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Development of a scalable procedure for fine calcium alginate particle preparation

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ABSTRACT

A simple and scalable two step method for the preparation of loaded calcium alginate fine powder was developed. Spray-dryer was used to prepare sodium alginate particles and all the spraying parameters were studied to optimize the powder characteristics. The particles were gelified successively by external gelation in a calcium chloride solution. Two model active pharmaceutical ingredients were encapsulated in alginate particles (i.e., bacitracin and bovine serum albumin). The obtained powders show the suitable characteristics for pulmonary drug delivery.

1. Introduction

The concept of microencapsulation, the process of enclosing a chemical (e.g., drug) in a microparticle [1], was first developed by the National Cash Register Co. in the 1930s [2]. Later on, this approach was proposed for medical [3–5] pharmaceutical [6,7] and biotechnological [8] applications, with different outcomes. In fact, while in the pharmaceutical field, microencapsulation has raised successful applications with several products on the market [9], the medical one still suffers from different issues and concerns (e.g., safety and efficacy) [10]. The scientific literature describes a large quantity of methodologies to embed drugs (e.g., small molecules, peptides, proteins, and genetic material) in a variety of wall materials [11]. However, most of these techniques are not easily scalable and only few of them (e.g., spray-drying) are consistent with the industrial requirements. Analogous considerations apply to the reagents, solvents and wall materials used in the process.

Alginate, a linear copolymer composed of α-L-guluronate (G) and α-D-mannuronate (M), is an example of non-toxic, biocompatible, and biodegradable polymeric material with many features exploitable in drug delivery [12]. Indeed, because of their peculiar chemical structure, the alternation of M and G homopolymer moieties, alginates are able to form insoluble gels in the presence of divalent or trivalent positively charged ions [13,14]. Alginate, as analogous polysaccharides, is particularly versatile in the encapsulation of unstable water-soluble macromolecules, such as proteins, because it creates a "friendly" hydrophilic environment that protects from denaturation [15].

Alginate beads are usually produced by external gelation [16–18], internal gelation [19–23] or a combination of both techniques [24]. External gelation can be obtained by simply dripping a sodium alginate solution in a calcium chloride solution. However, different techniques have been developed to form alginate droplets, e.g., alginate can be sprayed by air intake through a nozzle [18] or the nozzle can be equipped with a vibrating system that breaks the solution jet [17]. These techniques normally suffer from difficult scalability, even though some efforts have been done in this direction [25], and the obtained particles are generally large and not suitable for all applications. External gelation can also be performed by adding calcium chloride solution to an emulsion made of a sodium alginate solution dispersed in an organic solvent (e.g., isooctane and dichloroethane) [26]. The latter method is also hardly scalable and toxic solvents are needed to prepare the emulsion.

The internal gelation strategy consists in the use of a water-insoluble calcium salt that is initially dispersed in the sodium alginate solution [19–23]. The suspension is then emulsified in oil (e.g., canola and paraffin) in the presence of a surfactant. The emulsion is then acidified with acetic acid in order to solubilize the calcium ions for alginate bead gelation.

Abbreviations: G, α-L-guluronate; M, α-D-mannuronate; API, active pharmaceutical ingredient; d_{mv}, volume mean diameter; SEM, scanning electron microscopy; BCA, bicinchoninic acid; DSC, differential scanning calorimetry; MMAD, mass mean aerodynamic diameter.

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All the described methodologies possess a main limit, the difficulty or the impossibility to be scaled up and therefore their industrial use is restrained.

Therefore, the aim of this paper is to optimize an easily scalable method for the production of fine calcium alginate particles (~5 μm) useful for inhalation or to prepare composite microparticles for fragile active pharmaceutical ingredient (API) delivery [15]. This method was used to encapsulate two model drugs, namely bacitracin and bovine serum albumin.

2. Materials and methods

2.1. Materials

High mannuronic sodium alginate (M: 61%; G: 39%; polymer was comprised of blocks MM–GG–MM) was supplied by Stern Italia (Milan, Italy). Bacitracin was obtained from *Bacillus licheniformis* and calcium chloride dihydrate was purchased from Sigma–Aldrich Chemical (Milan, Italy). Bovine serum albumin was supplied by Merck (Darmstadt, Germany). All other chemicals and reagents were of the highest purity grade commercially available.

