Journal of Engineering and Technology for Industrial Applications

ITEGAM-JETIA

Manaus, v.6 n.25, p. 43-47, Sep/Oct, 2020

DOI: https://doi.org/10.5935/jetia.v6i25.702

ISSN ONLINE: 2447-0228

RESEARCH ARTICLE OPEN ACCESS

BEHAVIOR OF BIOMATERIALS IN RELATION TO THE CONSERVATION TIME AND TEMPERATURE: STABILITY TEST OF A BIOPRODUCT

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ARTICLE INFO

Article History
Received: September 23th, 2020
Accepted: October 20th, 2020
Published: October 30th, 2020

Keywords:
Biomaterials,
Bioproducts,
Stability test,
Bioactive,
Evaluation parameters.

ABSTRACT

All products before reaching the consumer's table go through a battery of analyzes, in order to check if these materials resist the weather, temperature, climate changes, friction, including transportation. Whether they really support physical, chemical and biological factors and also with the idea of estimating the useful life of these materials. Biomaterials are no different, all bioproducts are also subjected to resistance tests to reach industry, or long-term production. Among the numerous possibilities that exist for the application of biomaterials, recently the bioactive ones encapsulated in polymeric nanoparticles of controlled action stand out with their several alternatives of application, among them as natural insecticides, aiming at the reduction of conventional pesticides, including fungicides, used in the activation and controlled release of the drug. Thus, this research aims to develop a formulation and verify its behavior as a function of time and temperature (25 °C), as well as the application of different preservatives. , [phenoxyethanol-2-methyl-2H-isothiazolin-3-one-NE), citric acid and thymol] in order to verify its influence on the stability of the formulations as a function of time. Among all tested formulations, the one containing the preservative NE was the only one approved in all parameters.

I. INTRODUCTION

All products before reaching the consumer's table go through a battery of analyzes, in order to check if these materials resist the weather, temperature, climate changes, friction, including transportation. Whether they really support physical, chemical and biological factors and also with the idea of estimating the lifetime of these materials [1]. Biomaterials are no different, all bioproducts are also subjected to resistance tests to reach industry, or long-term production.

Biomaterials have an expressive representativeness in the health area, not being restricted only to the development of mechanical devices (robots - prototypes), but also in the creation of bioproducts with controlled action [2].

Recently, it has become normal to hear the term biomaterials, which are those applied to biological and health issues. For the development of biomaterials (bioproducts), joint action is required between health professionals (doctors, dentists, physiotherapists and speech therapists) and professionals in the exact area (engineers, chemists, physicists and biologists).

Among the countless possibilities that exist for the application of biomaterials, recently the bioactive ones encapsulated in polymeric nanoparticles with controlled action stand out with their several alternatives of application, among which as natural insecticides, aiming at the reduction of conventional pesticides, fungicides, as well as medicinal application with controlled activation and release. Figure 1 shows an illustrative example of a natural asset covered (encapsulated) by a biodegradable polymeric layer.

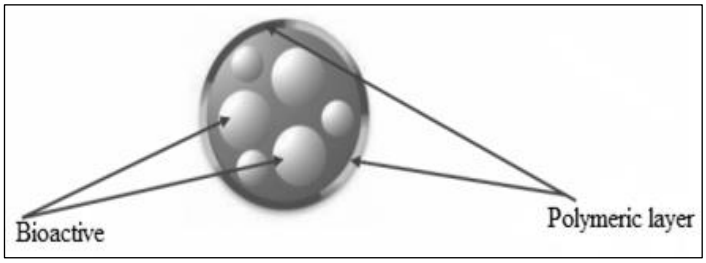

Figure 1: Illustrative image of a natural asset encapsulated by polymeric nanoparticles.
Source: Author, (2020).

Technological advances have allowed the development of biomaterials containing natural active substances [3]. Effective bioproducts, of low toxicity and low cost, as is the case of controlled release biocides based on the encapsulation of natural bioactives of various plant species rich in biologically active substances against fungi, bacteria, viruses, larvae, insects, agricultural pests and pathologies that affect human health [4-12].

One of the objectives of encapsulating biomaterials is to protect and prolong the durability of certain constituents of the formulation until the moment of its application [13], promoting a controlled action [14]. For this reason, this technique has been widely applied to encapsulate essential oils, as they are volatile substances susceptible to photodegradation.

