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CHARACTERIZATION OF SODIUM ALGINATE NANOEMULSIONS WITH *EUCALYPTUS CITRIODORA* ESSENTIAL OIL[1]

CARACTERIZAÇÕES DE NANOEMULSÕES DE ALGINATO DE SÓDIO COM ÓLEO ESSENCIAL DE EUCALYPTUS CITRIODORA

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ABSTRACT
The stability properties of nanoemulsions are influenced by emulsification conditions and the composition of formulations. Polymer nano-emulsions were prepared with the help of a mechanical homogenizer, in which five formulations of different concentrations were obtained in order to evaluate the stability with subsequent applications in controlled release systems. Viscosity, Particle Size and Encapsulation Efficiency were evaluated in order to obtain a stable emulsion. The N2 and N4 formulations, which were produced with 2:1 (N2) 1:2 (N4) oil and surfactant ratio and 1:1 (N2) 2:1 (N4) alginate and surfactant, stood out for having smaller Particle Size and more homogeneous size distribution. The N2 formulation obtained higher Encapsulation Efficiency, with a value of 68.2 ± 0.09%. It is assumed that the N2 nanoemulsion is the most favorable, because its formulation has a greater amount of surfactant making the interaction between the oil and the gum greater, thus having a better encapsulation capacity.

KEYWORDS: Alginate. Encapsulation. Essential oil.

RESUMO
As propriedades de estabilidade das nanoemulsões são influenciadas pelas condições de emulsificação e pela composição das formulações. Nanoemulsões poliméricas foram preparadas com auxílio de um homogeneizador mecânico, no qual foram obtidas cinco formulações de diferentes concentrações, no intuito de avaliar a estabilidade com posteriores aplicações em sistemas de liberação controlada. Foi avaliada a Viscosidade, o Tamanho de Partícula e a Eficiência de Encapsulamento visando obter uma emulsão

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estável. As formulações N2 e N4, que foram produzidas com 2:1 (N2) 1:2 (N4) razão óleo e surfactante e 1:1 (N2) 2:1 (N4) alginato e surfactante, se destacaram por apresentar menor Tamanho de Partícula e distribuição de tamanho mais homogêneo. A formulação N2 obteve maior Eficiência de Encapsulamento, com valor de 68,2 ± 0,09%. Supõe-se que a nanoemulsão N2 é a mais favorável, pois sua formulação possui uma maior quantidade de tensoativo fazendo com que a interação entre o óleo e a goma seja maior, assim tendo uma melhor capacidade de encapsulamento.

***PALAVRAS-CHAVE**: Alginato. Encapsulamento. Óleo Essencial.*

1. INTRODUCTION

Nanoemulsions (NE) are composed of mixtures of oils with surfactants that form emulsions with micellar size of 10-1000 nm, reflecting in their translucent aspect. As a result of their reduced size, they suffer less gravity, which gives them greater stability, and they present Brownian motion (DONS, FERRARI, 2016). NE are composed of three phases: aqueous phase and oil phase, often with the presence of an emulsifying agent acting at the interface, sometimes called surfactants or surfactants (CAPEK, 2004). structure of a nanoemulsion. Polymeric nanoemulsions have stood out due to their hydrophilic character, high water permeability, biocompatibility and tensile strength (BRUXEL, FERNANDA *et al.,* 2012). Natural polymers based on polysaccharides have been proposed as matrices for the formation of nanoemulsions, in order to improve the biocompatibility and biodegradability of the systems and improve the stability of nanoemulsions.

Some of these molecules, such as alginates, are able to interact with surfactant chains arranged around the oil droplets. (SALVIA-TRUJILLO, 2015). Eucalyptus is a plant belonging to the Myrtaceae family cultivated in all regions of Brazil, with about 800 known species. The species Eucalyptus citriodora has already been reported by several authors regarding its antimicrobial action (ELAISSI, *et al.*, 2011). The encapsulation of essential oils at the nanoscale represents a viable and efficient approach to increase the physical stability of bioactive compounds (WEISS *et al.,* 2009). EN appear as systems capable of effectively encapsulating, protecting and releasing these compounds (SALVIA-TRUJILLO and McCLEMENTS, 2016). The present work seeks an effective nanoemulsion in encapsulation for essential oil protection for application in controlled release systems.

