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PREPARATION OF CHITOSAN NANOPARTICLES MODIFIED WITH SODIUM ALGINATE WITH POTENTIAL FOR CONTROLLED DRUG RELEASE

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ABSTRACT

Chitosan nanoparticles modified with sodium alginate (CA) were synthesized using an ionic gelation method with pentasodium tripolyphosphate (STPP) as a crosslinking agent to evaluate their behavior as a drug carrier. Four samples were prepared (CA_1-CA_4) with different proportions of modifier agent alginate (0.5 and 1 mg / ml) and crosslinker STPP (1.5 and 2 mg / ml), maintaining a fixed concentration of chitosan (2.25 mg / ml). These nanoparticles were suspended in biological buffers to emulate the basicity and acidity conditions of the human gastrointestinal tract (pH 7.4 and 1.2 respectively). Hydrodynamic sizes of the synthesized nanoparticles were determined using dynamic light scattering (DLS). Based on these measurements, a hydrodynamic diameter of 152 nm was estimated for the best combination of chitosan-alginate-STPP. Tests were conducted to measure the encapsulation and controlled drug release capacity of the synthesized nanoparticles using rhodamine B as a tracer. From this evaluation, an encapsulation capacity of approximately 52% and values of dye release of 36% (pH 7.4) and 46% (pH 1.2) were observed, suggesting the potential of these nanoparticles for biomedical applications.

KEYWORDS: Nanoparticles, chitosan, alginate, ionic gelation, rhodamine, biomedicine.

PREPARACIÓN DE NANOPARTÍCULAS DE QUITOSANO MODIFICADAS CON ALGINATO DE SODIO CON POTENCIAL PARA LA LIBERACIÓN CONTROLADA DE MEDICAMENTOS

RESUMEN

Nanopartículas de quitosano modificadas con alginato de sodio (QA) fueron sintetizadas por el método de gelación iónica usando como agente entrecruzante tripolifosfato pentasódico (TPP) con el propósito de evaluar su comportamiento como excipiente de medicamentos. Se prepararon cuatro muestras de partículas (QA_1-QA_4) con diferentes proporciones del agente modificante alginato (0,5 y 1 mg/mL) y entrecruzante TPP (1,5 y 2 mg/mL), con una concentración fija de quitosano

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(2,25 mg/mL). Estas nanopartículas fueron suspendidas en *buffers* biológicos para representar las condiciones de basicidad y acidez del sistema gastro-intestinal humano (pH 7,4 y 1,2 respectivamente). El tamaño hidrodinámico de las nanopartículas fue determinado a través de un análisis de dispersión de luz dinámica (DLS). A partir de estas mediciones se estimó un diámetro hidrodinámico de 152 ± 68 nm para la mejor combinación de quitosano-alginato-TPP. Se realizaron pruebas para medir la capacidad de encapsulación y liberación controlada de medicamentos de las nanopartículas sintetizadas usando rodamina-B como trazador. A partir de esta evaluación se observó una capacidad de encapsulamiento del 52 % y valores de liberación de la molécula trazadora del 36 % (pH 7,4) y 46 % (pH 1,2), sugiriendo así el potencial de estas nanopartículas para aplicaciones biomédicas.

PALABRAS CLAVE: nanopartículas, quitosano, alginato, gelación iónica, rodamina, biomedicina.

PREPARAÇÃO DE NANOPARTÍCULAS DE QUITOSANO MODIFICADAS COM ALGINATO DE SÓDIO COM POTENCIAL PARA A LIBERAÇÃO CONTROLADA DE MEDICAMENTOS

