



POLYMERIC MICROPARTICLES IN SOLID DISPERSION FORM CONTAINING THE HORMONE TRIIODOTHYRONINE (T3) FOR TREATMENT OF HYPOTHYROIDISM: DEVELOPMENT, SYNTHESIS AND PHYSICAL-CHEMICAL AND MORPHOLOGICAL CHARACTERIZATION

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ABSTRACT

Hypothyroidism is a common metabolic disease, which presents a low concentration of thyroid hormone due to deficient synthesis and/or low sensitivity to hormones by its receptors, and its treatment is usually done with hormone replacement from Levothyroxine monotherapy (L-T4), a drug originated from the synthetic molecule of thyroxine that has a prohormone action. The active hormone for the thyroid gland is triiodothyronine (T3), but it is not widely used due to its rapid degradation in the body. Because of this, this work aimed to develop a solid dispersion with T3, this proposal being based on the expected increase in stability, therapeutic effect and, consequently, the half-life of the drug through microtechnology. The solid dispersion was synthesized with PEG 6000 and polysorbate 80 and prepared using the solvent evaporation method. Then, it was partially characterized physico-chemical and morphologically through analytical methods such as thermogravimetry (TG), differential scanning calorimetry (DSC), infrared absorption spectroscopy (IR), as well as by scanning electron microscopy (SEM), and such analyzes indicated the formation of an effective and innovative system for drug delivery.

Keywords: Solid dispersion, PEG 6000 (polyethylene glycol) and Triiodothyronine (T3).

RESUMO

O hipotireoidismo é uma doença metabólica comum, a qual se apresenta por baixa concentração de hormônio tireoidiano por síntese deficitária e/ou baixa sensibilidade aos hormônios pelos seus receptores, sendo seu tratamento normalmente feito com reposição hormonal a partir de monoterapia de Levotiroxina (L-T4), fármaco originado da molécula sintética de tiroxina que

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apresenta ação de pró-hormônio. O hormônio ativo para a glândula tireoide é a triiodotironina (T3), porém não é muito usado devido sua rápida degradação no organismo. Devido a isso, esse trabalho teve como objetivo desenvolver uma dispersão sólida com T3, sendo essa proposta baseada no esperado aumento de estabilidade, efeito terapêutico e, conseqüentemente, a meia-vida do fármaco através da microtecnologia. A dispersão sólida foi sintetizada com PEG 6000 e polissorbato 80 e elaborada a partir do método de evaporação do solvente. Em seguida, foi parcialmente caracterizada físico-quimicamente e morfológicamente através de métodos analíticos como termogravimetria (TG), calorimetria exploratória diferencial (DSC), espectroscopia de absorção no infravermelho (IR), bem como por através da microscopia eletrônica de varredura (MEV), sendo que tais análises indicaram a formação de um sistema eficaz e inovador para a liberação de fármacos.

Palavras-chave: Dispersão sólida, PEG 6000 (polietilenoglicol) e Triiodotironina (T3).

INTRODUCTION

Hypothyroidism is a metabolic disease, in which there is low synthesis of thyroid hormones by the thyroid gland and/or low sensitivity to hormones in their nuclear receptors. This pathology can be caused by several factors, such as: genetics, low dietary intake of iodine, deficient synthesis of pituitary hormones (TSH and TRH), low concentration of thyroglobulin and surgical trauma (OLIVEIRA et al, 2002).

Pharmacological treatment is carried out through the replacement of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), with T4 being the prohormone of T3, which is the biologically active form of the hormone (HALL, 2011). Typically, treatment is carried out by Levothyroxine monotherapy (L-T4) in which the active pharmacological ingredient is a synthetic molecule equivalent to thyroxine (BEVENGA et al, 2019). However, even with correct adherence to treatment and normal serum levels of thyroid and pituitary hormones, some patients report persistence of symptoms (CHAKERA et al, 2012), in addition to the fact that levothyroxine may require a gradual increase in doses, as there is a decrease in its absorption in the intestinal mucosa and its stability, which can be caused by food, gastrointestinal diseases and drug interactions (VINAGRE et al, 2010).

