CHAPTER 3 FOOD AREA

NANOENCAPSULATION OF RIBOFLAVIN IN BIODEGRADABLE POLYMERIC MATRICES USING NANOSPRAY DRYING

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Jaime Fonseca, M. R., Mancilla-Dávalos, V., López-Ordaz, P., & Yáñez-Fernández, J. (2023). Nanoencapsulation of riboflavin in biodegradable polymeric matrices using nanospray drying. In E. San Martín-Martínez (Ed.). *Research advances in nanosciences, micro and nanotechnologies. Volume IV* (pp. 141-154). Barcelona, Spain: Omniascience.

Abstract

In this work, riboflavin (vitamin B_2) was encapsulated in a matrix of alginate, chitosan, and a combination of both polymers, and the morphological properties of the nanocapsules and the release of riboflavin were studied. The nanocapsules were obtained through a nano spray dryer using alginate, and chitosan as protection or wall material, the concentration of the fed solution was 0.125 % (w/v) of each polymer with a 4 μm microfilter at a temperature of 120 °C. The structure of the nanocapsules was observed by Scanning Electron Microscopy (SEM), the SEM images were used to estimate the size of the nanocapsules by the image analysis methodology with ImageJ. The average size of the nanocapsules was 500-700 nm, observing a homogeneous spheroidal morphology. The interaction of the nanocapsules was determined using a nanozetasizer. The Z potential (ζ) of the alginate-chitosan nanocapsules ranged from 3.50 to 3.75 mV. The interaction of the carboxyl group (-COO) of alginate with the amino group ($-NH^{3+}$) of chitosan was observed by using FT-IR spectroscopy. In addition, the release of riboflavin was evaluated in an in-vitro system, showing that the alginate-chitosan mixture released riboflavin within the first 30 minutes.

Keywords: Nano drying, alginate, chitosan, nanoencapsulation, riboflavin, release.

1. Introduction

Vitamins are essential bioactive compounds for good health, they are defined as a group of micronutrients that are not usually synthesized by the human body [1]. Currently, there are more than 18 compounds classified as vitamins, divided into two large groups: fat-soluble (A, D, E, and K) and water-soluble such as thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9), cyanocobalamin (B_{12}) and C [2 − 4]. Vitamin deficiency can lead to serious diseases, such as scurvy, beriberi, ariboflavinosis, dermatitis, enteritis, and blindness, therefore, the consumption of vitamins is necessary to regulate metabolism and prevent diseases [5]. Riboflavin (vitamin B_2) is soluble in water and stable in acidic solutions [6 − 8] but can be degraded in alkaline solutions and is sensitive to light [9], non-toxic [10], and biocompatible [11]. The molecular formula of riboflavin is $C_{17}H_{20}N_4O_6$ and it consists of a heterocyclic isoalloxazine ring attached to the sugar alcohol ribitol, this molecule has a molecular mass of 376.36 g/mol (Figure 1). Riboflavin is used in food additives and supplements such as dyes and nutrients for human health [8], riboflavin is found in legumes, asparagus, spinach, lettuce, broccoli, cabbage, cereals, milk, eggs, and meat [12]. However, the obstacle that prevents the commercial use of riboflavin in the food industries its photosensitivity at a wavelength of 445 nm, causing a degradation of 79.9 % during the first 20 minutes [13, 14].