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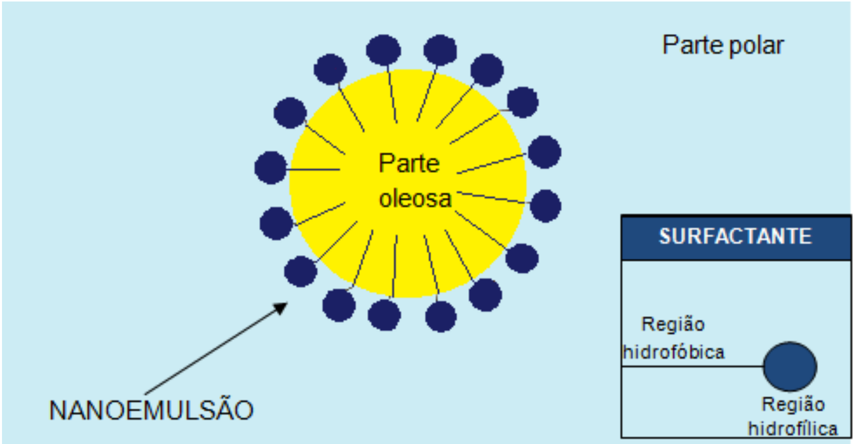
Figure 1- Structure of a nanoemulsion (SOURCE: Author's own)



2. Materials and methods

materials

Sodium Alginate (DINÂMICA), essential oil of Eucalyptus citriodora (FERQUIMA) was used, the surfactant used was Tween 80 ® . Water bath (CIENTEC), hot plates (QUIMIS), a high energy mechanical stirrer, the Ultra-stirrer (10,000-29,000 rpm) were used.

Preparation of nanoemulsions

Three types of sodium alginate solutions were prepared with different concentrations 2%, 1% and 0.5%. The nanoemulsions were prepared following the method of Fernandez *et al.,* (2004) with adaptations. The aqueous and oily phases were heated separately at a temperature of 75 ± 1°C, in a water bath (CIENTEC), then the oily phase was slowly poured into the aqueous with the aid of a syringe under variable agitation from 12,000 to 15,000 rpm. with the aid of a high energy mechanical stirrer (Ultra-stirrer) until the entire oily phase was reversed over the aqueous one, after this process the stirring continued at 22,000 rpm for 5 minutes, after this period of time the solution was taken to a magnetic stirrer until reaching room temperature (25 ± 5°C). Next, in Table 1, there are the relative formulations of the nanoemulsions.

Table 1 - relative composition of alginate nanoemulsions and citriodora eucalyptus essential oil and surfactant

Experimental Condition	sodium alginate	Surfactant	Oil	Surfactant and Oil	Alginate and Surfactant
N1	(2%) 100 mg	75 mg	75 mg	1:1	1.33:1
N2	(1%) 100 mg	100 mg	50 mg	2:1	1:1
N3	(1%) 100 mg	75 mg	75 mg	1:1	1.33:1
N4	(1%) 100 mg	50 mg	100 mg	1:2	2:1
N5	(0.5%) 100 mg	75 mg	75 mg	1:1	1.33:1

Characterizations of emulsions

The viscosity of the emulsions was evaluated in triplicate, through solutions with dilutions of 50%, 30%, 20%, 15% and 10%. The viscosity was measured by depositing the solution in an Otwald Viscometer, where the flow time was timed.

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The particle size of the emulsions was evaluated using the Zetasizer/Nanoseries 590 (MALVERN) device, with measurements in triplicate. The samples were diluted at a ratio of 1:100 and left under agitation for 24 hours before analysis.

The encapsulation efficiency of the emulsions was measured according to a modified methodology from Sebaaly *et al* ., (2015). Emulsion solutions were prepared in 1:4 ethyl alcohol, then the solutions were left to rest for 24 hours, there was a phase separation where the colorless part that represented the mixture of oil and alcohol was analyzed. The Encapsulation Efficiency (EE) was calculated from Eq. 1:

$$\frac{EE(\%)=M\,x100}{Mo} \qquad (1)$$

The determination was carried out by measuring the free concentration in the NE. It was determined by absorption spectroscopy in the UV-Vis region at a wavelength of 214 nm. A standard curve of Eucalyptus citriodora was prepared, so that it was possible to determine the concentration of oil in the medium through a calibration curve, represented by Eq. two:

$$Y=0.0024x + 0.0615 \qquad R^2=0.998 \qquad (2)$$

For the microscopic analysis, an optical microscope of the brand Olympus CX-31 was used, to observe the homogeneity and morphology of the dispersion. One drop of each formulation was placed on a glass slide for microscopy and covered with a coverslip. The slides were analyzed in 40x, 100x and 1000x objectives.