Therefore, there are other less common pharmacological treatments, such as the combined therapy of L-T4 and T3 (CHAKERA et al, 2012). Therapies using only synthetic or animal T3 are not frequent, as the hormone is biologically active and has a short half-life and consequently disintegrates more quickly (OLIVEIRA et al, 2002). Therefore, there is still a search for new pharmacotherapeutic strategies for the treatment of hypothyroidism (CHAKERA et al, 2012).

A pharmaceutical strategy to improve the absorption of lipophilic drugs is to use hydrophilic microcarriers or nanocarriers. One of these strategies is called solid scatter. In this system, a slightly soluble drug is dispersed in a hydrophilic microcarrier (DE AGUIAR, 2016), promoting higher dissolution and, consequently, better absorption of the drug incorporated into these carriers (LIRA, 2019). Solid dispersion is considered a drug delivery system, in which a drug is associated with a pharmaceutical release system, presenting advantages over unassociated drugs. This occurs by protecting the active ingredients and, thus, promoting greater

stability to them. This way, there can be greater absorption of the drug, increase the half-life, reduce toxicity, reduce adverse effects, and there is also the possibility of modulating its release in the body (SOUTO et al, 2018).

In view of the above mentioned, it is believed that the development of a solid dispersion containing the T3 hormone is a promising strategy to stabilize triiodothyronine, increase its release and half-life and, consequently, increase the effectiveness of the drug. Therefore, the aim is to obtain a drug release microsystem with better pharmacokinetics and greater stability than conventional pharmacotherapies used for hypothyroidism.

Therefore, this work aims to develop and characterize, according to physicochemical and morphological parameters, polymeric microparticles of PEG (polyethylene glycol) in the form of a solid dispersion containing the hormone triiodothyronine (T3), aiming at interest for future applications in the treatment of hypothyroidism.

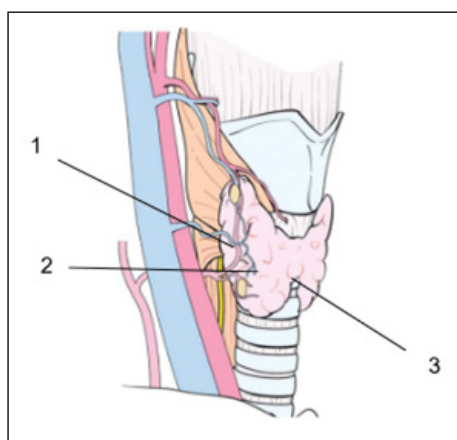
THEORETICAL REFERENCE

The thyroid is a bilobed endocrine organ anterior to the trachea (figure 1), composed of cuboidal epithelial cells, which have follicles, containing a secretory substance called colloid that presents thyroglobulin (Tg). This substance is a precursor of thyroid hormones – thyroxine (T4) and triiodothyronine (T3). Thyroid hormones are essential for the metabolic regulation of the human body. They are responsible for regulating the cardiovascular system, cellular metabolism, development of the central nervous system, as well as growth and protein synthesis (HALL, 2011).

Hypothyroidism is a disease characterized by low serum levels and low expression of thyroid hormones (HALL, 2011). The symptoms caused by this pathology are characteristic of hypometabolism, since these hormones are responsible for increasing cellular metabolic activity (BRENTA et al 2013).

Pharmacological treatments for this disease are through hormone replacement. The most common is the use of an oral medication containing L-T4 (synthetic T4 hormone) (BEVENGA et al, 2019). There are also combined L-T4 and T3 therapies, but it is normally indicated to attempt to regenerate normal thyroid tissue that has been damaged (CHAKERA et al, 2012). The use of synthetic or animal active hormone (T3) is not common, due to its shorter half-life (OLIVEIRA et al, 2002).

Figure 1. Thyroid anatomy. Right lateral-frontal view of the thyroid gland. 1 – Right lobe, 2 – Isthmus and 3 – Left lobe.



