



VETERINARY SHAMPOO WITH ANTIPARASITARY ACTION INCORPORATED WITH POLYMER NANOPARTICLES CONTAINING PRAZIQUANTEL: DEVELOPMENT AND STABILITY STUDY

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RESUMO

O estudo de parasitoses em animais domésticos tornou-se prioritário nas últimas décadas, já que existe uma relação de grande proximidade desses animais com o homem. Cuidados de saúde com esses animais devem ser inseridos na rotina, visando diminuir o risco de se tornarem vetores de algumas doenças, que compõe um problema de saúde pública no Brasil e em países subdesenvolvimento. Um dos antiparasitários mais utilizados é o praziquantel, um fármaco que atua diretamente na permeabilidade da membrana celular de cestódeos e trematódeos, provocando espasmos graves e paralisia nos músculos dos vermes. O fármaco, apesar de amplo espectro, apresenta uma estrutura extremamente permeável (caráter lipofílico), tem problemas devido à baixa solubilidade em água, fator limitante para uma boa biodisponibilidade do ativo, tendo seu efeito significativo em altas doses. Uma alternativa para o aumento da biodisponibilidade do fármaco é o uso de polímeros no desenvolvimento dos sistemas de liberação na forma de nanopartículas poliméricas. Além disso, o tratamento veterinário pode utilizar a "shampoterapia", como alternativa, pois ela permite uma terapia tópica com um tratamento dermatológico do animal devido a permeação de componentes ativos por todas as camadas da pele. Deste modo, no presente trabalho foi desenvolvida uma formulação inovadora de xampu transparente incorporada com nanopartículas de poli n-butilacrilato (PBCA) contendo praziquantel. O desenvolvimento do xampu foi realizado empregando as BPMPV (Boas Práticas de Manipulação de Produtos Veterinários), dando origem a 3 amostras: a primeira contendo apenas o xampu, a segunda com o fármaco praziquantel previamente dissolvido em álcool e a última com as nanopartículas poliméricas de praziquantel. A metodologia utilizada foi a de avaliação das características organolépticas, análises de pH, densidade e viscosidade, a fim de verificar se a formulação se mantém estável para utilização efetiva e segura. Ao final das análises, foi obtido um potencial produto inovador, que manteve as características de estabilidade, indicando que os parâmetros ideais para a finalidade do produto foram respeitados.

Palavras-chave: parasitoses, nanopartículas poliméricas, biodisponibilidade.

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ABSTRACT

The study of parasitosis in domestic animals has become a priority in recent decades, since there is a very close relationship between these animals and humans. Health care for these animals should be included in the routine, aiming to reduce the risk of them becoming vectors of some diseases, which is a public health problem in Brazil and in underdeveloped countries. One of the most widely used antiparasitics is praziquantel, a drug that acts directly on the permeability of the cell membrane of cestodes and trematodes, causing severe spasms and paralysis in the muscles of the worms. The drug, despite being broad-spectrum, has an extremely permeable structure (lipophilic character), has problems due to low solubility in water, a limiting factor for a good bioavailability of the active ingredient, having its significant effect at high doses. An alternative to increase the bioavailability of the drug is the use of polymers in the development of delivery systems in the form of polymeric nanoparticles. In addition, veterinary treatment can use "shampootherapy" as an alternative, as it allows a topical therapy with a dermatological treatment of the animal due to the permeation of active components through all layers of the skin. Thus, in the present work, an innovative formulation of transparent shampoo incorporated with poly n-butylcyan acrylate (PBCA) nanoparticles containing praziquantel was developed. The development of the shampoo was carried out using the GMP (Good Manufacturing Practices for Veterinary Products), giving rise to 3 samples: the first containing only the shampoo, the second with the drug praziquantel previously dissolved in alcohol and the last with the polymeric nanoparticles of praziquantel. The methodology used was the evaluation of the organoleptic characteristics, pH, density, and viscosity analyses, in order to verify if the formulation remains stable for effective and safe use. At the end of the analyses, a potential innovative product was obtained, which maintained the stability characteristics, indicating that the ideal parameters for the purpose of the product were respected.

Keywords: parasitosis, polymeric nanoparticles, bioavailability.

INTRODUCTION

The study of parasites in domestic animals has gained emphasis in recent decades, as these animals are closely related to humans. Health care for these animals must be included in the routine in order to reduce the risk of them becoming vectors of certain diseases (PIVOTO, et al., 2013).

