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## Use of alginate fluid gel microparticles to modulate the release of hydrophobic actives

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The use of hydrocolloids-based microparticles in the field of bioactives microencapsulation is an area of great interest and research. However, their industrial scale production and their application in food products holds many technological challenges. The objective of this study was to investigate the microencapsulation of tryptophan, used as model active, into alginate microparticles obtained using the fluid gel route, as an easy industrial method for their production. Several alginate fluid gels, loaded with different amounts of tryptophan, were produced and characterized by particle size, rheological properties and encapsulation efficiency, then in vitro release kinetics using a dialysis approach were studied. Although the produced materials were unable to highlight a correlation between operating parameters and release kinetics, alginate fluid gels showed the ability to slow down the release of the drug compared to a water solution of tryptophan. Obtained microspheres showed very small sizes, which makes them suitable for the enrichment and delivery of actives in food products.

Keywords: Fluid gel, rheological properties, encapsulation, industrial process, release kinetic.

### 1. Introduction

Across different sectors, like foods, agrochemicals and pharmaceuticals, there is a big interest in developing a new generation of microparticles able to show “smart” behaviours, including the controlled release of bioactive molecules overtime (Dubey et al., 2009; Roy et al., 2014; Caccavo et al., 2015). A large variety of natural polymers, especially polysaccharides and proteins, have been used during the last years for the encapsulation of bioactives. In particular, alginate is a natural polysaccharide having attractive characteristics for encapsulation and delivery of actives and drugs, due to its biodegradability, low toxicity, low cost and biocompatibility. It forms stable water-based gels, called hydrogels, under mild conditions in presence of multivalent-cations (Da Silva et al., 2014; Cardoso et al., 2016; Agüero et al., 2017). Several techniques have been used for the production and encapsulation of actives in alginate microparticles, including spray-drying, coacervation, extrusion and emulsion methods (Goh et al., 2012). However, all these approaches present some limitations, in terms of encapsulation of particular actives, for example due to the usage of high temperatures (spray-drying), presence of other materials, like emulsifiers and surfactants (emulsion), production of large particles and non-continuous production methods (extrusion) (Madene et al., 2006). In the last years, many studies have focused on the development of industrially feasible methods for the production of microparticles, particularly using the fluid gel route (Gouin, 2004; Garrec and Norton, 2012). Fluid gels are suspensions of gel particles in a continuous medium (usually water) that are produced by applying a shear environment to a polymer solution undergoing gelation (Norton et al., 1999). Alginate fluid gel particles can be easily obtained using continuous processes on a lab-scale; equipment able to provide a shear environment, like pin-stirrer, can produce alginate fluid gels in a reproducible and controlled way (Fernández Farrés et al., 2013). Studies have shown that fluid gels can find applications in the foods as stabilizers, thickeners, texturing materials and fat replacers (Le Révérend et al., 2010; Saha and Bhattacharya, 2010; Chung et al., 2014; Fernández Farrés et al., 2014). Because of the rise in population health problems and obesity the development of low-fat foods that should meet the “traditional food” sensory characteristics and costumers’ tasty expectations is a potential area for the application of fluid gels. In general, the addition of ingredients in foods should not affect their flavour, colour or sensory properties and the

addition of microparticles should not modify the food smoothness. As a consequence the addition of large particles is generally undesirable (Champagne and Fustier, 2007). Alginate fluid gels particles are not prone to be detected in mouth, due to their small particle dimensions (<10 µm) and “soft solid” behavior upon compression within the oral cavity (Fernández Farrés et al., 2014). However, alginate fluid gels particles have not been used so far as matrices for the encapsulation of bioactives. In order to fill the gap in the current literature, this work investigates the ability of alginate fluid gel to be used as material for the encapsulation and controlled release of tryptophan (TRP), used as model active. TRP was chosen because of its small dimension and low molecular weight (204.23 g/mol) in order to avoid its physical entrapment into the formed gel-network. Additionally, TRP presents a quite low water solubility (13.4 mg/mL at 25 °C (Yalkowsky, 1992)), which is an ideal characteristic to study the encapsulation of quite hydrophobic compounds; in fact, a small water solubility is a required characteristic to obtain homogeneous solutions for production of fluid gels. The viscosity and the particle size of fluid gels loaded with TRP were compared to the ones of unloaded fluid gels to clarify if the material properties are affected by the presence of the active. The encapsulation efficiency was determined and release experiments were conducted to assess the ability of the materials to control the release of the loaded active in controlled way overtime.