2.2. Alginate particle preparation

Sodium alginate particles were prepared by spray-drying using a Büchi Mini Spray Dryer B-290 (Büchi Italia S.r.l., Italy). Briefly, after nebulisation of a sodium alginate solution, the formed particles were separated from the air stream in the cyclone and collected in the glass vessel to recover the dry powder. The influence of spray-dryer parameters (i.e., inlet temperature, aspirator rate, feeding rate, and spray air flow) on particle characteristics has been investigated to individuate the ideal working conditions. Feeding solutions having different viscosities (Bohlin GEMINI rotational rheometer with a couette geometry SSC25) have been investigated as well. Viscosity measurements were carried out in triplicate at the controlled temperature of 20 ± 0.01 °C.

Calcium alginate particles were prepared by an external gelation strategy starting from the spray-dried sodium alginate particles. Briefly, sodium alginate particles were suspended in 20 mL acetonitrile and sonicated to allow a better suspension. This suspension was poured in a 1.2% (w:v) calcium chloride solution under magnetic stirring to allow particle gelation. Particles were recovered by filtration on PVDF filters (Millipore, 0.45 μm, Milan, Italy), washed three times with 20 mL distilled water and freeze-dried overnight. The preparation was performed in triplicate to assess reproducibility.

Calcium alginate microparticles have also been prepared in the presence of two model API, bacitracin and bovine serum albumin. Briefly, the API was dissolved in a sodium alginate solution prior to nebulisation using the optimized spray-drying settings. The loaded particles were then collected and gelified following the method previously described. Three different calcium chloride concentrations (1.2, 1.8 and 2.4%, w:v) were used to gelify bovine serum albumin microparticles to study the concentration effects on particle size, API loading, and *in vitro* release profile. Each batch was prepared in triplicate at a theoretical loading of 35% (w/w).

2.3. Alginate particle characterization

2.3.1. Particle size determination

An Accusizer C770 (PSS Inc., Santa Barbara, CA) using the technique "single particle optical sensing" was used to determine the mean particle size and distribution of the different alginate microparticle batches [27]. Since sodium alginate is soluble in water, sodium alginate particles have been analyzed using acetonitrile as dispersing medium and operating in gravity mode. In the case of calcium alginate particles, ultrapure water was used to suspend the powder. Both suspensions were sonicated for 1 min before analysis. The results were expressed as volume mean diameter (d_{mv}).

2.3.2. Morphological analysis

Sodium and calcium alginate particles have been observed by scanning electron microscopy (SEM) (Philips XL30 SEM, Heindoven, NL) to investigate their size, shape and surface properties. Samples were prepared by placing microparticle powder onto an aluminium specimen stub covered with a double sided carbon adhesive disc (Taab, Berks, UK). The samples were sputter coated with gold prior to imaging (EMITECH K-550X sputter coater Ashford, Kent, UK). Coating was performed at 20 mA for 4 min.

2.3.3. Encapsulation efficiency determination

Calcium alginate microparticles were treated with a phosphate buffer solution, PBS (100 mM, pH 7.4) to obtain calcium ion displacement from the hydrogel and alginate solubilization. The solution was then analyzed to determine the practical drug loading by microBCA (bicinchoninic acid) total protein assay method for bacitracin and for bovine serum albumin [28]. Measurements were performed in triplicate, and the error was expressed as standard deviation.

2.3.4. In vitro release studies

Bovine serum albumin *in vitro* release studies were carried out in triplicate with particles obtained at the three different calcium chloride concentrations. Release studies were performed in glass tubes at 37 °C using PBS (100 mM, pH 7.4) or Tris Base buffer (10 mM, pH 7.4) as release media. 10 mg of microparticles were used in order to maintain sink conditions. At predetermined intervals, 1 mL of supernatant was collected, refrigerated, analyzed by microBCA and replaced with fresh buffer.

