



Alginate/chitosan nanoparticles for encapsulation and controlled release of vitamin B₂

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ABSTRACT

This work aims at evaluating encapsulation and controlled release of vitamin B₂ from alginate/chitosan nanoparticles. Ionotropic polyelectrolyte pre-gelation was used as production method being chitosan and alginate used as main materials. Nanoparticles were characterized in terms of average size, polydispersity index (PDI), zeta potential and vitamin entrapment efficiency. The average size for alginate/chitosan nanoparticles was 119.5 ± 49.9 nm for samples without vitamin B₂ and 104.0 ± 67.2 nm with the encapsulation of vitamin B₂, presenting a PDI of 0.454 ± 0.066 and 0.319 ± 0.068 , respectively. The nanoparticles showed encapsulation efficiency and loading capacity values of $55.9 \pm 5.6\%$ and $2.2 \pm 0.6\%$, respectively. Release profiles were evaluated at different conditions showing that the polymeric relaxation was the most influent phenomenon in vitamin B₂ release. In order to study their stability nanoparticles were stored at 4 °C being particles sizes and PDI evaluated during 5 months showing the results that vitamin B₂-loaded nanoparticles are more stable (in terms of size and PDI) than nanoparticles without vitamin B₂.

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1. Introduction

Food industry pays special attention to vitamins due to their unique properties, e.g. essential for growth, development and normal maintenance of human organism [1,2]. In general, vitamins are defined as organic molecules of small dimension and low molecular weight, being divided into liposoluble and water-soluble vitamins according to their solubility [2,3]. Furthermore, humans do not have the capacity to synthesize vitamins, except vitamin D and B₃, meaning that they must obtain vitamins through external sources [1,2]. Vitamin B₂ is a flavin, water-soluble compound and is present in animal and plant cells (e.g. wheat bran, milk and milk products, eggs, meats and vegetables) [1,2,4].

One of the present challenges for the use of vitamins by food industry is their high sensibility and low stability to inadequate environmental conditions (e.g. temperature, oxygen, light and moisture), leading to the search for new strategies for their use. One of the presented solutions to keep stability of vitamins is nanoencapsulation [5,6]. Nanocapsules are constituted by an external cavity consisting of a polymeric membrane and an internal part composed by a liquid or polymeric matrix that contains the

active compound [7,8]. The selection of an appropriate method of encapsulation is an important step in order to have nanoparticles with the desired performance and functionality. The selected method will depend on the physicochemical character of the polymer, encapsulated bioactive compound and the desired properties of the nanoparticles (e.g. particle size, particle size distribution, surface area, shape, solubility, encapsulation efficiency and release mechanism) [9–11]. Besides, in order to improve the efficiency and stability of nanostructures, it is also essential to find adequate materials; in the particular case of foods, the replacement of non-food-grade materials by bio-based and biodegradable food-grade materials is of relevance. Biopolymers, such as polysaccharides, are one of the possibilities due to their distinct advantages of biodegradability, edibility and lack of toxicity [12].

Alginate is a hydrophilic polysaccharide extracted from marine brown algae of the *Phaeophyta* family. It is a linear biopolymer composed by two uronic acids, 1,4-linked-β-D-mannuronic acid (M) and α-L-guluronic acid (G), being carboxylic groups from uronic acids responsible by their negative charge. This biopolymer is a non-toxic, biocompatible, biodegradable and presents mucoadhesive properties, being approved for pharmaceutical and food applications [13–15]. The solubility of alginate in water depends on the associated cations, i.e. sodium alginate is soluble in water but when a solution with multivalent cations (e.g. calcium – Ca²⁺) is used the biopolymer can form a reversible gel [13,14,16].

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Chitosan is a cationic polysaccharide derived from the N-deacetylation of chitin, the second most abundant natural biopolymer. It is a linear copolymer composed by repeating units of 2-amino-2-deoxy- β -D-glucan with glycosidic linkages, where the amine groups confer to chitosan special properties (e.g. high charge density, readily available for chemical reactions and salt formation with salts) [13,17]. Chitosan presents three types of reactive functional groups: an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 position, respectively [18]. The position of free amino and N-acetyl groups is responsible by the solubility of chitosan; nevertheless, chitosan solubility is improved with aqueous acids (e.g. formic acid and acetic acid) [17,19,20]. Chitosan is biocompatible, biodegradable, non-toxic, with significant adsorption and mucoadhesive properties and antifungal activity. Due to these properties, chitosan has a great potential for food, environmental and pharmaceutical applications [16,19,21,22].