RESUMO

Nanopartículas de quitosana modificadas com alginato de sódio (QA) foram sintetizados pelo método de gelificação iónica como agente de reticulaçãotripolifosfatopentassódico (TPP), com o propósito de avaliar o seu desempenho como um excipiente de medicamentos. Se prepararam quatro amostras de partículas (QA_1-QA_4) com diferentes proporções de agente modificante alginato (0,5 e 1 mg / ml) e reticulação TPP (1,5 e 2 mg / ml), mantendo a concentração fixa de quitosano (2,25 mg / mL). Estas nanopartículas foram suspensas em buffers biológicos para representar as condições de basicidade e acidez do sistema gastrintestinal humano (pH 7,4 e 1,2 respectivamente). O tamanho hidrodinâmico das nanopartículas foi determinado por uma análise de dispersão de luz dinâmica (DLS). A partir destas medições se estimou um diâmetro hidrodinâmico de 152 nm para a melhor combinação de quitosana-alginato-TPP. Se realizaram testes para medir a capacidade de encapsulamento e libertação controlada de medicamentos das nanopartículas sintetizadas usando rodamina-B como um marcador de libertação. Deste Avaliação se observou uma capacidade de encapsulação Os valores de libertação de 52% e valores de libertação da molécula do marcador de 36% (pH 7,4) e 46% (pH 1,2), sugerindo assim o potencial destas nanopartículas para aplicações biomédicas.

PALAVRAS-CHAVE: Nanopartículas, quitosano, alginato, a gelificação iónica

1. INTRODUCTION

The development of new materials that respond to pharmaceutical requirements, such as increased selectivity of inert materials to improve drug delivery in affected areas, thereby avoiding the side effects caused by the main active ingredients in the medication, has increased the use of biopolymers such as polysaccharides, liposomes, and polyanhydrides, among others (Yang *et al*., 2015). They are a promising platform for drug release due to their excellent properties, such as stability, a high capacity to combine and release therapeutic macromolecules, and bioadhesion, which allows them to pass through epithelial barriers (nasal, intestinal, and ocular treatments) (Goycoolea *et al*., 2009). In addition to being highly safe, biodegradable, non-toxic, and biocompatible, polysaccharides are abundant in nature, and processes to extract them incur low costs (Shukla *et al*., 2013). They are polymeric carbohydrates whose structure includes repeated monosaccharide units connected by glucoside bonds. Since they have a high number of reactive groups in molecular chains, such as hydroxyl, carboxyl, and amino groups, they are easy to chemically and biochemically modify for use as drug carriers (Goycoolea *et al*., 2009).

One of the most widely studied polysaccharides is chitosan, which is obtained through deacetylation of the chitin found in the shells of shrimp. Chitin is the second-most abundant polysaccharide on Earth (Shukla

et al., 2013). Chitosan is a cationic polyelectrolyte which, in addition to the characteristics mentioned above, also shows antimicrobial properties and responds to external stimuli such as pH, temperature, and the medium's ionic strength, allowing for improved sensitivity of inert materials (Mohammed *et al*., 2013). Chemically, chitosan is composed of β-(1.4)-2-amino-2-deoxy-D-glucopyranose (deacetylated units) with or without minor residue of N-acetyl-D-glucosamine (acetylated units) (Chen *et al*., 2011). Another polysaccharide of interest is sodium alginate, which is an anionic polyelectrolyte extracted from brown algae and Pseudomonas and Azotobacter bacteria. Its properties allow it to be used as a gelling agent (Hay *et al*., 2013). Sodium alginate is chemically defined as a block copolymer made up of linear chains of D-mannuronic acid and L-guluronic acid, which are connected by 1→4 glocusidic bonds (Hay *et al*., 2013).

The use of these two polysaccharides to prepare nanoparticles guarantees that they may be used for drug release in areas which, given their size and the characteristics of the material, only these polysaccharides are suitable, broadening the margin for fighting cancer (Shukla *et al*., 2013; Raveendran *et al*., 2015). There are different methodologies for preparing the nanoparticles, including coprecipitation, chemical reticulation, thermal decomposition, coacervation, emulsification, and ionic gelation. The latter is of interest given that it is mainly used to prepare polysaccharide nanoparticles (Raveendran *et al*., 2015). Ionic gelation allows for crosslinking of polymer chains which are organized in nanostructures by intermolecular interactions which can be covalent or non-covalent and which can be achieved through the use of crosslinking agents that create this bond (Dong *et al*., 2013). This methodology avoids the use of processes requiring high temperatures for stabilization and toxic organic solvents, which can be costly (Dong *et al*., 2013; Raveendran *et al*., 2015).

