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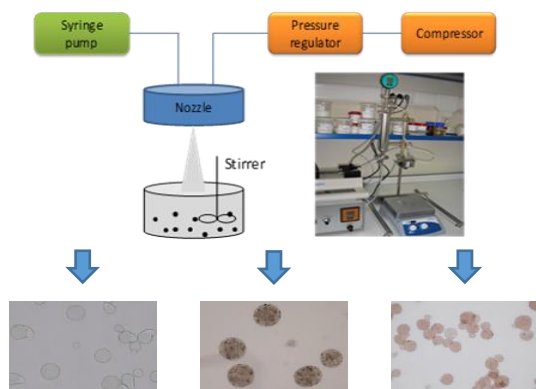
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## Microencapsulation by spray coagulation method: Study of the alginate crosslinking by different calcium salts

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The use of biopolymers such as alginate has been growing in the last decades due to properties such as non-toxicity and biodegradability. In this work, the microencapsulation of curcumin and safranin, used as model compounds for hydrophobic and hydrophilic active principles, respectively, was studied with alginate matrices by the spray coagulation technique. The alginate ionic crosslinking with three calcium sources (calcium chloride (CaCl<sub>2</sub>), calcium gluconate and calcium lactate) was considered. The obtained microcapsules were characterized in terms of load, encapsulation efficiency (EE), rehydration capacity, and particle size. The release profiles of both active principles were also studied. Through FTIR analysis the contribution of the calcium source, and the presence of the active principles inside the microcapsules, was identified, while the TGA evidenced an increasing of the thermal stability due to the alginate crosslinking effect, particularly when CaCl<sub>2</sub> was used. The overall results showed that CaCl<sub>2</sub> is the most effective calcium source for alginate crosslinking, while calcium lactate lead to a more gradual release of the active principles.

### Introduction

The use of microencapsulation techniques allows the protection of active principles through the coating with polymeric materials, resulting in systems with improved stability and controlled release. Nowadays, the concern with the toxicity of chemicals and the search for green processes and chemical systems motivate the development of environmental friendly alternatives. In this sense, the selection of chemical systems based on biopolymers such as alginate is an option widely studied. Alginate is a linear copolymer, generally extracted from brown algae, composed by (1, 4)-linked- $\beta$ -D-mannuronic (M) and  $\alpha$ -L-guluronic (G) acid blocks. One of the interesting properties of alginate is the gelation capacity in the presence of divalent cations, such as Ca<sup>2+</sup>, which is the most used cation for this end due to the required mild reaction conditions. Calcium chloride is one of the most used salts to promote the ionic crosslinking of alginate, however, it is often described as leading to a rapid and poorly controlled gelation [1]. The use of encapsulation methods such as spray-coagulation has great advantages comparatively to other conventional process (ex. processes based on double emulsions): (i) it allows the microparticles production in a single step; and (ii) does not require high temperatures like spray-drying method, which can compromise the encapsulation of thermosensitive substances.

In a general way, the microencapsulation studies are focused on a particular active principle, around which the chemical system and process are developed. A lack of information is noticed regarding systematic studies focused on model compounds, such as hydrophobic and hydrophilic models. Based on the stated considerations, the objective of this work is to study the microencapsulation of hydrophobic and hydrophilic model compounds using alginate systems, by using the spray-coagulation method. The selected hydrophobic active principle was curcumin and safranin was the hydrophilic one. In addition, the effect of the used crosslinker chemical structure on the alginate gelation was also studied, by testing the following calcium sources: calcium chloride (CaCl<sub>2</sub>), calcium gluconate (Ca-glu) and calcium lactate (Ca-lact).