2.3.5. Thermal analysis

In order to characterize bacitracin and bovine serum albumin microparticle thermal behaviors, differential scanning calorimetry (DSC) was performed by using a DSC821e (Mettler Toledo, Switzerland), equipped with a liquid nitrogen cooling system. The system was calibrated using an indium standard. Samples were hermetically sealed in aluminium pans and subjected to one heating cycle, from 25 to 300 °C, at a 5 °C/min rate, against an empty pan. DSC data were treated with STARe software, and the results were expressed as the mean of two independent measurements.

3. Results and discussion

3.1. Preparation method optimization

Among the different spray-dryer parameters, the inlet temperature surely has a strong influence on particle dimensions since it is determinant for droplet drying [29]. The inlet temperature has to be selected according to the used solvent as well as to minimize, as much as possible, the gap between inlet and outlet temperatures to reduce the final particle residual moisture [30]. The variation of the outlet temperature depends also on the aspirator flow rate, the feeding rate and feeding solution concentration [31].

In this work, sodium alginate was solubilized in ultrapure water and the inlet temperature must therefore be higher than 100 °C. Fig. 1 shows the alginate particle size distributions obtained with inlet temperatures in the range 135–145 °C. The highest temperature produced larger amount of aggregates increasing the d_{mv}. This behavior was explained by the increase in the difference between the inlet and the outlet temperatures (inlet, 145 °C; outlet, 70 °C) leading to higher residual moisture in the new-formed particles

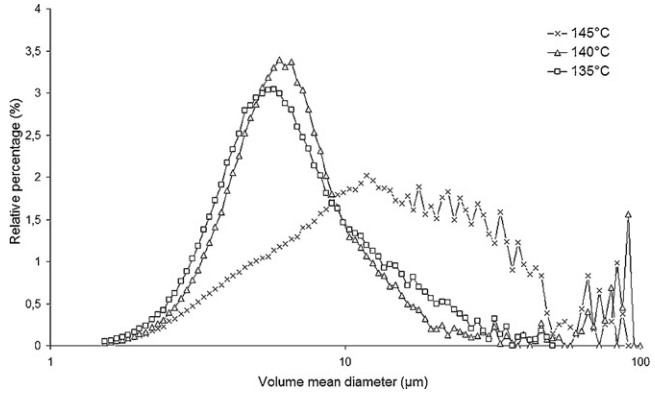

Fig. 1. Sodium alginate particle size distributions obtained varying the inlet temperature. Aspirator rate: 32 m^3/h; feeding rate: 2 mL/min; spray air flow: 473 L/h; feeding solution concentration: 0.4%.

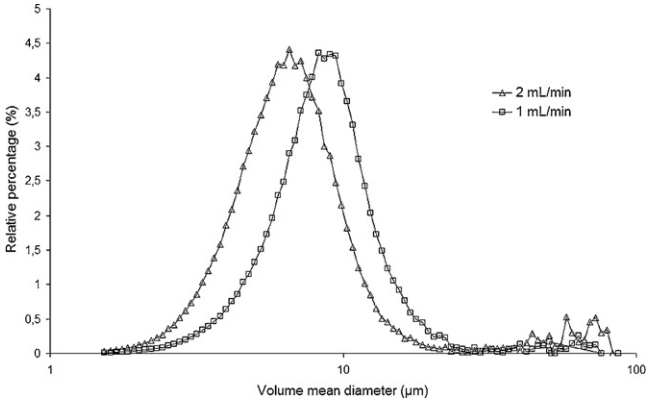

Fig. 3. Sodium alginate particle size distributions obtained varying the feeding rate. Inlet temperature: 140 °C; aspirator rate: 35 m^3/h; spray air flow: 473 L/h; feeding solution concentration: 0.4%.

[30]. The residual humidity could favor particle aggregation with a d_{mv} increase. At lower temperatures, the size distribution profiles were considerably narrower with a d_{mv} below 10 μm (Fig. 1).

The second investigated parameter was the aspiration rate, directly correlated with the quantity of warm air responsible for droplet drying. Lowering the rate, the particles in formation stay longer in the drying chamber, leading to a lower final moisture content. Nevertheless, the higher residence time could facilitate particle interaction or/and particle adhesion to the chamber walls thus reducing the yield. In fact, as shown in Fig. 2, the best particle dimension profile was obtained when working at a high aspiration rate (35–38 m^3/h).