The focus of this study was the preparation and characterization of biopolymer nanoparticles based on chitosan and modified with sodium alginate to evaluate their encapsulation capacity and release capacity of a model molecule (rhodamine B) as a function of the suspension medium's pH.

2. MATERIALS AND METHODS

2.1. Materials

The chitosan (85% deacetylation grade), rhodamine B (99%), and pentasodium tripolyphosphate (99%) were manufactured by the company Alfa Aesar. The sodium alginate was acquired from Danisco®. The reactives used to prepare the biological buffers, hydrochloric acid (37%), potassium acid phosphate (99%), sodium hydroxide, (99%), and potassium chloride (99%), were manufactured by Panreac.

2.2. Preparation of chitosan/alginate nanoparticles

The chitosan/alginate nanoparticles (CA) were prepared with the ionic gelation method, using pentasodium tripolyphosphate (STPP) as a reticulation agent (Goycoolea *et al*., 2009; Keawchaoon & Yoksan, 2011). **Table 1** shows the concentrations of chitosan, alginate, and STPP used to prepare the nanoparticles. The STPP and alginate solutions were made in distilled water, while the chitosan solution was prepared using an aqueous solution of 1% v/v acetic acid.

Table 1. Concentration of chitosan, alginate, STPP solutions used to synthesize biopolymer nanoparticles

SOLUTION	CONCENTRATION
Chitosan	2.25 mg/ml
STPP	2.0 mg/ml
	1.0 mg/ml
Alginate	0.5 mg/ml
	1.0 mg/ml

Using the concentrations shown in **Table 1**, four alginate/STPP solutions were prepared. 3 ml of each were then taken to be added at room temperature to four samples of 9 ml of the chitosan solution, maintaining constant agitation of 700 rpm for 10 minutes (Goycoolea *et al*., 2009). After the synthesis step, the nanoparticles were separated through centrifugation at 5500 rpm for 30 minutes, washed in deionized water, and lyophilized until a dry product was obtained. **Table 2** shows the different ratios of alginate-STPP used to synthesize the biopolymer nanoparticles.

Table 2. Alginate-STPP ratio used to synthesize nanoparticles based on chitosan (CA) using ionic gelation

Sample	Concentration (mg/ml)	
	Alginate	STPP
CA_1	0.5	1.5
CA_2	0.5	2.0
CA_3	1	1.5
CA_4	1	2.0

2.3. Preparation of chitosan/alginate nanoparticles with rhodamine B

In a typical preparation procedure, the model molecule rhodamine B was added to the STPP base solutions described in **Table 2** until a concentration of 0.06 mg/ml was reached. The sodium alginate solution was later added according to the ratios indicated in **Table 2**, and the chitosan/alginate nanoparticles were then synthesized following the synthesis steps described above. After the synthesis phase, the chitosan/alginate nanoparticles loaded with rhodamine B were separated by centrifugation at 5500 rpm for 30 minutes. They were then washed three times with deionized water to eliminate the free rhodamine B and lyophilized until a dry product was obtained.

2.4. Determination of hydrodynamic size and evaluation of encapsulation/release capacity of biopolymer nanoparticles

Dynamic light scattering (DLS) measurements were made using a Horiba LB-550 at room temperature to determine the hydrodynamic size of the chitosan nanoparticles modified with sodium alginate (CA). To do so, samples of the nanoparticles were suspended in biological buffers prepared at pH 1.2 and 7.4. These pH values were selected in order to simulate the acidity and basicity conditions that the particles could undergo in the gastrointestinal system. The basic buffer (pH 7.4) was prepared by mixing 100 mL of a potassium acid phosphate solution [0.1 M] and 78.2 mL of a sodium hydroxide solution [0.1 M]. The acid buffer (pH 1.2) was obtained by mixing 50 mL of a potassium chloride solution [0.2 M] and 85 mL of a hydrochloric acid solution [0.2 M] (Keawchaoon & Yoksan, 2011).