Thus, this research aims to develop a formulation, based on the creation of a biomaterial from oil encapsulated in biodegradable polymeric nanoparticles and developed based on gelatin and poly-caprolactone (PCL). It was evaluated as a function of time and temperature (25 °C), as well as the application of different preservatives, [phenoxyethanol-2-methyl-2H-isothiazolin-3-one-NE), citric acid and thymol] with the intention of verifying its influence on the stability of formulations as a function of time. In addition, the stability assessment was also monitored by estimating the encapsulation efficiency (EE), electrical conductivity, turbidity and pH measurements, and evaluating the organoleptic properties (color and odor), periodically monitored to select the most stable formulation.

II. MATERIALS AND METHODS

Usually the preparation of biodegradable formulations is composed of two phases, aqueous and organic. For the aqueous phase, 1 g of gelatin was solubilized in 100 ml of distilled water followed by stirring and heating to 50 °C. In another beaker, 0.3 g of Tween 80 was dissolved in 50 ml of distilled water with magnetic stirring until total solubilization. Then, Tween 80 was poured into the solubilized gelatin. For the organic phase, the PCL was solubilized in 5 ml of dichloromethane under magnetic stirring. 0.02 g of SPAN, 0.1 g of TACC and 0.075 g of natural Amazonian active substance homogenized in 5 mL of dichloromethane were used. After stirring, SPAN, TACC and the natural asset were poured into the solubilized PCL giving rise to the organic phase. After the magnetic stirring was completed, the turrax-type ultra-disperser was used to pour the organic phase into the aqueous phase for 30 s. Then, 0.0935 g of transglutaminase was added under magnetic stirring, adjusting the pH to 8. After the preparation of the synthesis, it was divided into three vials and a preservative was added to each solution. The preservatives used were NE, citric acid and thymol, so the formulations were kept in an incubator with a controlled temperature of 25 °C. To choose the formulation with the most stable preservative and with the greatest temperature resistance.

The stability evaluation of the formulations was performed using a pH meter from BEL, Model PHS-BW; Turbidimeter Brand MS TECNOPON Model TB-1000; Conductivimeter Brand BEL, Model W12D; UV-vis Global Trade Technology Model UV 5100. The evaluation of organoleptic properties (color and odor) was carried out in order to verify physical-chemical changes associated with possible changes. For the EE estimate, the empty and loaded nanoparticles were separated by centrifugation (DAIKI, model DTR 20.000) at 15.000 rpm for 15 min. The supernatant was used to determine the amount of free natural assets.

III. RESULTS AND DISCUSSIONS

Were evaluated (I) the formulation containing the encapsulated natural active and the preservative NE, (II) the formulation containing the encapsulated natural active and the citric acid preservative, (III) the formulation containing the encapsulated natural active and the preservative thymol. The purpose of stability assessment was to verify possible changes in relation to destabilization of the formulations, in addition to checking which formulation is more stable.

The stability tests were started in a centrifuge by means of a behavioral stability study, being evaluated the state of the formulations after centrifugation. The behavioral stability study aims to verify if the formulations support pressure, mobilization, temporal changes without phase separation [15]. The formulations were submitted to 15.000 rpm and no phase disjunction was observed.

After the completion of the behavioral study of the formulations by the centrifugation test, the stability analysis was performed at 25 °C, and the parameters evaluated were organoleptic properties (color and odor), turbidity, efficiency of encapsulation, pH and electrical conductivity. All parameters were analyzed over time.

All products need to undergo several stability assessments, industrial or manipulated. The initial stability tests aim to verify the product quality before release to the consumer. These tests are performed to guarantee its stability until the end of its validity, that is, for ensure that the product effectively performs the action for which it was created [16].

The organoleptic properties were the first aspect to be evaluated (color and odor), were analyzed in comparison with the initial characteristics of the standard formulation to verify changes in color and odor, in addition to phase separation, excessive turbidity and ruptures.

Regarding organoleptic properties, the formulations containing the citric acid and thymol preservatives showed changes in color and odor after 25 days, as shown in Figure 2.