In many cases, environmental contamination in public places, where pets are often taken for walks, is a risk factor for the transmission of parasites. The most common types are intestinal parasites, which are a public health problem in Brazil and in developing countries. These diseases are mainly caused by helminths which are endemic in some areas of Brazil (ROSALES, T.F. & MALHEIROS, A.F., 2017).

The World Health Organization (WHO) proposes several interventions based on the periodic administration of anthelmintics when an animal is affected by parasitosis. Once a diagnosis has been made, therapy for the worm can begin. One of the antiparasitic drugs used is praziquantel, an acetylated quinolone that has a broad spectrum against numerous species of cestodes and trematodes in dogs, cats and cattle. This drug acts by modulating the permeability of the parasites' cell membrane, causing severe spasms and paralysis in the worms' muscles (IBERO MAGISTRAL, 2019; SBP, 2020).

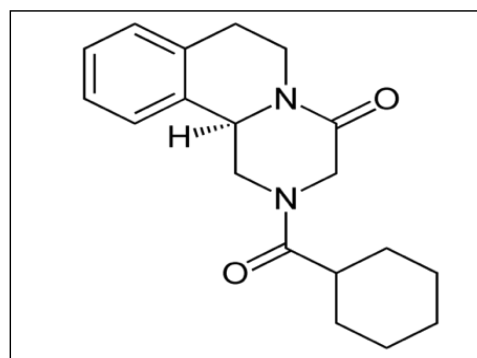
Praziquantel ((RS) -2- (;) -1,2,3,6,7,11b-hexahydro4H-pyrazino (2,1- α) isoquinolin-4-one), CAS 55268-74-1 (Figure 1) is a colorless, odorless drug with a characteristic bitter taste. It belongs to the tetrahydroquinoline class of organic compounds, with the chemical formula $C_{19}H_{24}N_2O_2$ and a molar mass of 312.4

g.mol⁻¹. It has a melting point in the range of 136-142°C, is a photosensitive drug and has low solubility in water (0.4mg/ml⁻¹), and high solubility in ethanol (97mg/ml), the Log P of the drug is 2.5 (FARMACOPEIA BRASILEIRA 6ª. ED, 2019).

The praziquantel molecule has a chiral carbon with two enantiomeric forms, presenting R-PZQ and S-PZQ, the S enantiomer is responsible for the bitter taste of the drug, ineffective pharmacologically, but related to adverse effects. The separation of the racemic mixture has some operational limitations related to the large volume of eluent restricting productivity (GOULART, L.M., 2021 & MARTINS, S. 2014).

The main problem with administering praziquantel to animals is the taste of the drug and the size of the tablets, often causing the animal to try to regurgitate the medicine (MARTINS, S. 2014).

Figure 1: Praziquantel molecule



Source: <https://upload.wikimedia.org/>

Although it has a broad spectrum, due to its structure the molecule is highly permeable, as it is lipophilic. With its low solubility in water, it is a limiting factor for good bioavailability, i.e. its effect is significant at high doses (CAMPOS, F.S, 2013).

The mechanism of action of PZQ is not fully understood, but it is believed to cause contractions in the parasite, as it acts on the influx of calcium, inhibiting the parasite's sodium and potassium pump. Some studies indicate that it increases the permeability of the helminth membrane, involving calcium and this generates an increase in the activity of the drug and muscle contraction resulting in paralysis (MARTINS, S. 2014).

An analysis of the physicochemical parameters of praziquantel allows us to predict the pharmacodynamic processes which are: absorption, distribution, metabolism, excretion and toxicity. Cell membranes are made up of a phospholipid bilayer in which the polar part is directed towards the aqueous part and the apolar part generates a very hydrophobic layer. For a substance to pass through the skin, the molecule must contain hydrophobic groups and be able to lose its hydrophilic sphere. The quinolone class is extremely influenced by its physicochemical properties, especially its degree of ionization (MARTINS, S., 2014).

The four main parameters involved in the analysis of the quinolone class are lipophilicity, the dissociation or ionization constant (pK), aqueous solubility and membrane permeability.

Lipophilicity

Lipophilicity, according to IUPAC, is the "representation of the affinity of a molecule or fragment for a lipophilic environment". This characteristic is quantified by the partition coefficient (log P) which corresponds to the logarithm of the ratio between the monomeric species of a compound in the organic phase and the concentration of the same species in the neutral form of the aqueous phase. The log P of PZQ is 2.5, which means that the drug has better permeability (usually between log P 0 and 3) (MARTINS, S. 2014).

