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## NSAID<sub>s</sub>: Design and Development of Innovative Oral Delivery Systems

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### Abstract

Recently, different technologies have been used to transform active pharmaceutical ingredients (APIs) into new dosage forms. Engineered drug delivery systems may modify biopharmaceutical properties of the API achieving either immediate or delayed release according to specific therapeutic needs. Particularly, preprogrammed release of oral formulations delivering the drug at expected times may be useful in chronotherapy of early morning pathologies. The conventional approach when dealing with such diseases is to administer NSAIDs two to three times daily. This approach does not allow to fit drug release with symptoms onset resulting in inefficient therapy and poor patient compliance. NSAIDs may be very effective if administered at least 4–6 h before the pain reaches its peak in the early morning. The solution could be to design delayed drug delivery systems allowing one administration before going to sleep acting in the early morning. This chapter highlights new approaches in developing controlled delivery systems of NSAIDs potentially useful to treat both acute and chronic inflammation. The chapter illustrates the versatility of laminar jet break-up technology (prilling) to produce gel beads able to control rate and time of drug delivery. A special focus will be on particle-engineering strategies, i.e., prilling and prilling technique in tandem with microwave or supercritical fluid-assisted drying.

**Keywords:** oral drug delivery, modified release dosage forms, chronotherapy, early morning pathologies, NSAIDs, prilling; hydrogels drying, dissolution, *in vivo* effectiveness

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## 1. Introduction

Any drug delivery system (DDS) may be defined as a system comprising:

- active pharmaceutical ingredients (APIs) and
- medical device or dosage form able to carry the drug inside the body.

To obtain a therapeutic response, the suitable amount of the drug must be delivered at the right time, in a safe and reproducible manner, to a specific target and at the required level. The distribution of the drug to tissues different from the sites of action and organs of elimination is a potential cause of toxicity.

Innovative drug delivery systems may overcome problems commonly related to conventional formulations. In fact, in the last few years, there is a continuous research in the development of engineered particles and systems for the targeted delivery and/or controlled release of APIs. These systems can protect the drug from degradation, realize controlled release, modify pharmacokinetics and biodistribution profiles, reduce clearance and side effects, and improve drug specificity. The administration of an old drug through an engineered delivery system may contribute to the improvement of the drug efficacy, safety, and patient compliance and may extend its conventional clinical applications [1, 2]. For example, in the treatment of both acute (i.e., postoperative pain, dental surgery) and chronic inflammatory diseases, immediate or delayed drug delivery systems are essential.

The development of delivery systems able to control and delay drug release is acquiring a growing interest with the recent advances in chronopharmacology. In fact, treating diseases affected by the circadian rhythms, such as “early morning pathologies” (EMPs) (rhinitis, rheumatoid arthritis, etc.) [3–5], requires a desired concentration of drug available at expected times [6, 7]. This effect may be assured by device releasing no drug within the time gap and delivering the optimal amount at certain point correspondent to symptoms peak [8–10]. Diseases with established circadian cycle show day–night patterns in the onset and symptoms exacerbation, usually with peaks in the morning and decrease throughout day [11]. Moreover, the plasma concentration of C-reactive protein and interleukin-6 has been shown to follow a circadian rhythm in chronic inflammatory-based diseases such as rheumatoid arthritis [12]. The main goals when dealing with such pathologies are as follows: stop inflammation, relieve symptoms, improve physical function and overall well-being, and reduce long-term complications.

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent one of the most used classes of drugs in the management of EMPs. These molecules inhibit COX and prevent further formation of prostaglandins and other related inflammatory mediators. Based on their mechanism of action, NSAIDs are useful as adjuvant therapy for the symptomatic management of the diseases, reducing inflammation and pain. NSAIDs employed to relieve symptoms are usually administered to patients through oral route at different moment of the day (after awakening, after lunch, and during the night) through immediate-release formulations. In this way, drugs are rapidly absorbed and explicate their action independently from the circadian

rhythms of hormones and cytokines responsible of EMP symptomatology. Such a conventional approach usually leads to ineffective therapy because drug liberation from the dosage form is not synchronized with the symptom peak resulting in an increase in side effects and, consequently, poor patient compliance. This explains why in the last few years a growing number of researchers have focused on the possibility to treat EMPs with a chronotherapeutic approach through oral route.

NSAIDs (i.e., indomethacin, aceclofenac, ketoprofen, flurbiprofen, and lornoxicam) are attractive API to be modified in their formulation obtaining chronotherapeutic drug delivery systems (ChDDSs) useful in the treatment of early morning pathologies [13]. For example, a pH-responsive dual pulse multiparticulate dosage form containing ketoprofen was developed and tested in rheumatoid arthritis. Results showed that this formulation was able to relieve circadian symptoms during midnight and early morning [14]. Levi et al. observed that the evening administration of indomethacin in a controlled release formulation was able to better control morning symptoms compared to the same formulation administered in other moment of the day [15].

Among different approaches used to design pharmaceutical dosage forms tailored to follow the human chronobiological rhythm [10, 16, 17], micro-technologies have been applied as innovative and efficient tool [18, 19]. Several methods and techniques are potentially useful for the preparation of polymeric microparticles, such as spray drying, fluid bed coating, solvent evaporation, coacervation phase separation, prilling, etc. The choice of a specific microencapsulation technology is related to the polymer nature, the chemical features of drug, the desired particles size, as well as to the reproducibility and ease to scale ability of the method [20].

### 1.1. Prilling technique

Among the different processes used for the preparation of ChDDSs, prilling or laminar jet breakup technique is an emerging process able to produce homogenous microparticles with high drug content and controlled release properties. Prilling is a vibration-based technology consisting in breaking apart a laminar jet of polymer solution into a row of monosized drops by means of a vibrating nozzle device (**Figure 1**) [21, 22]. Once the droplets are formed, the gelation/consolidation step follows in order to prevent either the aggregation of polymer droplets or the undesired leakage of encapsulated drugs. The chemical nature of the droplets (dispersed phase) determines the consolidation step, in which the droplets are transformed into solid microparticles known as gel-beads; this procedure can involve temperature modification, chemical reactions, and mainly ionic cross-linking (ionotropic gelation).

Several variables in prilling can affect droplet size and size distribution as polymer concentration and flow rate [23, 24], frequency of vibration, as well as falling distance. In fact, using high wavelengths of the jet breakup and reducing the falling distance to the hardening solution, the drop coalescence can be reduced. Thus, smaller nozzle diameters and higher frequencies increase the possibility of coalescence [22]. The frequency is usually kept as low as possible in order to avoid the formation of satellite droplets leading to a broader size range [21]. The



**Figure 1.** Prilling apparatus (Nisco Inc.) at the Department of Pharmacy, University of Salerno.

production of microparticles by the vibrating nozzle device is highly reproducible, is time-saving, and can be performed under aseptic and scaled-up conditions [25]. The acoustic jet excitation process involved in prilling was patented for production of uniform microspheres of alginate [26], collagen [27], and PLGA [28, 29]. The scale-up of the vibration process may be done by using a multinozzle system (**Figure 2**) without changing other process parameters such as flow rate and the vibration frequency [30]. However, the arrangement of the nozzles which must ensure equal jet formation and equal pressure drops between the nozzles is an



**Figure 2.** Multinozzle system BRACE GmbH [32].



important parameter [30]. The pilot apparatus using this technique is now being sold by Brace GmbH (Germany), Nisco Inc. (Switzerland), and Inotech AG (Switzerland) [22, 31].

The ionotropic gelation, a highly used consolidation step, is based on the ability of polyelectrolyte polymers (alginate, pectin, chitosan, and gellan gum) to cross-link in the presence of counter ions [32, 33]. Divalent cations ( $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Zn}^{2+}$ ) are suitable cross-linking agents with the exception of  $\text{Mg}^{2+}$  [32, 33]. The binding affinity depends on the chemical composition of the selected polysaccharide, which varies with the source, the characteristics of the polyvalent cations (i.e., ionic radius and coordination number), and the presence of water of hydration surrounding cross-linking ions [32].

After the production stage, hydrogels require to be dried to avoid chemical or microbiological degradation [34]. Drying processes may have a different impact on the final properties of the dried product (solute migration, polymorphism, damages by overheating, and structural modifications). Therefore, the selection of drying technique has a crucial role to determine the textural of the final product and to avoid altering its quality.

## 2. Prilling-based tandem techniques to produce NSAIDs controlled delivery systems

In this chapter, we described three different prilling-based tandem techniques to produce chronotherapeutic dosage forms delivering NSAIDs. The techniques are based on the combination of prilling with conventional drying, microwave-assisted drying, or supercritical fluid-assisted drying [35].

### 2.1. Prilling in tandem with conventional drying

To effectively formulate NSAIDs targeting EMPs, prilling microtechnology in combination with conventional drying was used. The traditional drying of hydrogels is based on the evaporation of the solvent; when the solvent inside the pores of the material evaporates, they are subjected to high tension, which causes the partial collapsing of the structure and the wet gel is changed in the so-called “xerogel.” Usually, this process is rapid, cheap, and easy to perform. Furthermore, recent studies [36–39] suggested that the opportune setup of some drying parameters may allow the production of materials with unique structural properties, i.e., microporous, micro-mesoporous, or micro-macroporous systems.

In this section, we described the combination of prilling technique with conventional drying to obtain xerogel-based beads loaded with ketoprofen, ketoprofen lysine salt, and piroxicam.

#### 2.1.1. Ketoprofen-loaded xerogels as new ChDDSs: design and *in vitro/in vivo* characterization

Ketoprofen is a NSAID commonly used in some EMPs as rheumatoid arthritis for its analgesic and anti-inflammatory features. Nevertheless, many collateral effects, such as gastric troubles, restrict its therapeutic benefit. Furthermore, its short biological half-life ( $t_{1/2} = 2.1\text{h}$ )

and the need of a high number of daily administrations enhance side effects especially for chronic therapy. For these reasons, ketoprofen is a good candidate for the development of controlled release formulations, able to provide drug release at predetermined rate and time, targeting intestine [21, 40].

2.1.1.1. Approach #1

With the aim to delay ketoprofen release as needed for chronotherapy of EMPs, we designed a gel-beads formulation for delayed delivery of ketoprofen release and *in vivo* absorption for several hours [41, 42].

2.1.1.1.1. Methods

The engineered particles were produced by prilling/ionotropic gelation technique using alginate as gastroresistant polymer carrier and zinc as cross-linking agent. Particularly, Zn<sup>2+</sup> was also selected for its anti-oxidant properties that may boost the efficacy of the anti-inflammatory drug encapsulated in the polymeric matrix [43, 44]. After the production, hydrated particles were then exposed to standard room conditions (22 °C; 67% RH) for 12–18 h until constant weight was reached obtaining xerogel beads. During the work, different experiments were conducted for the optimization of process parameters in order to obtain ketoprofen gel-beads with desired properties and performances (**Table 1**). Beads micromeritics, solid state, and drug release properties were studied using established method (SEM, DSC, USP 36 Paddle Dissolution).