The main objective of this work was the development and characterization of a biopolymer-based nanoparticle for the encapsulation of vitamin B₂ and assessment of vitamin release from it. The main challenges were the encapsulation of vitamin B₂ into an alginate/chitosan nanoparticle and their controlled release in different conditions. Although this type of nanoparticles has been studied, in this work the alginate/chitosan nanoparticles were applied to the encapsulation of vitamin B₂ aiming their application in food industry, which may be an added value for this field.

2. Materials and methods

2.1. Materials

Sodium alginate (Manutex RSX) with MW ≈ 15,900 Da and viscosity ≈ 200 cp (1% aqueous solution with Brookfield Model LV – 60 rpm at 25 °C) from *Laminaria hyperborea* was purchased from CP Kelco International, Ltd. (Portugal). Chitosan with 91.23% deacetylation degree was purchased from Golden-Shell Biochemical Co., Ltd. (China). Calcium chloride (CaCl₂) was obtained from Panreac (Panreac Quimica SA, Barcelona, Spain) and vitamin B₂ (MW = 376.36 g/mol) was obtained from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA).

Phosphate buffer saline (PBS) was purchased Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA), Potassium chloride was obtained from Merck (Darmstadt, Germany) and hydrochloric acid (36.5–38.0% purity) was obtained from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA).

2.2. Alginate/chitosan nanoparticles preparation

The development of alginate/chitosan nanoparticles was based on the methodology described by Sarmento et al. [16] with some modifications. Briefly, sodium alginate was dissolved in distilled water and chitosan was dissolved in 1% of acetic acid being the pH values of alginate and chitosan solutions initially set to 4.9 and 4.6, respectively. The pH values of the solutions were adjusted with sodium hydroxide (1 M) and hydrochloric acid (1 M). Through a preliminary study, the optimal concentrations (selected based in the particle size and polydispersity index (PDI) using dynamic light scattering) of alginate and chitosan were determined, being, respectively, 0.63 mg/mL and 0.4 mg/mL. Initially, 7.5 mL of 18 mM calcium chloride solution was dropped at a flow rate of 0.125 mL/min into a beaker containing 117.5 mL of alginate solution at a constant homogenization using an Ultra-Turrax (T 25, Ika-Werke, Germany) at 20,000 rpm. Then, 25 mL of chitosan solution were added dropwise into the previous solution with a stirring of 600 rpm and a flow rate of 0.278 mL/min. After the addition of chitosan solution, alginate/chitosan nanoparticles were maintained

at constant stirring during 30 min. For the water-soluble vitamin encapsulation, vitamin B₂ was dissolved in the alginate solution before addition of calcium chloride (CaCl₂) solution. Concentrations of vitamin B₂ were tested based on their maximum solubility in water, then the optimal concentration of vitamin B₂ was determined based in particle size and PDI, being 0.065 mg/mL. After preparation, the alginate/chitosan nanoparticles with or without vitamin B₂ were stored at 4 °C in solution.

2.3. Nanoparticle size, polydispersity index and zeta potential

Alginate/chitosan nanoparticles with and without vitamin B₂ were characterized in terms of size (by number distribution), the polydispersity index (PDI) and zeta potential. The particle size is the diameter of nanoparticles and PDI is the parameter that gave us the distribution of nanoparticles size. The PDI is dimensionless and indicate that the sample is or not monodisperse, in other words, has a very broad size distribution. The measurements of these parameters are based in a simple principle: illuminating of sample with a laser and analyzing the scattered light, where the detector position can be at either 173° or 90° depending on the model of Zetasizer Nano model, in our case were measured with a detection angle of 173°. The zeta potential (ζ) is other parameter that can be measurement by DLS and consist, essentially, in measurement of electrostatic/charge at the surface of the nanoparticle through a laser that passes the sample cell.