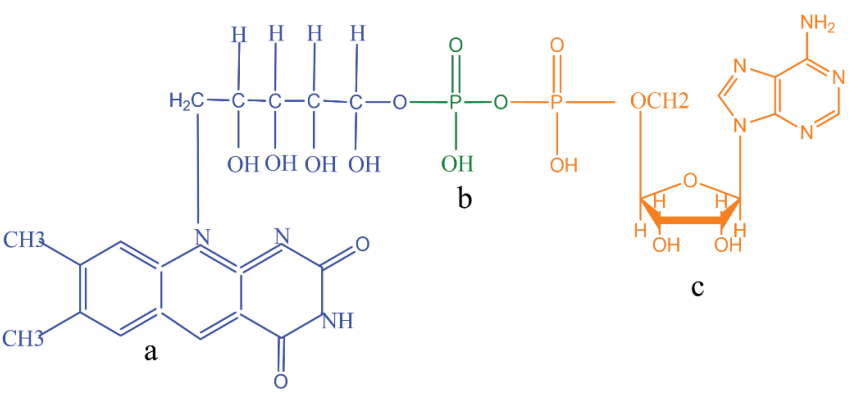

Figure 1. Chemical structure of riboflavin and its active forms, a) riboflavin; b) riboflavin phosphate (FMN); c) flavin adenine dinucleotide (FAD)

Encapsulation is a process used in the food and pharmaceutical industry to preserve bioactive compounds from environmental factors such as humidity, air, oxygen, light, and pH, increasing the useful life of the products [15]. The most

widely used biopolymers in the food and pharmaceutical industry are starch, pectin, maltodextrin, gelatin, alginate, cellulose acetate, ethyl cellulose, gum aarabic, K-carrageenan and chitosan [16 − 18]. Encapsulation can be carried out by nano spray drying, obtaining micro and nanometric scale particles. This technique is used in the food industry to transform liquids (solutions, suspensions, and emulsions) into solid powders such as milk, coffee, tomato, and glucose [19 − 22]. Nanoencapsulation performs nutrient entrapment at the nanoscale, providing much higher nutrient uptake and transport compared to microencapsulation [23, 24]. Therefore, the objective of this work was to develop riboflavin nanocapsules using alginate and chitosan as wall material. In addition, its morphology and particle size were characterized, and the controlled release of riboflavin was evaluated.

2. Methodology

2.1. *Materials and methods*

The materials used in the riboflavin nanoencapsulation were sodium alginate purchased by Sigma S.A. C.V. (Mexico), low-density chitosan, purchased from Sigma S.A. C.V. (Mexico), glacial acetic acid (ACS) provided by Fermont (Productos Químicos Monterrey S.A. C.V.) and riboflavin as active compound, purchased from Sigma S.A. C.V. (Mexico).

2.2. *Preparation of the solutions*

The solutions of the biopolymers from alginate and chitosan were prepared at a concentration of 0.125 % (w/v) separately, and then they were homogenized for 5 minutes using an ELMA brand (Germany) model D-78224 ultrasound equipment, with a frequency of 25 kHz. Finally, 0.185 mM M riboflavin was added.

2.3. *Riboflavin nanoencapsulation by nano drying*

Riboflavin nanoencapsulation was carried out using the Nano Spray Dryer B-90 brand equipment (BÜCHI, Switzerland), keeping the temperature at 120 °C according to the methodology reported by Kyriakoudi and Tsimidou [26]. The ratio of the concentration of alginate and chitosan was 0.125 and 0.25 % (w/v), respectively. The polymer solutions were fed to the nano dryer by a peristaltic pump at a flow rate of 7.5 ml/min using a nozzle of 4 μm.

2.4. *Zeta potential*

The Z potential (ζ) of the nanocapsules was determined using a Nano Zetasizer ZS unit (Malvern Instruments Ltd., Malvern, Worcestershire, UK), coupled to a Doppler laser velocimeter. The interaction of the nanocapsules was determined according to the methodology described by Porras *et al.* [27]. The samples were diluted to 43µL and placed in a cell with 2 electrodes that allowed the determination of the electrostatic charge of the particle.

2.5. *Characterization of nanocapsules by scanning electron microscopy (SEM)*

The morphology and size of the nanocapsules were analyzed using a scanning electron microscope (JSM-LV6390 JEOL, Japan). Samples were silver coated by sputtering (Desk IV, Denton Vacuum, USA) and viewed at 10,000 to 15,000X magnification establishing an accelerating voltage of 20 KV.

Furthermore, a study of nanocapsules stability was performed, changes were observed in the SEM and this process was carried out incorporating alginate and chitosan nanocapsules at different humidity environments of 33, 75 and 93 %, for 24 hours.