2.1.1.1.2. Results and discussion

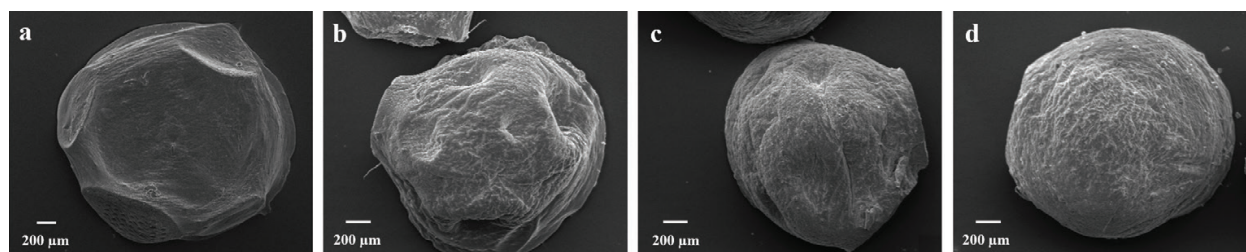
Formulation AK\_5 obtained with the highest ketoprofen content (alginate 2% w/w; ketoprofen–alginate ratio = 1:5) showed the highest value of both actual drug content (ADC 9%) and encapsulation efficiency (EE 53%). It is interesting to point out that ADC and EE were related to the drug/polymer ratios suggesting that the incorporation of a great quantity of ketoprofen (into the feed solutions) could induce, during the gelation process, the establishment of intermolecular interactions (as hydrophobic or hydrogen bonding) able to stabilize the “egg-box” structure, leading to a more strong cross-linked matrix. All K-loaded beads exhibited a remarkable reduction in mean diameter in comparison to blank beads with diameters’ values

Alginate concentration = 2.0% (w/w)	Gelling cations = Zn <sup>2+</sup>
Drug/polymer ratio = 1:20–1:10–1:5	Gelling time = 2 min
Ø nozzle = 400 µm	pH gelling solution =1.5
N = 0.350 kHz A = 100%	Flow rate = 5 mL/min
Composition of the aqueous feed solutions, prilling parameters (nozzle diameter Ø, frequency N and amplitude A of vibration, and flow rate), gelling solution variables (divalent cation for ionotropic gelling, pH of the gelling solution and cross-linking time).	

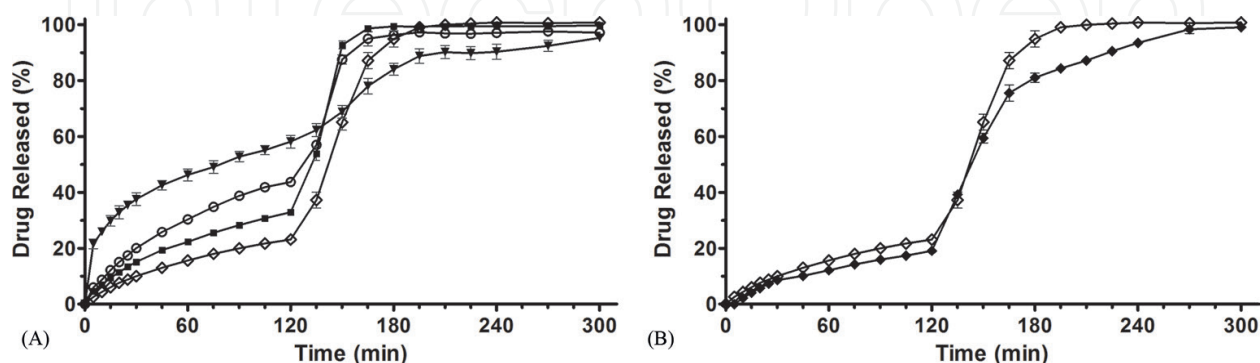
**Table 1.** Optimized prilling operative conditions.

in the range of 1.7–1.8 mm. Stabilization of the polymer matrix influenced beads shape after the drying process. In particular, SEM analyses confirmed that K-loaded beads had almost spherical shape according to K loading (**Figure 3**).

Pilot dissolution tests of all formulations were performed by conventional vessel methods (USP Apparatus 2, paddle) [45] using a classic pH change method, providing essential information and recommended in various guidelines [46] as a first choice for the *in vitro* dissolution testing of controlled/modified release formulations [47]. Results showed that AK\_5 exhibited a gastroresistant profile followed by a slow and extended release of K in SIF (**Figure 4A**). Probably, the intermolecular interactions combined to the high zinc ability to cross-link alginate moieties, led to a strong polymeric matrix for AK\_5 able to better control the drug diffusion. The dissolution profile of this formulation was deeply studied using the USP apparatus 4 (flow-through, open-loop configuration), because, as reported in literature, it is able to accurately mimic *in vivo* hydrodynamics and consequently better predict the real *in vivo* behavior of oral formulations [48, 49]. USP apparatus 4 analysis (**Figure 4B**), confirmed the enteric release ability of AK\_5 beads showing in SIF a slower ketoprofen release compared to USP 2 analysis. The observed differences were mainly due to the particular hydrodynamic conditions realized by the USP 4 apparatus (no agitation mechanism and constant exposure to the formulation to a laminar flow) that are very similar to the *in vivo* gastrointestinal conditions [50, 51].



**Figure 3.** SEM microphotographs of dried Zn-alginate beads formulations: (a) blank beads, (b) AK<sub>20</sub>, (c) AK<sub>10</sub>, and (d) AK<sub>5</sub>.



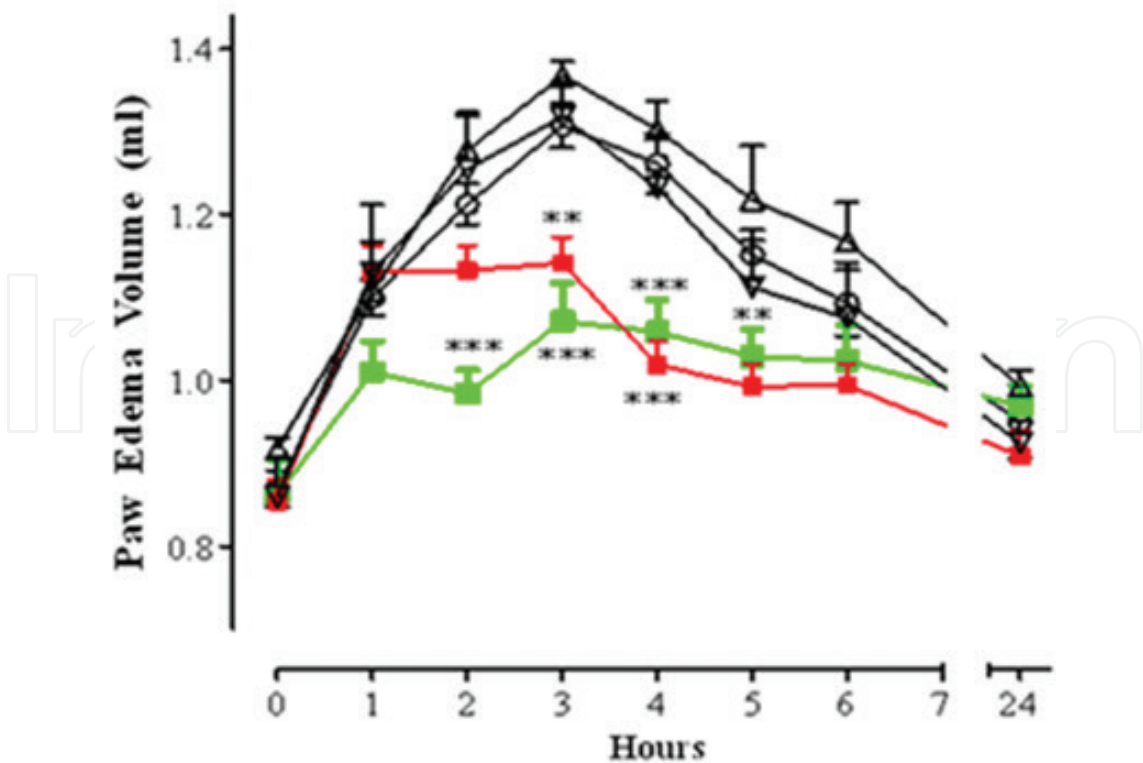
**Figure 4.** (A) Release profiles of K raw material (▼) and K-loaded formulations AK<sub>20</sub> (○), AK<sub>10</sub> (■), and AK<sub>5</sub> (◇), performed by USP Apparatus 2; (B) release profiles comparison of AK<sub>5</sub> obtained by USP Apparatus 2 (◇) and USP Apparatus 4 (◆), using a pH change assay. Mean ± SD; (n=6).



To verify whether the *in vitro* drug release may be correlated to a prolonged anti-inflammatory effectiveness, AK\_5 (ketoprofen equivalent dose of 3 mg/kg) was tested *in vivo* using a carrageenan-induced acute edema in rat paw (male Wistar rats). Its activity was compared to the activity of pure drug. In detail, samples were administered to rats at the time 5, 3, and 0.5 h before the injection of the phlogistic agent, and paw volume was measured plethysmographically at the time zero, each hour for 6 h, and at 24 h.

As shown in **Figure 5**, AK\_5 exhibited a prolonged anti-inflammatory effect expressed as paw volume reduction, after oral administration in rats in comparison with the control. Unformulated ketoprofen (3 mg/kg) reduced rat paw edema only with a contemporary administration (0.5 h before carrageenan injection), without any effect at t=3 or 5 h before the phlogistic agent injection. Blank zinc-alginate beads, administered to rats at the same time points, did not interfere with the inflammatory process. AK\_5 administered 3 or 5 h before edema induction still showed a significant anti-inflammatory activity reducing maximum paw volume (3–4 h) in response to carrageenan injection, therefore reflecting its drug delayed release (**Figures 4 and 5**). Moreover, the prolonged *in vivo* anti-inflammatory effect may be related to the mucoadhesive features of alginate particles able to reduce the ketoprofen absorption in the gastrointestinal tract [52].

Being the optimized formulation AK\_5 able to delay *in vivo* K release and absorption up to 6–7 h, it can be considered a potential chronotherapeutic system for the delivery of K. Opportunely timing its administration is possible to match the disease rhythms and to control early morning symptoms in inflammatory-based EMPs.



**Figure 5.** Edema volume reduction obtained administering AK\_5 and pure K per os to rats 5h (■\_AK\_5 and -V-\_K) and 3h (▲\_AK\_5 and -Δ-\_K) before carrageenan injection compared to control (○-); mean ± SD (n=8). \*\*P ≤ 0.01, \*\*\*P ≤ 0.001 compared with control.