3. Results and discussion

Characterizations of Viscosity, Particle Size, Encapsulation Efficiency and Optical Microscopy were performed. It is expected that the lower the viscosity, the smaller the corresponding particle diameter and that the nanoemulsion has a greater oil retention. Figure 2 shows the flow time of the five samples that increase with the concentration. It can be noted that the nanoemulsions N2, N3 and N1 respectively have higher viscosity values, the higher the viscosity, the greater the stability and lifetime of the sample, so N2 has a better Viscosity profile. N2 containing a ratio of 2:1 surfactant and oil and 1:1 alginate and surfactant. Sample N5 has a lower viscosity value, which gives it instability. According to the literature, low-mass surfactants have high mobility at the interface, thus readily adsorbing to droplet surfaces, reducing the surface or interfacial tension between them (ARTIGA-ARTIGAS *et al.,* 2018).

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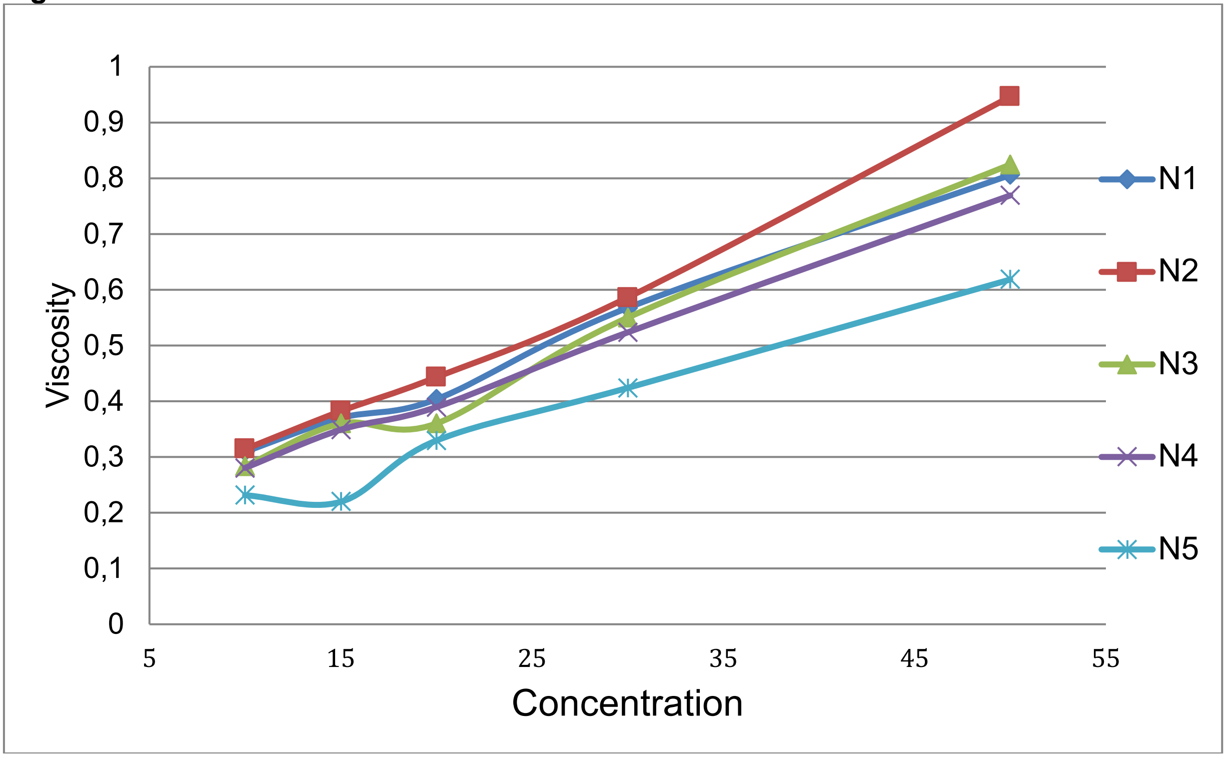
Figure 2- Flow time of nanoemulsions at different concentrations.



Optical microscopy testing was performed 8 weeks after sample preparation.

The analysis by optical microscopy allowed visualizing the morphology of the micelles, as shown in Figure 3. It is observed that among the systems studied, the systems (N1, N2, N3, N4, N5.) exhibited some homogeneity, it was possible to detect the micelles formed in different emulsification reactions and the presence of Brownian motion. Tween 80, due to its larger nonpolar chain, is able to make the stabilization interface between the oil and Alginate in a more successful way. (ABREU *et al* ., 2020).