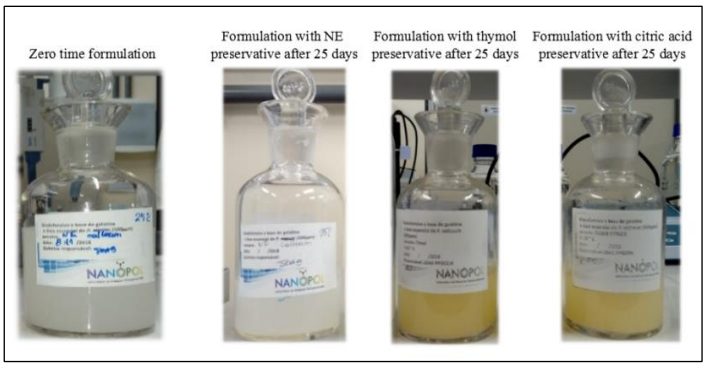

Figure 2: organoleptic properties, the formulations.
Source: Author, (2020).

These results suggest a possible proliferation of microorganisms [17]. As they also indicate that preservatives were not appropriate. However, the formulation containing the preservative NE did not change in the same evaluation period. Therefore, the formulation containing this preservative was the only one approved in the study of organoleptic properties. Corroborating the signs of destabilization verified by the evaluation of the organoleptic properties of formulations containing citric acid and thymol preservatives, the turbidity analyzes of these formulations also showed changes. It is known that the destabilization of biodegradable systems is related to physical, chemical and biological factors [16, 18-19]. However, in the formulation containing the preservative NE, no significant changes were observed in the turbidity values capable of compromising the stability of the bioproduct, corroborating the evaluation of organoleptic properties, as shown in Figure 3.

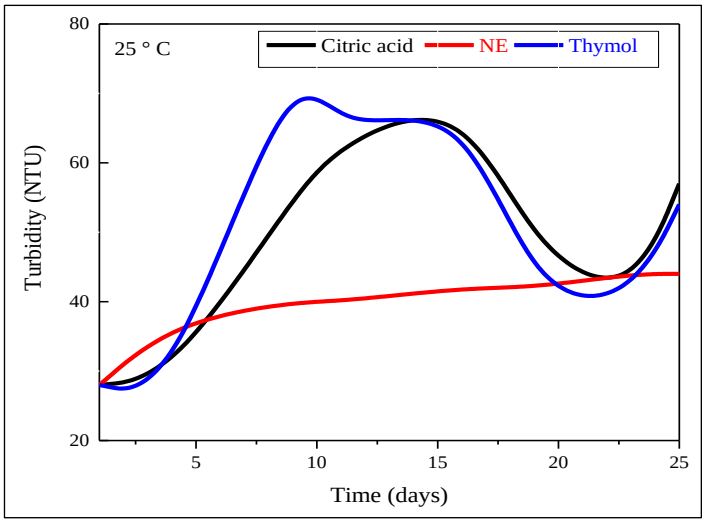

Figure 3: Turbidity (NTU).
Source: Author, (2020).

All formulations showed encapsulation efficiency (EE) greater than 90%, indicating that the combination of materials applied to encapsulate the natural asset was effective. The formulation containing the citric acid preservative showed an initial EE of (97 ± 2) % and a final value of (91 ± 2) %. The formulations containing the preservative NE and thymol showed an initial EE of (95 ± 2) % and, at the end of the analysis, (90 ± 2) %. The formulation containing the preservative thymol was also above average. Figure 04 shows the variation of the EE in an evaluation period of 25 days (25 ºC) for formulations containing preservatives NE, citric acid and thymol.

Polymeric biomaterials are considered efficient when their encapsulation efficiency is greater than 70% [20]. If we consider only the analysis of the EE, all formulations would indicate stability, since they presented high EE. For this reason, it is important to associate the stability assessment with other analyzes (such as organoleptic assessment and turbidity) and, as previously seen, the preservatives citric acid and thymol were not able to maintain the stability of the formulations, even with high EE.

Although the formulations containing the citric acid and thymol preservatives have shown destabilization proven by the organoleptic assessment and turbidity, this fact has not compromised the EE, probably because it is a bilayer carrier system, promoting greater protection of the natural asset. Some studies report that biodegradable polymeric systems that have two layers are more resistant to extrinsic factors than those that have only one layer [21-22].

