (-Δ-) microwave irradiation; 2.00% (w/w) alginate solution dried at level I (-■-) and level IV (-▲-) microwave irradiation. Mean ± S.D. (n = 6).

interactions between ketoprofen and polymer matrix via Ca^{2+} and a reduction in the level of alginate chains interactions (Wong et al., 2005). These phenomena were more intense when irradiation was prolonged.

DSC thermograms of blank beads dried both at room conditions and by MW treatments are reported in Fig. 7. Air-dried beads exhibited a melting peak at 182 °C before decomposition of the polymeric material and a broad endothermic signal at 95 °C peculiar of the loss of water (Fig. 7a). MW dried blank beads (Fig. 7b and c) exhibited melting endothermic peaks with peculiar pattern shifted to higher temperature between 190 °C and 202 °C, directly correlated with the regime of MW irradiation.

Ketoprofen as crystalline raw material exhibited melting point at 97 °C (Fig. 8a). Ketoprofen loaded beads dried at room conditions (Fig. 8b) exhibited two very broad peaks. The first presented its minimum at 95 °C peculiar of the loss of water from the polymer matrix; the second at 185 °C related to the endothermic melting process of the polymer matrix. The disappearance of drug melting peak and detection of an endothermic melting peak at higher temperature (300 °C) before decomposition of the polymer material confirmed that drug–polymer interaction occurred, as previously suggested by FTIR spectra.

The thermograph of drug loaded beads dried by MW level III (Fig. 8d) shows a tiny endothermic peak at 95 °C associated with the melting of residual crystalline ketoprofen present on the beads surface as showed by SEM microphotographs. In MW level IV dried beads (Fig. 8e) it is clear the absence of the endothermic peak of the fusion characteristic of the crystal raw material due to the total amorphization of the drug when exposed to MW treatments for prolonged period (Cirri et al., 2009; Moneghini et al., 2008) as also confirmed by SEM and XRD analysis. Moreover, thermographs were all characterized by endothermic peaks due to melting of polymer matrix and decomposition of the samples (Fig. 8c–e).

3.3. Release studies

Release profiles of ketoprofen from beads obtained by different drying processes are shown in Fig. 9.

As previously reported (Del Gaudio et al., 2009), beads dried at room conditions showed the typical behaviour of alginate known as enteric polymer therefore releasing less than 20% of the drug in simulated gastric fluid (SGF – pH 1.2) even when formulated with the lowest polymer concentration. Total release of the drug was obtained in simulated intestinal fluid (SIF – pH 6.8). Drug release rate was dependent on alginate concentration increasing in accordance with Ca^{2+} depletion from alginate matrix that led to a complete liberation of the drug from 5 to 7 h for 1.50% and 2.00% (w/w) alginate beads, respectively. Moreover, a time lag was observed in the SIF release profile increasing with the polymer concentration. This phenomenon may be strictly related to the number of "egg box" inside the beads that is directly proportional to the concentration of the polymeric material used.

Beads dried at 105 °C exhibited a burst drug release effect in the first 15 min in SGF, in accordance to the presence of solid ketoprofen on the beads surface. In this medium, the drug release ranged between 28% and 36% depending on polymer concentration. After the initial burst effect beads exhibited a release profile analogous to those dried at room temperature. These observations suggest that the same diffusion controlled drug release mechanism still holds and release rate is dependent on swelling/degradation of alginate matrix in SIF.

Drug release behaviour for MW dried beads was found to be dependent on both alginate concentration and time of exposure to microwaves.

Unlike beads dried at room condition, release profiles of MW treated beads at levels II–IV did not exhibit any time lag and are typical for conventional orally release formulations. The presence of solid ketoprofen and cracks on the beads surface of every batch dried at MW irradiation (see SEM images in Figs. 2 and 4) allows an immediate release of the drug and a faster and continuous penetration of the dissolution medium inside the alginate matrix in both SGF and SIF. In the former, the amount of ketoprofen released was in the range 45–50%, whereas complete release of the drug was obtained in about 2 h in SIF. Since, levels II–IV MW irradiation produce beads with strongly similar structure (see SEM and IR data) release profiles were not significantly different both in SGF and SIF.

A different behaviour was observed for beads exposed to MW at level I. Drug release was significantly reduced in SGF whereas it was prolonged and sustained until 6 h in SIF, as shown in Fig. 9. A number of parameters should be examined to explain differences in ketoprofen release profiles such as beads morphology, porosity, amount and kind of solid state of drug on beads surface (crystals or amorphous) as well as ketoprofen-polymer interactions, as previously shown by SEM, DSC and FTIR data. The non-porous matrix and the structure integrity of these beads are able to protect the drug in acidic medium. Prolonged/sustained release of ketoprofen was achieved in SIF, where about 6 h were needed to obtain total release, probably due to the interaction between drug and polymer matrix promoted by MW irradiation at level I (Nurjaya and Wong, 2005).