2.6. *Fourier Transform Infrared (FTIR)*

The FTIR spectra were obtained by means of a FTIR-spectrophotometer (Shimadzu Fourier Transform Infrared Spectrophotometer IRAffinity-1, Corporation Kyoto, Japan). For each sample 32 scans were performed in a range of wavelengths from 400 cm^{-1} to 4000 cm^{-1} and a resolution of 4 cm^{-1} using a 36X objective with a 2 mm diameter diamond crystal.

2.7. *Riboflavin release*

To release riboflavin, 0.015 g of the nanocapsules were taken and placed in a 2000 Dalton dialysis membrane, 11.5 mm in diameter, with a volume of 2.0 ml of distilled water. The riboflavin was released by adding a volume of 50 ml. The released concentrations of riboflavin were measured by UV-Vis spectroscopy (Multiskan GO from Thermo Scientist, Japan) at a wavelength of 200-450 nm following the modified methodology described by Abraham [28].

2.8. *Design of experiments and statistical analysis*

Experiments were performed using a 2k factorial design (k=3). Significant differences ($p<0.05$) were verified by ANOVA using Design Expert software (version 7.1.6, Minnesota, USA). All measurements were performed by triplicate.

3. Results and discussion

3.1. *Alginate-chitosan interaction at different pH*

The ζ potential is a measure of the stability of the nanocapsules, particles with a zeta potential greater than +30 mV and less than −30 mV are considered generally stable [29, 30]. In addition, the ζ potential also provides information on the surface charge of the nanocapsules. Figure 2 shows the effect of pH on the ζ potential of polymeric materials. In the alginate-chitosan solution, the ζ potential values reached their greatest interaction, allowing an adhered and resistant structure. The greatest interaction between alginate-chitosan was with an electrical potential of -35 mV for alginate and 35mV in chitosan at pH 3.3. These results contributed to the formation of nanocapsules as observed in (Figure 3). Azevedo *et al.* [30], reported similar values (−30.9±0.5 and −29.6±0.1 mV) for nanoparticles without and with riboflavin, respectively, indicating that from these values the nanoparticles have good stability [29]. The zeta potential values are significantly different ($p<0.05$), which shows that the riboflavin charge can influence the ζ potential of the nanocapsules; this behavior is generated by the positive charge of riboflavin in solutions at low pH [31].

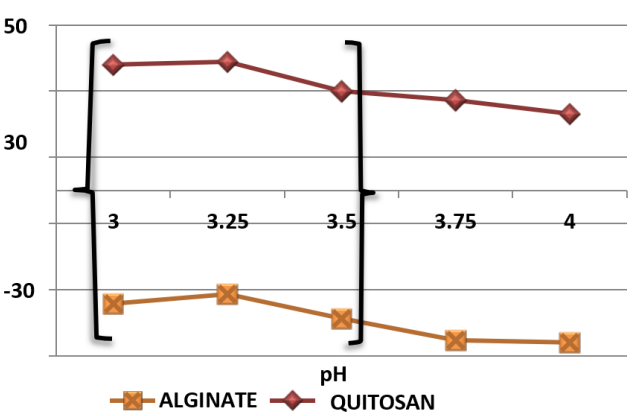

Figure 2. Variation of electrostatic charges as a function of pH for alginate-chitosan.

3.2. *Morphology of riboflavin (vitamin B_2) nanocapsules*

The nanocapsules were analyzed by SEM showing a spherical morphology (Figure 3) with an average size of 514.41±161.74 and 572.98±323.35 from alginate and chitosan, respectively. The average size of nanocapsules with riboflavin was 493.87±161.74 nm and 615.94 ± 168.80 nm when alginate and chitosan were used as a wall material, respectively, while alginate-chitosan-riboflavin nanocapsules were an average size of 621.06±270.94.

The sizes vary depending on the polymer that is being used, it is observed that when incorporating riboflavin, the size of the chitosan nanocapsules increases, however with alginate the size decreased, this may be due to a greater interaction of riboflavin and alginate, developing more compact nanocapsules after drying.