## 2. Materials and methods

### 2.1 Materials

Sodium alginate (ALG) and L-tryptophan (TRP) (reagent grade, ≥98% (HPLC)) were purchased from Sigma-Aldrich® (Sigma–Aldrich Company Ltd., Dorset, UK). Calcium chloride (CaCl<sub>2</sub>, anhydrous, 93%) was purchased from Alfa Aesar™ (USA). All materials were used without further purification. Milli-Q water was made using an Elix® 5 distillation apparatus (Millipore®, USA) and was used for all water-based preparations.

### 2.2 Gel preparation

#### 2.2.1 Blank fluid gels

Firstly, a solution of 2% (w/w) was prepared by dissolving ALG in distilled water at 95°C for 30 min under stirring to ensure a complete powder dissolution (Fernández Farrés and Norton, 2014). Thereafter, the solution was cooled at room temperature (R.T.). Secondly, a CaCl<sub>2</sub> solution having a concentration of 0.35% (w/w) was prepared by dissolving CaCl<sub>2</sub> at R.T.. All used concentrations were calculated on the final weight of the material. Alginate Fluid Gels (AFG) were prepared using a pin-stirrer vessel (Het Stempel, HL): the alginate solution was pumped into the pin-stirrer using a peristaltic pump (Masterflex L/S Peristaltic, DE), while the CaCl<sub>2</sub> solution was injected using a syringe pump (Cole-Parmer Single-syringe, US) through a stainless steel needle having an internal diameter of 1.25 mm.

#### 2.2.2 Tryptophan loaded fluid gels

Firstly, a solution of 2% (w/w) was prepared by dissolving ALG in distilled water at 95°C for 30 min under stirring to ensure complete powder dissolution. After the solution was cooled at R.T.. A second solution was prepared by firstly dissolving a calculated amount of TRP (0.025%, 0.05% or 0.10%) in water at R.T.. After the complete dissolution of TRP, 0.35% of CaCl<sub>2</sub> was added and dissolved at R.T.. All used concentrations were calculated on the final weight of the material. Tryptophan Fluid Gels (TFG) were then prepared using a pin-stirrer vessel as described in section 2.2.1 (Blank fluid gels).

### 2.3 UV-Vis measurements

The UV absorbance of water solutions of TRP was measured using an Orion AquaMate 8000 UV-Vis spectrophotometer (Thermo-Scientific®, UK) at 278 nm wavelength and correlated to the corresponding concentration by using the Beer-Lambert equation.

### 2.4 Encapsulation efficiency

The encapsulation efficiency (EE%) of TRP in AFG particles was determined by ultracentrifugation: a weighted amount of sample (approximately 15g) was centrifuged using a Sigma 3K-30 refrigerated centrifuge (Sigma®, D), equipped with a 12150 rotor, at 21,000 rpm at 21°C for 45 minutes. A weighted amount of supernatant (approximately 1.5 g) was placed in a 100 mL volumetric flask and filled with distilled water. The concentration of the active in the supernatant solution was quantified spectrophotometrically as described in section 2.3 (UV-Vis measurements). The EE% was calculated using equation 1:

$$EE \% = (W_f/W_i) \times 100 \quad (1)$$

where,  $W_f$  is the amount of encapsulated TRP detected by UV and  $W_i$  is the quantity of TRP added during sample preparation (Piacentini, 2016).

## 2.5 Rheological properties

Rheological properties of fluid gels were determined by shear viscosity tests using a rotational rheometer (Kinexus™, Malvern®, UK) equipped with a 40 mm diameter sand blasted plate geometry. The analysis were carried out at 25°C using a shear rate ramp of 31 points between 0.01 and 100 s<sup>-1</sup>. Measurements were performed in triplicate.