For this characterization a dynamic light scattering (DLS) apparatus (Zetasizer Nano ZS, Malvern Instruments, UK) equipped with a He-Ne laser at a wavelength of 633 nm was used. All measurements were performed at 25 °C. Each measurement of size and PDI was performed with a detection angle of 173° and zeta potential measurements with an angle of 17°, being the zeta potential values calculated by Smoluchowski's model. For all size, PDI and zeta potential measurements at least three replicates were obtained.

NanoSight NS500 (NanoSight Ltd., UK) was also used to measure the size of nanoparticles and to analyze the nanoparticles concentration by size. For measurements the samples were diluted to a concentration suitable for the analysis (according to the equipment instructions) (NanoSight, 2010). The dilutions were made with distilled water. The diluted samples were injected into the sample chamber fitted with a 640 nm diode laser. The software Nanoparticle Tracking Analysis (NTA) 2.0 Build 127 was used for capturing and analyzing the data.

2.4. Morphology

The surface morphology of the nanoparticles with and without vitamin was evaluated by transmission electron microscopy (TEM) (ZEISS 902 A, Zeiss, Germany) operating at a voltage of 80 kV. Before loading of samples into the microscope (direct deposition), the samples were drop-cast onto a carbon coated copper grid and dried during 1 min at room temperature.

2.5. Encapsulation efficiency and loading capacity

Encapsulation efficiency (EE) and loading capacity (LC) were determined after separating the nanoparticles with encapsulated vitamin from the supernatant with free vitamin. The separation was performed using an Amicon® Ultra-0.5 centrifugal filter device (Amicon® Ultra – 0.5 mL 3 K device, Millipore Corp., Ireland). Briefly, 0.5 mL of sample was added to the Amicon® and was centrifuged at 14,000 × g during 10 min. After centrifugation a filtrate with free vitamin and a concentrate with nanoparticles with encapsulated vitamin were obtained. These were used for EE and LC determination. To calculate EE, the filtrate was assayed spectrophotometrically at 437 nm, which corresponds to the

maximum absorbance peak of vitamin B₂ [23], and the amount of free vitamin was calculated using an appropriate calibration curve: $y = 33.77x - 0.0049$ ($R^2 = 0.999$) being y and x the absorbance and amount of free vitamin B₂, respectively. LC was determined by the weight of dried nanoparticles (0.5 mL of sample corresponds to approximately 0.7 mg of nanoparticles). EE and LC were calculated as follows [6,16]:

$$EE\% = \frac{Vit_{total} - Vit_{free}}{Vit_{total}} \times 100 \quad (1)$$

$$LC\% = \frac{Vit_{total} - Vit_{free}}{Nps_{total}} \times 100 \quad (2)$$

where Vit_{total} represents the total amount of vitamin; Vit_{free} the amount of free vitamin in filtrate and Nps_{total} the total weight of nanoparticles.

2.6. Release profile

Dialysis methodology was used in order to evaluate the release profile of encapsulated vitamins from nanoparticles [24–26]. The release experiments were conducted at 37 and 25 °C and the release media utilized were PBS and Tris-HCl for pH 7 and 2, respectively. The procedure of dialysis was as follows: 5 mL of nanoparticles (approximately 7 mg of nanoparticles) with encapsulated vitamin were added into a dialysis membrane (molecular weight cut-off 3500 Da; Cell-Sep H1, Membrane Filtration products, USA) and the sealed membrane was then placed into 50 mL of medium (PBS or Tris-HCl) at 37 or 25 °C, under magnetic stirring. At determined time intervals, 1 mL of samples were taken from the medium and replaced by fresh medium to maintain the original volume. All the experiments were performed in triplicate.

2.6.1. Release kinetics

The release profile of encapsulated vitamin in nanoparticles was evaluated using Berens and Hofenberg model [27] that considers contributions from Fickian diffusion and polymeric relaxations of the hydrophilic matrix:

$$M_t = M_{t,F} + M_{t,R} \quad (3)$$

being M_t the total amount of sorption per unit weight of polymeric nanoparticles at time t ; $M_{t,F}$ the contributions of Fickian diffusion; and $M_{t,R}$ the contributions of polymeric relaxations.

