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Development of gelatin nanoparticles for the treatment of pathologies caused by *Paraccocideoides brasiliensis*

Desenvolvimento de nanopartículas de gelatina para o tratamento de patologias causadas por *Paraccocideoides brasiliensis*

Desarrollo de nanopartículas de gelatina para el tratamiento de patologías causadas por *Paraccocideoides brasiliensis*

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Aline Cristiane de Oliveira Silva
ORCID: https://orcid.org/0000-0002-0065-9863
University of Vale do Paraíba, Brazil
E-mail: aline.cos83@gmail.com
Thainara Alves Gouvea
ORCID: https://orcid.org/0000-0001-6749-7371
University of Vale do Paraíba, Brazil
E-mail: thaigouvea20@gmail.com
Jéssica Aparecida Ribeiro Ambrósio
ORCID: https://orcid.org/0000-0003-0457-6624
University of Vale do Paraíba, Brazil
E-mail: jessicaacdc.ja@gmail.com
Milton Beltrame Junior
ORCID: https://orcid.org/0000-0002-3535-6512
University of Vale do Paraíba, Brazil
E-mail: beltrame@univap.br
Priscila Maria Sarmeiro Correa Marciano Leite
ORCID: https://orcid.org/0000-0002-7880-125X
University of Vale do Paraíba, Brazil
E-mail: prileite@univap.br
Flávia Villaça Morais
ORCID: https://orcid.org/0000-0001-8576-9684
University of Vale do Paraíba, Brazil
E-mail: flavia@univap.br
Andreza Ribeiro Simioni
ORCID: https://orcid.org/0000-0002-3087-7431
University of Vale do Paraíba, Brazil
E-mail: simioni@univap.br
Erika Peterson Gonçalves
ORCID: https://orcid.org/0000-0002-2064-9929
University of Vale do Paraíba, Brazil
E-mail: erika@univap.br

Abstract

Introduction: *Paraccocideoides brasiliensis* (Pb) is a fungal pathology that causes serious lesions in the mouth in the form of ulcerations that destroy the gingiva, palate, reflecting on the immune system. For the treatment of Paracoccidioidomycosis (PMC) the choice of drug is based on the degree of the disease, with Amphotericin B (AB) being the most used due to its mechanism of action and interaction with the fungal membrane, causing its destruction. However, with all the history of AB toxicity, there is a need to reduce the serum levels of the drug in the body. The use of nanoparticles as controlled release systems (DDS) is an alternative for this purpose. The objective of this article was to develop gelatin nanoparticles for the treatment of pathologies caused by Pb. Method: In this work, gelatin nanoparticles were processed by the two-step desolvation method and loaded with AB. The nanoparticulate system was characterized by scanning electron microscopy (SEM) and dynamic light scattering (DLS). Finally, the effectiveness of DDS was evaluated against Pb yeasts by Disk Diffusion Test. Results: the synthesized particles presented suitable sizes and shapes for application in DDS with an average size of 169.4 and 172.0 nm, for the free and encapsulated system, respectively. Biological model tests of Pb showed positive results indicating that lower concentrations of AB can be efficiently administered if nanostructured DDS is used. Conclusion: This study is promising for future applications of AB-loaded gelatin nanoparticles for the treatment of pathologies caused by *Paraccocideoides brasiliensis*.

Keywords: *Paraccocideoides brasiliensis*; Amphotericin B; Drug delivery system; Nanoparticles.