#### 2.1.1.2. Approach #2

These preliminary results have been the starting point to further improve technological properties of gel-beads' formulations in terms of ketoprofen loading and release performances. Therefore, in the following study, we designed a more complex system based on core/shell particles (double-layered particles) consisting of a Zn-pectinate core loading NSAID and an enteric shell of Eudragit S100 (anionic copolymer of methacrylic acid and methyl methacrylate insoluble in acid pH) [53]. Among different commercially available pectins, we focused our attention on amidated low methoxy (ALM) pectin (esterification degree 24% and amidation degree 23%) that, showing high hydrophobic interactions between pectin chains and internal hydrogen bonding between amide groups, is able to stabilize the egg-box structure [54]. Pectins are usually classified on the basis of their degree of esterification (DE) and divided in high methoxyl (HM\_DE > 50%) and low methoxyl pectin (LM\_DE < 50%). The latter are the most suitable for the development of oral controlled drug delivery systems. In addition, as reported in the literature, the presence of amide groups in low methoxyl pectin enhances the gel-forming ability, the stability under different pH conditions improving, consequently, the possibility to control drug release [55].

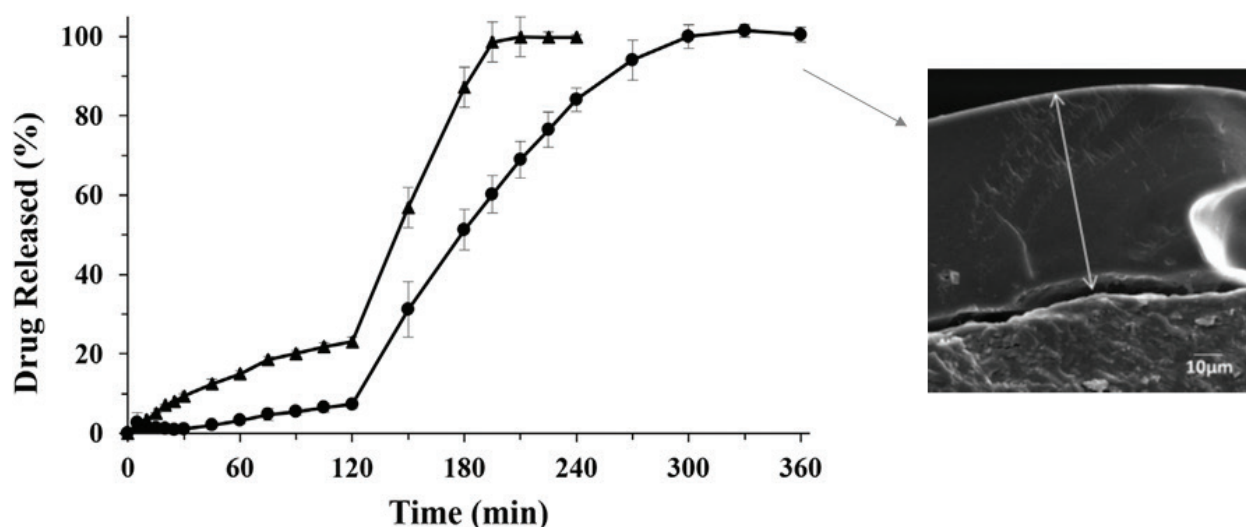
##### 2.1.1.2.1. Methods

Ketoprofen-loaded core particles based on Zn-pectinate matrix were produced by prilling technique using optimized operative conditions [53]. Beads micromeritics, solid state, and drug release properties were studied using established method (SEM, DSC, USP 36 Paddle Dissolution).

##### 2.1.1.2.2. Results and discussion

Formulation BK\_5 (Pectin 6% w/w, drug/polymer ratio = 1:5), obtained with the greatest amount of drug, showed the best morphology and the highest EE value (86.7%). In fact, as also observed for alginate beads loaded with ketoprofen [41], the drug and its amount play an important role during the gel matrix formation conditioning and therefore affect technological and mechanic properties of the final dosage form.

To obtain information about *in vitro* release performances, this formulation was tested through a USP apparatus II using a classic pH change method. As expected, monolayered Zn-pectinate beads were not properly effective in retaining drug in simulated gastric fluid (22.2% of the drug was released in 120 min). Their solubility and swelling properties in aqueous media allow a prompt release of NSAID after the pH change of the dissolution fluid (complete drug release was achieved in 180 min). The chronotherapeutic system was obtained by coating zinc-pectinate core (BK\_5) with a Eudragit S100® shell. The core-shell system BK\_5/E40, obtained applying coating in the amount of 40% (w/w), showed a strong delay of ketoprofen release in SGF followed by a slow and controlled release in SIF (**Figure 6**). This system was able to significantly reduce ketoprofen release in acidic environment from 22.2% (BK\_5) up to 7.3% and to prolong its release in simulated intestinal medium. In fact, after the pH change, drug release was completed in approximately 3 h (t=300 min) rather than in 1 h (t= 180) as for BK\_5. Only after the dissolution of the enteric shell in SIF, the zinc-pectinate core is exposed to the fluid and pectin chains begin the hydrolysis, releasing slowly the loaded drug. This profile appears feasible and potentially effective for chronotherapy of early morning pathologies.



**Figure 6.** Drug release profiles of core-shell system BK5/E40 (-●-) compared to monolayer formulation BK5 (-▲-) and SEM picture of a cryo-fractured core-shell particle BK5/E40 with magnification of the core-shell structure.

### 2.1.2. Ketoprofen lysine salt-loaded beads: design and characterization

Although several studies have been reported on polysaccharide hydrogels entrapping the nonsoluble form of ketoprofen [21, 41, 53, 56], few data are available on its L-lysine salt showing better pharmacokinetics and tolerability, enhancement in the rate of absorption, reduction of the onset of therapeutic effect, as well as improvement of the gastric tolerance [57]. The major issue encountered when dealing with such API is the high aqueous solubility preventing the formulation of delivery systems with high encapsulation efficiency values and, above all, effective controlled release properties [58].

Thanks to the expertise grew up in the last few years, we proposed alginate and ALM pectin as carriers and release tailoring agents for the development by prilling of chronotherapeutic system loaded with KL. As highly soluble drugs in hydrogels exhibit poor drug entrapment and ineffective control in drug release, many formulation variables have been investigated to find the optimal ones.

#### 2.1.2.1. Methods

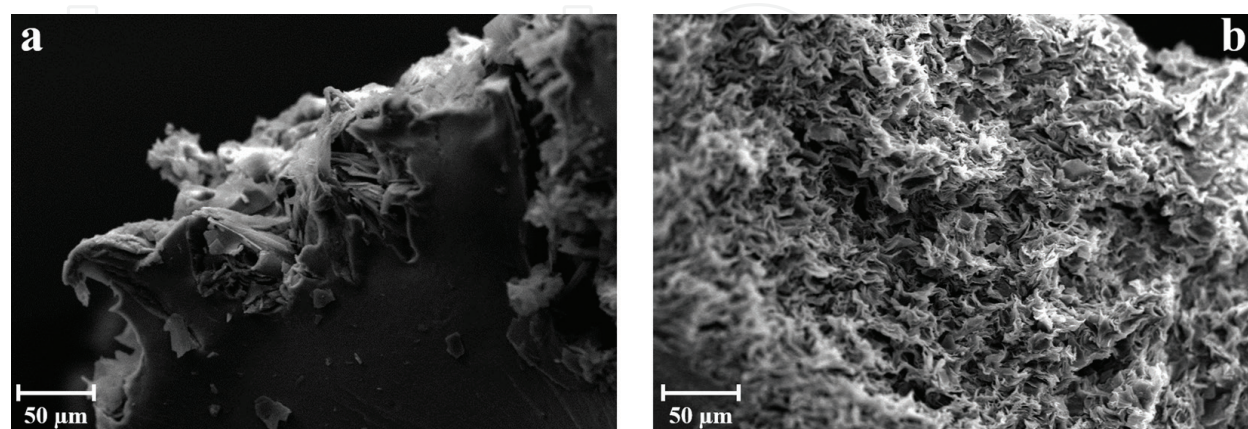
Beads were prepared using  $\text{Zn}^{2+}$  as a gelling agent and optimizing cross-linking conditions as follows: temperature (4–5°C), pH (1.5) and gelling time (2 min). Different formulations were produced setting polymer concentration at 2.0% and 6.0% w/w for alginate (A) or pectin (B), respectively, and varying drug-polymer ratio as follows: 1:20 (KL\_20), 1:10 (KL\_10) and 1:5 (KL\_5). Drying process of the hydrated beads was conducted by exposing them to standard room conditions as previously described. Beads micromeritics, solid state, and drug release properties were studied using established method (SEM, DSC, USP 36 Paddle Dissolution).

### 2.1.2.2. Results and discussion

ADC and EE values were related to the specific polymeric material used (A or B) and drug polymer ratio. The higher the drug polymer ratio, the higher the EE. KL encapsulation was higher in pectin (81–94%) than in alginate (39–49%) beads. This effect could be related to the low molecular weight of pectin able to improve the hydrogel structure [59, 60]. The produced particles were cryofractured and analyzed by SEM (**Figure 7**) with the aim to study the effect of alginate or pectin on the inner structure. Pectin-made beads showed a uniform and more compact inner structure with regard to alginate-based particles. Indeed, KL crystals resulted effectively included in the pectin matrix, whereas, for alginate beads, they resulted being distributed on the external layer (**Figure 7**). This effect may be due to the formation of several intermolecular hydrogen bonds and hydrophobic interactions between KL and pectin amino residues able to deeply stabilize the egg-box structure [61, 62]. The strong hydrogel network can determine a high shrinkage of the volume after drying process, leading to a significant reduction in mean diameter of the beads.