Figure 2: Partition coefficient equation

$$\text{Log } P = \log_{10} \frac{(\text{compound})_{\text{org}}}{(\text{compound})_{\text{water}}}$$

Source: Adapted from Quinolones in Medicinal Chemistry.pdf

Ionization constant

Quinolones have three ionizable groups: the carboxyl group at position 3, and the N-1 (proton at low pH) and N-4 azotes of the piperazilino group at position C-7. lipophilicity. Knowledge of these dissociation constants is important because they reflect the ionization state of the compounds at any given pH, which in turn is important for determining the ability of the compounds to be absorbed in the gastrointestinal tract or to cross biological membranes at a given pH value. This acid-base dissociation affects the biological activity, absorption and solubility of the compounds (MARTINS, S. 2014)

Membrane permeability

The action of drugs at the target site depends directly on the adequacy of the absorption process. The ability to dissolve depends on the pH of the medium, which allows only uncharged molecules to efficiently cross membrane barriers. Praziquantel has high lipophilicity but low solubility, i.e. it easily crosses the membrane but cannot dissolve well enough for effective absorption (MARTINS, S. 2014).

Solubility

Solubility is of great importance, because once a drug has been administered, it needs to dissolve in the physiological aqueous medium, in its free form, so that the drug can act on the target site and achieve its effect. Thus, low solubility, such as that of praziquantel, reflects low bioavailability. Some studies show that all quinolones are relatively soluble at pH between 4 and 9 (MARTINS, M.S. 2014).

To date, no intravenous formulations have been developed, since quinolones predominate in zwitterionic forms, where solubility is minimized, and since blood is an aqueous suspension, their use is limited. One of the alternatives for increasing this solubility is to use liposomes or encapsulate the drug in nanometric release systems (MARTINS, S. 2014).

Nanotechnology and polymeric nanoparticles

Nanotechnology manipulates matter on an atomic and molecular scale and its aim is to create materials and processes that are different from ordinary materials. Nanometers (units of nanometric systems) correspond to one billionth of a meter and their composition varies according to their chemical composition, size, shape and surface. The principle of this science is that materials on the nanoscale can have different chemical and physicochemical properties to materials on a larger scale (ASSIS, B.A & GUIMARÃES, M.G., 2018).

Nanoparticles can be produced by two processes: bottom-up, in which the system is formed from atoms and molecules that are added until they form an organized structure, and top-down, in which the structure is formed by reducing large material to the desired nanometric scale. One of the nanometric systems is polymeric nanoparticles. They act as carriers for drugs and other active molecules. Their diameter is between 10 and 1000nm. Depending on the process used in the preparation method, nanospheres or nanocapsules can be obtained.

In nanospheres, the drug can be retained, adsorbed or dispersed in the polymeric matrix. In nanocapsules, the drug is dissolved in an oily core or adsorbed on the polymer wall. Nanoparticles are gaining prominence because they help minimize problems in existing pharmacotherapy, increasing efficacy and reducing possible side effects or toxicity. In addition, they have the potential to protect encapsulated components from degradation (MORAES, 2009).

Some advantages in the application of polymeric nanoparticles as carriers include: a) the possibility of modulating the diameter of the nanoparticle; controlling and sustaining the release of the drug at the target site, altering distribution and elimination from the body, increasing therapeutic efficacy and reducing side effects; c) the possibility of controlling the degradation process; d) good physical, chemical and biological stability (ASSIS, B.A & GUIMARÃES, M.G., 2018).

The topical application of these nanosystems is being widely studied and aims to increase the stability of the active ingredients. The advantages of topical application are: improved permeation of therapeutic agents into the skin, especially drugs that are very liposoluble but have low water solubility, as they increase the concentration gradient across the skin. They also improve the stability of the active drug, reduce possible side effects such as skin irritation and targeting, minimizing the risk of systemic exposure (ASSIS, B.A & GUIMARÃES, M.G., 2018).

To overcome these limitations, various strategies are being studied to modify the pharmacological properties of drugs. One of these strategies is the use of polymers in the development of release systems in the form of nanoparticles, which enable prolonged release, targeting of the drug inside areas of inflammation and other routes of administration (CAMPOS, F.S, 2013).

One of the technologies that can be used is polymeric nanoparticles. They are used therapeutically on a nanometric scale (10 - 1000 nm). The choice of nanoparticle system depends on the intended action, the type of drug to be used and the route of administration, as these factors affect the choice of suitable excipients, requiring a rigorous stability and compatibility study (FRANCO, N. A., 2013).