Resumo

Introdução*: Paraccocideoides brasiliensis* (Pb) é uma patologia fúngica que causa lesões graves na boca na forma de ulcerações que destroem a gengiva, palato, refletindo no sistema imunológico. Para o tratamento da Paracoccidioidomicose (PMC) a escolha do fármaco ocorre pelo grau da doença, sendo a Anfotericina B (AB) a mais utilizada devido ao seu mecanismo de ação e interação com a membrana do fungo, ocasioando a sua destruição. Porém, com todo histórico de toxicidade da AB, surge a necessidade de diminuir os níveis séricos do fármaco no organismo. O uso de nanopartículas como sistemas de liberação controlada (DDS) é uma alternativa para este fim. O objetivo deste artigo foi desenvolver nanopartículas de gelatina para o tratamento de patologias causadas por Pb. Método: Neste trabalho, nanopartículas de gelatina foram processadas pelo método de dessolvatação em duas etapas e carregadas com AB. O sistema nanoparticulado foi caracterizado por microscopia eletrônica de varredura (MEV) e espalhamento dinâmico de luz (DLS). Por fim, a eficácia do DDS foi avaliada contra leveduras de Pb pelo Disk Diffusion Test. Resultados: as partículas sintetizadas apresentaram tamanho e formas adequadas para aplicação em DDS com tamanho médio de 169,4 e 172,0 nm, para o sistema livre e encapsulado, respectivamente. Os testes em modelo biológico de Pb apresentaram resultados positivos indicando que concentrações mais baixas de AB podem ser administradas de forma eficiente se o DDS nanoestruturado for utilizado. Conclusão: Este estudo é promissor para futuras aplicações de nanopartículas de gelatina carregadas com AB para o tratamento de patologias causadas por *Paraccocideoides brasiliensis*.

Palavras-chave: *Paraccocideoides brasiliensis*; Anfotericina B; *Drug delivery system*; Nanopartículas.

Resumen

Introducción: *Paraccocideoides brasiliensis* (Pb) es una patología fúngica que provoca graves lesiones en la boca en forma de ulceraciones que destruyen la encía, paladar, repercutiendo en el sistema inmunológico. Para el tratamiento de la Paracoccidioidomicosis (PMC) la elección del fármaco se basa en el grado de la enfermedad, siendo la Anfotericina B (AB) la más utilizada por su mecanismo de acción e interacción con la membrana fúngica, provocando su destrucción. Sin embargo, con toda la historia de toxicidad de AB, existe la necesidad de reducir los niveles séricos de la droga en el cuerpo. El uso de nanopartículas como sistemas de liberación controlada (DDS) es una alternativa para este fin. El objetivo de este artículo fue desarrollar nanopartículas de gelatina para el tratamiento de patologías causadas por Pb. Método: En este trabajo, las nanopartículas de gelatina fueron procesadas por el método de desolvatación en dos pasos y cargadas con AB. El sistema de nanopartículas se caracterizó mediante microscopía electrónica de barrido (SEM) y dispersión de luz dinámica (DLS). Finalmente, se evaluó la efectividad de DDS frente a levaduras Pb mediante el Disk Diffusion Test. Resultados: las partículas sintetizadas presentaron tamaños y formas adecuados para su aplicación en DDS con un tamaño promedio de 169,4 y 172,0 nm, para el sistema libre y encapsulado, respectivamente. Las pruebas de modelo biológico de Pb mostraron resultados positivos que indican que se pueden administrar de manera eficiente concentraciones más bajas de AB si se usa DDS nanoestructurado. Conclusión: Este estudio es prometedor para futuras aplicaciones de nanopartículas de gelatina cargadas con AB para el tratamiento de patologías causadas por *Paraccocideoides brasiliensis*.

Palabras clave: *Paraccocideoides brasiliensis*; Anfotericina B; Sistema de administración de fármacos; Nanopartículas.

1. Introduction

Paracoccidioidomycosis (PCM) is a systemic mycosis originally described by Adolfo Lutz in 1908, native to Latin America, with the highest incidence recorded in South American countries (Brazil, Argentina, Colombia and Venezuela). In Brazil, most cases have been reported in the south, southeast and central-west regions. Paracoccidioidomycosis is endemic among rural populations, affecting males in the productive age group (30-60 years) and is related to agricultural activities. The etiologic agent is a thermodimorphic fungus (*Paracoccidioides brasiliensis*) (Araújo et al., 2020; Turissini et al., 2017). Paracoccidioidomycosis represents an important public health problem due to its high disabling potential, in addition to causing premature deaths (Griffiths et al., 2019).