Compared to other works using similar biopolymers, the size of nanocapsules developed proved to be according to those reported by other authors, which range between 700 and 4000 nm [32, 33]. On the other hand, Goycoolea *et al.* [34] reported that insulin-loaded alginate/chitosan nanoparticles ranged from 200 to 300 nm. Furthermore, Sarmento *et al.* [35] indicated that when the pH decreases from 5.2 to 4.7, the mean particle size decreased. However, an important part of the alginate initiates an aggregation process which may contribute to the increase in mean particle size [35, 36]. Besides Goycoolea *et al.* [34] and Sarmento *et al.* [35], observed that the alginate load increase could contribute to the instability of the nanocapsules and, consequently, increase their size.

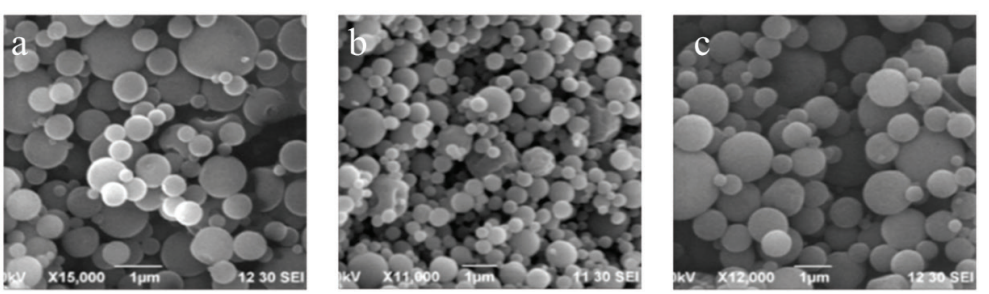

Figure 3. Micrographs of nanocapsules a) alginate; b) chitosan; c) alginate-chitosan.

On the other hand, Figure 4 shows the differences in the stability of the nanocapsules at different humidity levels (33 and 75 %) for 24 h. Therefore, it is observed that alginate and chitosan nanospheres are stable up to 75 % humidity in the first 24 hours of exposure, however at 93 % humidity the nanospheres of alginate (figure not shown) and chitosan were hydrated and agglomerated.