A study carried out by Souza (2011) developed solid nanoparticles containing praziquantel and analyzed them for 60 days at 4°C, in the face of a freeze-drying process. The analysis showed that the formulation was stable for this period under these temperature conditions, with a more effective action of the drug and a reduction in hepatotoxicity (CAMPOS, F.S, 2013).

According to the article "Development of polymeric nanoparticles containing praziquantel and coated with polysorbate 80", polymeric nanoparticles can promote an increase in the solubility of praziquantel, its bioavailability, as well as improving the stability of the nano-particulate drug. In this article, the authors propose the synthesis of polymeric nanoparticles made of PBCA containing praziquantel and coated with polysorbate 80 for use in the pharmaceutical and veterinary fields (SILVA, A.R.G. & GUIMARÃES, M., 2019).

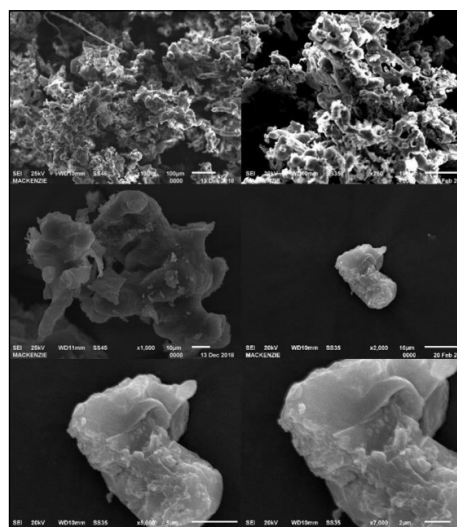
In veterinary treatment, one of the alternatives that can be used is "shampootherapy". In this case, the shampoos developed allow topical therapy with dermatological treatment of the animal, allowing greater permeation of the active components through all layers of the skin. Dermo-cosmetic formulations promote greater hydration of the coat, lipid replacement of the epidermal barrier, keratinocyte recovery and increased protection, among other specific benefits. With this, it is possible to associate the treatment of the animal's skin with the treatment of any disease, speeding up

and prolonging the healing process (SOFT CARE).

Drugs incorporated into nanoparticles generally increase their absorption and reduce their toxicity. However, nanoparticles must meet the following conditions to produce their ideal effect (transport): low toxicity, long life and easy degradation by the body (SILVA, A.R.G. & GUIMARÃES, M., 2019). In this work, PBCA, a biodegradable, biocompatible, bioadsorbable and bioadhesive polymer was used with 1 mL of a solution of praziquantel/EtOH:H₂O (8:2). After infrared, thermogravimetric and differential thermal analysis, the authors concluded that there was evidence of the development of poly(n-butyl) nanoparticles that incorporated praziquantel satisfactorily, showing the qualitative aspects of the nanosystem (SILVA, A. R. G. & GUIMARÃES, M., 2019).

The aim of this study is therefore to develop a shampoo for veterinary use with polymeric nanoparticles containing praziquantel. To date, praziquantel is basically found on the market in oral formulations (tablets, capsules, cookies) in its pure, non-vectorized form, despite its partial gastrointestinal absorption. This form of administration does not always produce satisfactory therapeutic results for the animals, making it a problem for treatment. With this in mind, the veterinary product to be developed is expected to optimize the treatment of patients, since it will be applied in the form of baths, characterizing an innovation in the use of the drug.

Figure 3: Structure of PBCA-PZQ nanoparticles by SEM



Source: (SILVA, A. R. G. & GUIMARÃES, M., 2019).

MATERIAL AND METHOD

An experimental study was conducted focusing on the evaluation of a base shampoo formulation for the incorporation of drugs, along with the integration of polymeric nanoparticles containing the drug praziquantel. The samples were subjected to a series of analyses to evaluate organoleptic characteristics, such as appearance, color and odor, as well as various physicochemical parameters, including pH, density and viscosity. The simultaneous execution of the tests with the base, the shampoo with the pure

drug and the shampoo with added polymeric nanoparticles of praziquantel made it possible to attribute the changes observed to the active components used.

Preparation of nanoparticles

Polymeric nanoparticles can be prepared by polymerizing monomers (e.g. alkyl cyanoacrylates) to form polymers called polyalkylcyanoacrylates (PACA) (SOUTO, 2012). The polymerization reaction of alkyl cyanoacrylates takes place in three stages:

1st - nucleation, which consists of the activation of the monomer and the generation of reactive carbons;

2nd - the propagation or growth phase of the polymer and, finally,

3rd - the end of the reaction phase, which results in the nanospheres or nanocapsules. Nanospheres have a polymeric matrix in which the drug is retained. Nanocapsules have a polymer coating around an oily core, in which the drug may be dissolved or absorbed (ANDRIEUX et al., 2009).