To determine the biopolymer nanoparticles' encapsulation capacity, two calibration curves were created for concentration of rhodamine B versus absorbance as a function of the suspension medium's pH. These measurements were made using a UV-Vis Spectro UV-2650 spectrophotometer at a wavelength of 554 nm, which has been reported in the literature to determine type-B rhodamine in an aqueous solution (Peng, Voelcker, Kumar & Griesser, 2007). To plot said curves, two stock solutions of rhodamine B were prepared in the biological buffers at a concentration of 0.03 mg/ml. Once the calibration curves were complete, the chitosan-alginate particles which showed the smallest hydrodynamic size were selected. They were then loaded with rhodamine B as is described above. Next, the resulting supernatant from the centrifugation isolation of the loaded nanoparticles was taken, and a UV-Vis spectrophotometer reading was made to determine the non-encapsulated rhodamine B concentration. Based on this value, and knowing the initial quantity of rhodamine B added to the system, the percent encapsulation of the nanoparticles was calculated using **Equation (1)** (Keawchaoon & Yoksan, 2011):

$$\text{Encapsulation}(\%) = \frac{\text{Rhodamine mass– B encapsulated}}{\text{Rhodamine mass – B initial}} * 100 \qquad (1)$$

To determine the rhodamine B release capacity, the nanoparticles loaded with the dye were suspended in the biological buffers and placed in an incubator at 37°C with constant agitation at 150 rpm for a period of 48 h. During this process, 5 mL aliquots of the solution were taken at different time intervals and then immediately replaced with an equal amount of fresh buffer solution (Goycoolea *et al.*, 2009). Each aliquot taken was centrifuged at 5500 rpm for 10 minutes, then the supernatant was analyzed in the UV-vis spectrophotometer to determine its absorbance, which indicated whether the model molecule was released from the nanoparticles as an effect of the suspension medium's pH (Makhlof *et al.*, 2011). The percent of rhodamine B released was calculated using the following equation (Keawchaoon & Yoksan, 2011):

$$\text{Rhodamine – B Liberated}(\%) = \frac{m_{\text{rhodamine (t = 0)}} - m_{\text{rhodamine (t = n)}}}{m_{\text{rhodamine (t = 0)}}} \qquad (2)$$

Where:

$m_{rhodamine\ (t = 0)}$: Mass of rhodamine B contained in the nanoparticle at time 0.

$m_{rhodamine\ (t = 0)}$: Mass of rhodamine B contained in the nanoparticle at time n.

3. RESULTS

3.1. Hydrodynamic size

Figure 1 shows the frequency histograms obtained for the hydrodynamic size measurements of the chitosan-alginate (CA) particles suspended in a biological buffer at pH 7.4. Based on these measurements, it was observed that the particles with smaller hydrodynamic sizes were obtained using the lowest concentration level of alginate (0.5 mg/ml) and of the crosslinking agent STPP (1.5 mg/ml) with an average hydrodynamic size of 152 nm for the CA_1 sample at pH 7.4. The CA_2, CA_3, and CA_4 samples showed average hydrodynamic sizes of 396 nm, 725 nm, and 670 nm, respectively.

The ionic gelation method leads to the formation of particles due to electrostatic interactions from the dissociation of the reagents in the aqueous solutions. During the process, the following groups appear: $-NH_3^+$ belonging to the chitosan structure, $P_3O_{10}^{5-}$ and $HP_3O_{10}^{4-}$ belonging to the STPP structure, and $-COO^-$ belonging to the alginate (Goycoolea *et al.*, 2009). The success of this crosslinking depends on the negative charge of the functional groups of the STPP and the positive charge of the amino groups in the chitosan. The results provided by the DLS analysis show high values for hydrodynamic size for each suspension, caused by the high availability of crosslinking groups present in the suspension. It was observed that the amount added to the reagents participating in the process affected the hydrodynamic size of the different preparations, and when the data was compared, the following behavior is observed: $CA_3>CA_4>CA_2>CA_1$, determined at pH 7.4. This results show the trend among the different concentrations of each preparation.