As expected, drug release rate was also dependent on the polymer concentration, since the higher the concentration of the polymer solution used to manufacture the beads, the lower was the release rate of the drug, as shown in Fig. 10.

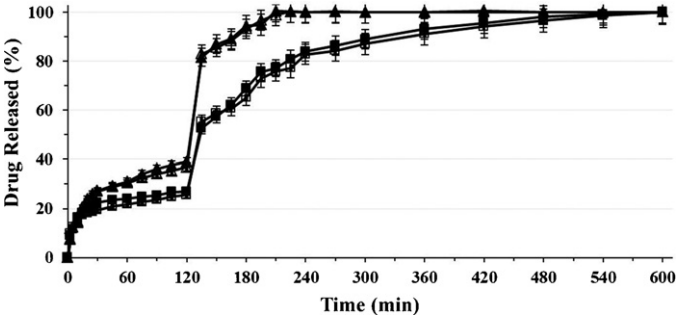

Fig. 11. Release profiles of dried beads formulated with 1.75% (w/w) alginate solution loaded with 8% (w/w) ketoprofen at MW level I (-□-) and MW level IV (-Δ-); 25% (w/w) ketoprofen loaded beads dried at MW level I (-■-) and MW level IV (-▲-). Mean ± S.D. (n = 6).

A reduction of about 5% and 10% of drug release rate in SGF was observed between beads formulated with 1.50% and 2.00% (w/w) of alginate and dried at level I and level IV, respectively. A tougher alginate matrix seems to slow down diffusion of the drug.

On the contrary, similar drug release profiles were obtained by dissolution studies of beads manufactured with the same polymer solution (i.e. 1.75% w/w) but loaded with different drug amounts (from 8 to 25% (w/w)), as shown in Fig. 11. Drug content did not influence drug release profiles nor release rate; in fact, dissolution profiles were almost superimposable in both SGF and SIF.

4. Conclusions

This study showed that prilling in combination with microwave treatments is a useful and simple tandem technique to prepare dextran-based dried beads with spherical shape and narrow particle size distribution. When ketoprofen, a low melting point BCS class II NSAID, was used as model drug, an encapsulation efficiency higher than 93% was achieved. Microwave treatments demonstrated to be an interesting alternative to the traditional drying methods, allowing to achieve dried beads through faster and controllable dehydration kinetics and to retain shape and size distribution of the hydrated particles.

Moreover, MW treatments under different time and irradiating regimes are able to modulate drug release from alginate beads. Interestingly, MW level I irradiation led to beads with sustained and prolonged drug release whereas MW level II-IV irradiation led to beads suitable for conventional immediate release from oral dosage forms. MW irradiation is able to affect the extent of solid state interaction between drug and alginate matrix via Ca^{2+}, the porosity of the beads and the grade of amorphization of the residual drug located on the surface of the beads, as shown by results of SEM, FTIR, DSC and X-ray analyses.

Compared to convective dried beads, where the drug release mechanism is based on Ca^{2+} depletion from alginate matrix, amorphization of ketoprofen by MW exposition might explain some of the differences in drug release profiles. Moreover, drug release rate can be also modulated by concentration of the polymer solution used to manufacture the beads. In fact, the higher was the alginate concentration, the slower was the release rate of the encapsulated drug. At the same time, both drug content and encapsulation efficiency were not affected by MW exposition.

Therefore, microwave irradiation compared to conventional heating/drying methods offers several advantages such as faster drying kinetics leading to energy saving with low operative costs and the possibility to modulate drug release and dissolution profiles as a function of the irradiation level.

The tandem technique, based on prilling and microwave assisted drying seems to be highly suitable for beads manufacturing in the pharmaceutical industry.

Acknowledgments

The authors would like to thank Professor Piolannelli (University of Salerno) for his support in the X-ray diffraction experiments.