In the work by Silva et al. (2019), nanoparticles were developed from the biodegradable polymer PBCA due to its ease of synthesis. In addition, the particles were coated with polysorbate 80, which is part of a class of non-ionic surfactants and excipients widely used in various pharmaceutical and cosmetic formulations. They act by increasing the solubility of drugs in suspensions with low or no solubility.

Initially, 10mL of 0.1M HCl solution with 0.1000g of Dextran® was pipetted into an erlenmeyer flask and placed on a magnetic stirrer. After the Dextran® had completely dissolved, 100 µL of Histoacryl® (n-butyl cyano acrylate) monomer was added to the reaction medium. A period of 1 hour was waited and 1mL of a solution of praziquantel/EtOH:H₂O (8:2) was added, keeping the dispersion under agitation for a further 3 hours. After a total of 4 hours of stirring, a 0.1 M NaOH solution was added until the nanoparticle dispersion was neutralized (pH = 7.0 ± 0.3). After neutralization, the dispersion containing the nanocarrier was filtered using filter paper to remove monomer agglomerates that had not been polymerized (SILVA, et.al., 2019).

Formulation

The product developed was a base shampoo for the incorporation of a medicinal active, using good handling practices. A shampoo containing non-ionic surfactants and anionic surfactants was manipulated to promote cleanliness and allow the addition of praziquantel in the form of polymeric nanoparticles.

The ingredients were weighed according to Table 1. First, sodium lauryl ether sulfate, triethanolamine lauryl sulfate and amide 90 (coconut fatty acid diethanolamine) were added to a glass container. Then purified water and methylparaben, previously solubilized in q.s. of ethyl alcohol, were added. After this, the pH was adjusted with citric acid solution and the viscosity corrected with the addition of sodium chloride.

Table 1: Transparent shampoo formulation developed

| Components | Composition (%) | Function |
|--------------------------------|-------------------|---------------------------------|
| Sodium lauryl ether sulfate | 21,0 | Anionic surfactant |
| Triethanolamine lauryl sulfate | 5,0 | Surfactant with detergent power |
| Amide 90 | 4,0 | Thickener and emollient agent |
| Methylparaben | 0,2 | Preserving agent |
| Citric acid (10% sol.) | q.s. pH 7,0 a 7,5 | pH regulator |
| Sodium chloride | q.s. | Viscosity agent |
| Purified water | q.s.p. 100mL | vehicle |

Source: the authors.

Accelerated stability studies

After preparing the shampoo base, a set of 12 samples were separated, 3 of which were subjected to an environment with incidence of light, 3 to an environment without incidence of light, 3 in an oven and 3 in a refrigerator. The tests were carried out 0 (D₀), 7 (D₇), 14 (D₁₄), 21 (D₂₁) and 28 (D₂₈) days after preparation. The appearance, color, odor, pH, density and viscosity were evaluated during the test periods.

Organoleptic characteristics analysis

The organoleptic characteristics (color, odor and appearance) of the samples, which had previously been subjected to different conditions, were analyzed by comparing them with the results obtained on the day of handling (D₀), as detailed in Table 2.

Viscosity analysis

Viscosity was determined using a rotary viscometer (Brookfield viscometer). This instrument has cylinders of different diameters (spindles) in which the appropriate cylinder is used depending on the viscosity of the fluid being analyzed. After manipulation, the viscosity was measured using spindle 2 and 60 RPM.

pH analysis

The pH was analyzed using a Digimed DM22 digital pH meter. On the day of handling, the base formulation of the shampoo was neutralized (to pH ±7.0) with a 10% (w/v) citric acid solution.

Density analysis

A pycnometer was used to assess density. Basically, the empty instrument was weighed and the weight recorded. It was then filled with purified water, avoiding the introduction of bubbles, and carefully dried on the outside for weighing and writing down the weight. Finally, the same was done with the sample for subsequent density calculations using the mathematical equation below:

Figure 4: Density calculation equation

$$Density_{sample} = \frac{Pyc_{sample} - Pyc_{empty}}{Pyc_{water} - Pyc_{empty}}$$

Source: the authors.