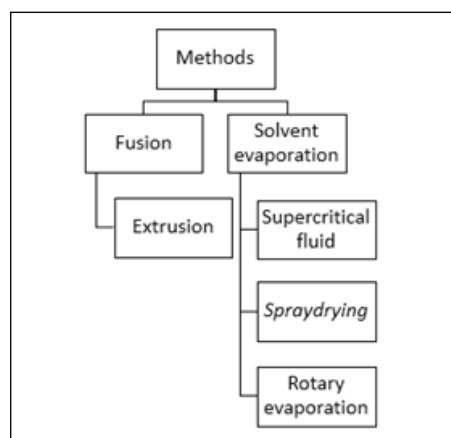
Source: Adapted from MOHEBATI (2012)

Drug delivery systems are pharmacotechnical strategies to improve stability, solubility, bioavailability and even reduce the adverse effects of some drugs (MORAES, 2008). Solid dispersion is one of these strategies, normally applied to lipophilic drugs, as they have low bioavailability and low solubility in water. When added to water-soluble or hydrodispersible microcarriers presented in a solid state, the systemic effect of this hormone improves (DE AGUIAR, 2016).

The choice of carrier for solid dispersion depends on some factors, such as: solubility in water, compatibility with the drug, lack of pharmacological activity, low toxicity and good stability in dispersion, in addition to requiring ANVISA approval and proof of its security (DE AGUIAR, 2016). Thus, carriers are divided into four generations, the first generation being crystalline carriers, the second generation being amorphous carriers – such as PEG (polyethylene glycol) and PVP (polyvinylpyrrolidone), which have better thermodynamic stability (KIM, 2011); the third generation characterized by the addition of one or more surfactants to improve the stability of the system; and the fourth generation characterized by the use of water-insoluble polymers, responsible for controlling the release of lipophilic and low half-life drugs (DE AGUIAR, 2016).

The preparation of solid dispersions occurs mainly using two methods: melting and evaporation of the solvent, which have subdivisions as shown in figure 2 (LIRA, 2019).

The choice of the method is made with the aim of achieving greater stability of the system, therefore it is necessary to evaluate the physicochemical characteristics of the drugs and carriers, considering that some carriers require vigorous heating and may inactivate thermosensitive drugs. To validate the quality and effectiveness of the solid dispersion, it needs to be characterized through its physicochemical properties. The most commonly used methods for the physicochemical and morphological characterization of these structures are: differential scanning calorimetry (DSC), thermogravimetry (TG), X-ray diffraction, infrared spectroscopy (IR), scanning electron microscopy (SEM), solubility and dissolution of solid dispersion, among others (DE AGUIAR, 2016).



Source: Adapted from LIRA (2019)

METHODOLOGICAL PROCEDURE

PREPARATION OF THE BLANK AND T3 SOLID DISPERSION

The development and preparation of the blank solid dispersion (DSB) and the solid dispersion containing T3 (DST3) were carried out in the Semi-industrial Laboratory of the Pharmacy course. For DSB, 18g of PEG 6000 (microcarrier), 2g of polysorbate 80 (non-ionic surfactant) and 30 ml of acetone (organic solvent) were used. The preparation was made by fusing PEG 6000 with polysorbate 80 in a round-bottom flask, using a hot water bath from the rotary evaporator. Acetone was then added and the flask was attached to the rotary evaporator until the solvent was completely evaporated.

Likewise, the preparation of DST3 used the same components as DSB, however, after fusing PEG 6000 and polysorbate 80, 5.9µg of T3 solution were added and then 30ml of acetone. The flask was then coupled to the rotary evaporator until the solvent was completely evaporated.

To prepare both, after removing the flask from the system, it was mandatory to wait for the system to cool and solidify before removing the material by scraping and homogenizing by pulverization, with the aid of a mortar and porcelain pestle. They were then sent to the MackGraphe laboratories for characterization analyses.

PHYSICAL-CHEMICAL AND MORPHOLOGICAL CHARACTERIZATION

ORGANOLEPTIC ANALYSIS

The analyzes were carried out using the method of subjective observation of the developed samples, observing the macroscopic characteristics, as well as the shape, color and odor, comparing with the characteristics of the excipients used (polysorbate 80 and PEG 6000).

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

For the analysis, DSB and DST3 samples were evaluated, as

described in items 3.1 and 3.2, and then sent to the MackGraphe laboratories where the analyzes were conducted using the TM3000 microscope. (Figure 3).

