# Formulation strategy to improve the encapsulation efficiency of thyme aqueous extract in alginate beads

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## INTRODUCTION

The delivery of drugs or active compounds represents a challenge in the pharmaceutical and nutraceutical field when they are susceptible to external factors or to the physiological processes that limit and reduce their bioavailability, biological half-life, and tissue uptake [1]. Microencapsulation could be a good strategy to overcome these drawbacks, and ionotropic gelation can be a useful option to deliver the bioactive substances thanks to its mild process conditions and the possibility of selecting biocompatible, biodegradable, and non-toxic materials as gelling polymers. However, the necessity to cure microparticles in an external water phase results in low encapsulation efficiency and loading capacity due to the loss of the drug into the aqueous solution [2]. This is especially true with small and highly soluble drugs or in the case of proteins, amine-based drugs, and vaccines.

This study aimed to improve the encapsulation efficiency of alginate-based microparticles containing a thyme aqueous extract, rich in polyphenols and useful for its antioxidant, antimicrobial and antifungal activity, by the use of several co-carrier excipients.

## EXPERIMENTAL METHODS

### Encapsulation of thyme aqueous extract

Three basic microparticle formulations composed of thyme aqueous extract and low-viscosity alginate were prepared. All the solutions had the same alginate concentration (2.5% w/w) but different thyme extract content in order to guarantee final products which differed from each other for the ratio between thyme and alginate: 70:30 (TA1), 50:50 (TA2) and 30:70 (TA3). Each formulation (about 20 g) was dripped into a gelling bath (500 mL calcium chloride aqueous solution, 100 mM). The newly formed beads were maintained under curing for 15 minutes; then, they were recovered by filtration, washed with deionized water, and dried for 1 hour by dynamic drying in a fluid bed dryer under airflow at 27 °C.

According to the visual aspect and morphology properties of the beads, the most promising basic formulation was selected as a reference and modified with the addition of co-carrier excipients (medium and high viscosity alginate, soy protein, flaxseed flour, maltodextrin, modified starch, carrageenan iota, pectin, chitosan at low molecular weight, shellac gum, diutan gum, or hydroxyethylcellulose) to improve the encapsulation efficiency. All the new formulations were the starting point to obtain beads by ionotropic gelation according to the procedure previously described.

### Characterization

A general characterization, including encapsulation efficiency (EE) based on spectrophotometric determination of thyme content (282 nm), process yield (PY), dimensional and morphological analysis, was carried out on all prepared beads; a deeper investigation (SEM analysis, flowability, swelling study in water, HCl pH 1.0 and phosphate buffer at pH 6.8 (PBS), release test and thermogravimetric analysis) was carried out on the most performing formulation and the reference.

## RESULTS

The microparticulate systems obtained by the treatment of the TA1, TA2 and TA3 formulations showed morphological properties that were strictly correlated to their composition. The surface appearance, average diameter and shape factor were related to the amount of alginate in the dried beads. The increase of polymer amount (TA1 < TA2 < TA3) was responsible for forming beads characterized by larger diameter, more regular shape, and more homogeneous surface. Moreover, the formulation composition also affected the PY (TA1 < TA2 < TA3) but did not significantly change the EE. According to the results, TA3 was selected as the reference mainly for the PY (73.56%) and aspect/morphology characteristics. Nevertheless, EE was not satisfying because it was about 57%. The thyme encapsulated was halved compared to the initial one because it tended to diffuse out of the microparticles into the gelling bath, driven by the concentration gradient. The use of co-carrier excipients was not always satisfactory, only the beads (coded as TACh) obtained by dripping the thyme-alginate solution into a calcium chloride (100 mM) acetic acid (1%) gelling bath containing chitosan (0.2% w/w) gave highly ameliorated EE (70.43%) compared to that of TA3 (57.24%). The acidic nature of the gelling bath was responsible for the increased availability of $H^+$ ions that reduced the proportion of ionized carboxylic groups in the alginate structure and, consequently, its solubility as well as the network porosity. Moreover, the positively charged aminic sites of chitosan were able to react with the remaining groups -COOH of the alginate, creating a more packed shell around the droplets/beads [3].