## 2.6 Particle Size Distribution

The particle size distribution (PSD) of fluid gels was evaluated using a Mastersizer-2000 (Malvern®, UK). Few drops of sample were placed into the mixing chamber and stirred for 10 min at 1250 rpm before performing the analysis to disrupt any possible macro-aggregation. PSD was evaluated as numerical particle sizes percentage. Measurements were performed in triplicate.

## 2.7 Drug release analysis

In vitro release studies of TRP from AFGs were performed by enclosing a known amount of material (approximately 2.5 g) into a cellulose dialysis tube (Sigma-Aldrich Company Ltd., Dorset, UK, width 43 mm, M.W. cut-off of 14000 Da). Dialysis membranes were soaked in distilled water for 10 min before filling with TFG. Tests were conducted at room temperature (thermostated at 21.5 °C) under stirring (150 rpm) using 500 mL of water as release medium. At regular intervals, aliquots of 2 mL were withdrawn, measured using the spectrophotometer and then poured back into the release medium. Each analysis was carried out in triplicate. Tests were conducted for a total of 5 hours each, since after that time no changes in concentration were observed in the release medium. The same analysis was performed also on a TRP water solution, prepared by dissolving 0.10% (w/w) of TRP in water.

## 3. Results and discussion

In the first part of the experimentation, three different samples of alginate fluid gels loaded with tryptophan (TFG) were prepared by changing the TRP concentration to 0.025%, 0.05% and 0.10% (w/w) (TFG-0.025, TFG-0.05, and TFG-0.10), to study the EE% of TRP as function of its concentration. Additionally, an Alginate Fluid Gel (AFG) formulation without TRP was prepared, in order to study the effect of TRP presence and its concentration on the rheological behavior and PSD of AFG microparticles. Afterwards, a second set of experiments was performed on TFG to evaluate their TRP release kinetics overtime.

### 3.1 Encapsulation Efficiency

The EE% of TRP in AFG was determined as described in section 2.4 (Encapsulation efficiency) and results are reported in Figure 1:

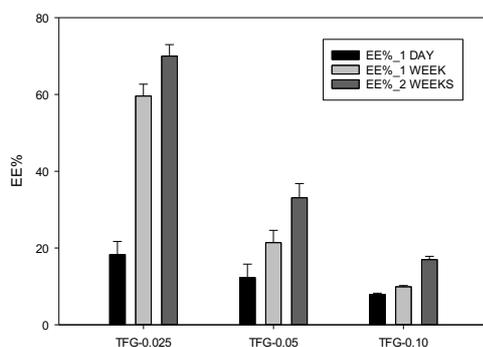


Figure 1- EE% of TRP in AFG as a function of storage time. Results are shown as mean  $\pm$  st.dev. (n=3).

The EE% of TRP in AFG was monitored over a 2 weeks period to assess if any changes occurred as a function of the storage time ( $t_s$ ), where  $t_s$  was the time elapsed from the sample production. It is possible to notice that the EE% increased as a function of  $t_s$ . It can be suggested that TRP diffuses into alginate microparticles, in order to fit their internal core that can be described as a more hydrophobic environment if compared to the continuous water phase surrounding particles. In fact, TRP is not very hydrophilic (water solubility of 13.4 mg/mL at 25 °C (Yalkowsky, 1992)) and would preferentially be surrounded by alginate polymer chains. Several studies were conducted in the past revealing hydrophobic interactions between hydrocolloids polymer chains, including alginate and milk proteins, and hydrophobic molecules, like vanillin and sodium dodecyl sulphate (Chobpattana

et al., 2002; Yang et al., 2008; Yang et al., 2009). The diffusion of TRP into particles as a function of time can be explained considering a similar effect, which justifies the increase of EE% overtime.

### 3.2 PSD and Viscosities

Production parameters, apart from TRP concentration, were not changed and they are here reported: 2% ALG concentration, 0.35% CaCl<sub>2</sub> concentration, pin-stirrer shaft speed of 1000 rpm and 4 min of residence time in the pin-stirrer. Mastersizer curves, reported in figure 2A, reveals that all materials have the same PSD, independently from the used concentration of TRP. Particle sizes were in the range between 0.2 μm to 7 μm, with particles mean size around 0.3/0.4 μm, and they had comparable dimensions with particles of AFGs produced without TRP. Viscosity profiles did not present substantial changes due to the presence of TRP, as can be noticed from figure 2B. Low percentages of TRP were used for materials preparation if compared to the percentage of other formulation constituents (i.e. ALG and CaCl<sub>2</sub>). It is possible to conclude that the TRP addition, in the range of used TRP concentrations, does not affect the particle size and the rheological behavior of AFG..