In turn, for most cases, only one main polymer relaxation affects the transport and thus the equations can be simplified using $n = 1$ [26]. This way the Fickian and relaxation contributions are expressed by the following equations:

$$M_{t,F} = M_F \left[1 - \frac{6}{\pi^2} \times \exp(K_F t) \right] \quad (4)$$

$$M_{t,R} = M_R [1 - \exp(K_R t)] \quad (5)$$

being,

$$M_t = M_F \left[1 - \frac{6}{\pi^2} \times \exp(K_F t) \right] + M_R [1 - \exp(K_R t)] \quad (6)$$

where,

$$K_F = \frac{4\pi^2 D}{d^2} \quad (7)$$

and M_F the total mass of compound release at equilibrium by unrelaxed polymer; M_R the total mass of compound release at equilibrium by relaxation process; K_R the respective relaxation rate constant; d nanoparticles diameter; and D the diffusion coefficient.

The non-linear estimation module of Statistica® 7 (Statsoft, Tulsa, OK, USA) was used in order to determine the suitable of equations in describing vitamin release data over time.

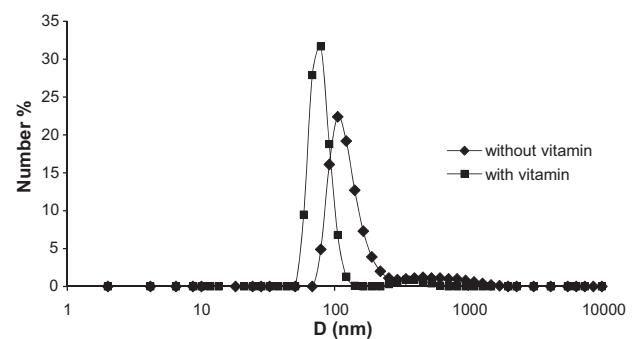


Fig. 1. Size distributions by number for alginate/chitosan nanoparticles without and with vitamin B₂.

2.7. Stability

Freshly prepared samples were used for stability measurements. In order to evaluate the stability of nanoparticles throughout time, nanoparticles with and without vitamin were stored at 4 °C in solution state being their size and PDI measured during five months.

2.8. Statistical analyses

Data analyses were performed using Microsoft Windows Excel 2003 and GraphPad Prism (Version 5.00 (Trial), edition 2007, GraphPad Software, Inc., La Jolla, CA, USA). Dispersion of data was reported in terms of the mean standard deviation (SD) from at least three values. Analysis of variance (ANOVA) followed by Tukey's multiple-comparison test ($\alpha = 0.05$) were employed to assess the statistical significance of differences between groups of data. Differences in experimental results were considered to be statistically significant at 95% confidence level ($p < 0.05$).

The quality of the regressions was evaluated on the basis of the determination coefficient, R^2 , the squared root mean square error, RMSE (i.e., the square root of the sum of the squared residues (SSE) divided by the regression degrees of freedom) and residuals visual inspection for randomness and normality. R^2 was obtained directly from the software.

3. Results and discussion

3.1. Diameter, polydispersity index (PDI) and zeta potential

The diameter average of alginate/chitosan nanoparticles was 119.5 ± 49.9 nm for samples without vitamin B₂ and 104.0 ± 67.2 nm with vitamin B₂, being the PDI values of 0.454 ± 0.066 and 0.319 ± 0.068 , respectively (Fig. 1). The decrease of diameter values is not significantly different ($p > 0.05$) showing that encapsulation of vitamin does not influence the size of the nanoparticles, however the PDI values for alginate/chitosan nanoparticles without and with vitamin B₂ are significantly different ($p < 0.05$) decreasing when the vitamin B₂ is loaded in alginate/chitosan nanoparticles. The lower values of PDI suggest that nanoparticles with vitamin B₂ are more homogeneous than samples without vitamin. This behavior can be explained by interactions between vitamin B₂ and biopolymers used. Vitamin B₂ when mixed with alginate solution at pH 4.7, acquire a cationic form interacting strongly with negatively charged alginate [28]. We believe that this strong ionic interaction generated between vitamin B₂ and alginate is contributing to the decrease of both size and PDI in nanoparticles containing vitamin B₂; also, the influence of hydrogen bonding and van der Waals forces that vitamin B₂ molecules can establish with alginate and chitosan must not be discarded [16]. The size of nanoparticles was also determined by NTA