After the optimization of these two parameters (T inlet: 140 °C and aspirator rate: 35 m^3/h), the influence of the feeding rate has been investigated. As previously mentioned, the aggregation observed by varying this parameter can be ascribed to the increase in the gap between the inlet and outlet temperatures. In fact, by using a high feeding rate, the volume to dry increases and, therefore, also the energy necessary to dry the droplets. Hence, to avoid aggregation phenomenon, the feeding rates were kept between 1 and 2 mL/min. As observed in Fig. 3, both feeding rates led to narrow particle size distributions, however 2 mL/min is preferable as it consents to obtain a slightly lower d_{mv} and to halve process duration.

The last optimized instrumental parameter was the spray air flow. Theoretically, at higher air flow rate, the liquid is dispersed into smaller droplets producing particles with smaller sizes [32]. As shown in Fig. 4, particle size distributions at 357 and 473 L/h were very similar. As stated before, smaller droplets and then particles should be obtained using higher spray air flow. Anyhow, the size distribution profile is slightly shifted to smaller d_{mv} when a 357 L/h flow is used. It can be speculated that higher rates (i.e., 473 L/h) confer higher speed to the formed droplets with their potential impaction on the spray chamber walls or among droplets. The consequences of this event should be the loss of material and increase of particle dimensions following droplet fusion.

A non-instrumental parameter, namely the feeding solution concentration, was investigated as well. As shown in Fig. 5, particle size was reduced as lower alginate concentrations were employed.

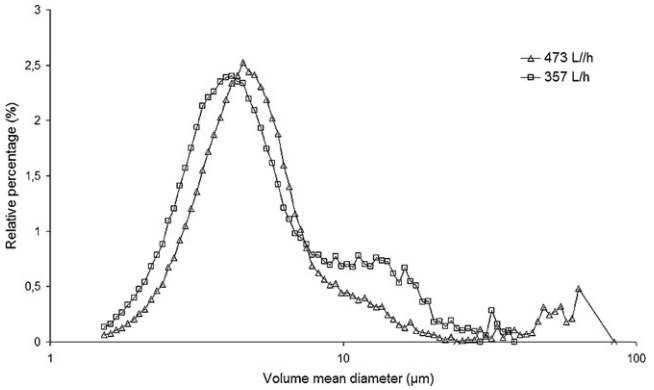

Fig. 4. Sodium alginate particle size distributions obtained varying the spray air flow. Inlet temperature: 140 °C; aspirator rate: 35 m^3/h; feeding rate: 2 mL/min; feeding solution concentration: 0.4%.

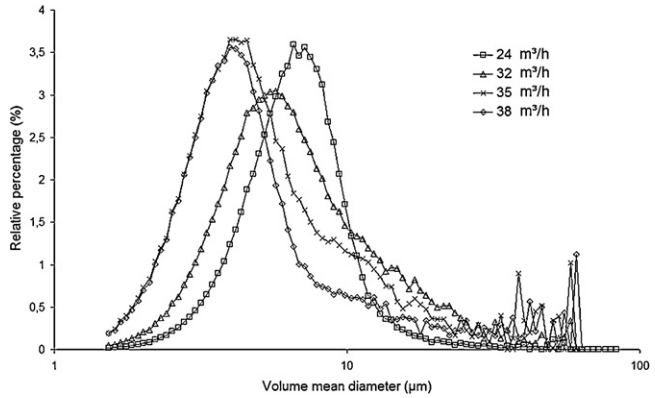

Fig. 2. Sodium alginate particle size distributions obtained varying the aspirator rate. Inlet temperature: 140 °C; feeding rate: 2 mL/min; spray air flow: 473 L/h; feeding solution concentration: 0.4%.