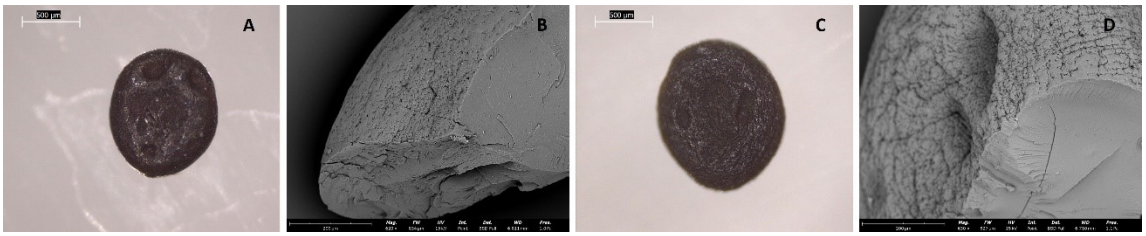


**Figure 1: Stereomicroscope and SEM images of the external surface and cross-section of TA3 (A, B) and TACh (C, D) dried microparticles.**

TACh dried microparticles were larger in diameter than TA3 (1126.02 ± 78.77 µm *vs* 1000.10 ± 57.42 µm) because of the presence of the polymeric blend alginate-chitosan. The internal surface of both TA3 and TACh microparticles was smooth and homogeneous (Fig. 1). On the contrary, SEM images showed that the TACh external surface was more wrinkled and organized than that of TA3 one, due to the strong polyelectrolytic interaction between alginate and chitosan. In both cases, pores, fractures, or ruptures were not visible, indicating a structural integrity of the systems. The surface irregularities influenced the flowability properties of both batches of dried beads, which were poor for TA3 (angle of repose 50°) and passable for TACh (43°). The thermal profile (data not shown) of TACh was in line with TA3 one, even if there was a slight difference in the residual humidity percentages (7.79% for TA3; 6.24% for TACh).

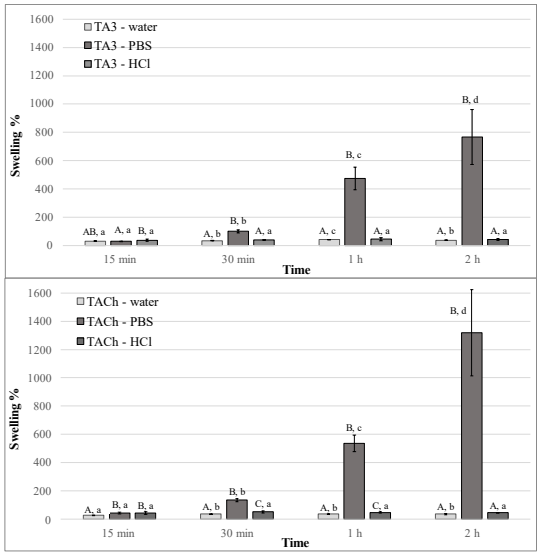


**Figure 2: Swelling behavior of TA3 and TACh microparticles in water, PBS and HCl. Different uppercase letters over the bars indicate significant differences among the fluids at the same time intervals ($p \leq 0.05$), while distinct lowercase letters over the bars demonstrate significant differences between the time intervals of the sample in the same fluid ($p \leq 0.05$).**

The dried beads were characterized by similar swelling behavior in HCl and water (Fig. 2): in both cases, they immediately took up the fluids, increasing their weight (the swelling percentage was about 40%), which was then kept constant for the next steps of the study. In PBS, the swelling ability of the beads was pronounced, and the gap among the samples became wide, mainly after 2 hours. In this fluid, the swelling behavior was due to the $Ca^{2+}/Na^{+}$ exchange as regards the alginate egg-box structure, to which the decrease of the alginate-chitosan interactions was added in the case of TACh beads [4].

Similar thyme release profiles characterized TA3 and TACh beads: after 15 minutes from the beginning of the test, more than 20% of the extract for TA3 and more than 34% for TACh were released, and the whole process ended in about 1 hour (Fig. 3). The influence of chitosan was not significant for the totality of the release process, but only for its initial stages. Being diffusion the predominant mechanism of the thyme release in the early phases of the process, the highest thyme content in TACh beads increased the starting concentration gradient between the beads and the release medium and promoted the rapid outcome of the extract.

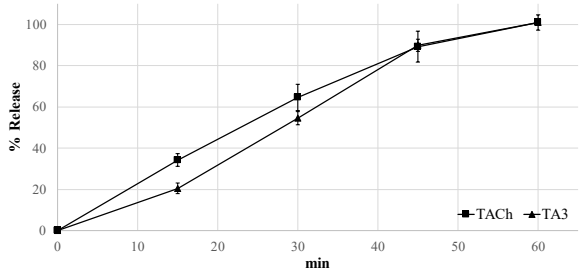


**Figure 3. *In vitro* thyme release profiles of TA3 and TACh beads.**

## CONCLUSION

The addition of the chitosan as co-carrier excipient in alginate beads allows an increase in the encapsulation efficiency and, consequently, in the thyme content without altering the principal characteristics of the systems.

## REFERENCES

1. Tan *et al*., ACS Nano, 13(8), 9016–9027 (2019).
2. Lim *et al*., Biomaterials Science, 1(5), 486-493 (2013).
3. Martins *et al*., International Journal of Biological Macromolecules, 57, 174-184 (2013).
4. Pasparakis *et al*., International Journal of Pharmaceutics, 323(1-2), 34-42 (2006).