Fig. 2. Transmission electron microscopy (TEM) image of alginate/chitosan nanoparticles without vitamin B₂ (scale bar = 2 μ m, magnification = 7000 \times).

in order to confirm the results obtained by DLS. The values of the mean diameter \pm standard deviation and the mode were respectively 168 ± 97 and 101 nm, for nanoparticles without vitamin B₂, and 210 ± 109 nm and 107 nm for nanoparticles with vitamin B₂. The sizes obtained by NTA are consistent with the results obtained by DLS, confirming the nanoscale range of the developed nanoparticles.

When compared with others works (using similar methodologies and materials) the developed nanoparticles were shown to be smaller. Sarmento et al. [16] obtained sizes ranged between 700 and 4000 nm, Zhang et al. [29] had a mean size around 750 nm for insulin-loaded alginate/chitosan nanoparticles and Goycoolea et al. [30] developed nanoparticles with diameter values ranging between 200 and 300 nm. The same happens for other systems for encapsulation of vitamin B₂, e.g. El-Ghaffar et al. [31] obtained particles with significantly higher sizes (1.40 ± 0.05 mm).

Zeta potential indicates if samples will have a good colloidal stability, being particles with zeta potentials above +30 mV or below -30 mV usually considered as being stable [32]. Besides that, zeta potential also provides information on the surface charge of nanoparticles. The values obtained were -30.9 ± 0.5 and -29.6 ± 0.1 mV for nanoparticles without and with vitamin B₂, respectively, showing that nanoparticles have a good colloidal stability. The zeta potential values are significantly different ($p < 0.05$) showing that vitamin-loading can influence the zeta potential of the nanoparticles; this behavior can be explained by the positive charge of vitamin B₂ in solutions with low pH [28].

3.2. Morphological observation

Fig. 2 shows that alginate/chitosan nanoparticles have a spherical shape being the particle sizes in the same order of magnitude of values obtained by DLS and NTA. Moreover, Fig. 2 shows that particles' size distribution was not homogeneous, in agreement with the PDI values obtained.

3.3. Encapsulation efficiency and loading capacity

The ability of alginate/chitosan nanoparticles to encapsulate vitamin B₂ was evaluated through the determination of encapsulation efficiency (EE) and loading capacity (LC). The values of EE and LC obtained were $55.9 \pm 5.6\%$ and $2.2 \pm 0.6\%$, respectively. Other systems have shown higher values of EE for vitamin B₂ (80–100%), however the size of those systems is significantly higher (1.4 ± 0.05 mm) when compared with the size of the particles

obtained in this work [31]. For other alginate/chitosan nanoparticles where different bioactive compounds (e.g. insulin) were encapsulated different results were observed: Zhang et al. [29] obtained EE between 58 and 80% and LC of 9.6%, however the mean size obtained were greater than the mean size obtained in the present work; Sarmento et al. [16] obtained EE values between 70 and 90% and LC in the range 5–15%, being the mean size of the particles between 700 and 4000 nm; and Goycoolea et al. [30] obtained EE values similar to those obtained in this work (~50%), but also in this case the particles size are in the nanometric scale (200 and 300 nm). This way, alginate/chitosan nanoparticles obtained in with this work presents good values of EE and LC.

3.4. Release profile

The release behavior of vitamin B₂ from alginate/chitosan nanoparticles was performed at 25 and 37 °C and at pH 2 and 7, aiming at understanding how these conditions could influence particles' behavior at different external environment conditions (e.g. gastrointestinal conditions and acidic and alkaline food products).