RESULTS AND DISCUSSION

During the development of new products, the stability study generally contributes to the development of the ideal formulation, as well as the materials that should be used in its packaging, in order to highlight possible improvements to improve the formulation, estimate the shelf life and information, assist in monitoring the organoleptic, microbiological and physicochemical stability and this produces reliability and safety in the products, also exposing the conditions that accelerate changes that can occur over time (BRASIL, 2004).

As established in ANVISA Resolution RDC No. 318/19 and in accordance with SDA/MAPA Normative Instruction No. 15/2005, this study was designed to examine the possible physical and chemical changes in the drug base and the product during storage under different controlled conditions. This included ambient temperatures (20 ± 5) °C, with light incidence and without light, oven (40 ± 2) °C and refrigeration (5 ± 3) °C.

Both RDC 318/19 and IN 15/2005 do not specify the ideal values for the Relative Standard Deviation (RSD) for the accelerated stability analysis of pharmaceutical products, however, internally, we have established the criterion of considering values below 5.0% as ideal. This metric serves as a parameter for assessing the consistency and stability of samples under various storage conditions over time.

Organoleptic Characteristics analysis

After describing the organoleptic aspects obtained on the day of preparation, the physical and visual characteristics were analyzed over the weeks. The color of the samples was compared by placing them next to each other under the same incidence of light. The odor was sensed to check that there were no deviations or unpleasant/strong odors and the results are shown in Tables 2 and 3.

Table 2: Organoleptic characteristics observed in D₀

| Analysis | Sample 1 | Sample 2 | Sample 3 |
|------------|----------------|----------------------------|----------------|
| Appearance | Viscous liquid | Viscous liquid | Viscous liquid |
| Color | Translucent | Slightly milky | Translucent |
| Odor | Neutral | Characteristic of the drug | Neutral |

Legend: Sample 1 - Blank; Sample 2 - shampoo with pure praziquantel; Sample 3 - shampoo with praziquantel nanoparticles.

Source: the authors

Table 3: Organoleptic Characteristics Test

| Organoleptic Characteristics Test | | | | |
|-----------------------------------|----|-----|----|----|
| Sample/ Condition | EL | EWL | R | G |
| | 7 | 14 | 21 | 28 |
| Sample 1 | C | C | C | C |
| Sample 2 | C | C | C | C |
| Sample 3 | C | C | C | C |

Legend: Sample 1 - Blank; Sample 2 - Shampoo with pure praziquantel; Sample 3 - Shampoo with praziquantel nanoparticles. (EL: Environment with incidence of light; EWL: Environment without incidence of light; G: Greenhouse; R: Refrigerator) *C: Compliant. **Source:** the authors

The organoleptic analysis of D₇, D₁₄, D₂₁ e D₂₈ showed compliance with the parameter defined on the day of handling. There was no change in the color of any of the samples, showing that they remained stable in terms of this parameter under all the conditions. In the odor analysis, it was noticeable that the samples containing pure praziquantel, especially the one submitted to the oven, had a stronger odor than the others, probably due to the presence of the pure drug. The odor is similar to that of the isolated drug. Under conditions of lower exposure to light and ambient temperatures, the Relative Standard Deviation (RSD) values were significantly lower, indicating improved stability of the base.

Viscosity analysis

Table 4: Viscosity observed in D₀

| Sample | Viscosity (Pa.s) |
|----------|------------------|
| Sample 1 | 0,275 |
| Sample 2 | 0,052 |
| Sample 3 | 0,150 |

Legend: Sample 1 - Blank; Sample 2- shampoo with pure praziquantel; Sample 3 - shampoo with praziquantel nanoparticles.

Source: the authors.

During the viscosity analysis, on the days D_{14} , D_{21} e D_{28} , the apparatus showed a certain instability, and all the samples had a Relative Standard Deviation (RSD) of $>5.0\%$, not meeting the specification. It is therefore suggested that a new stability study be carried out in order to have a more accurate assessment of the viscosity parameter.

pH Test

Table 5: Results of the pH analysis of the samples D_0

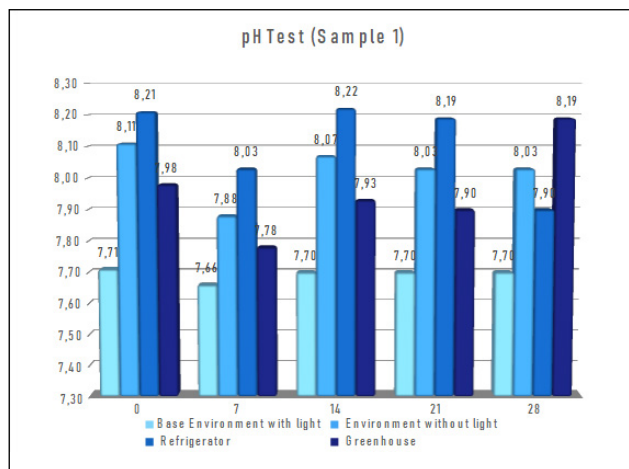
| Samples | pH |
|----------|-----|
| Sample 1 | 7,5 |
| Sample 2 | 7,8 |
| Sample 3 | 7,7 |

Legend: Sample 1 - Blank; Sample 2 - shampoo with pure praziquantel; Sample 3 - shampoo with praziquantel nanoparticles.