Figure 3. Scanning electron microscopy device

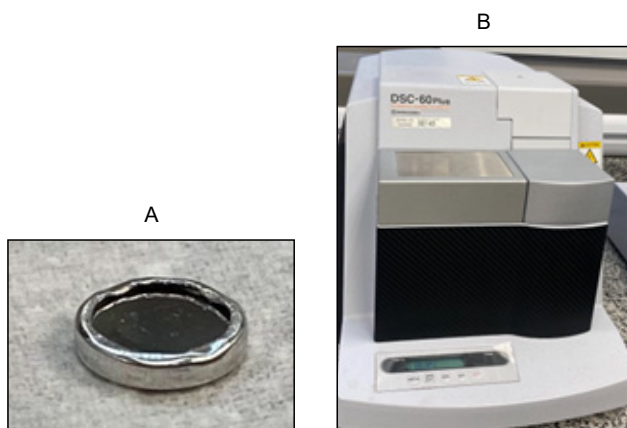


Source: The authors

DIFFERENTIAL EXPLORATORY CALORIMETRY (DSC) ANALYSIS

DSC analyzes were carried out on a DSC-60 Plus (Figure 4), in the range of 25 to 600°C, using aluminum crucibles with varying mass for each sample. In the equipment, the samples were placed in one of the aluminum crucibles present in the equipment and heated until their total degradation, with observation of the energy flow during the period. The samples were initially prepared and pulverized as described in items 3.1 and 3.2.

Figure 4. (A) Aluminum crucible with sample (B) DSC device



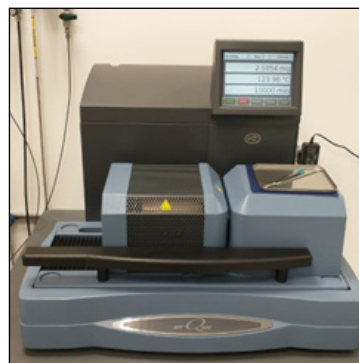
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CHARACTERIZATION BY THERMOGRAVIMETRY

Characterization by thermogravimetry (TG) is a thermoanalysis carried out using a balance, which measures the loss or gain of

mass in relation to time and/or temperature. This technique is mainly used for physical-chemical characterization of the sample (DENARI, 2013). The analysis was carried out with the SDT Q600 equipment (Figure 5).

Figure 5. Thermogravimetry device



Source: The authors

CHARACTERIZATION BY ABSORPTION SPECTROSCOPY IN THE INFRARED REGION (IR)

The samples were pulverized and taken for analysis in the IRAffinity-1S device (Figure 6) and the infrared analysis was carried out using the ATR ZnSe (zinc selenide) crystal technique, in which the surface of the white solid dispersion and with T3 was studied.

Figure 6. Spectrometer device

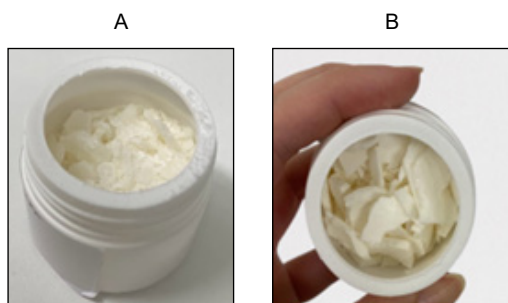


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RESULTS AND DISCUSSION

Organoleptic characterization is done through the analysis of the color, odor and appearance of the sample. This method is simple, but effective for analyzing the general and macroscopic characteristics of the obtained products (DE SOUZA, 2019).

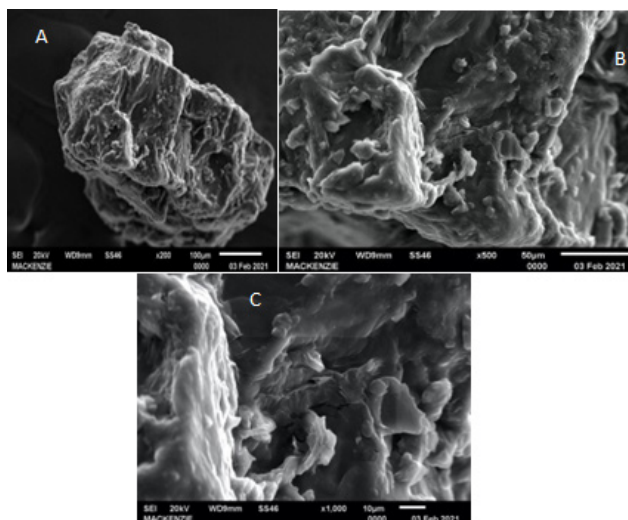
The solid dispersions DSB and DST3 presented the appearance of amorphous structures of white to yellowish color, with a slightly sweet odor, and these characteristics possibly observed were observed due to the use of polysorbate 80 to help in the solubilization of the hormone to PEG (figure 7).