A mathematical model (LSM, Eq. (6)) was applied to the experimental data (Fig. 3), and it is shown that the model-generated values are in good agreement with vitamin B₂ release experimental data. This suggests that this model can be used to describe the physical mechanism involved in vitamin B₂ release from alginate/chitosan nanoparticles. From the parameters presented in Table 1 it can be seen that the transport mechanism involved in vitamin B₂ release does not involve Brownian motion alone, i.e. it does not strictly follow Fick's behavior, but is governed by both Fickian and relaxation phenomenon also called by Case II transport, with only one main relaxation of the nanoparticles. Also, regression analysis resulting of the LSM fitting showed that this model adequately describes the experimental data with relatively good regression quality ($R^2 > 0.90$) and that almost all parameters were estimated with good precision.

Distinct release behaviors were observed for the tested conditions; explained by alginate and chitosan chemical structure the strength of electrostatic interactions between biopolymers at different pH values can lead to different release behaviors. The pK_a value of alginate is around 3.5 [33], therefore at this pH the carboxylic groups are half protonated and consequently partially uncharged, which means that the electrostatic interactions between alginate and vitamin B₂ or chitosan are weaker [23]. When submitted to pH 7, a similar behavior can occur due to the pK_a (7) of chitosan [33]. This leads to a change of nanoparticles' structure that promotes the release of vitamin B₂ due to polymer relaxation.

Regarding the Fick's component of transport, the total mass release from nanoparticles by Fick's mechanism (M_F) is higher at 25 °C. This result can be explained by alginate and chitosan gelling properties, because the temperature is an external parameter that induces these properties. Alginate and chitosan can exhibit a solution or gel form when temperature is below or above 32 °C, respectively, being this temperature defined as the lower critical solution temperature (LCST) [34,35]. In addition to that, when a hydrophilic bioactive compound is loaded in alginate/chitosan particles and the temperature is below the LCST, the total mass of bioactive compound release can increase. However, when temperature is above the LCST, alginate and chitosan exhibit a gel form and preventing the release of the loaded component [34,36]. Considering the effect of pH on the mass release via Fick's mechanism, the experimental results show that for temperature 25 °C the M_F at pH 7 and 2 are similar. Nonetheless, at 37 °C the M_F is lower at pH 2 and this fact can be explained based on the effect of pH in vitamin B₂ and the biopolymers used. As already mentioned, the vitamin B₂ acquire a cationic form at pH 2 interacting strongly with negatively charged alginate which slow down the diffusion of vitamin