References

Acierno, D., Barba, A., d'Amore, M., 2004. Heat transfer phenomena during processing materials with microwave energy. Heat Mass Transfer 40, 413–420.
Almeida-Prieto, S, Blanco-Méndez, J., Otero-Espinar, F.J., 2004. Image analysis of the shape of granulated powder grains. J. Pharm. Sci. 93, 621–634.
Almeida-Prieto, S., Blanco-Méndez, J., Otero-Espinar, F.J., 2006. Microscopic image analysis techniques for the morphological characterization of pharmaceutical particles: influence of process variables. J. Pharm. Sci. 95, 348–357.
Araszkiewicz, M., Koziola, A., Lupinskaa, A., Lupinskia, M., 2006. Temperature distribution in a single sphere dried with microwaves and hot air. Drying Technol. 24, 1381–1386.
Bergese, P., Colombo, I., Gervasoni, D., Depero, L.E., 2003. Microwave generated nanocomposites for making insoluble drugs soluble. Mater. Sci. Eng. C 23, 791–795.
Brandenberg, H., Widmer, F., 1998. A new multinozzle encapsulation/immobilization system to produce uniform beads of alginate. J. Biotechnol. 63, 73–80.
Chappard, D., Degasne, I., Huré, G., Legrand, E., Audran, M., Baslé, M.F., 2003. Image analysis measurements of roughness by texture and fractal analysis correlate with contact profilometry. Biomaterials 24, 1399–1407.
Chee, S.N., Johansen, A.L., Gu, L., Karlsen, J., Heng, P.W.S., 2005. Microwave drying of granules containing a moisture-sensitive drug: a promising alternative to fluid bed and hot air oven drying. Chem. Pharm. Bull. 53, 770–775.
Cirri, M., Maestrelli, F., Mennini, N., Mura, P., 2009. Physical–chemical characterization of binary and ternary systems of ketoprofen with cyclodextrins and phospholipids. J. Pharm. Biomed. Anal. 50, 683–689.
Del Gaudio, P., Colombo, P., Colombo, G., Russo, P., Sonvico, F., 2005. Mechanisms of formation and disintegration of alginate beads obtained by prilling. Int. J. Pharm., 1–9.
Del Gaudio, P., Russo, P., Rosaria Lauro, M., Colombo, P., Aquino, R., 2009. Encapsulation of ketoprofen and ketoprofen lysinate by prilling for controlled drug release. AAPS PharmSciTech 10, 1178–1185.
Gainotti, A., Losi, E., Sonvico, F., Baroni, D., Massimo, G., Colombo, G., Del Gaudio, P., 2006. The effect of residual water on antacid properties of sucralfate gel dried by microwaves. AAPS PharmSciTech 7, E1–E6.
Hegedus, Á., Pintye-Hódi, K., 2007. Comparison of the effects of different drying techniques on properties of granules and tablets made on a production scale. Int. J. Pharm. 330, 99–104.
Jamuna-Thevi, K., Zakaria, F.A., Othman, R., Muhamad, S., 2009. Development of macroporous calcium phosphate scaffold processed via microwave rapid drying. Mater. Sci. Eng. C 29, 1732–1740.
Koch, S., Schwinger, C., Kressler, J., Heinzen, C., Rainov, N.G., 2003. Alginate encapsulation of genetically engineered mammalian cells: comparison of production devices, methods and microcapsule characteristics. J. Microencapsul. 20, 303–316.
Loh, Z.H., Liew, C.V., Lee, C.C., Heng, P.W.S., 2008. Microwave-assisted drying of pharmaceutical granules and its impact on drug stability. Int. J. Pharm. 359, 53–62.
Marini, F., Bucci, R., Ginevro, I., Magrì, A.L., 2009. Coupling of IR measurements and multivariate calibration techniques for the determination of enantiomeric excess in pharmaceutical preparations. Chemom. Intell. Lab. Syst. 97, 52–63.
Moneghini, M, Bellich, B., Baxa, P., Princivalle, F., 2008. Microwave generated solid dispersions containing Ibuprofen. Int. J. Pharm. 361, 125–130.
Mujumdar, A.S., 2006. Handbook of Industrial Drying, Principles, Classification and Selection of Dryers, third ed. CRC Press by Taylor & Francis.
Nurjaya, S., Wong, T.W., 2005. Effects of microwave on drug release properties of matrices of pectin. Carbohydr. Polym. 62, 245–257.
Sakai, T., Hoshino, N., 1980. Production of uniform droplets by longitudinal vibration of audio frequency. J. Chem. Eng. Jpn. 13, 263–268.
Sakai, T., Sadataka, M., Saito, M., Matsushita, K., 1985. Studies of disintregation of liquid column between prodiction of uniform size droplets by vibration method. In: ICLASS-85, 7B , pp. 2–15.
Sánchez-Dasi, M.J., Garrigues, S., Cervera, M.L., de la Guardia, M., 1998. On-line solvent recycling: a tool for the development of clean analytical chemistry in flow injection Fourier transform infrared spectrometry. Determination of ketoprofen. Anal. Chim. Acta 361, 253–260.
Schneider, L.M., Hendriks, C.D., 1964. Source of uniform liquid droplets by vibration method. Rev. Sci. Instrum. 35, 1349–1350.
Schubert, H., Regier, M., 2005. The Microwave Processing of Foods. CRC Press by Taylor & Francis Group, LLC, p. 345.

Serp, D., Cantana, E., Heinzen, C., Von Stockar, U., Marison, I.W., 2000. Characterization of an encapsulation device for the production of monodisperse alginate beads for cell immobilization. Biotechnol. Bioeng. 70, 41–53.

Soong, D., Shen, M., 1981. Kinetic network model for nonlinear viscoeleatic properties of entangled monodisperse polymers. I. Steady state flow. J. Rheol. 25, 259–273.

Wong, T.W., Chan, L.W., Kho, S.B., Heng, P.W.S., 2005. Aging and microwave effects on alginate/chitosan matrices. J. Control. Release 104, 461–475.

Wong, T.W., Chan, L.W., Kho, S.B., Sia Heng, P.W., 2002. Design of controlled-release solid dosage forms of alginate and chitosan using microwave. J. Control. Release 84, 99–114.