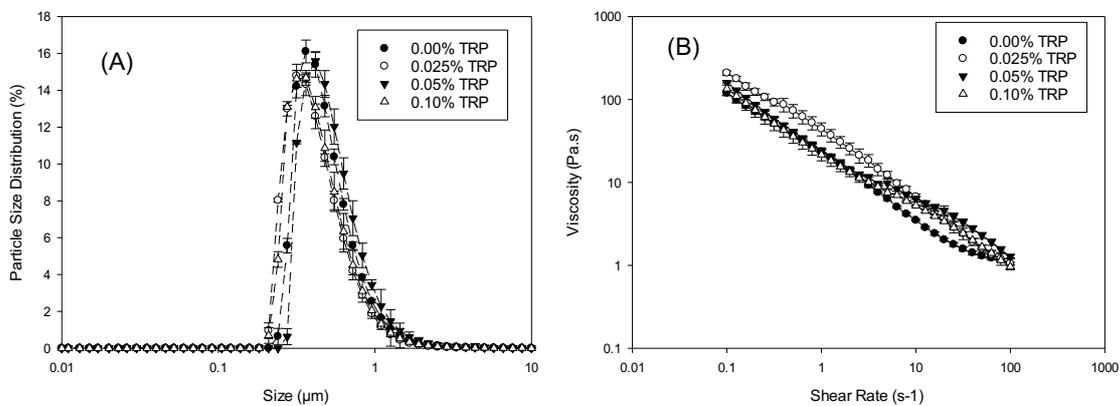


Figure 2- TRP loaded alginate fluid gels: (A) Particle size distribution; (B) Viscosities. Results are shown as mean ± st.dev. (n=3).

### 3.3 In vitro release studies

TFG-0.025, TFG-0.05, TFG-0.10 were tested for in vitro release studies as described in section 2.7 (Drug release analysis). Curves are reported in figure 3:

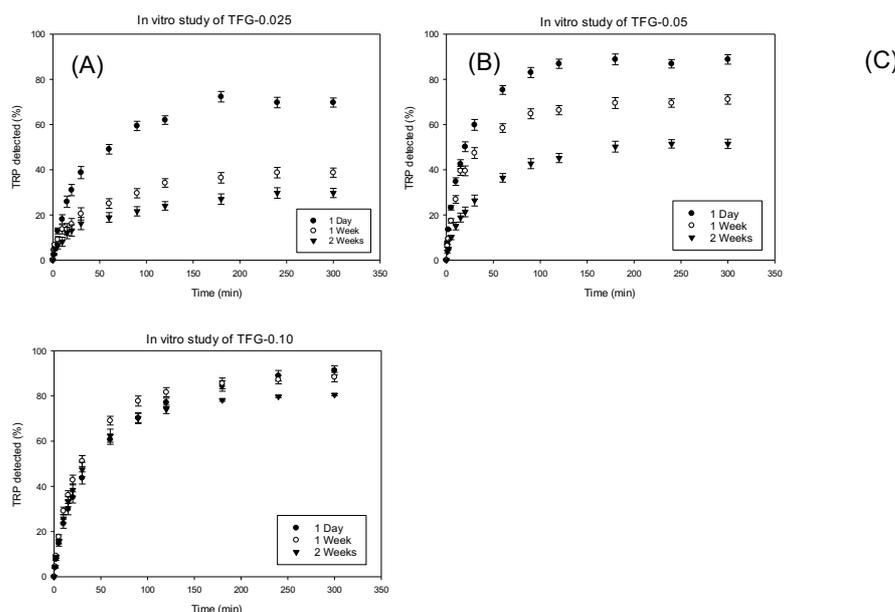


Figure 3 – In vitro release studies as function of the storage time: (A) TFG-0.025; (B) TRP-0.05; (C) TRP-0.10. Results are shown as mean  $\pm$  st.dev. ( $n=3$ ).