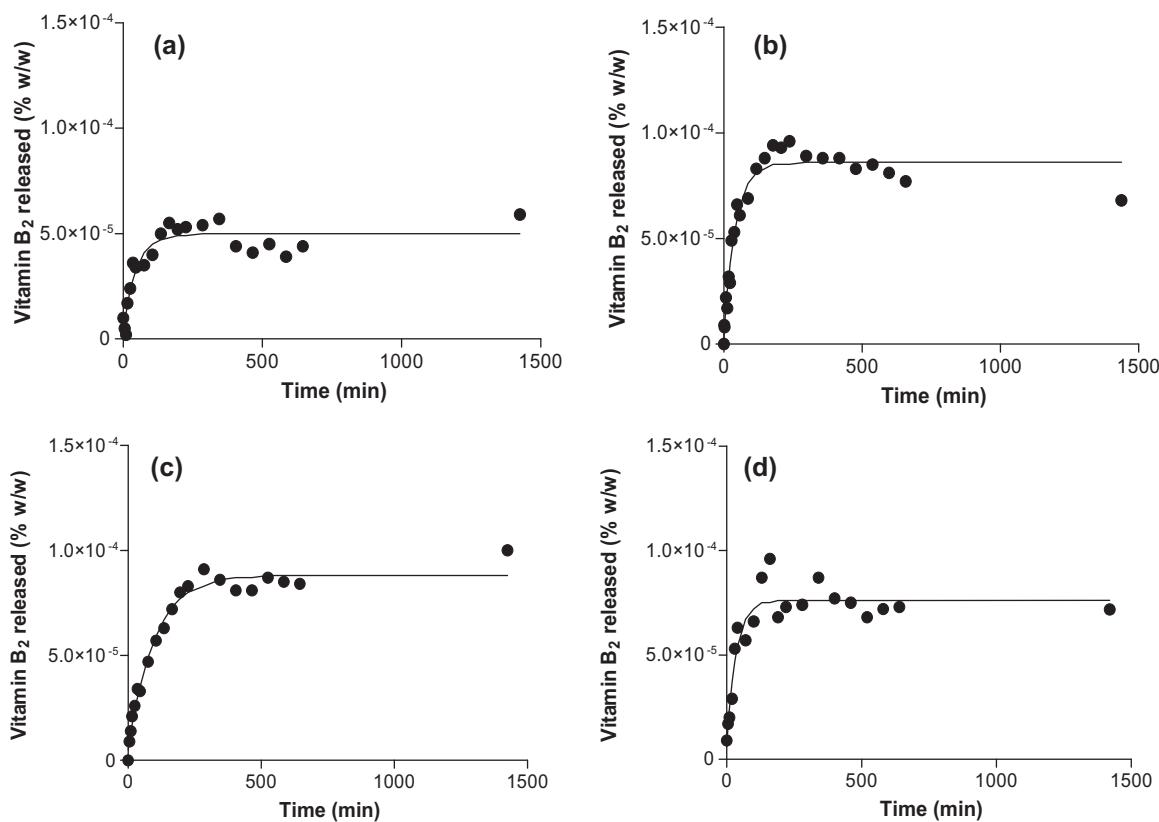


Fig. 3. Fitting of Eq. (6) to vitamin B₂ controlled release experimental data from alginate/chitosan nanoparticles ((·) experimental results and (—) model-generated values): (a) at 37 °C and pH 7; (b) at 37 °C and pH 2; (c) at 25 °C and pH 7; (d) at 25 °C and pH 2.

B₂ into dissolution medium and consequently M_F lower [28,31]. Other explanation for the lower M_F at pH 2 can be explained by the change of alginate structure at low pH's, which can lead to a shrink of the structure decreasing the release of vitamin B₂ encapsulated [37]. Nevertheless, the values of diffusion coefficient (D) determined through Fickian rate of diffusion (K_F , Eq. (7)) are very similar for all the tested conditions.

The total mass released from nanoparticles by relaxation (M_R) presents a similar behavior when compared with total mass release by Fick's, being higher at 25 °C; happening the same for the relaxation rate constant (K_R). Considering the effect of pH in relaxation parameters (M_R and K_R) it was observed that at 37 °C, M_R is higher at pH 2 and at 25 °C M_R is higher at pH 7 and for both temperatures the K_R value is higher at pH 2. When alginate/chitosan nanoparticles are placed in Tris-HCl buffer (pH 2), the negatively charged carboxylate groups of alginate begin to protonate to form uncharged –COOH groups. This reduces the degree of crosslinking due to the decrease of ionic and electrostatic interactions among the alginate and chitosan chains within the nanoparticles [23], this phenomenon may

result in the loss of nanoparticle structure with subsequent faster release of vitamin B₂.

When compared the parameters M_F and M_R , the experimental results have shown that for all tested conditions M_R is higher than M_F . Being so, it can be concluded that Case II of transport (i.e. relaxation) is the mechanism prevailing in release profile of vitamin B₂ from alginate/chitosan nanoparticles.

3.5. Stability

Fig. 4 shows the average diameter values and PDI of alginate/chitosan nanoparticles during 5 months. Results showed that vitamin B₂-loaded nanoparticles are more stable in size than nanoparticles without vitamin B₂, being the PDI of alginate/chitosan nanoparticles with vitamin B₂ lower ($p < 0.05$) than that of nanoparticles without vitamin. This fact can be explained by interactions between vitamin B₂ and biopolymers used; vitamin B₂, besides the ionic interactions that can be established with

Table 1

Fitting the non-linear model (Eq. (6)) to experimental data of the release profile. Evaluation of the quality of the regression on the basis of RMSE and R^2 .