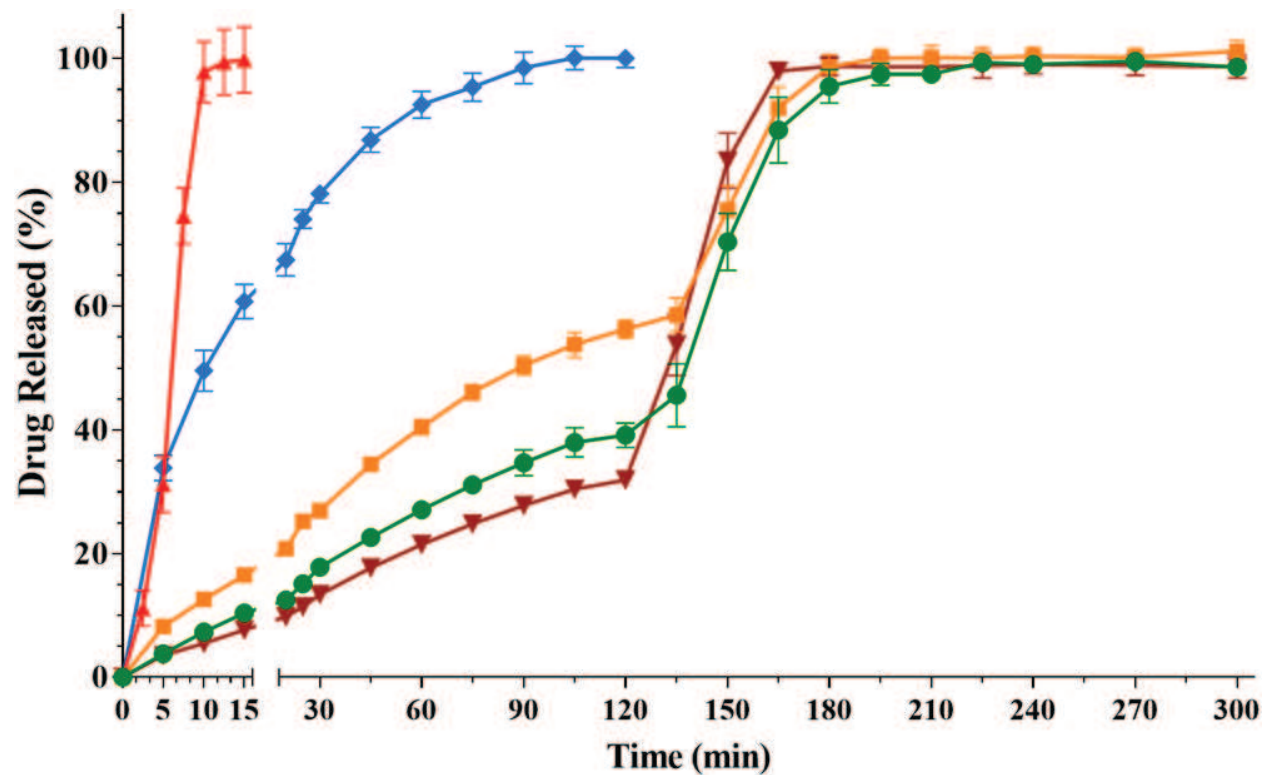
Results of dissolution tests (**Figure 8**) revealed that all KL-alginate beads provided fast and complete drug release in acidic medium, without any lag time, in 120 min. On the contrary, all pectin-based beads were able to achieve KL sustained release. The specific drug release profile from each formulations' series perfectly matches with beads morphology and structure data obtained by SEM investigation. The faster release of KL-alginate beads in SGF was related to KL crystals on the particle's surface and to the higher permeability of alginate matrix. Instead, Zn-pectinate beads showed a reduced swelling and erosion process in gastric environment as effect of their more compact texture [53, 63]. These data are consistent with recent literature studies, confirming the higher suitability of ALM-pectin to produce xerogel beads with improved technological properties compared to alginate [64, 65].

To improve delayed and sustained release performances, BKL\_5 was selected as starting material for the preparation of a drug delivery platform made up by DR® capsules and BKL\_5 (BKL\_5/DR caps). Although gelatin capsules disaggregate after few minutes in simulated biological



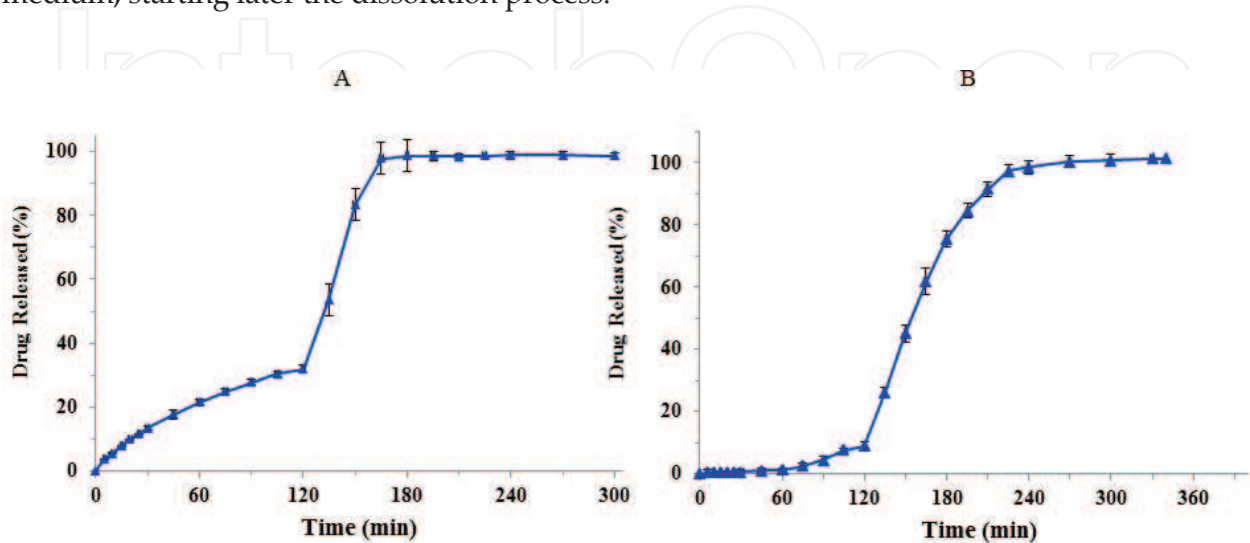
**Figure 7.** SEM microphotographs of cryo-fractured xerogel beads: (a) AKL\_5 and (b) BK\_5.





**Figure 8.** KL release profiles of formulations AKL\_5 (◆-), BKL\_20 (■-), BKL\_10 (●-), and BKL\_5 (▼-) compared to KL raw material (▲-).

fluids, the selected capsules (DR®) can prolong the disaggregation step up to 75–90 min delaying the hydration process of BKL\_5 beads in SGF. The optimized platform (BKL\_5/DR caps) significantly reduced KL dissolution in SGF and showed a slower release in intestinal environment (**Figure 9**). This platform reduced KL release in SGF from 31.9 to 8.8% and prolonged its release in intestinal simulated fluid until 4.5 h (t=270 min) compared to 1 h (t=180 min) as observed for BKL\_5. Only after the disaggregation of the capsule’s body, beads are exposed to dissolution medium, starting later the dissolution process.



**Figure 9.** KL release profiles of BKL\_5 (A) compared to the optimized platform BKL\_5/DR caps (B).



Platform BKL\_5/DR caps may be potentially useful for the oral administration of highly soluble NSAIDs such as ketoprofen lysinate in the chronotherapeutic treatment of EMPs.

### *2.1.3. Piroxicam-loaded core/shell beads as new ChDDS: design and characterization*

Piroxicam (PRX) is a NSAID of the oxicam class largely used to treat EMPs. PRX is included in the class II of the Biopharmaceutical Classification System as ketoprofen; however, it possesses different physical–chemical properties such as higher melting point and ampholytic nature. Thus, it may exist in different ionic forms at physiological pH [66]. PRX is rapidly and completely absorbed following oral administration (recommended dose in adults is 20 mg daily) and exhibits a long half-life (24–48h). However, its elimination is impaired in some elderly people [67], who represent the majority of patients affected by rheumatic diseases, resulting in a high inter-individual variability in the steady state plasma levels. Therefore, sustained release dosage forms capable of steady release of PRX over prolonged periods may overcome such variability due to erratic drug elimination [68].

In the case of PRX, we investigated the possibility to develop multiparticulate beads based on a blend of natural polysaccharides (monolayered particles) or a combination of polysaccharides with other polymers (double-layered particles). As discussed, beads based on single polysaccharide matrix may exhibit a premature release of the drug in the upper part of the GIT, making necessary the use of additional technological actions such as an enteric coating [53] or an envelopment in capsular devices [58]. However, a gel-matrix based on the combination of two or more polymers may be very effective [69].

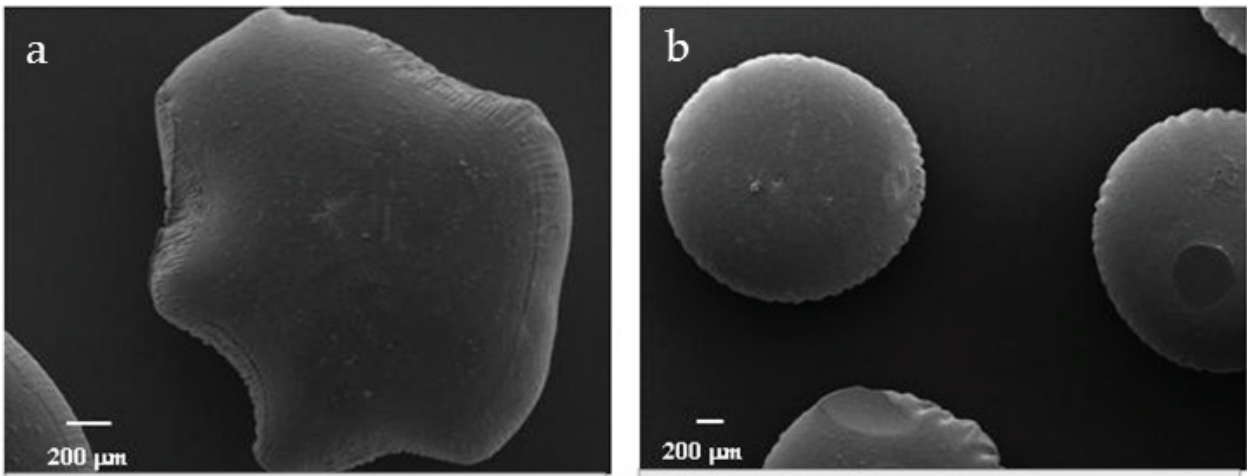
#### *2.1.3.1. Methods*

Two series of piroxicam-loaded particles were produced using pectin/alginate (B/A) blends or pectin alone as carrier material. Several formulations were designed varying polymer total concentration between 4.0 and 6.0% (w/w), pectin alginate ratio from 10:0 to 10:2 and setting drug/polymers ratio at 1:20 (PRX\_20) or 1:15 (PRX\_15). Other operative conditions were set accordingly to previous experiments in order to obtain spherical hydrated beads with tough polymer matrix, smooth, and regular surface [69]. Beads micromeritics, solid state, and drug release properties were studied using established method (SEM, DSC, USP 36 Paddle Dissolution).

#### *2.1.3.2. Results and discussion*

High encapsulation efficiency (EE ranging from 62 to 93%), correlated to the increase of the polymer concentration in the feed, was achieved especially for BPRX\_20, formulated with pectin alone. SEM analyses (**Figure 10**) highlighted that no crystals of piroxicam were visible on the surface, suggesting the drug complete entrapment within the polymeric matrix. Good micromeritics and encapsulation efficiency proposed the pectin-based formulation BPRX\_20 as a starting point to develop a more efficient technological platform applying the two step core–shell approach previously tested for ketoprofen.