Pharmacological measures consist of antifungals with effect on *P. brasiliensis*. The choice of the drug to be administered depends on various circumstances such as clinical severity, previous history of therapeutic resistance, gastro-intestinal drug absorption capacity, association with comorbidities, and treatment adherence. *P. brasiliensis* is sensitive to most antifungal drugs, including sulfonamides, azoles (ketoconazole, fluconazole, itraconazole, posaconazole, voriconazole), amphotericin B (AB), and terbinafine (Mahajan, 2014; Ambrósio et al., 2014; Orofino-Costa et al., 2017)

Sulfamethoxazole-trimethoprim or itraconazole can be used in the mild or moderate localized forms (Borges et al.,

2014; Mendes et al., 2018) being amphotericin B reserved for the disseminated and severe forms, as in the central nervous system (CNS) (Hahn & Hamdan, 2000; Alonso et al., 2022).

The mechanism of action of AB is related to the interaction of ergosterol (a component of the fungal cell membrane), in this way AB will form small holes in the fungal membrane, increasing its permeability and causing the escape of ions and metabolites, mainly ions of potassium (Baghirova & Kasumov, 2021; Lee & Lee, 2018)

Due to AB affinity for ergosterol, the toxic effect it produces due to its affinity for cholesterol, which is directly related to the human cell membrane, making it extremely nephrotoxic (Kamiński, 2014). Thus, it is crucial to improve the therapeutic options.

Amaral et al. (2009) described efficient treatment of PCM in lungs with amphotericin B loaded nanoparticles coated with dimercaptosuccinic acid (DMSA), emphasizing lung-targeting role of DMSA (Amaral et al., 2009).

Jannuzzi et al. (2018) describe the use of peptide antigens, such as *P. brasiliensis* P10 incorporated into poly(lactic-co-glycolic acid) (PLGA) nanoparticles. The authors report that the nanoparticulate system demonstrated an effective immune response against fungal infections when combined with antifungal drugs, thus increasing therapeutic efficacy against PCM (Jannuzzi et al., 2018).

Cunha-Azevedo et al. (2018) studied PLGA-DMSA nanoparticles loaded with itraconazole for treatment of PCM (Cunha-Azevedo et al., 2018). So, nanobiotechnology enables the development of new pharmaceutical formulations for controlled delivery of drugs, which can avoid many of the problems related to low efficacy and side effects of traditional drugs. Some of the advantages of using nanoparticles for drug delivery include enhanced stability, controlled release, and enhanced bioavailability (Mitchell et al., 2021).

To investigate whether this approach reduces the side effects of AB while simultaneously providing a controlled drug delivery system, AB-loaded gelatin nanoparticles were developed by the two-step desolvation method.

The present study aimed to characterize this nanoparticulate system in terms of its physicochemical properties and to evaluate its antifungal activity against Paracoccidioides brasiliensis (strain Pb18).

To our knowledge, this study is the first to report a project of a drug delivery system with AB functionalized in gelatin nanoparticles for the treatment of pathologies caused by *Paraccocideoides brasiliensis*.

2. Methodology

2.1 Materials

Gelatin Porcine Skin type A Bloom 300 and Amphotericin B were purchase from Sigma-Aldrich (St. Louis). Organic solvents in analytical grade were purchased from Synth (Brazil) and used as received.

2.2 Fabrication of AB-loaded gelatin nanoparticles

Gelatin nanoparticles (NPG) were prepared by a two-step desolvation method reported by Carvalho et al., (2018), Ambrósio et al. (2019), de Souza et al. (2021) and Trindade et al. (2022) with minor modifications.