Figure 7. Sample of (A) DSB and (B) DST3

Source: The authors

The scanning electron microscopy (SEM) technique was carried out with the aid of a conventional optical microscope with an electron beam, this is because this beam allows the visualization of smaller particles, which is the case of solid dispersion that presents particles in the range of micrometers. Scanning electron microscopy is essential as it provides knowledge about the three-dimensional morphology of particles (DEDAVID et al, 2007).

The first analysis conducted by SEM aimed to analyze the morphological characteristics of PEG6000. It was possible to observe that there are no fragments on the surface of the material and there are microgranules scattered on the surface, which gives the material a rough appearance, as seen in the images below (figure 8).

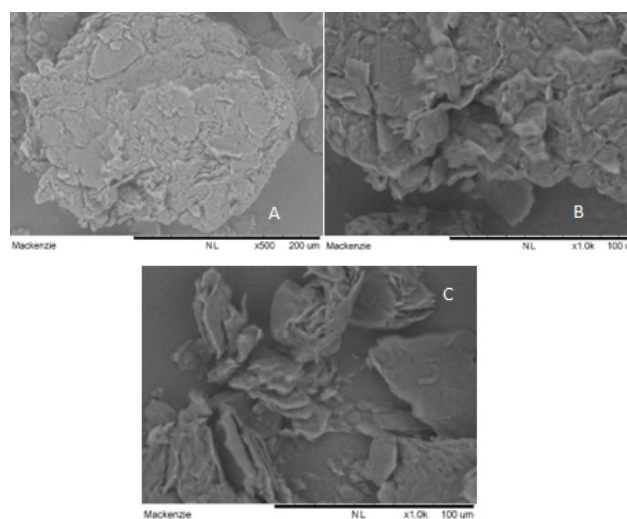
Figure 8. Electron microscopy (SEM) images of PEG 6000 - (A) PEG 200x magnification; (B) PEG 500x magnification; (C) PEG 1000x magnification

Source: The authors

After analyzing the excipient, present in greater quantities in the formulation, the analysis of the blank solid dispersion (DSB) was carried out. This was the sequence conducted to verify the change generated by the process through the comparative method

of the blank dispersion sample and the isolated PEG sample (figure 8).

When observing the next images (figure 9) it is possible to notice the differences in relation to the images in figure 8, morphologically proving that there was a change between the isolated PEG 6000 and the blank solid dispersion. It is possible to observe that there are several particles and fragments of the material, which have a surface with a flaky appearance generated by small agglomerated fragments. However, when analyzing an isolated particle, the difference in the surface is notable, which presents a lamellar appearance, formed by overlapping fragments, which can be caused by the fusion process of PEG 6000 with polysorbate 80.

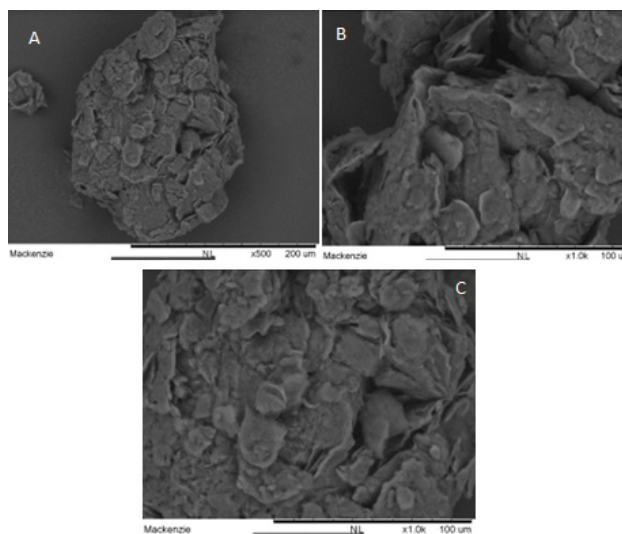
Figure 9. Electron microscopy images of the DSB (A) DSB front view 500x magnification; (B) DSB side view 1000x magnification; (C) DSB side view 1000x magnification.