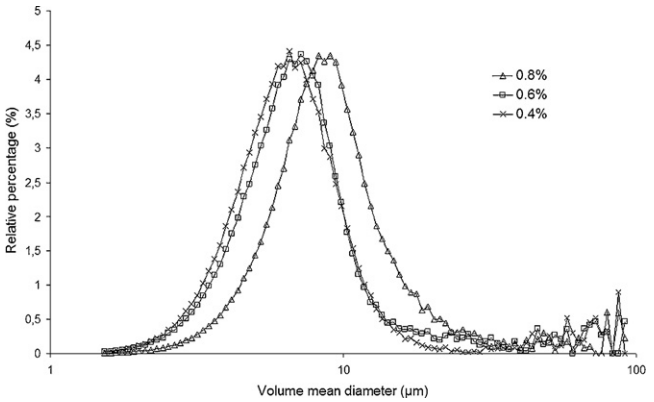

Fig. 5. Sodium alginate particle size distributions obtained varying the feeding solution concentration. Inlet temperature: 140 °C; aspirator rate: 35 m^3/h; feeding rate: 2 mL/min; spray air flow: 357 L/h.

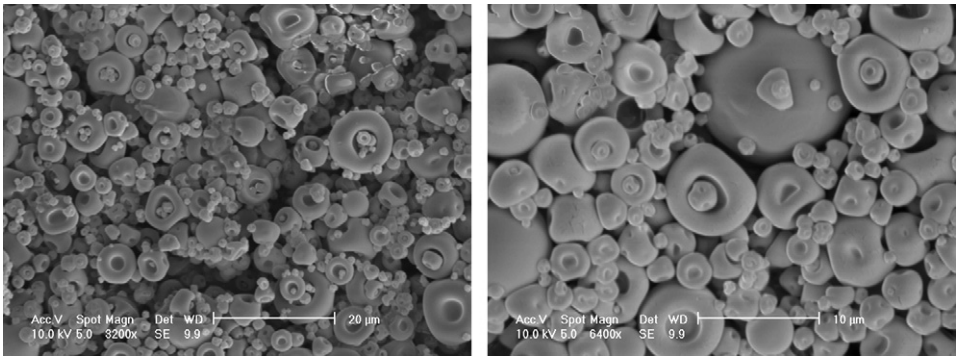

Fig. 6. Sodium alginate particles observed by SEM after optimizing the spray-drying parameters.

Table 1
Viscosity data of sodium alginate solutions.

Sodium alginate concentration (w/v%)	Viscosity ± standard deviation (cP)
0.4	8.25 ± 0.09
0.6	14.1 ± 0.33
0.8	22.9 ± 0.58

As shown in Table 1, the viscosity of the solution linearly increases with the polymer concentration ($r^2 > 0.9860$). As reported in literature, high solution viscosity can preclude movements in the droplet favoring the formation of a solid membrane around a still liquid core. This phenomenon inevitably influences the powder properties such as particle size, density and morphology [29,33,34]. Commonly, particle size is directly affected by the feeding solution concentration. This relationship can be expressed by the following equation:

$$d_{ae} = \sqrt[6]{\frac{\rho_p}{\rho_0}} \cdot \sqrt[3]{\frac{c_f}{\rho_0}} \cdot d_D$$

where d_{ae} is the mass median aerodynamic diameter (MMAD), ρ_p the particle density, ρ_0 is 1 g/cm^3, c_f the concentration of the feed solution and d_D is the diameter of the droplet [29]. According to this equation, the MMAD will be controlled mainly by the feed solution concentration and by the droplet dimensions. Therefore, atomization is a crucial step in the spray-drying process and viscosities should be maintained below 250 cP to avoid the formation of strands and to obtain spherical droplets [35]. In this study, diluted sodium alginate solutions (0.4, 0.6, and 0.8%), with viscosity below 250 cP, were employed (Table 1).

In light of these considerations, sodium alginate particles were prepared from a 0.4% (w/v) alginate solution using the following parameters: inlet temperature, 140 °C; aspirator rate, 35 m^3/h; feeding rate, 2 mL/min; spray air flow, 357 L/h. Sodium alginate particles appeared as not perfectly spherical and with mean dimensions below 10 µm (Fig. 6). The observed morphology can be explained by the rapid evaporation and by the high Peclet number that generally characterizes polymers such as alginate [29,33]. A high Peclet number (much larger than 1) is observed when solvent evaporation is fast while the solute diffusion from the droplet boundary to its center is slow and, therefore, the solute will concentrate in the periphery of the droplet. These two factors lead to the rapid formation of a thin membrane at the surface of the droplet that, according to its properties, will or will not crumple giving rise to wrinkled or spherical particles of low density. Considering all these characteristics, the optimized sodium alginate particles appear promising candidates as carriers for inhalation.