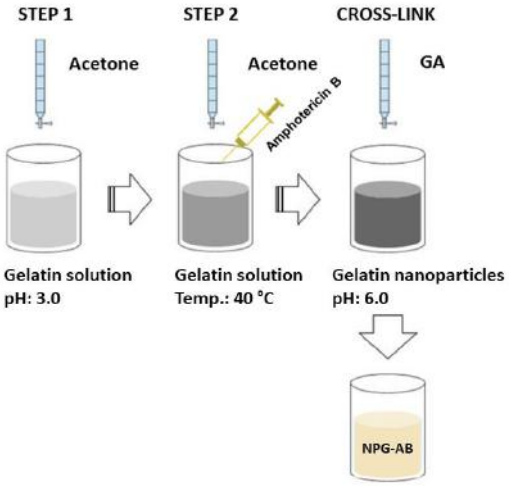
Briefly, 200 mg of gelatin was dissolved in 25 mL of distilled water under constant heating at 40° ± 1°C. After total solubilization, 25 mL of acetone was quickly added as a desolvating agent, causing the precipitation of high molecular weight gelatin molecules, which is used due to the strong bonds provided by cross-linking (Graziola et al., 2016). The supernatant was discarded and the molecules with the highest molecular weight of gelatin were redissolved by adding 25 mL of distilled water under constant heating and magnetic stirring.

AB dissolved in 500 µL of dimethylsulfoxide (DMSO) was added to the aqueous solution of the polymeric phase, followed by the dropwise addition of 75 mL of acetone to form NPs. Immediately after the end of the acetone dripping, 1.0 µL

of an aqueous solution of glutaraldehyde (25% v/v) was added as a crosslinking agent. The reaction medium was stirred for 60 minutes at room temperature.

The solution was then incubated overnight at room temperature, at a concentration of 1 $mg.mL^{-1}$ AB. The unencapsulated AB was removed by centrifugation (Eppendorf Centrifuge 5804 R) at 5000 rpm for 20 minutes and washed three times with acetone solution (75%). Subsequently, the pellet was suspended with 2.5 mL of acetone solution (75%) and stored at 4 ºC. This procedure produced the AB-encapsulated gelatin nanoparticles (NPG-AB). Obtaining empty gelatin nanoparticles (NPG) followed the protocol described above, except for the step of adding the AB solution to the reaction medium (Figure 1).

Figure 1: Gelatin nanoparticle synthesis route.



Source: Authors.

2.3 Scanning electron microscopy (SEM)

The size and external morphology of NPG and NPG-AB were examined by Scanning Electron Microscopy (SEM) by the secondary electron method on an EVO-MA10 microscope (Zeiss) with tungsten filament. The sample was prepared for the test in a sample holder where a drop of solution dispersed under ultrasound in a minimum amount of water was deposited. Drying was carried out by evaporating the sample holder at room temperature for 24 hours. A thin film of gold was then deposited on the plasma.

2.4 Steady state measurements

Cary 50 BIO-Varian (Inc. Scientific Instruments) was used for absorption measurements. The spectrophometric analyses were done with a that was background corrected using matched quartz cuvettes with scanning over the wavelength range from 300 to 480 nm. To establish linearity of the proposed methods seven calibration curves were constructed at seven concentrations levels within the range of 0.5 – 5.0 $\mu mol.L^{-1}$. In the spectrophotometric analyses the Beer's law was obeyed. Least square regression analysis was done for the data. One-way analysis of variance (ANOVA) and a Lack-of-fit test ($p = 0.05$) were used to determine whether the linear model adequately explains obtained data.

2.5 Colloidal stability

Loaded and unloaded nanoparticles were characterized in terms of diameter, ζ-potential and stability over time by laser light scattering technique using dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS, Malvern, UK). 0.1 mL of the AB-loaded gelatin nanoparticles were suspended with 10 mL MilliQ water via vortexation and ultrasonication for 10 min for the particle size measurement at a scattering angle of 173° at room temperature. The polydispersity index (PDI) was calculated using the equipment software for DLS measurements. For the colloidal stability and size changing of the particle, the shelving effect upon the nanoparticulate system characteristics (size distribution and zeta potential) was probed by five measurements collected at distinct storage times. The samples were storage at 4 °C and kept on dark condition. Analyses were carried out in time function for 90 days.