Source: the authors.

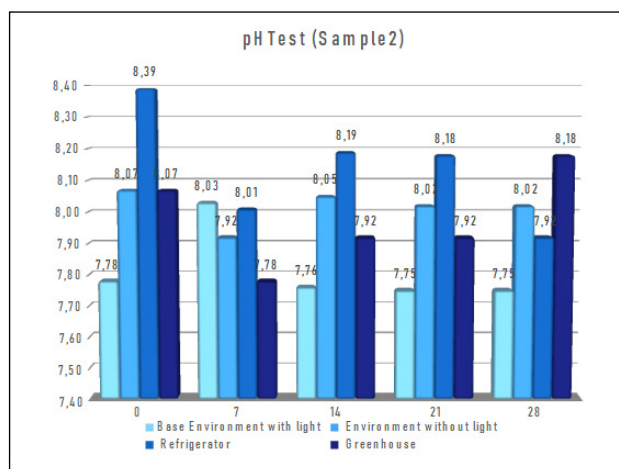
According to Briones (2004), the pH of a healthy dog's skin can vary between 5.8 and 6.4, with an average of 6.1, and by analyzing various shampoos in his article, the author showed that the pH of commonly used shampoos, whether for therapeutic purposes or not, varies between 5-8. During the weekly analysis, it was possible to notice that the samples varied between 7.5 and 8.3, indicating little variation, i.e. no signs of degradation processes. However, it would be appropriate to adjust the pH so that it is closer to 5-6, which is the physiological pH value for dogs, in order to avoid irritation and allergic processes. The pH values obtained in subsequent weeks were evaluated in comparison to the results obtained at D_0 , as detailed in Graphs 1, 2 and 3.

Graph 1: pH test (Sample 1)



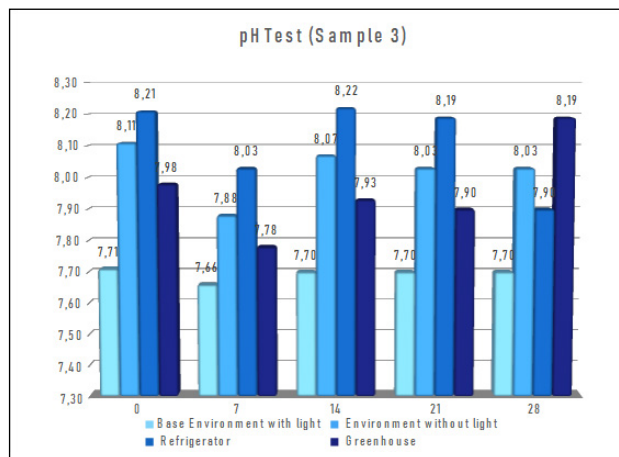
Legend: Sample 1 White Shampoo.
Source: the authors.

Graph 2: pH test (Sample 2)



Legend: Sample 2 Shampoo with pure praziquantel.
Source: the authors.

Graph 3: pH test (Sample 3)



Legend: Sample 3 - Shampoo with praziquantel nanoparticles.
Source: the authors.

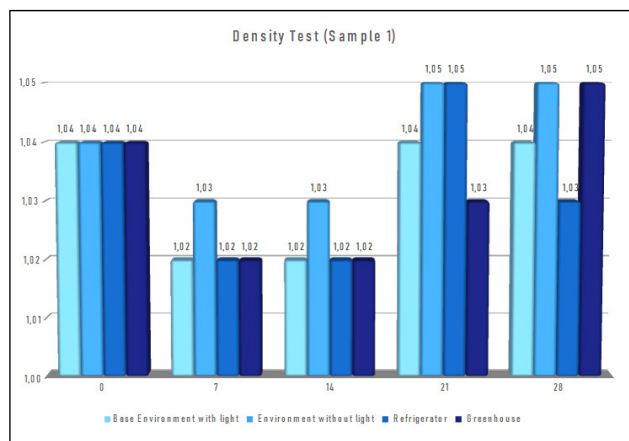
The pH test indicated variations in (DPR) for different storage conditions. Sample 1 showed the highest DPR in an oven (1.9%), which suggests that exposure to heat may be inducing the degradation of the base's sensitive components, such as sodium lauryl sulfate. Sample 2 showed the greatest deviation under refrigeration (2.2%) and in an oven (1.9%), indicating that extreme conditions do not guarantee good stability; the lowest DPR was at room temperature with protection from light (0.7%). Finally, sample 3 showed the same behavior, indicating greater variation in the oven (1.9%) and refrigerator (1.7%), and was best stored at room temperature with protection from light (0.3%).