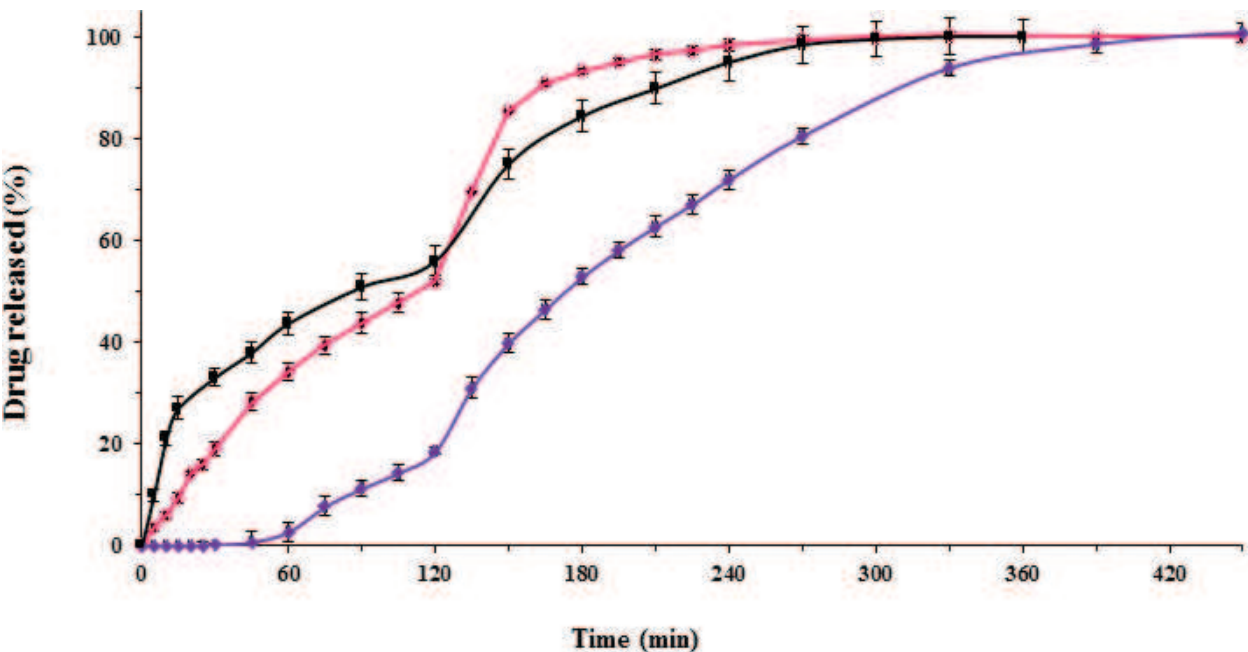
Beads BPRX\_20 were coated by immersion with Eudragit® S100 to obtain an enteric coating able to avoid the erosion of the core pectin matrix and protect the drug in acidic medium [69].



**Figure 10.** SEM microphotographs of pectin/alginate (a) and pectin beads (b) loaded with PRX produced by prilling and dried at room conditions.

Core/shell beads BPRX\_20/E40 showed a drug release profile typical of gastro-resistant oral dosage forms, releasing less than 20% of piroxicam in SGF. In SIF, this formulation released approximately 50% of the loaded drug after 1 h achieving complete PRX release in approximately 5 h (**Figure 11**).

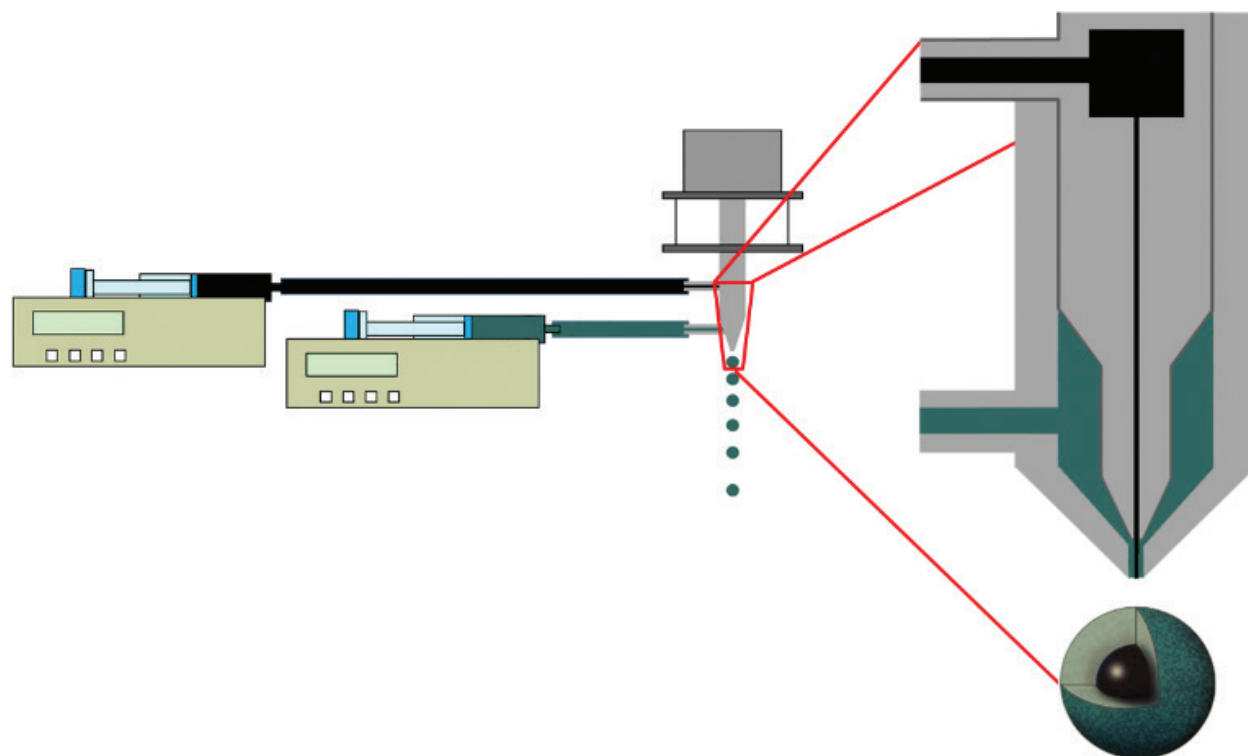
On the basis of these results, we can conclude that prilling technique in combination with an enteric coating is a very promising and simple two-step method to formulate core/shell beads as chronotherapeutic agents.



**Figure 11.** Release profiles of uncoated BPRX\_20 (◇) and Eudragit®-coated beads formulations BPRX\_20\_40 (◆), with regard to pure piroxicam (■). Mean ± SD; (n=6).

Core-shell particles may also be obtained using a novel single step approach based on coaxial prilling technique. Core-shell systems composed of alginate (A) in the outer layer and pectin/piroxicam (B/PRX) as core were designed [70]. This innovative method employs multiple concentric nozzles to produce a smooth coaxial jet comprising polymer annular shell and core material, which are broken up by acoustic excitation into uniform core-shell droplets and gelled into a cross-linking solution. Alginate and pectin/PRX suspensions were pumped out through coaxial nozzles with inner and outer diameters of 400 and 600  $\mu\text{m}$ , respectively. Using volumetric flow rate of 10 ml/min, vibration frequency of 350 Hz and 100% amplitude of the vibration, the two viscous polymers suspensions subjected to prilling immediately formed droplets, which were dripped in a gelling  $\text{Zn}^{2+}$  bulk ( $\text{Zn}^{2+}$  concentration 10% w/v, pH 1.5 and cross-linking time 8 min). Schematic illustration of the core-shell process is shown in **Figure 12**.

Results showed that satisfying encapsulation efficiency values were obtained for the produced formulations (EE 58–93%). However, it is interesting to point out that EE for coated beads was related to shell integrity. In fact, formulation A/B\_PRX10 (core/shell ratio 4:1 and PRX/pectin ratio 1:10) showing the most homogeneous coating presented very high EE (86.3%). The formation of complete alginate shell was able to prevent the piroxicam leaking from the droplets into the gelling medium during the polymer cross-linking phase; accordingly, a better entrapping of the drug within the core was obtained. The critical factor to obtain beads with regular shell was found to be the ratio between the nozzle viscosity of inner and outer polymer solutions ( $\mu_c/\mu_s$ ). In fact, when nozzle viscosities ratio was less than 4, particles showed



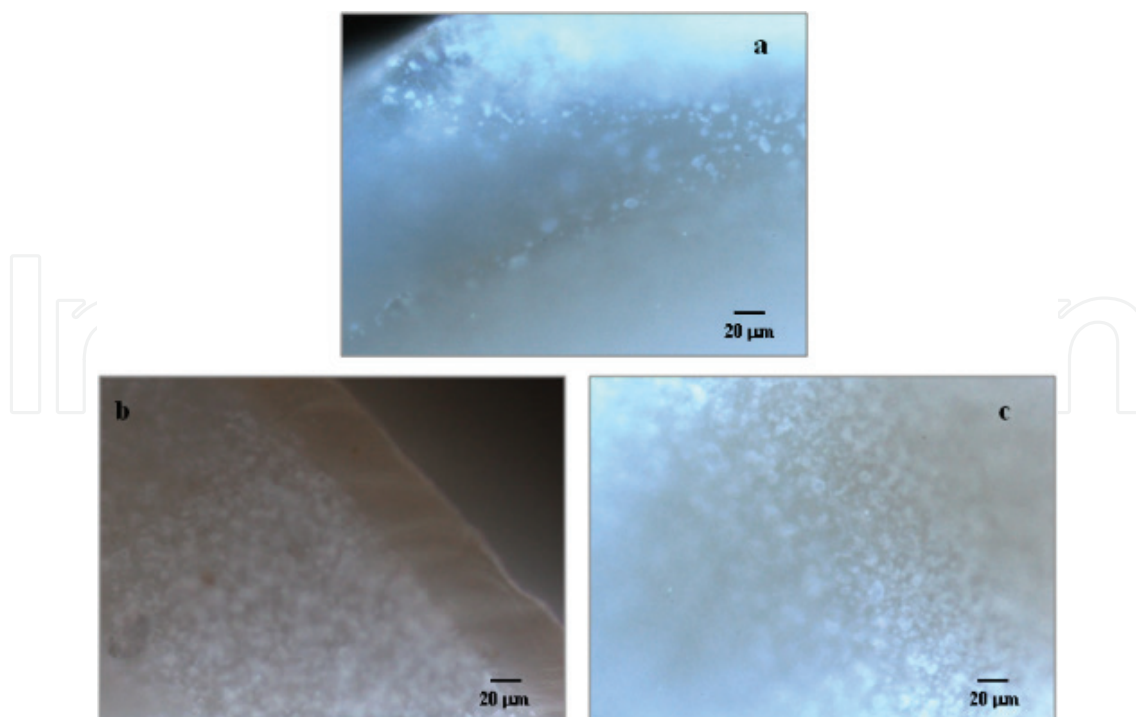
**Figure 12.** Schematic reproduction of prilling technology in coaxial configuration.

irregular or uncompleted alginate coating around the pectin core, whereas values over six matched with completely layered beads. During the droplet formation, the outer solution must be able to completely enclose the inner one. Therefore, the decrease in the alginate concentration and, subsequently, the decrease in the nozzle viscosity determine a regular core/shell beads formation [71].

To better understand the influence of drug content on polymeric matrix, cryofractured beads were also analyzed by fluorescent microscopy (FM). FM images of PRX-loaded pectin beads exhibited fluorescent spots due to crystals of piroxicam homogeneously encapsulated within the Zn-pectinate matrix (**Figure 13a–c**). FM analysis also proved that PRX core/shell beads, with B/A mass ratio of 4:1, presented the most homogeneous coating and, interestingly, showed that drug was confined into the particle inner matrix without any leaching into the alginate shell.