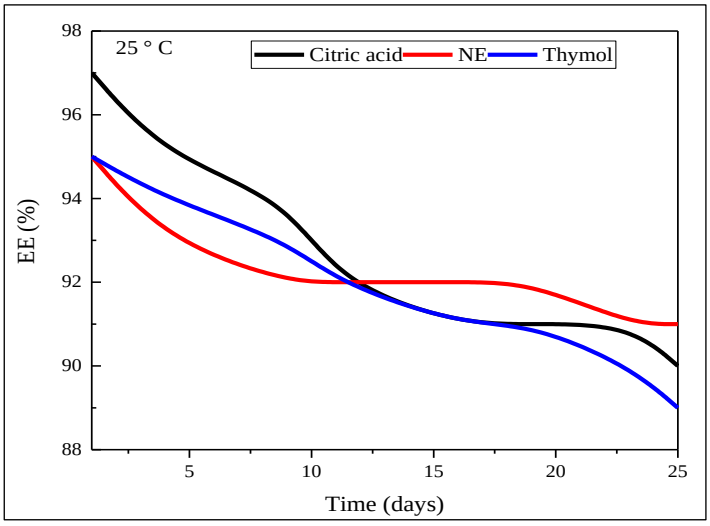

Figure 4: Encapsulation efficiency (EE).
Source: Author, (2020).

During the 25 days of analysis of the stability of the formulations, the pH values decreased, but remained above 6.3. Based on this result, it can be suggested that the natural asset remained protected by the bilayer during the evaluation period (as demonstrated by the high percentage of EE), since lower pH values indicate exposure of the asset to the medium (pH = 5, 5) and destabilization of formulations.

All formulations showed initial pH 8 and, in the final analysis, the results were: formulation containing preservative NE, pH = 6.7; formulation containing citric acid preservative, pH = 7.3 and formulation containing thymol preservative, pH = 6.5.

As shown in Figure 05, all formulations showed a decrease in pH over the 25 days of analysis. This fact is related to the exposure of the bioactive to the aqueous medium, resulting from the degradation of the capsules, causing a decrease in EE, as previously seen. Among all the formulations evaluated, the one that contained the preservative NE was the one that showed the lowest pH variation after the fifth day of analysis. However, as previously mentioned, a single isolated evaluation is not enough to analyze the stability of a formulation, several parameters must be applied for the results to be efficient.

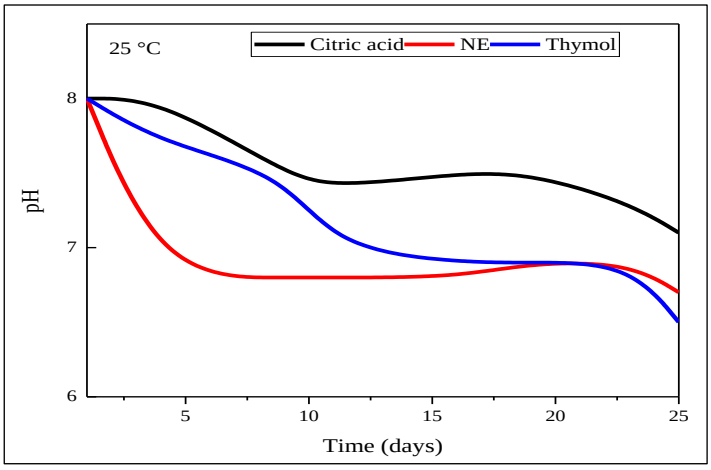

Figure 5: pH.
Source: Author, (2020).

However, the decrease in pH values is not only related to the exposure of the natural asset in the aqueous medium, since the pH of the asset is acidic and, naturally, from its exposure to the medium, the formulation will tend to its pH. The decrease in pH values can also be related to the proliferation of microorganisms in the formulation, such as bacteria and fungi, which naturally changes the pH [23]. In addition, extreme pH changes are suggestive of instabilities, as they indicate degradation of the polymeric material.

The formulation containing the citric acid preservative showed electrical conductivity in the range of 1.9 to 4.0 mS. The formulation containing the preservative thymol showed electrical conductivity from 0.8 to 1.3 mS. The formulation containing the preservative NE showed electrical conductivity between 2.1 and 2.7 mS, as shown in Figure 6. These values corroborate the evaluations of organoleptic properties, turbidity and EE, as unexpected changes in electrical conductivity are indicative of instabilities [15].