The preparation CA_1 showed lower values of alginate and STPP concentration for the formation of nanoparticles, which were observed in the results obtained from the DLS reading. CA_1 was therefore profiled as the most appropriate for application in medical treatments for various illnesses that require completely free circulation of the nanoparticles through the body for selective and controlled release of the medication given its smaller size, as well as the advantages of biopolymer particles such as their good compatibility, bioadsorption, and low side effects (Masalova, *et al.*, 2013).

Chitosan is a polycationic polysaccharide sensitive to conformational changes such as the effect of the pH of the suspension medium (Harris *et al.*, 2008). This is due to the fact that its chemical structure contains amino groups and hydroxyl groups, which can form hydrogen bonds with the water molecules that surround it in an aqueous solution. When the chitosan particles are dispersed in an acid medium, the hydrogen bonds can dissociate due to the protonation of the amino groups, causing the particle to swell and thereby increasing its hydrodynamic size (Cerchiara *et al.*, 2015). In order to corroborate the change in hydrodynamic size of the synthesized chitosan-alginate particles as a function of the suspension medium's pH, samples of these particles were suspended in the buffer prepared at pH 1.2. **Figure 2** shows the results obtained in the frequency histograms of the hydrodynamic size presented by the particles in the acid medium. Based on this analysis, a significant increase was observed in the hydrodynamic size of all of the samples, estimating average sizes of 585 nm for sample CA_1, 527 nm for sample CA_2, 1046 nm for sample CA_3, and 926 nm for sample CA_4.

3.2. Encapsulation capacity

Since the concentration ratio between alginate and STPP used to prepare sample CA1 was the one that showed the smallest hydrodynamic size at pH 7.4, these same proportions were used to prepare the nanoparticles loaded with the dye rhodamine B. In order to detect the percentage of rhodamine B encapsulated in the biopolymer nanoparticles, the absorbance of a sample of supernatant from the centrifugation of the nanoparticles loaded with rhodamine B was determined using the UV-Vis at a wavelength of 554 nm. Using the calibration curve created for the absorbance of rhodamine and **Equation (1)**, the percent encapsulation of the dye was estimated in triplicate. **Table 3** provides a summary of these results, based on which an average value of 51.8% ± 0.27 of rhodamine B encapsulation was estimated in the chitosan-alginate nanoparticles.

Figure 1. Frequency histograms at pH 7.4 for hydrodynamic size readings of particles based on chitosan and modified with sodium alginate. a) sample CA_1; b) CA_2; c) CA_3; and d) CA_4.