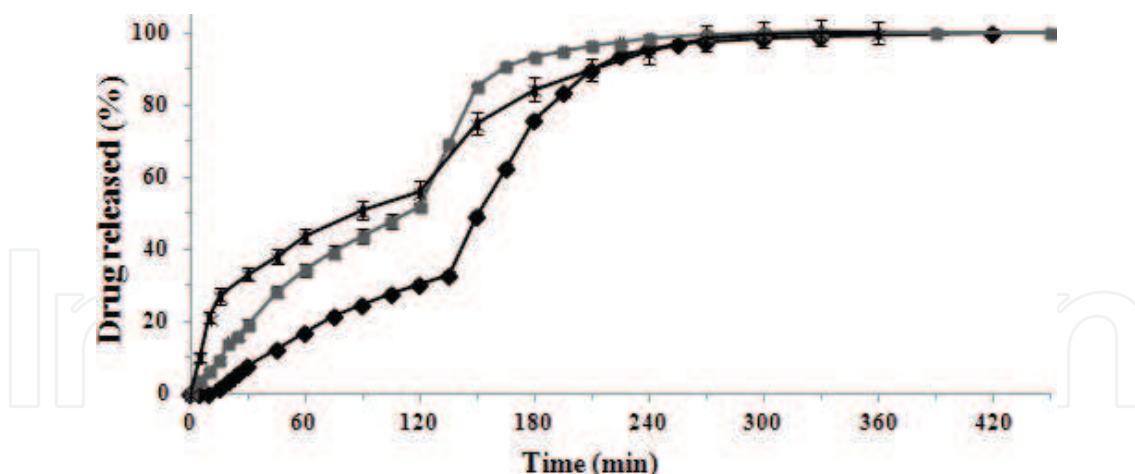
As reported elsewhere, Refs. [72–74] monolayered pectin beads act as a fast release formulation delivering approximately 50% of PRX in simulated gastric fluid and allowing the total drug release in SIF, after pH change, in approximately 2 h (**Figure 14**). On the contrary, core shell beads A/B\_PRX10 release approximately 30% of PRX in SGF, achieving 100% in 5 h. The alginate shell decreases the initial burst effect, delaying both particles dissolution and drug diffusion in the simulated gastric fluid.

This study demonstrated that co-axial prilling technique can be used as a novel single-step approach for the manufacturing of core–shell particles containing NSAIDs. The most critical process parameter to obtain uniform double-layered particles was identified in the ratio between



**Figure 13.** Fluorescent microscopy pictures of cryofractured PRX-loaded formulations: (a) uncoated Pct beads and (b and c) B/A beads.





**Figure 14.** Release profiles of PRX raw material (\*-), monolayered pectin beads (-■-) and core/shell beads A/B\_PRX10 (-◆-), performed using a pH change assay. Mean  $\pm$  SD (n = 6).

inner and outer solution viscosities. The optimized core/shell (A/B\_PRX10) beads can be considered either as a self-consistent dosage form or as a formulation to be hosted in suitable gastro-resistant capsule with the aim to further delay drug release meeting the requirements of EMPs.

## 2.2. Prilling in tandem with microwave-assisted drying

Hydrogel drying can also be conducted using alternative methods to conventional techniques. Each drying process has a different impact on structural characteristics of the dried beads especially on release properties. Particularly, in recent years, microwaves (MW) assisted heating has gained great interest in many applications with special reference to pharmaceutical processing. Several benefits can be obtained from technologies such as short processing time, better products uniformity and yields, energy saving, reduced production costs, as well as the ability to confer unique structural characteristics to the end materials. Recently, MW heating technology has been shown to be useful in the design of single-unit dosage forms such as solid dispersion, granules, and tablets [75–78]. Moreover, in the case of dextran-based formulations (i.e., alginate, pectin, and chitosan), MW irradiation leads to obtain xerogels with modified drug release properties due to the extent of polymer cross-linking and drug-excipient complexation [79, 80].

The efficiency of MW-assisted drying process is strongly influenced by dielectric, thermal, and other physical properties, as well as by moisture content of the irradiated material [81–84]. Under the influence of microwaves, the highly polarizable H<sub>2</sub>O molecules can rotate rapidly in an attempt to align themselves with the alternating field, determining the frictional heat that promotes water evaporation. This forms the basis of microwave-assisted drying.

In view of these considerations, it is expected that MW heating is suitable to dry dissipative materials as carbohydrate-based hydrated beads manufactured by prilling. Therefore, the feasibility of joining prilling and MW-assisted treatments as a tandem technique was investigated to produce alginate-based xerogels with tailored NSAIDs release. Particularly, in this case, we selected two NSAIDs: ketoprofen (K) and piroxicam (PRX).



### 2.2.1. Release characteristics of ketoprofen-loaded beads obtained by prilling-dielectric treatments

As described by Auriemma et al. ketoprofen-loaded beads were obtained using prilling in tandem with dielectric treatments [85].

#### 2.2.1.1. Materials and methods

Hydrated beads loaded with ketoprofen were manufactured by prilling and dried using MW-assisted heating under different radiation conditions (MW, power level I–IV) as control beads dried by air-bulk heating (tray oven) at 105°C and air-bulk room conditions were prepared. During the research, we studied the effects of different MW irradiation levels on particles micromeritics (i.e., morphology, size distribution, matrix porosity, and solid state of the loaded drug) and drug release behavior using established method (SEM, DSC, USP 36 Paddle Dissolution).

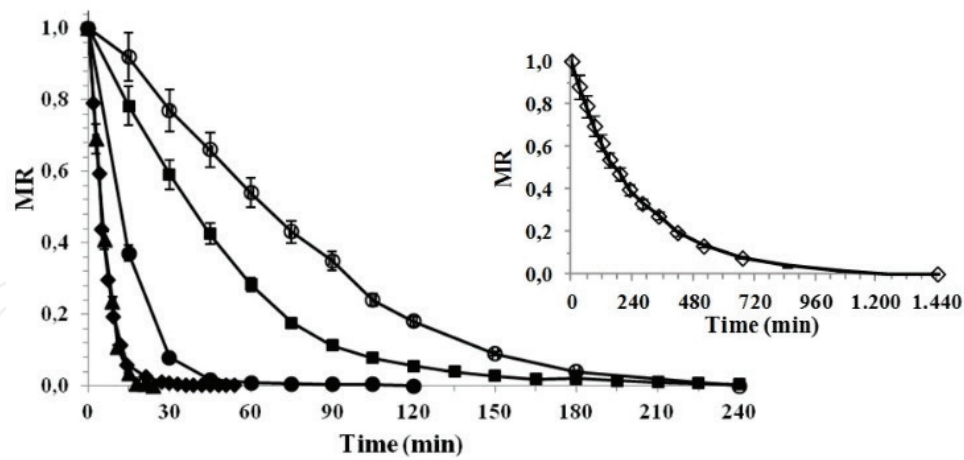
#### 2.2.1.2. Results and discussion

Drug content and encapsulation efficiency were not influenced by the drying method. Particularly, ADC increased from 8 to 25% in accordance with the increasing of drug/polymer ratio (1:10 up to 1:3), and EE was remarkably high (over 93%) without any variation due to the drying process. In addition, as shown in **Figure 15**, the regime of MW irradiation had an important role on drying curves. MW irradiation process conducted at levels IV and III showed a drying rate essentially superimposable in the first 15 min. This might be due to the penetrative and volumetric heating nature of microwaves interesting the water migration from the inside out of the particles [86]. On the contrary, weaker irradiations needed prolonged times to eliminate water from particles. Conventional heating methods require, instead, even more prolonged process times (12–18 h at room conditions and 7 h for tray oven). This effect is probably due to the high thermal capacity and the low thermal diffusivity of hydrated beads.

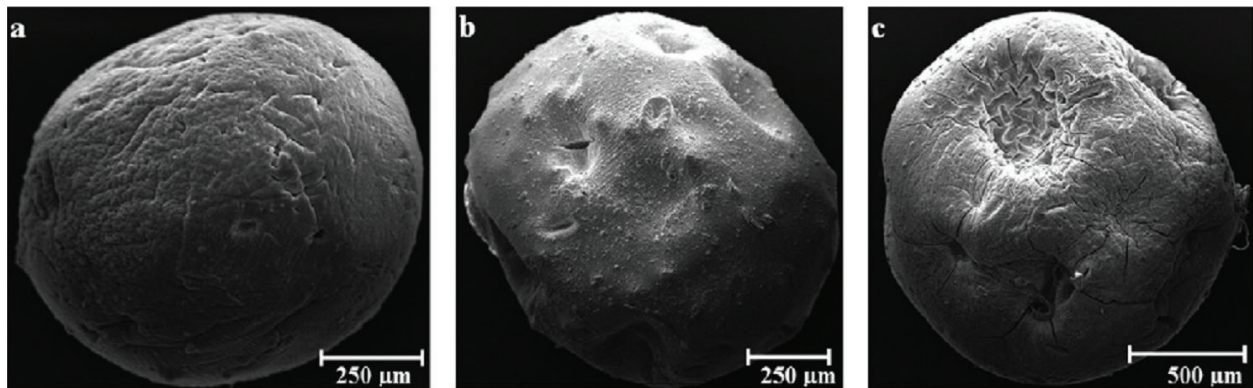
Morphological analysis showed main differences in particle mean diameters for beads dried at different regime of MW irradiation. In fact, beads dried in MW continuous regime (level IV) exhibited the highest mean diameter (around 1.4 mm), while beads exposed to the weakest irradiation (level I) presented the lowest mean diameter (1.3 mm). Surface roughness as well as the number of cracks and craters on beads surface increased when level IV MW regime was used as shown by SEM analysis (**Figure 16b, c**).

Moreover, ketoprofen was in the amorphous state when drying was conducted at MW level IV due to the harsh regime of irradiation [87], whereas only few crystals without any trace of amorphous drug on the particle surface were observed for MW level II dried beads, as confirmed by X-ray analysis (**Figure 17**).