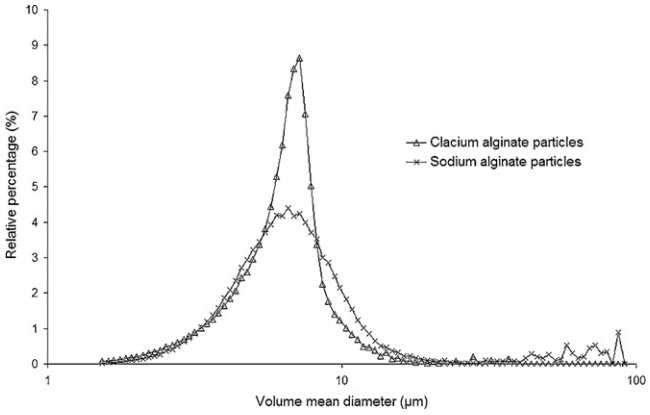

Fig. 7. Sodium and calcium alginate particle size distributions.

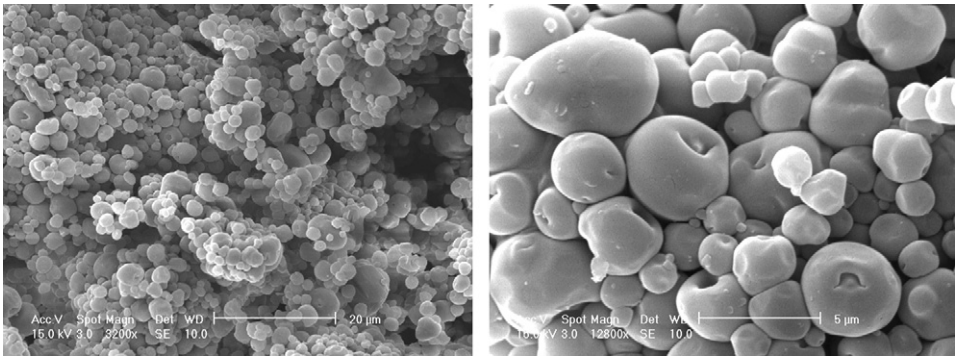

Fig. 8. Calcium alginate particles obtained by external gelation observed by SEM.

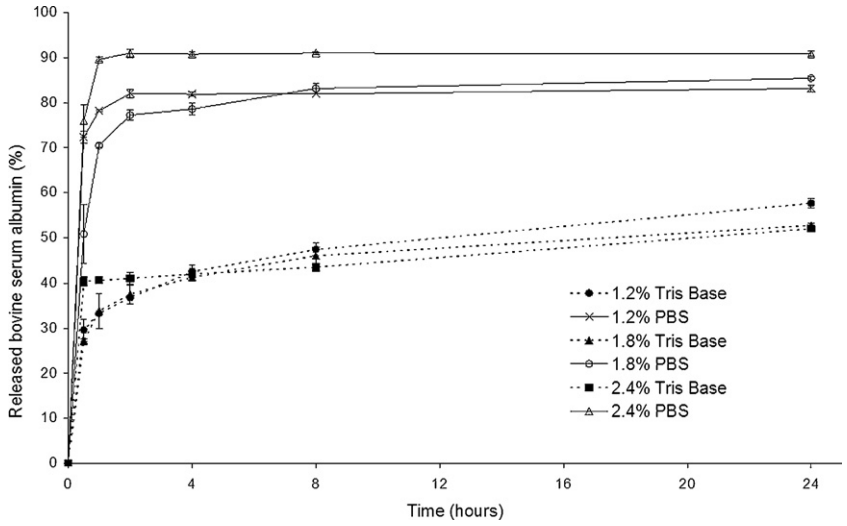

Fig. 9. Bovine serum albumin *in vitro* release profiles from calcium alginate microparticles in Tris Base buffer and PBS at 37 °C. Particles were obtained by external gelation at three different calcium chloride concentrations (1.2, 1.8, and 2.4; w:v).