Source: The authors

Then, the DST3 sample was analyzed, which presents characteristics similar to DSB. This can be justified by the low concentration of active pharmacological ingredient, as expected because it is used in a drug release system. As seen in the images in figure 8, the characteristics of the sample are similar, in the front view of the sample it is possible to observe an irregular surface, which analyzed together with the side view of the sample is complemented by the presence of a lamellar aspect of the solid dispersion and without evidence of the active pharmacological ingredient on the surface, which is an indication of encapsulation of the active ingredient by the excipients.

The thermal analysis was conducted using differential scanning calorimetry (DSC) and thermogravimetry (TG), techniques conducted to evaluate the physical property of the sample as a function of temperature, controlling the temperature change under a specified atmosphere. DSC and TG analyzes were conducted separately. The results found in the DSC were exemplified in table 1 and graphically in figure 11.

Figure 10. Electron microscopy images of DST3 (A) DST3 frontal view 500x magnification; (B) DST3 side view 1000x magnification; (C) DST3 side view 1000x magnification.

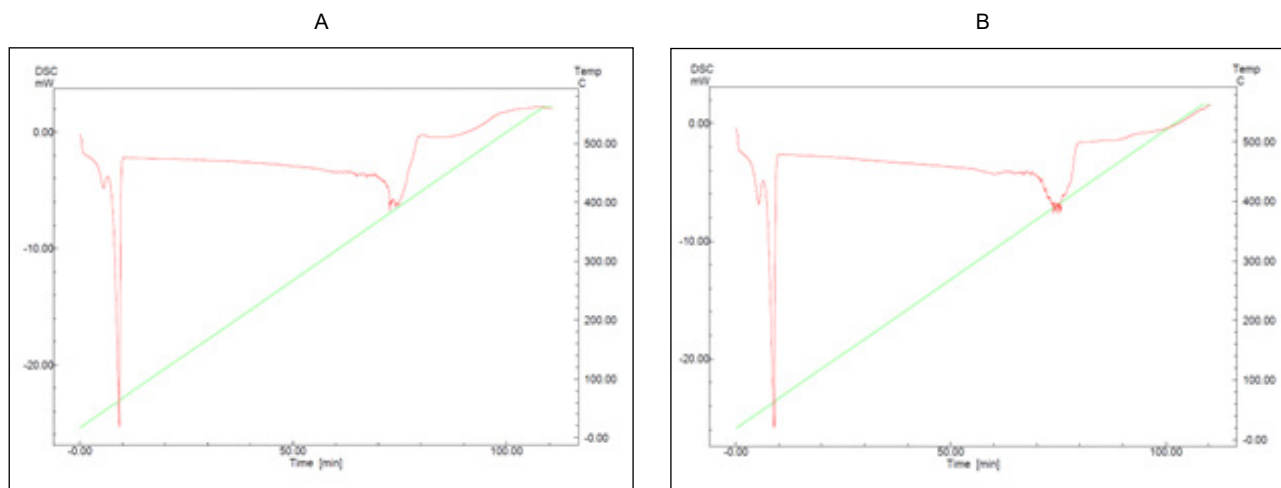


Source: The authors

Table 1. Comparison of DSC results between DSB and DST3

Peaks DSC	1° Peak	2° Peak	3° Peak
DSB	38,31°C a 44,55°C	49,9°C a 61,65°C	379,44°C a 381,32°C
DST3	39,33°C a 44,88°C	49,90°C a 61,46°C	369,36°C a 389,21°C