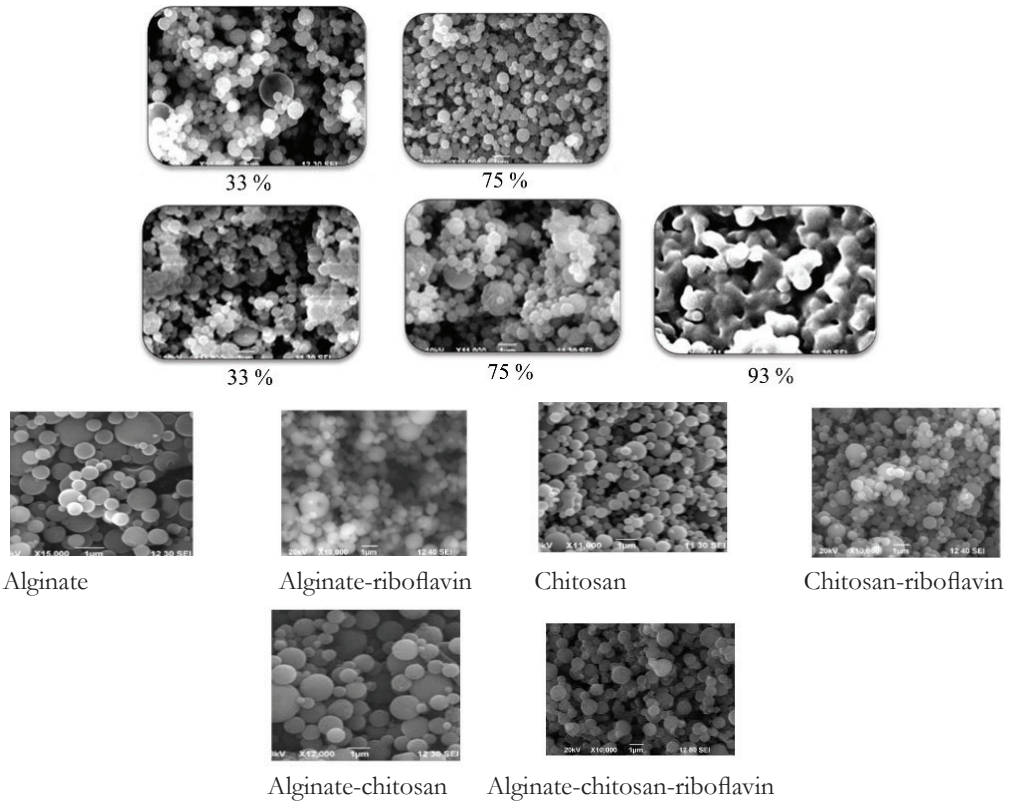

Figure 4. Micrographs of the nanocapsules at different humidity percentages after 24 h, stability analysis.

3.3. *FTIR*

FTIR was used to characterize the intermolecular interactions between alginate-chitosan-riboflavin. The spectra of the alginate-chitosan-riboflavin combination are shown in Figure 5. In the spectrum, the peaks of the carboxyl group of the alginate can be observed near 1605 cm^{-1} due to its symmetrical shape and stretch vibration (COO^-). However, at 1414 cm^{-1} the asymmetric presence and stretching vibration of the COO- are manifested. The chitosan spectrum shows a protonated amino peak at 1562 cm^{-1}, this peak agrees with that reported by Sarmento *et al.* [35] and Neiras *et al.* [38]. The chitosan- alginate interaction shows a displacement in the amino group at 1562 cm^{-1}, in addition, the presence of the carbonyl group (C=O) is observed due to the C6 stretching of the primary alcohol expressed in the 1069 cm^{-1} peak, this peak agrees with that reported by Thawatchai *et al.* [39]. Sarmento *et al.* [35] reported that chitosan under 75 % deacetylation conditions presents a spectral peak of 1641 cm^{-1} corresponding to the amide bond. The interaction of

alginate-chitosan in the nanocapsules was expressed in the 1579 cm^{-1} peak, characteristic of the amide groups that are formed in both polymers [39, 40]. The spectral peaks observed in the alginate-chitosan-riboflavin combination at 2916-3000 cm^{-1} are attributed to the stretching vibration of the –OH group. This peak often overlaps with the N- H bond of the amino group. The peaks observed ranged from 1579, 1410, and 1033 cm^{-1} and are attributed to C-O stretching vibration asymmetry and C-O symmetry of chitosan, respectively [41].

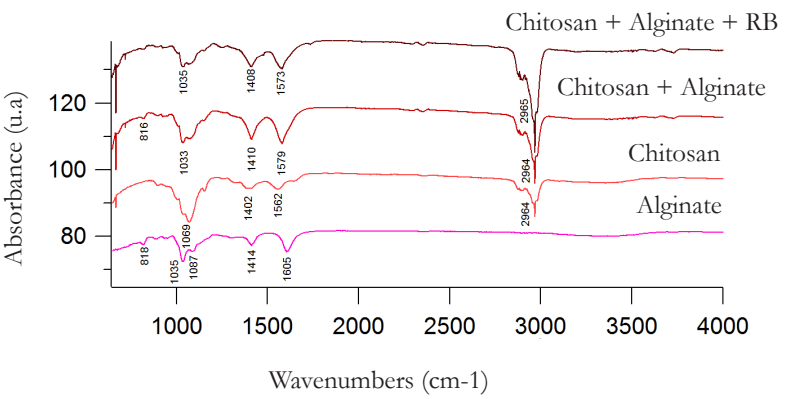

Figure 5. Infrared spectra of the alginate-chitosan-riboflavin combination.