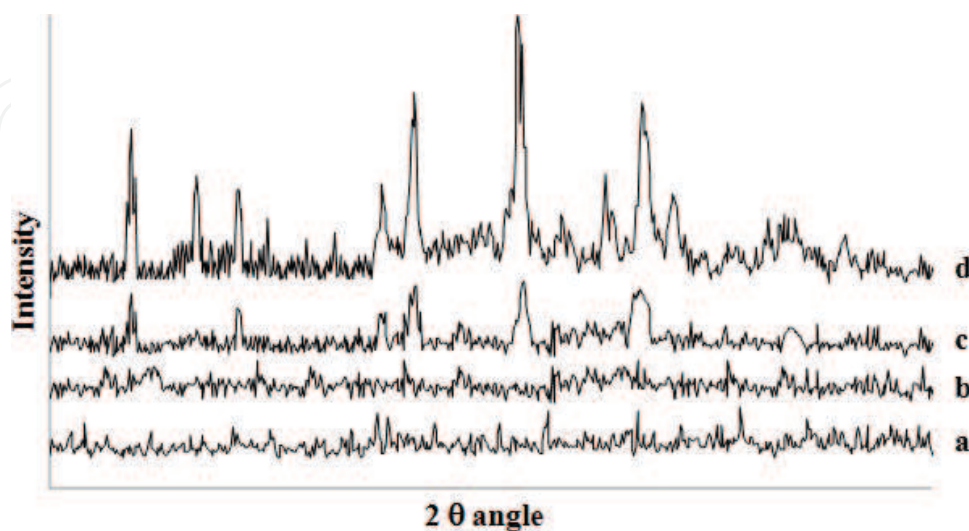
Regarding release properties, dielectric treatments provided a modulation of drug release (**Figure 18**). While beads dried at room conditions showed the typical behavior of enteric formulations, MW-treated beads at level IV acted as a conventional release formulation exhibiting a fast drug release without any lag time. In this case, the presence of solid ketoprofen and cracks on the bead surface (SEM images in **Figure 16**) allow an immediate release of the drug (45–50% in SGF) and a faster and continuous penetration of the dissolution medium inside the



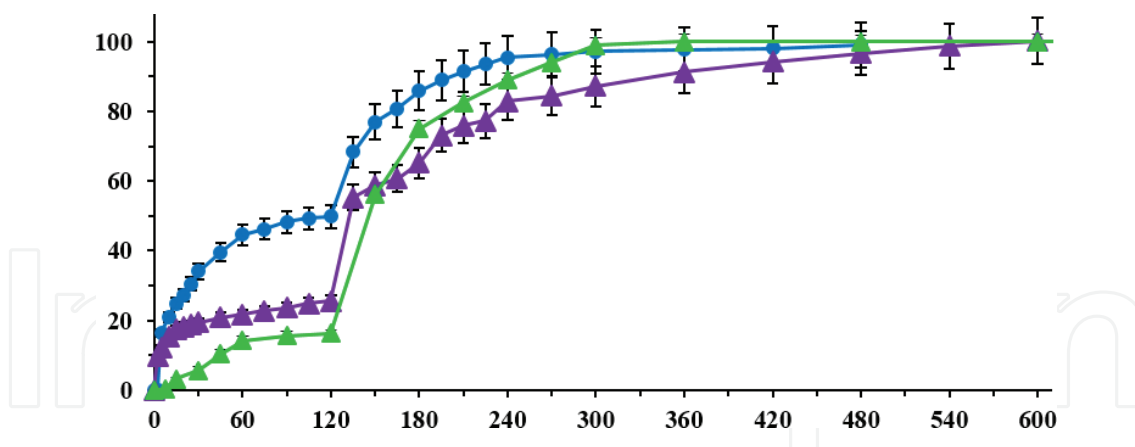
**Figure 15.** Drying curves of ketoprofen-loaded beads produced with 1.75% (w/w) alginate solution dried at different microwave irradiation levels: IV (-▲-); III (-◆-); II (-●-); I (-■-); tray oven drying at 105°C (-○-) and room conditions drying (-▲-) are also reported. Mean  $\pm$  SD; (n=6).



**Figure 16.** SEM microphotographs of K-loaded alginate beads dried in different conditions: (a) at room conditions, (b) level I, and (c) level IV microwave irradiation.



**Figure 17.** (a) X-ray diffraction patterns of blank alginate beads; K-loaded alginate bead treated at different microwave power levels: (b) IV; (c) III; and (d) pure crystalline ketoprofen.



**Figure 18.** Release profiles of dried beads formulated with 1.75% (w/w) alginate solution and loaded with ketoprofen 8% (w/w) dried at room temperature (-▲-) and by MW treatments at: level I (-▲-) and level IV (-●-). Mean  $\pm$  SD; (n=6).

alginate matrix in both SGF and SIF (complete release in about 2 h in SIF). A different behavior 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 **Figure 18**. The non-porous matrix and the structure integrity of the dried beads obtained with level I irradiation seem able to protect the drug in acidic medium and to achieve a prolonged/sustained release of ketoprofen in SIF, probably due to the interaction between drug and polymer matrix promoted by MW irradiation at level I [79].

#### 2.2.2. Release characteristics of piroxicam-loaded beads obtained by prilling-dielectric treatments

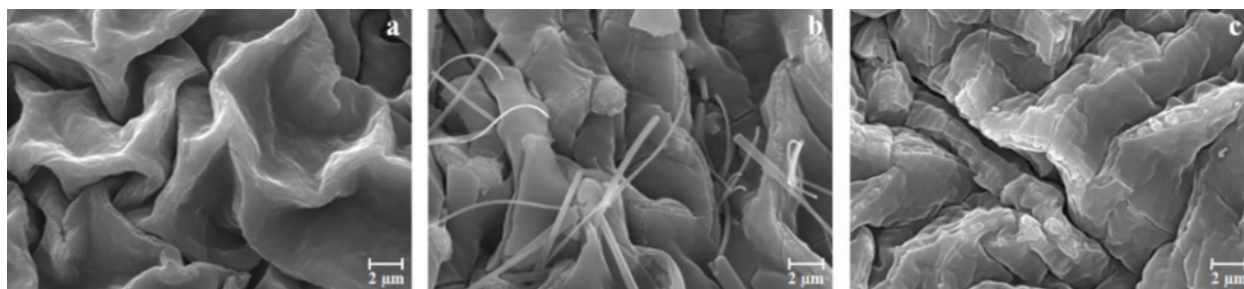
Comparable results were obtained applying prilling/MW tandem technique to produce alginate beads loaded with piroxicam.

##### 2.2.2.1. Results and discussion

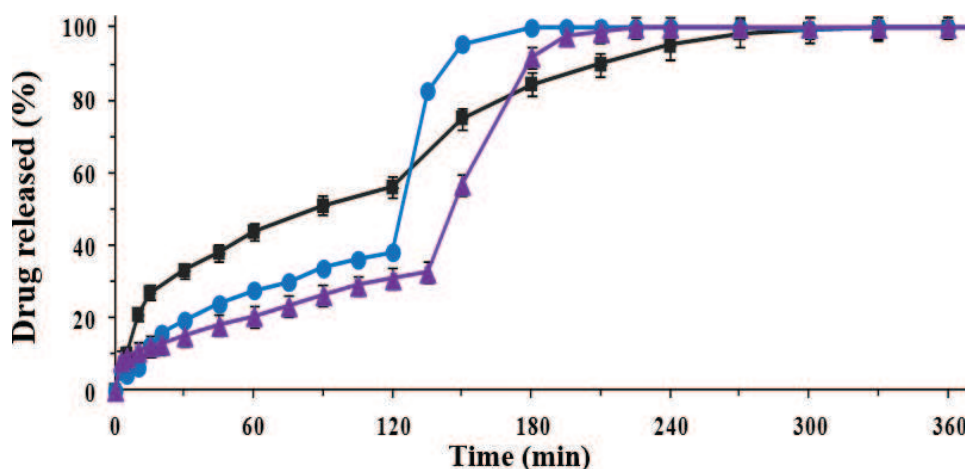
Overall results from particles' technological analysis showed high values of encapsulation efficiency (over 85%) and a very narrow dimensional distribution.

SEM analyses showed that MW at levels III-IV drying process led to beads with small spots of crystalline piroxicam coming out of the surface (**Figure 19b** and **c**), whereas unspotted surface was obtained by MW irradiation at level I (**Figure 19a**). As reported in the literature, amorphous piroxicam shows a strong tendency to crystallize in either cubic ( $\beta$ ) or needle form ( $\alpha$ ) [88]. The highest level of irradiation (MW level IV) allowed to obtain only the cubic form of crystalline PRX (**Figure 19c**). However, when MW level III was applied, both polymorphs ( $\beta$  cubic and  $\alpha$  needle forms) were identified (**Figure 19b**).

Results of dissolution tests showed that the highest MW level (IV) produced a piroxicam intestinal dosage form with total PRX liberation in less than 1 h in simulated intestinal fluid. On the contrary, the lowest MW level (I) allowed to obtain a more sustained drug release profile (**Figure 20**). Differently from alginate/ketoprofen beads, where the presence of the drug (in amorphous or crystalline state) on the bead surface affected the release rate [85], in this case, drug release was not influenced by cubic or needle crystalline forms.



**Figure 19.** SEM microphotographs of beads' surface (alginate 1.75% w/w; PRX 7% w/w) after MW irradiation at level I (a), III (b), and IV (c).



**Figure 20.** Drug release profile of piroxicam-loaded beads (alginate 1.75% w/w and PRX 7% w/w) dried by MW at level I (-▲-) and level IV (-●-) compared to piroxicam raw material (-■-). Mean±S.D. (n = 6).

The obtained results suggest that surface and inner characteristics of the alginate/piroxicam beads (roughness, network of cracks, and a fraction of crystalline piroxicam on beads surface) are strongly able to modulate the drug dissolution.

On the basis of these results, we can state that prilling/MW tandem technique can be used as a simple method to formulate alginate beads with tailored NSAIDs release depending on drug characteristics and MW irradiation level. Dielectric treatments compared to conventional methods offer 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 without affecting drug loading.

### 2.3. Prilling in tandem with supercritical fluid-assisted drying

Another approach to develop dried beads with modified drug release behavior is represented by the use of prilling technique in tandem with supercritical fluids. Supercritical drying processing has also gained wide acceptance as an alternative to conventional drying techniques. Particularly, supercritical antisolvent extraction (SAE) overcomes the problems encountered with traditional drying methods and may preserve the nanoporous structure of the wet gel-beads leading to aerogels. Among the many possible supercritical fluids (SFs), carbon dioxide



(CO<sub>2</sub>) is the most widely used. It has readily accessible critical points and as a process solvent offers the additional benefits of being nontoxic, nonflammable, environmentally acceptable, inexpensive, and can be used at a mild critical temperature suitable for processing thermally labile compounds. One major challenge for the preparation of alginate-based aerogels is to eliminate the liquid solvent from the gel, while avoiding the collapse of the already existing nanoporous structure with the subsequent shrinkage of the dried gel. Supercritical CO<sub>2</sub>-assisted drying is the most appropriate drying technique able to overcome these problems [89]. However, SC-CO<sub>2</sub> shows only a very limited affinity with water; therefore, in principle water-based solution cannot be treated. Aerogel production technology comprises a series of steps such as formation of a polysaccharide water solution (hydrogel), gelation of the sol induced by cross-linking promoters, replacement of water by a solvent (usually ethanol or acetone) and, finally, solvent elimination [90]. Various aerogel forms (monolithical cylinders, spheres, membranes, tubes, etc) may be produced by different techniques (moulding, extrusion, and milling); however, beads are preferred for applications in pharmaceuticals.

### *2.3.1. Ketoprofen-loaded aerogel beads produced by tandem prilling/SAE*

Recently, we designed and developed alginate-based aerogel beads by using prilling in combination with SC-CO<sub>2</sub> processing [73]. As above mentioned, water-based “materials” cannot be dried directly with SC-CO<sub>2</sub> due to the low solubility of water in the supercritical phase. To overcome this limitation, we tested either gelling process in alcoholic solution or solvent exchange pretreatment of hydrate beads to allow successful elimination of the solvent and to obtain aerogel formation. For this study, ketoprofen (K) was used as a model drug. Beads were produced by prilling and cross-linked by calcium cations either in ethanol (sol → alcogels) or in aqueous solutions (sol → hydrogels). In the latter case, solvent exchange was necessary to replace water by ethanol obtaining alcogels that were successively subjected to solvent extraction by SC-CO<sub>2</sub> leading to aerogels. Aerogel beads were designed to obtain a prompt release of the loaded drug and, consequently, a rapid onset of the analgesic effect, as required in postoperative pain [91, 92], dental surgery [93], renal and ureteral acute colic [94, 95].