Density analysis

The density tests showed that the samples remained within the values expected from similar product specifications. Comparing the density values from the seventh day to the twenty-eighth

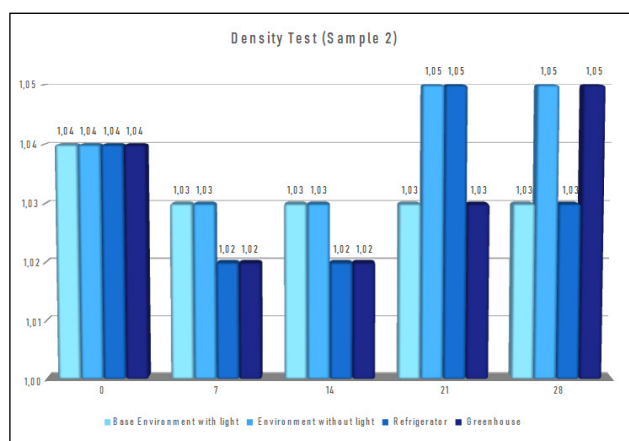
day (Graphs 4 to 6), it can be seen that there were no significant changes in the samples.

Graph 4: Density test (Sample 1)



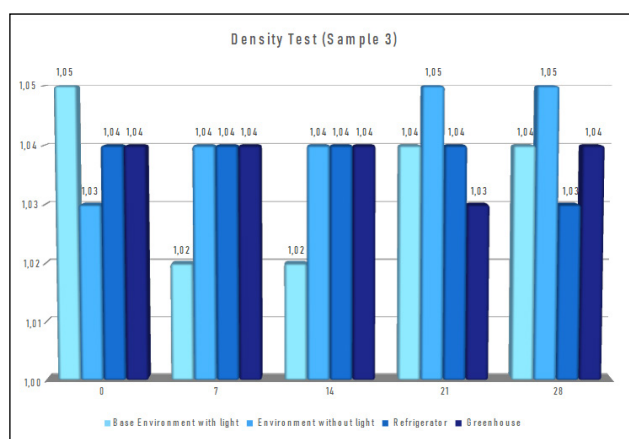
Legend: Sample 1 White Shampoo. **Source:** the authors.

Graph 5: Density test (Sample 2)



Legend: Sample 2 - Shampoo with pure praziquantel. **Source:** the authors.

Graph 6: Density test (Sample 3)



Legend: Sample 3 - Shampoo with praziquantel nanoparticles.

CONCLUSION

The study of nanoparticulate release systems has been currently gained emphasis in the dermatology field. These products are essential because they act as carriers, enabling the incorporation of active ingredients with low solubility in other vehicles, facilitating permeation and increasing bioavailability.

The developed shampoo will be indicated for veterinary use in dogs for the treatment and prevention of parasites with nanoparticles of the drug praziquantel. The availability of the active ingredient, which up to now has only been found in oral formulations and is an obstacle to administration, will be facilitated as the proposed treatment will be applied to the animal in the form of baths.

During the accelerated stability study, the veterinary shampoo incorporated with nanoparticles containing praziquantel proved to be a stable product at room temperature and in the absence of light exposure, preserving its physicochemical characteristics. By comparing the analyses, it can be seen that Sample 2, containing the pure drug, was susceptible to degradation under thermal stress. Therefore, the polymeric nanoparticles containing praziquantel have been shown not to cause physicochemical instability to the product, indicating both pharmaceutical and marketing potential.

The product could be available in partnerships with compounding pharmacies, pharmacies specializing in veterinary care or businesses interested in animal care and this type of treatment offered by the product.

It is therefore extremely important to carry out studies on the incorporation of nanoparticles (drug-delivery systems) in order to learn about their behavior in different drugs and vehicles, enabling new forms of treatment.

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