Figure 11. Result of DSC analysis of samples (A) DSB; (B) DST3



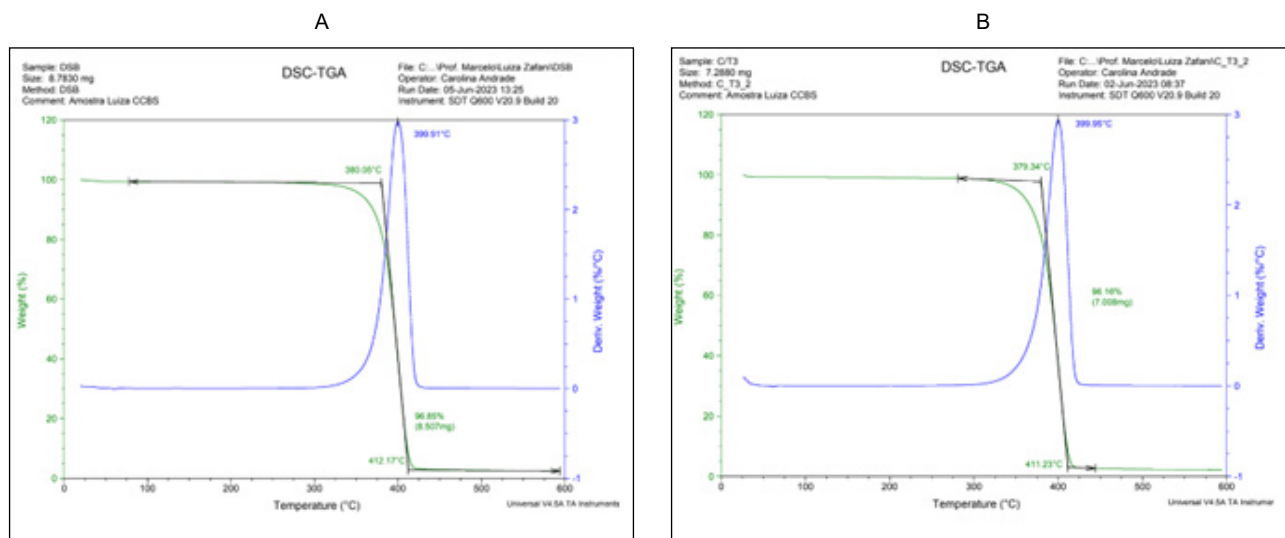
Source: The authors

As observed by comparing both DSB and DST3 samples, it is possible to observe that there is thermostability of the basic formulation, since the sample presents endothermic peaks where the sample melts. Both analyzes present similar activity, which is indicative of the stability and incorporation of the drug into the basic formulation (microcarrier), as there is no change in the behavior of

the blank DSC analysis in relation to DST3.

Thermogravimetry (TG) was another thermal analysis conducted to evaluate the stability of the basic formulation comparing to the other samples. When observing figure 12, it is also possible to verify that the DSB and DST3 analyzes present similar results, indicative of the thermostability of the formulation and incorporation of the drug.

Figure 12. Result of TG analysis of samples (A) DSB; (B) DST3



Source: The authors

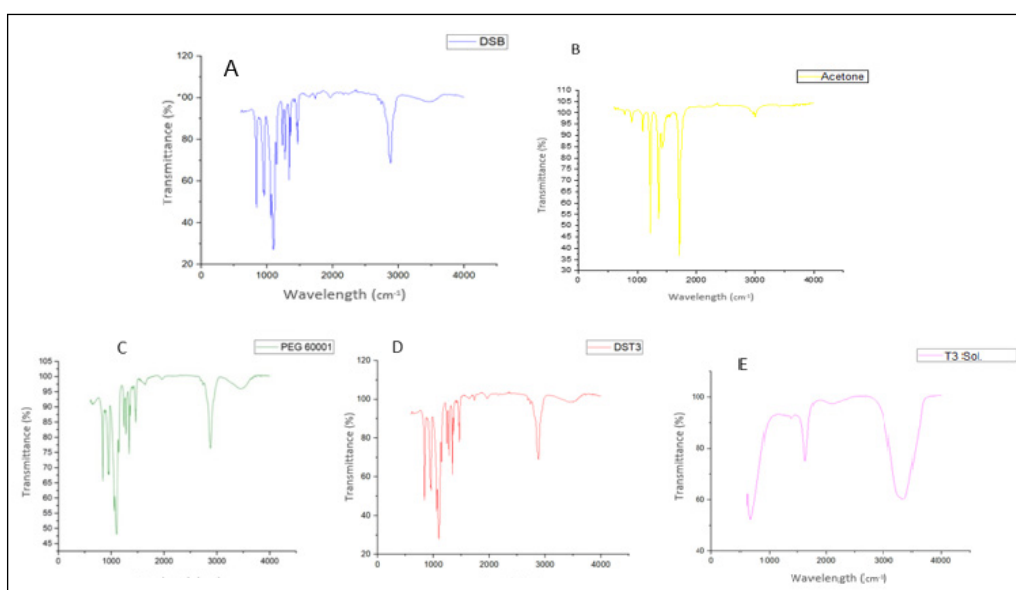
Absorption spectroscopy in the infrared region is a technique carried out by measuring the electromagnetic radiation emitted or absorbed by the analyzed sample, this analysis is carried out with the aid of a spectrometer for physical-chemical and morphological analysis of DSB and DST3 solid dispersions (LEITE et al, 2012).