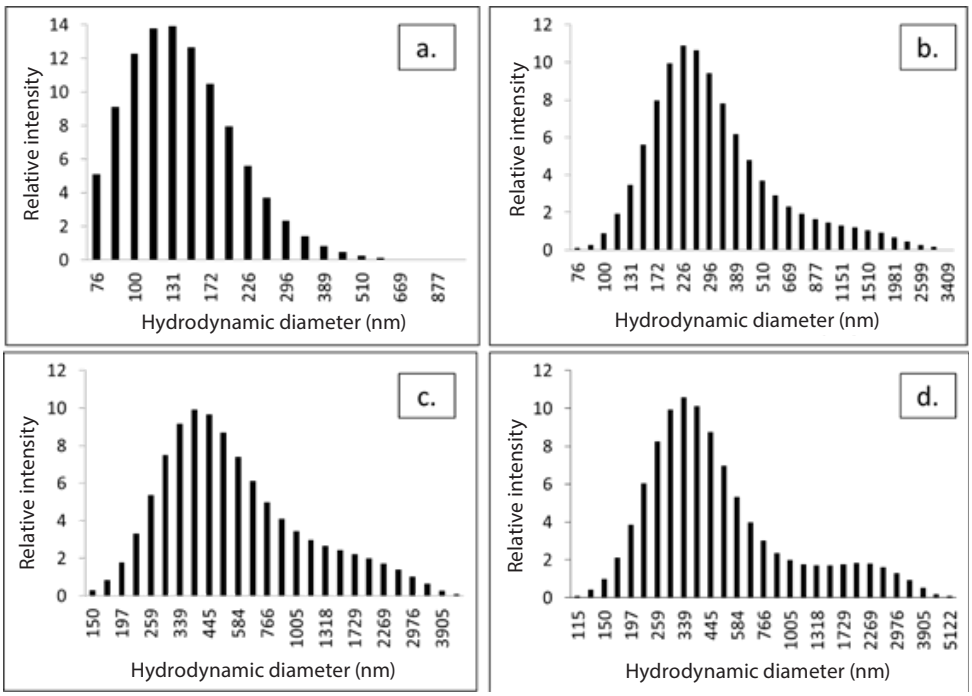


Figure 2. Frequency histograms at pH 1.2 for hydrodynamic size readings of chitosan-alginate particles. a) sample CA_1; b) CA_2; c) CA_3; and d) CA_4.

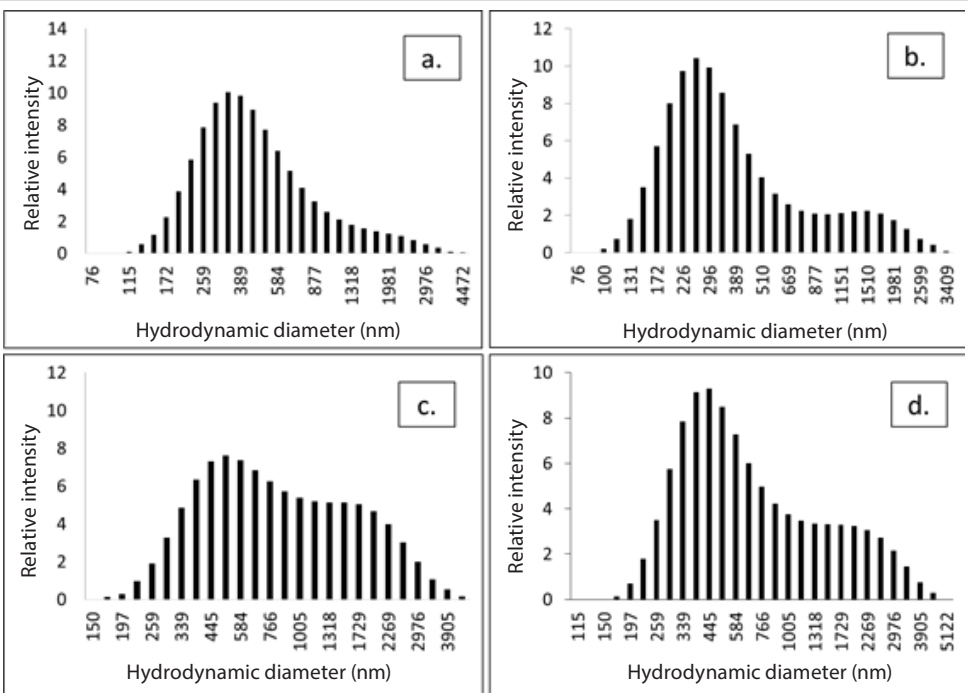


Table 3. Percent encapsulation

Supernatant absorbance	Supernatant concentration (mg/ml)	% encapsulation
2.790	0.028796	52.01
2.793	0.028880	51.86
2.800	0.029079	51.53

Rhodamine B is a compound used as a tracer, and its structure contains carboxylic groups which are easily protonizable and show a predominant form in acid conditions of keeping the ring open, allowing for repulsion from the protonizable amino groups in the chitosan's structure since the medium used for nanoparticle preparation is acidic given the quantity of acetic acid used to make the chitosan soluble. These repulsions cause the low effectiveness of encapsulation. However, the values reported in this study match those reported by other researchers. In 2009, Goycoolea *et al*. reported insulin encapsulation percentages between 47% and 52% for chitosan and alginate nanoparticles prepared using the same method (Goycoolea *et al*., 2009), while Mukhopadhyay *et al*. obtained 85% insulin encapsulation (Mukhopadhyay *et al*. 2015).