3.4. *Riboflavin release*

Riboflavin release evaluation was performed for each biopolymer: alginate, chitosan, and their combination, separately. Release of alginate-encapsulated riboflavin began within the first hour (Figure 6a). After the first hour, a linear release trend was observed until the third hour, therefore, no significant change was observed, achieving a stable concentration in the solution within a release rate of 0.1215 mM/min. In the chitosan nanocapsules (Figure 6b) the release of riboflavin was observed after 20 minutes and after three hours the highest riboflavin release rate was reached (0.2064 mM/min). Likewise, in the biopolymer mixture (Figure 6c) a linear trend was observed starting at 30 minutes up to 3 hours and later reaching a maximum release rate of riboflavin of 0.1746 mM/min.

According to Azevedo *et al.* [30], in the transport mechanism for the release of riboflavin, not only the Bro5wnian movement is involved, that is, it does not follow the Fick behavior, but it is more related to the relaxation phenomenon, also called Case II transport. Thomas *et al.* [37] proposed a strong dependence of the solubility parameter and diffusion coefficient on concentration. However, they recognized that some independent material property must control the

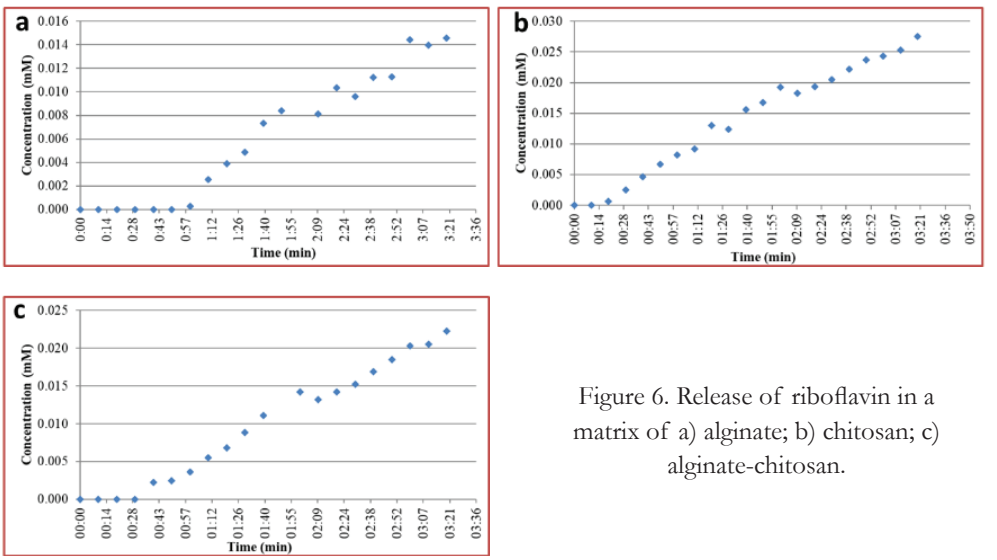

Figure 6. Release of riboflavin in a matrix of a) alginate; b) chitosan; c) alginate-chitosan.

velocity of the front and suggested time-dependent rupture and disentanglement of molecular chains as possible processes. This phenomenon may result in the loss of the structure of the nanocapsules with a more rapid release of riboflavin. When alginate/chitosan nanoparticles are mixed, the negatively charged carboxylate groups of the alginate begin to protonate to form uncharged COOH groups. It reduces the degree of electrostatic interactions between the alginate and chitosan chains within the nanocapsules [32, 37].

4. Conclusions

In this work, it was possible to form nanocapsules with homogeneous spheroidal morphology with a size of 500-700 nm through nano spray drying. Chitosan nanocapsules showed the fastest release of riboflavin with a release rate of 0.206 mM/min, compared to the mixture of alginate-chitosan. On the other hand, the alginate-chitosan nanocapsules presented a higher retention at 30 minutes with retention (0.175 mM/min). It indicates that a mix of alginate-chitosan allows a more delayed release than chitosan nanocapsules.

Acknowledgment and Funding

The authors thank CONACYT for the scholarship awarded, as well as the financial support provided for project 2542765 in the 2022 academic modality postdoctoral stay.

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