### Materials and methods

**Microencapsulation.** The first step for the microencapsulation process corresponded to the preparation of the active principles aqueous suspension (for curcumin), and solution (for safranin), both with a concentration of 4% (w/w). For the curcumin suspension, 20 ml of distilled water were mixed with 100 $\mu$ l of Tween80<sup>®</sup> and 100 mg of curcumin. The mixture was homogenized during 20 minutes by using a homogenizer (CAT Unidrive X, Germany) at 11000 rpm. Afterwards, 800 mg of alginate were added to the suspension, which was then kept under stirring until the alginate dissolution was complete. For the safranin aqueous solution, 20 ml of distilled water and 100 mg of safranin were stirred until the complete dissolution of safranin was reached. After, 800 mg of alginate were added and the solution was kept under stirring until its full dissolution. 250 ml of the coagulation solution (using one of the three calcium sources), with concentration of 4% (w/v), were prepared. Then, the curcumin suspension or safranin solution, was introduced into a syringe and placed on the syringe pump of the atomization system (Nisco VarJ30 system (Zurich, Switzerland)). The flow rate and the pressure of the atomizing gas (nitrogen) were established at 0.3 ml/min and 0.1 bar, respectively. At the end of the atomization, the coagulation stage was maintained for further 4 hours at room temperature, in order to promote the microspheres (Mic) crosslinking. Following, the obtained Mic were recovered by filtration, washed twice with distilled water and freeze-dried for further tests. Empty Mic (EMic, Mic without active principle) were also produced.

**Mic characterization.** The Mic samples were characterized by optical microscopy (OM), using the optical microscope Ni-U (Nikon Eclipsei) equipped with a digital camera and a NIS-Elements Documentation software, in order to access their morphology. The encapsulation efficiency (EE), load, dry residue, rehydration capacity of the freeze-dried Mic were evaluated. The release profile of curcumin was evaluated on ethanol, while for safranin water was used. The Mic particle size was evaluated by laser diffraction, using the equipment Malvern Mastersizer 3000, equipped with a dispersion unity Hydro MV (Malver, United Kingdom). The FTIR spectra were obtained on a ABB Inc. FTIR, model MB3000 (Quebec, Canada) in transmittance mode by using KBr pellets with a sample concentration of 1% (w/w). Spectra were recorded between 550

and 4000  $\text{cm}^{-1}$  at a resolution of 16  $\text{cm}^{-1}$  and by co-adding 32 scans. Thermogravimetric analysis (TGA) was done by using a NETZSCH model TG 209F3 Tarsus (Selb, Germany) equipment. The used temperature range was from 32 to 600°C, with a heating rate of 10°C.min<sup>-1</sup>. The experiments were made under nitrogen flow (50 mL/min).

## Results

**Optical Microscopy.** The OM analysis allowed to check the spherical conformation of the EMic, being also noticed a predominance of a pear shape, mainly for the Mic crosslinked with CaCl<sub>2</sub> and Ca-glu. The curcumin Mic had a spherical conformation, and it was possible to observe the presence of small curcumin crystals on their surface. Regarding the safranin Mic, a predominant spherical conformation was observed. However, the occurrence of Mic deformation was also noticed, especially for the Mic crosslinked with CaCl<sub>2</sub>.

**Encapsulation Efficiency.** The EE was estimated based on the quantification of the active principle loss to the crosslinking solution during the coagulation stage. The results, shown in Table 1, revealed that all the samples presented high EE values. When CaCl<sub>2</sub> is used, higher EE was obtained, followed by Ca-glu and Ca-lact. For curcumin Mic, due to its hydrophobicity it wouldn't be expected a significant loss for the aqueous solution, during the coagulation stage. Regarding safranin Mic, the high values were not expected, once safranin is a hydrophilic compound with a good chemical compatibility with the coagulation aqueous solution, being thus expected a higher loss during the coagulation stage.

**Dry Residue and Rehydration Capacity.** The dry residue (%) allows the quantification of the Mic solid content, based on the weight measured before and after the freeze drying. The results presented in Table 2 showed that a higher dry residue was obtained for the Mic crosslinked with Ca-glu, followed by the Mic crosslinked with CaCl<sub>2</sub> and finally the Mic crosslinked with Ca-lact.

Regarding the rehydration capacity, this parameter is related with the Mic ability to absorb water and recover the initial shape. The results (Table 3) evidenced a higher capacity for the Ca-glu crosslinked Mic, in all the cases, followed by the Ca-lact Mic and finally, the CaCl<sub>2</sub> ones.