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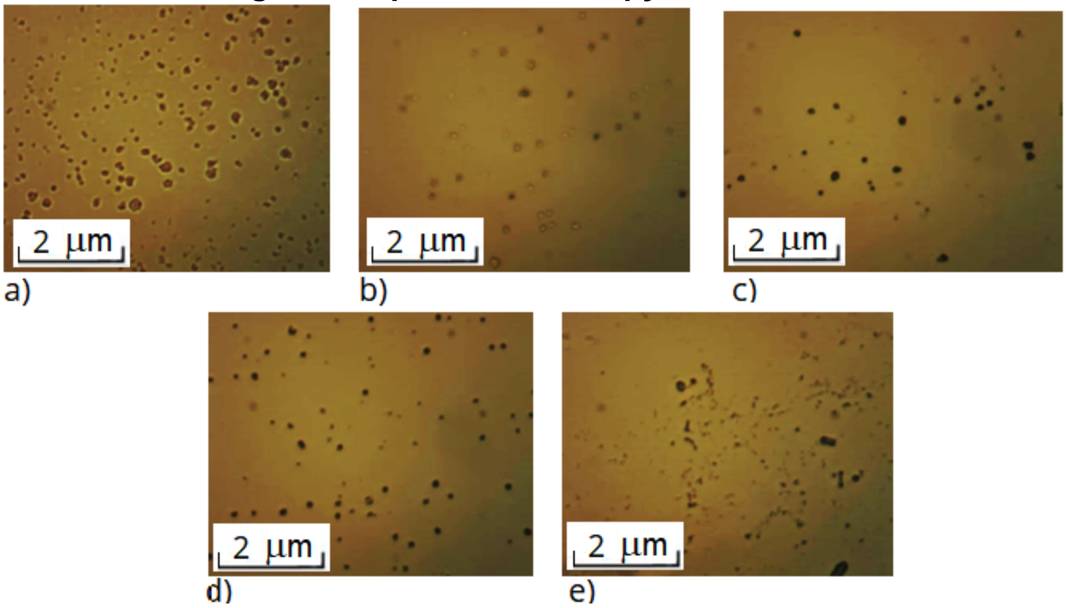
Figure 3: Optical Microscopy of Emulsions



a) N1 (1:1 Surfactant and Oil, 1.33:1 Alginate and Surfactant), b) N2 (2:1 Surfactant and Oil, 1:1 Alginate and Surfactant), c) N3 (1:1 Surfactant and Oil 1.33:1 Alginate and Surfactant), d) N4 (1:2 Surfactant and Oil, 2:1 Alginate and Surfactant), e) 1:1 N5 Surfactant and Oil. Alginate and Surfactant 1.33:1)

Regarding the particle size results, taking into account the experimental conditions and the proportion of each formulation, the following data shown in Table 2 was obtained .

Table 2 - Relationship between particle size, reaction conditions and proportion of oil, surfactant and gum.

Experimental Condition	Surfactant and Oil	Alginate and Surfactant	Particle Size (nm)	AND IS%
N2	2:1	1:1	313 (94.8%); 88.15 (5.2%)	68.2±0.09
N4	1:2	2:1	309.8 (100%)	41.0±0.1
N5	1:1	1.33:1	371 (80.8%); 5362 (19.2%)	54.6±0.07

As can be seen, the nanoemulsions showed a droplet diameter between 88.15 and 5362 nm, in agreement with the evaluated formulations. Table 2 shows the mean values of the peaks for the NE of the experimental conditions N2, N4 and N5. The NE N2 and N5 showed a bimodal distribution, with two peaks representing a portion of particles with different average size, with a small fraction of 88.15 nm (5.2%) and with a majority profile of 313 nm (94.8%). for N2. For N5, a fraction of 5362 nm (19.2%) and with a predominant profile of 371 nm (80.8%). NE N4, on the other hand, showed a smaller overall size in a unimodal distribution with an average particle size fraction of 309.8 nm.

The smallest droplet sizes were obtained in formulations N2 and N4 where it is observed that the surfactant Tween 80 was used in both. When Tween 80 is used, smaller particle sizes are obtained, which means that there was less coalescence or a lower degree of Ostwald maturation. The degree of encapsulation was calculated as a function of the oil content added in the emulsion and is shown in Table 1. The NE2 and NE5 samples stand out with values of 68.2 ± 0.09 and 54.6 ±0.07, respectively. These formulations have the highest amount of surfactant, which makes the interaction of the aqueous and oil phase greater than the other formulations, providing greater stability.

4. CONCLUSIONS

The sample that achieved the best results in the physicochemical tests was the NE2 nanoemulsion, as it has stable viscosity values, Particle Size between 88.15 and 313 nm and an Encapsulation Efficiency value greater than 68.0% as already discussed due to the higher amount of surfactant. Sodium alginate nanoemulsion with *Eucalyptus citriodora* essential oil proved to be a good alternative for encapsulation and for essential oil protection for use in delivery systems, providing stable nanoemulsions.

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