The analysis was conducted using the IRAffinity-1S device (figure 6), which obtains absorption results in the infrared region of the sample, studies carried out in the region of 4000 to 500 cm^{-1} at room temperature. The samples were placed on the horizontal attenuated total reflectance (ATR) accessory, which reflects the characteristics arranged on the surface of the sample.

For better interpretation, in addition to analyzing the blank solid

dispersion (DSB) and PEG6000 separately, acetone analysis was also performed. Through the graphs it is possible to observe the similarity between the curves between DSB and PEG6000 and the non-similarity with the acetone graph, which may suggest that acetone as a solvent did not change the transmittance properties of the sample. This is justified due to its high volatility, that is, the solvent is evaporated through the rotary evaporation process without leaving residues in the sample. This analysis seeks to show possible changes present in the solid dispersion systems, but it is possible to verify from the graphs that in DSB and DST3 there was total evaporation of acetone, in addition to analyzing the T3 solution.

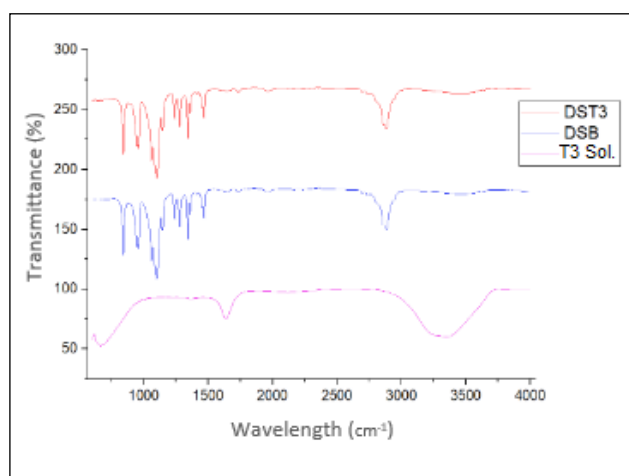
Figure 13. IR analysis result (A) DSB analysis; (B) PEG 6000 analysis; (C) Acetone analysis; (D) DST3 analysis; (E) T3 solution analysis.



Source: The authors

To evaluate by comparative method, the FTIR graphs of the DSB, DST3 and T3 solution samples were displayed in figure 11. This makes it possible to observe the incorporation of T3 by solid dispersion, taking into account the similarity of the graphs and maintenance of its functions, in addition to the suppression of specific bands in the T3 solution. It is also possible to observe the predominant characteristic with PEG6000, which is the component with the highest concentration in the formulation, and this may be indicative of incorporation of the drug into the base.

Figure 14. FTIR comparison graph of DSB, DST3 and T3 solution.



Source: The authors

FINAL CONSIDERATIONS

Drugs that have low stability in the physiological environment can benefit from different drug delivery systems, such as solid dispersions that feature microcarriers, which increase drug stability. This increase guarantees a greater therapeutic effect. Considering that the T3 hormone is not used because it is quickly degraded in the body, this strategy is potentially important for the possible use of this drug in medicines.

Considering the objectives proposed by this work to develop and characterize physicochemically and morphologically such samples, it was possible to obtain results that indicate stability of the formulation and the increase of the stability of the active pharmacological ingredient, through its incorporation.

Therefore, the results presented by the physicochemical and morphological characterization are indicative of the stability of the solid dispersion and compatibility with the active pharmacological ingredient, considering that the base remained stable after its incorporation. However, the study developed is a prototype for future projects and, therefore, the concentration of T3 can be adjusted in future studies by the research group, through preclinical trials. Therefore, the project presented satisfactory results in the first stage of development, indicating a stable formulation and increased stability of the drug.

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