The increase in the values of electrical conductivity in systems containing natural assets encapsulated in biodegradable polymeric materials is related to the presence of a large amount of free charge (ions) in the solution. Thus, the increase in electrical conductivity is related to the exposure of the asset in the solution and, consequently, to the decrease in EE and destabilization of the formulation. In some cases, these electrical conductivity values can be changed later, depending on the temperature at which the formulation is packaged. The decrease in pH values can be correlated to the values of electrical conductivity, since this decrease is related to exposure to greater amounts of bioactive substances and, consequently, to increased charges [24].

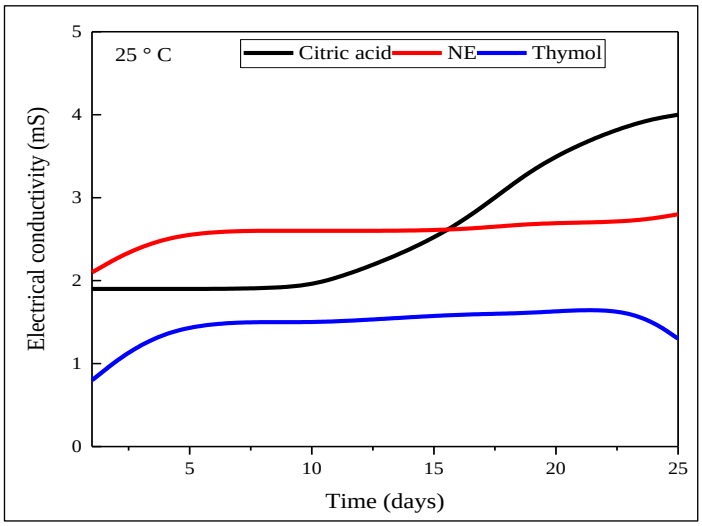

Figure 6: Electrical conductivity (mS).
Source: Author, (2020).

The next stage of the research aimed to choose the most stable formulation using all parameters previously evaluated for 25 days and at 25 ºC, namely: EE, pH, turbidity and electrical conductivity. The values of the parameters obtained in the evaluation are presented in Table 1. Of the formulations evaluated, the one that showed greater stability (that is, the one that was approved in all the evaluated parameters) was the one that contained the preservative NE. The systems containing the other preservatives did not pass all the evaluation parameters. Therefore, the bioproduct containing the preservative NE was selected for further study.

Table 1: Evaluation and preservative parameters.

Preservative	**Turbidity (NTU)**
NE	28 – 44
Citric acid	28 – 57
Thymol	28 – 54
Preservative	**EE (%)**
NE	95 – 90
Citric acid	97 – 91
Thymol	95 – 90
Preservative	**pH**
NE	6,7
Citric acid	7,3
Thymol	6,5
Preservative	**Electric conductivity (mS)**
NE	(2,1 - 2,7)
Citric acid	(1,9 - 4)
Thymol	(0,8 - 1,3)

Source: Author, (2020).

IV. CONCLUSIONS

The present work successfully developed a formulation based on biodegradable polymeric materials based on PCL/gelatin containing encapsulated natural assets and maintained at a specific temperature, based on the Brazilian climate. Initially, the formulations were developed and evaluated over time based on the best preservative, that is, the formulation with the preservative that contributed to keeping the biomaterial more stable within the evaluation parameters. This system was the one that contained the preservative NE. The stability data analyzed showed that the synthesis with the preservative NE is promising for future applications and that its initial durability remained stable in 25 days of handling. The system presented stability at 25 °C. It is hoped that this research can contribute to the proposal of an alternative formulation to be used as a low toxicity and effective bioproduct.

V. AUTHOR'S CONTRIBUTION

Conceptualization: Joab de Souza Arouche.
Methodology: Joab de Souza Arouche.
Investigation: Joab de Souza Arouche.
Discussion of results: Joab de Souza Arouche.
Writing – Original Draft: Joab de Souza Arouche.
Writing – Review and Editing: Joab de Souza Arouche.
Resources: Joab de Souza Arouche.
Supervision: Joab de Souza Arouche.
Approval of the final text: Joab de Souza Arouche.

VI. ACKNOWLEDGMENTS

Thanks to the Federal University of Amazonas - UFAM, to Nanopol - UFAM, where this study was carried out, and to FAPEAM for the financial support for the development of this research.

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