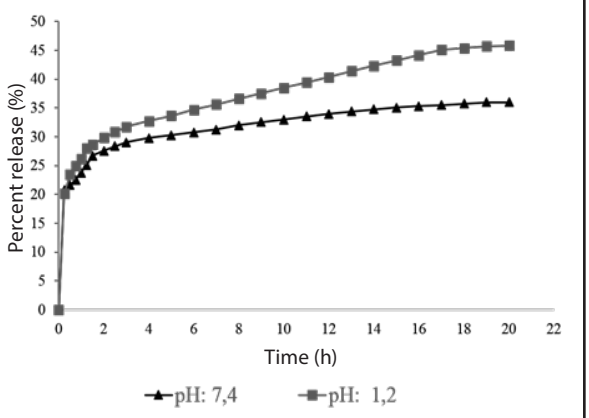
3.2. Liberation capacity

The nanoparticles loaded with rhodamine B were prepared to study the controlled release of a model molecule. After they were synthesized, they were washed with 150 ml of distilled water, then centrifuged at 5500 rpm. Finally, the absorbance of the supernatant was read. This process was repeated until the absorbance read was approximately zero, guaranteeing the non-presence of rhodamine B, which had adhered to the surface of the nanoparticle. The nanoparticles were suspended in the buffer solutions at pH 7.4 and 1.2, and the steps cited in section 2.3 were followed. The data obtained were tabulated and interpreted in the dispersion graph in **Figure 3**.

Figure 3 shows that chitosan nanoparticles modified with alginate (CA_1) are sensitive to the pH of the medium in which they are suspended since controlled release of the model molecule was achieved, reaching 36% release of the initial quantity of encapsulated rhodamine in the suspension medium whose pH was 7.4, and 46% release in the suspension medium whose pH was 1.2, after 20 hours of analysis. During the first 5 hours, it was observed that the release rate in both mediums was high, with more rhodamine B released at pH 1.2 during that period. These data show that the flow of rhodamine B from the nanoparticle is favored by the difference of pH shown by the suspension medium. Therefore, the release speed was higher at the beginning of the analysis, and the longer the nanoparticles were left suspended in the same medium, the release speed began to decrease. It is probable that the release would be much greater at an acid pH due to the protonation presented in this medium of the rhodamine's carboxylic groups and the chitosan's amino groups, causing repulsions that favor release of the model molecule.

These data differ from those obtained by other researchers who, when releasing insulin in an acid medium, obtained a release value of 50.7% (Goycoolea *et al*., 2013), and when simulating a continuous gastrointestinal system, passing the nanoparticles through an acid medium and then a basic medium (pH 1.2 and 7.4, respectively), obtained a total value of 84% insulin release (Mukhopadhyay *et al*., 2015).

Figure 3. Release kinetics of rhodamine B encapsulated in the chitosan-alginate nanoparticles (CA_1) as a function of dispersion time in the biological buffers prepared at pH 7.4 and 1.2



4. DISCUSSION AND CONCLUSIONS

During the preparation of the chitosan nanoparticles modified with sodium alginate, it was clear that in order to obtain sizes smaller than 200 nm, low ratios of concentrations must be prepared when adding the crosslinking agent and the modifier agent given the formation of electrostatic complexes derived from the functional groups present. The nanoparticles prepared using the ionic gelation methodology showed a size of 152 nm for the preparation of CA_1, which is within the range reported in the majority of studies. This size is appropriate for use in medical treatments, which require that the nanoparticles be undetectable by the reticuloendothelial system, ensuring that in future in vivo research, it will remain in the bloodstream for a sufficient period of time.

The prepared nanoparticles allow for 52% encapsulation of the initial rhodamine, showing their retention capacity. This value allows chitosan nanoparticles modified with sodium alginate to be considered as a means of medication encapsulation.