2.6 Encapsulation efficiency (EE%)

Encapsulation efficiency was evaluated by UV–Vis spectroscopy (Cary 50 BIO-Varian) at 419 nm. The drug encapsulation rate was evaluated by 3 mg of freeze-dried nanoparticles initially dissolved in 2 mL of dimethylsulfoxide (DMSO), and 0.5 mL samples were diluted with 2% sodium dodecyl sulfate (SDS) in phosphate buffered saline (PBS) and magnetically stirred for 1 h at 28 °C. The supernatant was collected, and its AB content was quantified by spectroscopy techniques. The absorption measurements were carried out with a Cary 50 BIO-Varian, which was background corrected using matched quartz cuvettes with scanning over the wavelength from 300 to 480 nm. The AB concentrations were measured from standard curve, and 2% SDS in PBS was used as reference solvent. Encapsulation efficiency was calculated from Eq.(1).

$$EE\ (\%) = ((Dt - Ds) \times 100)/Dt \qquad \text{Equation (1)}$$

where, EE is the encapsulation efficiency, Dt is theoretical loading of drug and Ds is the drug concentration in the supernatant.

2.7 In vitro drug release

For the in vitro drug release, briefly, 50 mg of particles containing AB was dispersed in 50 mL of 2% SDS phosphate buffered saline, pH 7.4, at 37 °C. and at predetermined time intervals, an aliquot (1.5 mL) was withdrawn, replaced with the fresh one, and analyzed by UV–Vis spectrophotometer at 419 nm. The amount of drug released (DR) was determined by the equation (2).

$$DR\ (\%) = Dt/D0 \times 100 \qquad \text{Equation (2)}$$

where D(t) (mg) represents the amount of drug released at time t, and D(0) (mg) the amount of drug loaded. All studies were conducted in triplicates.

2.8 In vitro experiments

2.8.1 Cell and culture description

Yeast cells of *Paracoccidioides brasiliensis*, isolate 18, (Pb18) were maintained in glass tubes containing solid YPD medium at 4 °C. Yeast culture was obtained by inoculating two yeasts in 20 mL of YPD liquid medium (0.5% yeast extract, 1% peptone, 0.5% dextrose, sterilized by autoclaving) and incubation for 4 days, under agitation, at 36 °C.

2.8.2 Disk diffusion test

For the beginning of the experiments, the yeasts were collected by centrifugation (12,000 rpm, 10 minutes), washed twice in saline solution (0.9% NaCl, sterilized by autoclaving). For the yeasts to be disassembled, after washing, they were subjected to two successive passages through needles of different gauges, 25x8 and 25x7, and the cell suspension was collected after resting for 5 to 10 min. Cell concentration was obtained by counting in a Neubauer chamber, in the presence of the vital dye methylene blue, and adjusted with the same solution to $1x10^6$ cells/mL. One milliliter of culture containing $1x10^6$ Pb18 yeast cells was inoculated into petri dishes containing 20 mL. Cells were spread using a Drigalsky loop. Sterile 6 mm diameter filter papers were added to the inoculated yeasts, on which 10 µL of different concentrations of amphotericin B and nanoencapsulated amphotericin B were added.

2.8.3 Statistical analysis

The tests were performed in quadruplicate, and all data were submitted to ANOVA followed by the Tukey test. Microsoft Excel was used to generate the charts. All data are expressed as the mean ± standard deviation (SD). A probability value of $p < 0.05$ was considered significant in this study.

3. Results and Discussion

The two-step desolvation process has been the most used technique to obtain gelatin nanoparticles, and this technique is based on the addition of a desolvating agent to the aqueous solution of gelatin to dehydrate the gelatin molecules, resulting in the conformational change of the helix triple (Ahsan & Rao, 2017a).

Ahsan and Rao (2017) report that gelatin denaturation is a reversible phenomenon and that its solubilization after the first desolvation step confirms the ability of gelatin to re-solubilize after removing the desolvating agent (Ahsan & Rao, 2017b). The use of smaller amounts of acetone in the second desolvation step is important when it is necessary to preserve the tri-helical structure. However, to produce gelatin nanoparticles by the desolvation process it becomes feasible at higher levels of acetonation.

In this work, the gelatin nanoparticles for AB encapsulation were prepared by two-step desolvation methodology. This method is advantageous because it is nontoxic, rapid in reaction rate, and produces very small particles (Hong et al., 2020).

The SEM studies indicated that the obtained nanoparticles were almost spherical in shape as shown in Figure 2.

Figure 2: SEM images of nanoparticles: (a) NPG, (b) NPG-AB.