**Release Profile.** The release profile of both, curcumin and safranin compounds, was lower when CaCl<sub>2</sub> was used (5.70% of curcumin in ethanol, and 4.15% of safranin in distilled water), followed by the Ca-glu Mic (8.30% for curcumin and 4.42% for safranin). Ca-lact crosslinked Mic give rise to systems with higher release (12.10% for curcumin and 4.76% for safranin). The curcumin's release was restricted by the formation of curcumin crystals inside the Mic. For safranin Mic, gradual release was achieved during the evaluation period, without reaching a plateau indicating that the analysis should be extended for longer periods.

The overall results revealed that the alginate crosslinking can be influenced the calcium salts dissociation and the calcium diffusion to the Mic inner. The conditions used on this study were chosen in order to guarantee the calcium salts dissociation (once the solutions pH was above the pK<sub>a</sub> values of the calcium salts). Regarding the calcium diffusion to the Mic inner, for the system alginate/CaCl<sub>2</sub>, the molar ratio between the calcium ions (Ca<sup>2+</sup>) and the alginate G residues (Ca<sup>2+</sup>/G) was 45, which means

that the CaCl<sub>2</sub> solution at 4% (w/v) has a Ca<sup>2+</sup> concentration 45-fold higher than the required for total alginate crosslinking, favouring this reaction. In the same manner, for the Ca-lact, the Ca<sup>2+</sup>/G ratio was 25, but for this calcium salt the complete dissociation of Ca<sup>2+</sup> was not achieved, due to interaction between the negatively charged alcohol groups on the lactate complex and the Ca<sup>2+</sup> [2], which decreased the dissociation capacity, resulting on a less effective alginate crosslinking. Regarding the Ca-glu, the Ca<sup>2+</sup>/G ratio was 11, and the dissociation was complete, being more efficient than the Ca-lact

Table 1. Encapsulation Efficiency ((w/w) %).

Mic	Crosslinking agent		
	CaCl <sub>2</sub>	Ca-glu	Ca-lact
Curcumin	99.3%	98.5%	97.9%
Safranin	98.5%	97.6%	97.4%

Table 2. Dry Residue ((w/w) %).

Mic	Crosslinking agent		
	CaCl <sub>2</sub>	Ca-glu	Ca-lact
EMic	5.9 %	6.6 %	5.7 %
Curcumin	8.7 %	10.2 %	9.9 %
Safranin	9.5%	11.1%	10.2%

Table 3. Rehydration Capacity ((w/w) %).

Mic	Crosslinking agent		
	CaCl <sub>2</sub>	Ca-glu	Ca-lact
EMic	12.1%	39.6 %	15.5%
Curcumin	11.5%	23.7%	15.2%
Safranin	18.2%	42.9%	32.6%

**Particle Size Analysis.** The particle size analysis showed that the larger particle size, based on the volume distribution, was obtained for the Ca-glu based system (mean sizes of 131.46, 78.85 and 91.46  $\mu\text{m}$  for curcumin, safranin and empty Mic), while the CaCl<sub>2</sub> based system gave rise to the smallest ones (mean sizes of 91.65, 60.10 and 80.31  $\mu\text{m}$ , respectively for curcumin, safranin and empty Mic).

**FTIR and TGA analysis.** The FTIR analysis was used to access the effectiveness of the encapsulation of curcumin and safranin on the alginate matrix, and also evaluate the contribution of the crosslinkers in the Mic's chemical structure. The spectra analysis evidenced the curcumin and safranin presence, proving their encapsulation, and the contribution of the calcium crosslinkers was also identified. The TGA was used to evaluate the thermal stability of the Mic. Pure alginate, curcumin and safranin were also analyzed. The degradation profile obtained showed an increase of the thermal stability due to alginate crosslinking, once the active principles degradation shifted to higher temperatures due to the crosslinked polymeric matrix protection, particularly when calcium chloride was used.

## Conclusions

Analyzing the overall results obtained for the EE, it can be concluded that the CaCl<sub>2</sub> is the most effective calcium source for alginate crosslinking, being followed by calcium gluconate and finally calcium lactate. From the point of view of the curcumin and safranin release profile, calcium lactate lead to a more gradual release than the other two calcium sources.

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