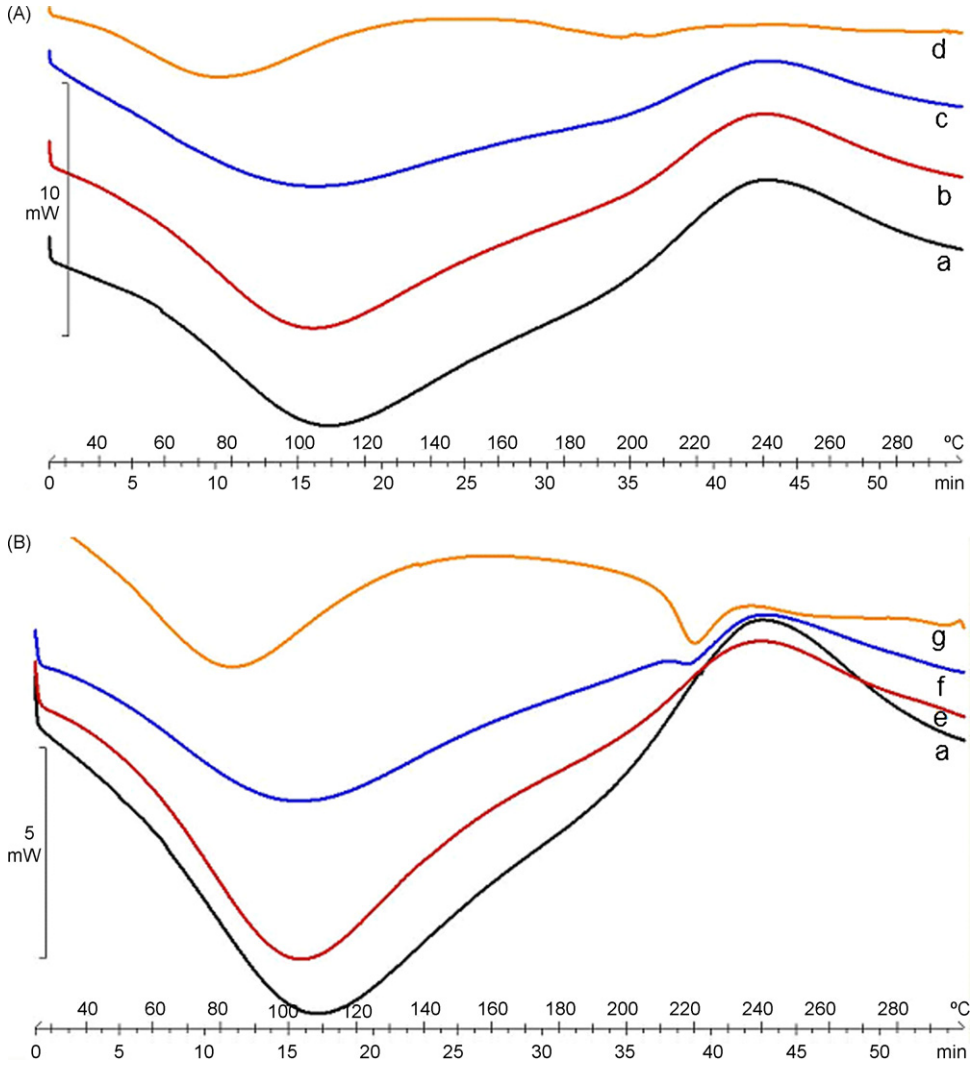

Fig. 10. DSC data of formulations and raw materials. Panel A, (a) calcium alginate microparticles, (b) bacitracin loaded microparticles, (c) bacitracin/calcium alginate microparticle physical mixture, (d) bacitracin; Panel B, (a) calcium alginate microparticles, (e) albumin loaded microparticles, (f) albumin/calcium alginate microparticles physical mixture and (g) bovine serum albumin. Exotherm up.