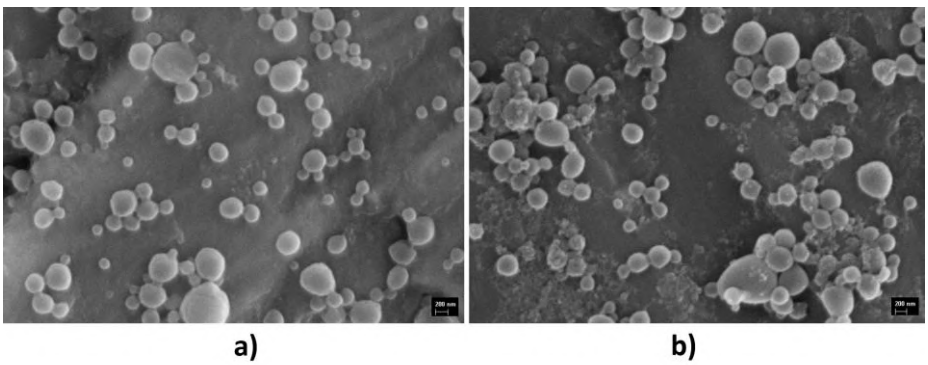


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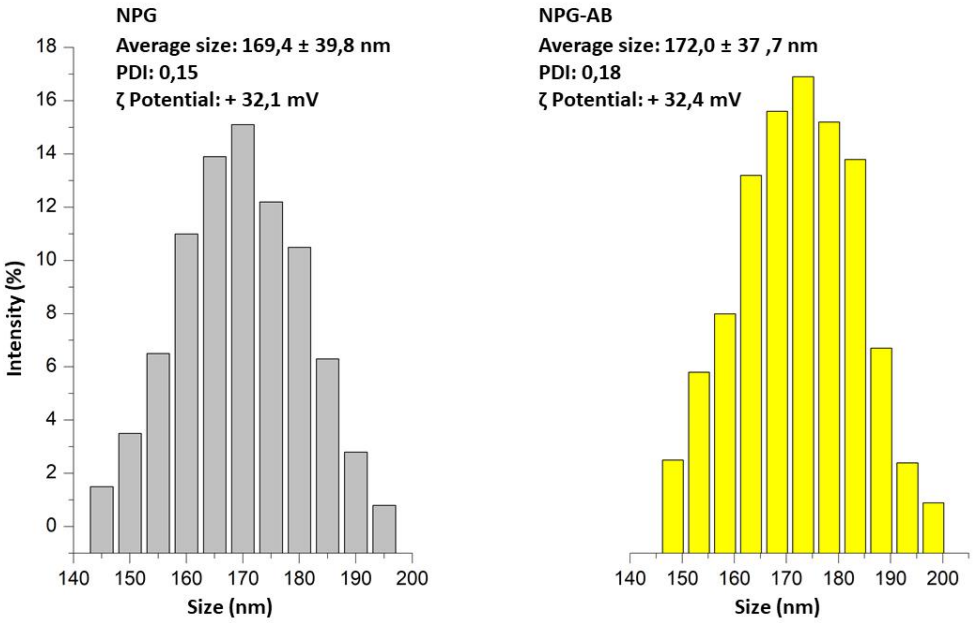
Figure 2 shows that the particles formed have spherical shapes as predicted by Ambrósio et al. (2019) showing that the two-step desolvation process was efficient to obtain the NPGs. The use of glutaraldehyde as a stabilizing agent for the

cross-linking of gelatin nanoparticles proved to be efficient (Satar et al., 2017), the amine groups present in the gelatin structure are responsible for the interaction with the glutaraldehyde molecules resulting in the crosslinking of the gelatin particles through bonds between their hydroxyl and amine groups, giving them a spherical shape (Joy et al., 2016).

The higher initial concentration of gelatin in the desolvation process favors denaturation, facilitating the process of breaking the triple helix (Shamarekh et al., 2020). The structural reorganization of gelatin forming the nanospheres is concentration-dependent, so the higher initial gelatin concentration, low acetone concentration, and low glutaraldehyde concentration in the cross-linking step are indicated to preserve the gelatin structure during the nanoparticle synthesis process for specific applications (Subara et al., 2018).