As can be noticed even the in vitro tests were strongly influenced by  $t_s$ ; the quantity of TRP detected in the release medium decreased as a function of  $t_s$  for the same material. In addition, none of TFG formulations was able to completely release 100% of the TRP added during sample preparation as can be noticed in figure 3. To better understand the release behavior of TRP within AFG, the non-encapsulated percentages of TRP and the percentages of TRP detected into the release medium after 5 hours of in vitro studies have been reported in Table 1 and Table 2, respectively.

Table 1: Percentages of non-encapsulated TRP in TFG

Sample	% TRP non-encapsulated (1 Day)	% TRP non-encapsulated (1 Week)	% TRP non-encapsulated (2 Weeks)
TFG-0.025	81.7	40.4	30.0
TFG-0.05	87.7	78.6	66.9
TFG-0.10	92.1	90.1	83.0

Table 2: Percentages of TRP detected after 5 hours in in vitro release studies of TFG

Sample	% TRP detected (1 Day)	% TRP detected (1 Week)	% TRP detected (2 Weeks)
TFG-0.025	69.8	38.7	29.7
TFG-0.05	88.8	71.1	51.5
TFG-0.10	91.3	88.4	80.5

Comparing data from tables 1 and 2 can be noticed that the percentages of TRP able to diffuse out of AFG formulations were always lower than the percentages of non-encapsulate TRP. In addition, the amount of TRP detected in the release medium decreased as a function of  $t_s$ . Considering that the EE% increased as a function of  $t_s$ , as reported in section 3.1 (Encapsulation Efficiency), it is possible to conclude that only the TRP not encapsulated in the microparticles was able to diffuse from the formulation. This can explain why not all the 100% of TRP within AFG was never detected in the release medium of in vitro studies. It can be concluded that TRP cannot be released from microparticles. This behavior may be due to hydrophobic interactions between the TRP molecule and ALG polymer chains. However, molecular modelling experiments should be conducted in order to assess these interactions.

The diffusion time of TRP from AFG was compared to the diffusion time of TRP from a water solution and the results are compared in Figure 4:

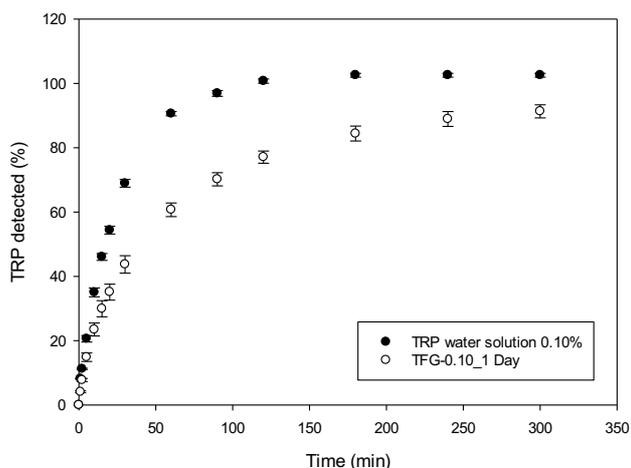


Figure 4- In vitro release studies of 0.10% (w/w) TRP water solution and TFG-0.10 (1 day of storage time). Results are shown as mean  $\pm$  st.dev. (n=3).

As can be noticed the AFG formulation was able to slow down the diffusion of the active when compared to its diffusion from a water solution. This effect can be probably related to the higher viscosity of AFG.

#### 4. Conclusions

The ability of Alginate Fluid Gels (AFG) to be used as matrices for the encapsulation of Tryptophan (TRP) was investigated. The results showed that its EE% is a function of  $t_s$  and that TRP cannot diffuse out of alginate microparticles, under the conditions used for the in vitro release tests. Further experiments should be conducted to verify the ability of AFG to release TRP under different conditions, like gastric or physiological environment. Nevertheless, this study showed the ability of AFG to slow down the diffusion of TRP in in vitro studies when compared to a TRP water solution, suggesting that more studies should be conducted in order to investigate the usage of these materials for the development of formulations to control the diffusion of actives overtime.

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