The behavior of the release curve observed for these nanoparticles evidenced that they are sensitive to the pH of the suspension medium and can be used in medical treatment as a means of transportation and release of medications. Since the amino groups present in chitosan and the carboxylic groups present in rhodamine are protonized in acidic conditions, electrostatic repulsions are produced, which facilitate release of the loaded molecule in acidic conditions similar to gastric conditions.

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REFERENCES

Cerchiara T., Abruzzo A., Di Cagno M., Bigucci F., Bauer-Brandl A., Parolin C., Vitali B., Gallucci M., Luppi B. (2015) Chitosan based micro and nanoparticles for colon targeted delivery of vacomycin prepared by alternative processing methods. *European Journal of Pharmaceutics and Biopharmaceutics*, 92, pp. 112-119.

Chen, M., Liu Y., Yang W., Li X., Liu L., Zhou Z., Wang Y., Li R., Zhang Q. (2011), Preparation and characterization of self-assembled nanoparticles of 6-O-cholesterol-modified chitosan for drug delivery. *Carbohydrate Polymers*, 84, April, pp. 1244-1251.

Dong Y., Kiong Ng W., Shen S., Kim S., Tan R. (2013), Scalable ionic gelation synthesis of chitosan nanoparticles for drug delivery in static mixers. *Carbohydrate Polymers*, 94(2), May, pp. 940-945.

Goycoolea F., Lollo G., Rumañan-López C., Quaglia F., Alonso M. (2009). Chitosan-Alginate blended nanoparticles as carriers for the transmucosal delivery of macromolecules. *Biomacromolecules*, 10(7), pp. 1736-1743.

Hay I., Wang Y., Rehman Z., Rehm B., Moradali M. (2013). Microbial alginate production, modification and its applications. *Microbial biotechnology*, 6(6), August, pp. 637-650.

Harris R., Paños I., Acosta N., Heras A. (2008). Preparation and characterization of chitosan microspheres for controlled release of tramadol. *Journal of Controlled Release*, 132 (3), pp.e76.

Keawchaoon L., Yoksan, R. (2011). Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids and surfaces B: *Biointerfaces*, 84(1), May, pp. 163-171.

Makhlof A., Tozuka Y., Takeuchi H. (2011). Design and evaluation of novel pH-sensitive chitosan nanoparticles for oral insulin delivery. *European Journal of Pharmaceutical Sciences*, 42(5), April, pp. 445-451.

Masalova O., Kulikouskaya V., Shutava T., Agabekov V. (2013). Alginate and chitosan gel nanoparticles fore efficient protein entrapment. *Physics Procedia*, 40, pp. 69-75.

Mohammed M., Williams P., Tverezovskaya O. (2013). Extraction of chitin from prawn shells and conversión to low molecular mass chitosan. *Food Hydrocolloids*, 31(2), June, pp. 166-171.

Mukhopadhyay P., Chakraborty S., Bhattacharya S., Mishra R., Kundu P. (2015), pH-sensitive chitosan/alginate core-shell nanoparticles for efficient and safe oral insulin delivery. *International Journal of Biological Macromolecules*, 72, January, pp. 640-648.

Raveendran S., Palaninathan V., Nagaoka Y., Fukuda T., Iwai S., Higashi T., Mizuki T., Sakamoto Y., Mohanan P.V., Maekawa T., Kumar S. (2015), Extremophilic polysaccharide nanoparticles for cancer nanotherapy and evaluation of antioxidant properties. *International Journal of Biological Macromolecules*, 76, May, pp. 310-319.

Shukla S., Mishra A., Arotiba O., Mamba B. (2013). Chitosan-based nanomaterials: A state-of-the-art review. *International Journal of Biological Macromolecules*, 59, August, pp. 46-58.

Yang J., Han H., Zheng H., Dong H., Liu J. (2015), Preparation and application of micro/nanoparticles based on natural polysaccharides. *Carbohydrate Polymer*s, 123, June, pp. 53-66.

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