3.2. Calcium alginate microparticle characterization

Calcium alginate particles, prepared by simple external gelation, were characterized for their dimensions and morphology. In fact, it is important to determine if this process affects particle characteristics and, in particular, their size. Sodium and calcium alginate size distribution profiles are reported in Fig. 7. As it can be observed, the d_{mv} did not change significantly following alginate gelation (d_{mv} = 6.59 μm). However, the distribution profile was narrower resulting in a more homogeneous particle population. The shrinkage can be explained by the substitution of sodium by calcium ions and the resulting smaller knits with respect to sodium alginate. In addition, the gelation process did not alter particle morphology (Fig. 8).

In the same way, loaded gelified particles were characterized by a narrow size distribution with a d_{mv} of 8.86 ± 3.36 μm and 7.48 ± 3.62 μm for bacitracin and bovine serum albumin, respectively. Both particle morphology and dimensions were not influenced by the presence of the drug and should be suitable for peptide delivery into the lungs. In addition, the calcium chloride solution concentration in the range 1.2–2.4% (w:v) had no effect on particle dimensions. Bacitracin and bovine serum albumin loaded particle encapsulation efficiencies were 29.5 ± 13.35% and 76.7 ± 0.13%, respectively. These efficiencies correspond to practical drug contents of 10.5% and 27.2%. These results are satisfying if considering that the gelation process occurred in a large volume of water and the hydrophilic easily soluble drug diffused into the external aqueous phase. Moreover, high drug contents are difficult to achieve owing to the particle small dimensions. These results also evidenced the influence of API molecular weight on the loading capacity. In fact, encapsulation efficiency is much higher for bovine serum albumin (Mw ~ 66 kDa) than for bacitracin (Mw ~ 1.4 kDa) because of its lower diffusion coefficient in the hydrogel.

In vitro release studies were carried out in two different buffers. In fact, PBS, largely used to reproduce physiological conditions, is well known to favor calcium release from calcium alginate hydrogel and should accelerate API release [36,37]. Therefore, Tris Base buffer was also used to mimic *in vivo* environment. Fig. 9 shows bovine serum albumin *in vitro* release profiles. As it can be observed, calcium chloride concentration had a small effect on the release profile as the behavior is comparable for all three concentrations used. In the case of Tris Base buffer, the release resulted incomplete and reached a plateau at 24 h maintained for at least 1 week (data not shown). In Tris Base buffer, alginate degradation is very limited and it can be hypothesized that interactions between bovine serum albumin and alginate hindered and/or retarded the complete release. In PBS, the profile observed is very different. In fact, *in vitro* release is almost complete after just a few hours of incubation at 37 °C. This behavior is clearly ascribable to the PBS capacity to transform calcium alginate in sodium alginate. *In vivo*, the release profile should be different and slower from the behavior observed in PBS since the phosphate ion concentration is lower. However, *in vivo*, calcium alginate will dissolve progressively releasing the whole API content.

Fig. 10 shows the DSC data of calcium alginate microparticles, drug loaded microparticles, bacitracin, bovine serum albumin, and the physical mixtures of the drug and alginate microparticles. As it can be observed, both APIs (Fig. 10d and g) are characterized by a broad endothermic band due to water vaporization and protein denaturation. As far as calcium alginate microparticles (Fig. 10a) are concerned, the thermogram showed an initial loss of water (broad endotherm with maximum ~110 °C) followed by the decomposition of the polymer (exothermic event with maximum ~240 °C) [38,39]. Comparing drug loaded microparticles (Fig. 10b and e) and drug/calcium alginate microparticle's physical mixtures (Fig. 10c and f), no significant differences in the thermal behavior were observed asserting that, in these experimental conditions, no interactions were evidenced. In fact, both profiles corresponded to the sum of the drug and the calcium alginate microparticle DSC data.

4. Conclusion

A simple and scalable two step method for the preparation of fine calcium alginate powders was developed. Spray-drying is largely employed in the industrial field, while alginate external gelation might be optimized to be carried out in a continuous process. Continuous production lines have difficulties to reach pharmaceutical industries but are very challenging since they allow to reduce time-to-market and production cost with respect to batch production [40,41]. It is speculated that the presented method, with little improvements, could be successfully employed in the industrial production of API loaded calcium alginate microparticles.

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