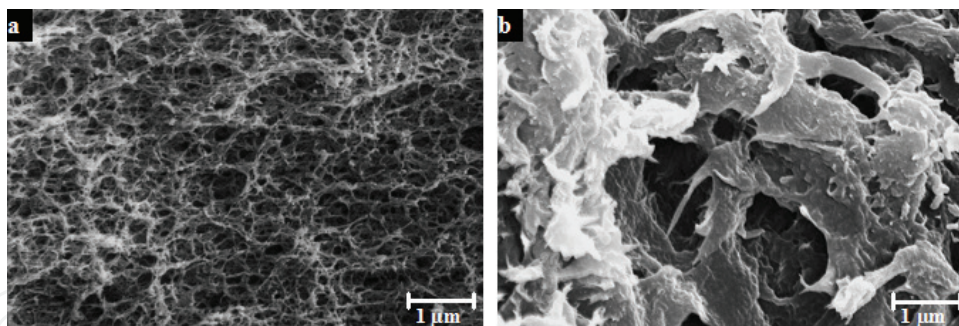
#### *2.3.1.1. Methods*

Two series of beads were manufactured using 0.3 M CaCl<sub>2</sub> in water or in ethanol as cross-linker solutions. All hydrated beads showed spherical shape (SC,  $0.98 \pm 0.01$ ), smooth and regular surface and a mean diameter ranging between 3.81 and 3.97 mm. Tiny white solid spots were present inside all loaded particles, whereas unloaded beads were almost transparent. Supercritical CO<sub>2</sub> drying was performed by SAE laboratory apparatus at 150 bar and 37°C.

#### *2.3.1.2. Results and discussion*

In the selected drying conditions, reduced particle shrinkage was observed and the internal porous texture of the parent hydrogel was preserved, as illustrated in the SEM image (**Figure 21**), where the nanofibrous alginate network is well visible. SC-CO<sub>2</sub> drying determined a reduction of beads diameters ranging between 2.70 and 3.11 mm. As a comparison, drying process was also conducted using a homogenous amount of hydrated beads exposed





**Figure 21.** SEM microphotographs of ketoprofen-loaded alginate beads inner matrix dried using different processes: supercritical CO<sub>2</sub> treatment (a) and room conditions (b).

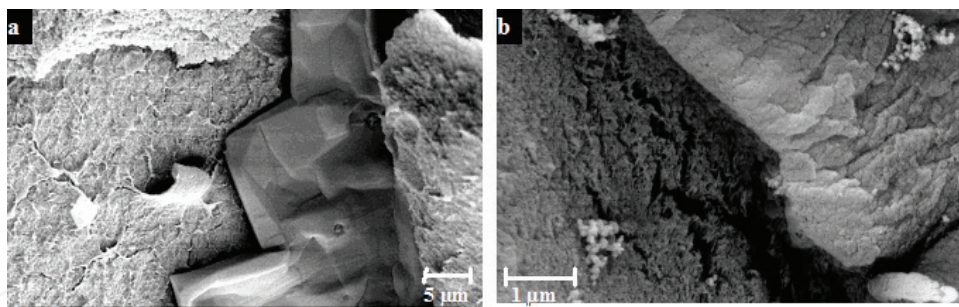
to room conditions and, alternatively, to air-bulk heating (tray oven) at 105°C. As expected, both convective processing methods took long times (tray oven 4- h; room conditions 12–18 h). Moreover, an extensive volume shrinkage was observed when convective drying methods were used (diameter 1.8 mm with SD lower than 3%) due to the collapse of the polymer matrix during the slow solvent evaporation.

Encapsulation efficiency values were greater for beads subjected to conventional drying than for those treated with supercritical carbon dioxide without relevant differences between room conditions and tray oven. As previously reported, the transport phenomena involved in the supercritical fluid extraction may cause the extraction of small quantities of drug [96]; this effect may explain the observed decrease in EE. In detail, convective dried beads exhibited EE around 52 or 94% depending on ethanol or aqueous cross-linking, respectively. In the same gelling conditions, SC-CO<sub>2</sub>-treated beads showed EE around 6 or 59%.

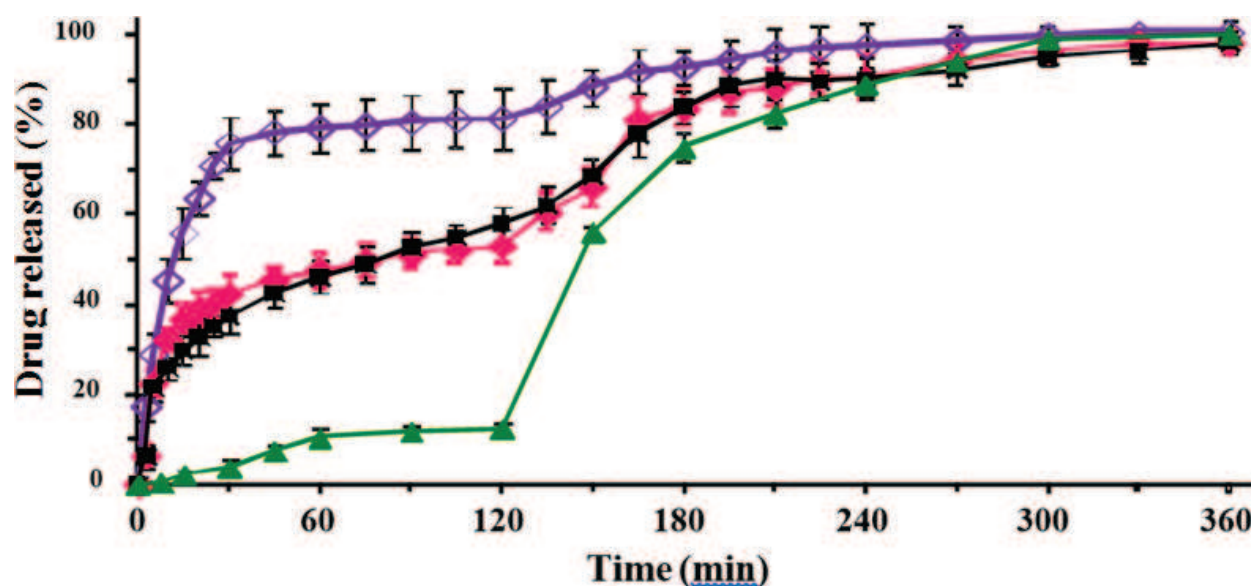
The inner matrix structure was deeply influenced by different drying techniques. Supercritical treatment produced a nanoporous alginate matrix characterized by a network of nanopores with diameters around 200 nm. Convective drying leads to a compact matrix with less and larger pores where the nanofibrous structure was completely lost, as shown in **Figure 21b**. It is well known that one of the major challenges in aerogel production from hydrogels is the solvent elimination that must occur without inducing the collapse of the porous structure; in this regard, the fast elimination of ethanol from alcogels by SC-CO<sub>2</sub> processing is able to prevent the collapsing of the parent gel structure [97, 98].

More interestingly, the procedure for producing the alcogel-influenced habitus of solid K into the inner matrix, as shown by SEM images (**Figure 22**) of cryo-fractured dried particles. Cross-linking alginate in ethanol (**Figure 22a**) leads to crystal clusters of K drug embedded into the aerogel, beads from aqueous cross-linking, and water replacement by ethanol (**Figure 22b**) showed only nanometric particles of amorphous K.

The dissolution profile of K from SC-CO<sub>2</sub>-treated beads (**Figure 23**) presented an enhanced burst effect in SGF (more than 75% of the encapsulated drug in 30 min in acidic medium) for aerogels obtained cross-linking the particles in aqueous CaCl<sub>2</sub> and then replacing water with ethanol. Formulations cross-linked in ethanol as well as crystalline material released less than 40% of K in the same time. The considerable increase of the drug dissolution rate for



**Figure 22.** SEM microphotographs of cryo-fractured ketoprofen-loaded alginate beads dried by supercritical CO<sub>2</sub> previously cross-linked in ethanol (a) or water (b).



**Figure 23.** Release profiles of K-loaded alginate beads dried by SC-CO<sub>2</sub> treatment previously cross-linked in ethanol (-♦-) or water (-◇-), exposed to room conditions (-▲-) in comparison to pure ketoprofen (-■-). Mean  $\pm$  SD; (n=6).

formulations cross-linked in water can be explained by the high porosity of the aerogel and by the increased surface-volume ratio due to nanometric dimensions of the solid amorphous ketoprofen [99] embedded into the nanoporous structure.

In conclusion, prilling process in tandem with SC-CO<sub>2</sub>-assisted drying allows to produce alginate-based aerogel. Drug loading capacity and encapsulation efficiency reached approximately 59%. Interestingly, after SC-CO<sub>2</sub> drying, particles keep constant spherical shape and narrow dimensional range and retain the porosity of the parent hydrated gel-matrix. The highly nanoporous drug carrier with high surface area provides enhanced and controlled fast release of ketoprofen. Formulations cross-linked in water, and then replacing water with ethanol, improve significantly the dissolution rate of K, especially in simulated gastric fluid. High enhancement of the drug dissolution can be very useful in favoring drug liberation and absorption in order to obtain a rapid onset of the therapeutic effect as required in acute inflammation. Alginate-based aerogel produced by prilling in combination with SC-CO<sub>2</sub>-assisted drying may be proposed as fast dissolving formulation for slightly soluble NSAIDs belonging to BCS class II.

### 3. Conclusions

Particle engineering through prilling-based techniques can be applied to widely used NSAIDs as ketoprofen and piroxicam to develop new drug delivery systems, which meet current therapeutic input and health demands. This approach allows to fit specific therapeutic needs of inflammatory-based diseases. Particularly, prilling technique in tandem with dielectric treatment at high MW level or with supercritical (SC-CO<sub>2</sub>) drying allows to produce porous microparticles with high surface area able to enhance the dissolution rate of slightly soluble NSAIDs (BCS class II). These systems providing a fast drug release may promote a rapid therapeutic effect after oral administration, as required in acute pain and inflammation. Instead, using prilling in combination with conventional or dielectric drying at low MW levels, it is possible to obtain engineered particles with slower NSAIDs release compared to conventional and commercially available formulations. By opportunely selecting carrier materials or gelling conditions or using additional technological actions such as enteric coating or capsular devices, it is possible to obtain chronotherapeutic drug delivery systems useful to treat EMPs. The potential benefits in chronotherapy of these systems were also confirmed using an *in vivo* model of inflammation. The control and strong delay of NSAIDs release make these drug products suitable to be taken at bed time and act in the early morning hours.

### Author details

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