T (°C)	pH	R ²	M_F	D (m ² /s)	M_R	K_R (min ⁻¹)
37	7	0.871	7.00E-06 (78.13%)	0.10E-12 (0.0%)	4.70E-05 (0.0%)	4.70E-05 (99.86%)
	2	0.950	5.00E-06 (80.77%)	0.15E-12 (25%)	8.40E-05 (0.0%)	2.40E-02 (21.26%)
25	7	0.980	1.30E-05 (0.0%)	0.45E-12 (0.0%)	8.31E-05 (0.0%)	1.02E-02 (18.06%)
	2	0.900	1.59E-05 (68.55%)	0.10E-12 (0.0%)	6.99E-05 (0.0%)	2.90E-02 (32.91%)

Estimates' precision is evaluated using the SHW% (in parenthesis). T, Temperature; M_F , equilibrium amount of sorption in unrelaxed polymer; M_R , Equilibrium sorption of the relation process; K_R , relaxation rate constant; D , diffusion coefficient.

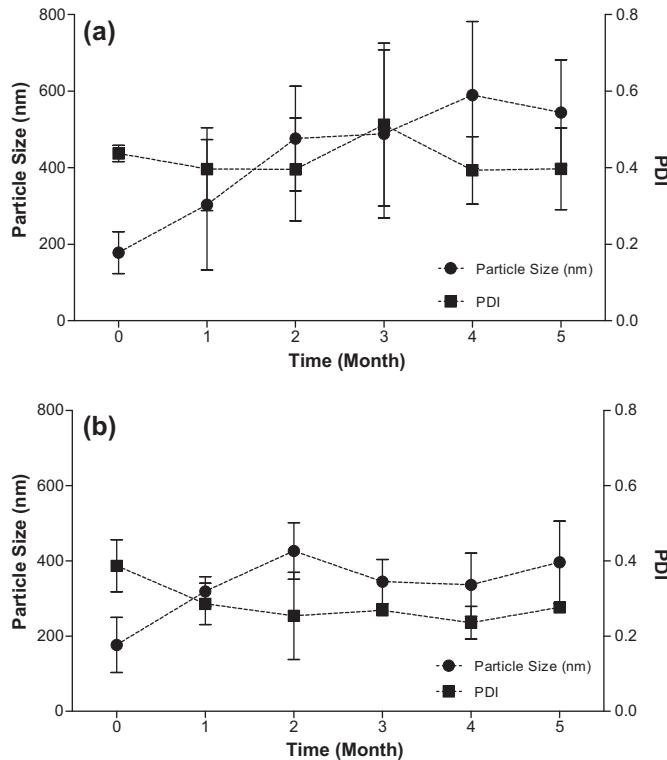


Fig. 4. Stability of alginate/chitosan nanoparticles: (a) without vitamin B₂; (b) with vitamin B₂.

alginate, can also interact via Van der Waals forces and hydrogen bonding with alginate and chitosan [16].

Nanoparticles were developed at pH 4.6 near the alginate pK_a value, being so the negatively charged carboxylate groups of alginate starts to protonate forming uncharged –COOH groups over time and the electrostatic interactions between alginate and chitosan became weaker [23]. As a result, the alginate can precipitate or aggregate [38] or the nanoparticle structure change [23] contributing to increase of the particle size.

It was also observed that the pH of medium increased from 4.6 to 5.4; this significant increase can be due to the change of alginate charge (explained above). This fact may also contribute to the instability of the nanoparticles and, consequently, the increase of nanoparticles' size [30,38].

Fig. 4 shows that alginate/chitosan nanoparticles without vitamin B₂ were more sensitive to alginate protonation and pH changes than alginate/chitosan nanoparticles with vitamin B₂, leading to higher values of size and PDI.

4. Conclusion

In this work has been demonstrated that encapsulation of vitamin B₂ in alginate/chitosan nanoparticles can be achieved using a pre-gelation ionotropic method, obtaining EE and LC values of ~55% and ~2%, respectively. The release profiles showed that alginate/chitosan nanoparticles could be used in the retention of Vitamin B₂, being this system stable for at least five months. This work indicates that these nanostructures may be used in different food and/or beverage products for human nutrition, while contributing to increase vitamin stability in food matrices.

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