The results of the DLS measurements show a monomodal and broad peak of particle size distribution for both samples with a mean diameter of 169.4 ± 39.8 and 172.0 ± 37.7 nm, for the NPG and NPG-AB respectively, which shows the uniformity of the particles (Figure 3).

Figure 3: Size distribution of the nanoparticle measured DLS from (a) gelatin nanoparticles; (b) NPG-AB.



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Particle uniformity/homogeneity was also confirmed by the polydispersity index (PDI ranges from 0.0 for a perfectly uniform sample with respect to particle size; to 1.0 for a highly polydisperse sample with various particle sizes of populations). The analysis of the synthesized NPG's found a PDI of 0.15 ± 0.01 and for the NPG-AB it was found a PDI of 0.18 ± 0.02, which shows indices of monomodal polydispersity and system homogeneity.

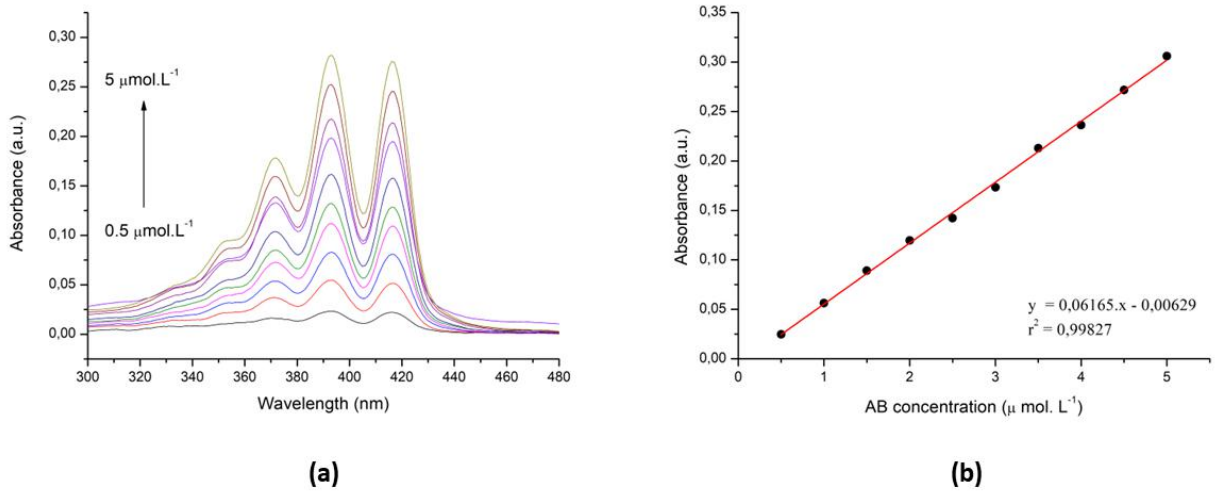
In drug delivery applications using nanoformulations, a PDI of 0.3 and below is considered acceptable and indicates a homogeneous population (Danaei et al., 2018).

The variation in size and PDI of nanoparticulate carrier systems can affect drug encapsulation and delivery (Azimi et al., 2014). Using the two-step desolvation methodology, the low molecular weight gelatin chains were discarded in the first desolvation step and, later, the high and uniform molecular weight gelatin molecules were used to produce nanoparticles, which ensured the lowest PDI, indicating that two-step desolvation is recommended for obtaining AB-encapsulated gelatin-based systems.

The surface potential of NPG was found at a value of +32.1 mV and for NPG-AB +32.4 mV, the positive charge of the Zeta potential confers greater stability on the NPG (Pompeu et al., 2018). In both cases the potential remained stable for 90 days.

The amount of drug adsorbed on the particles was determined by UV-Visible spectrometry (Figure 4).

Figure 4: (a): Absorption spectra of AB at different concentrations (0.5 - 5.0 µmol.L^{-1}). In DMSO; (b): plot of AB concentration vs. absorbance.



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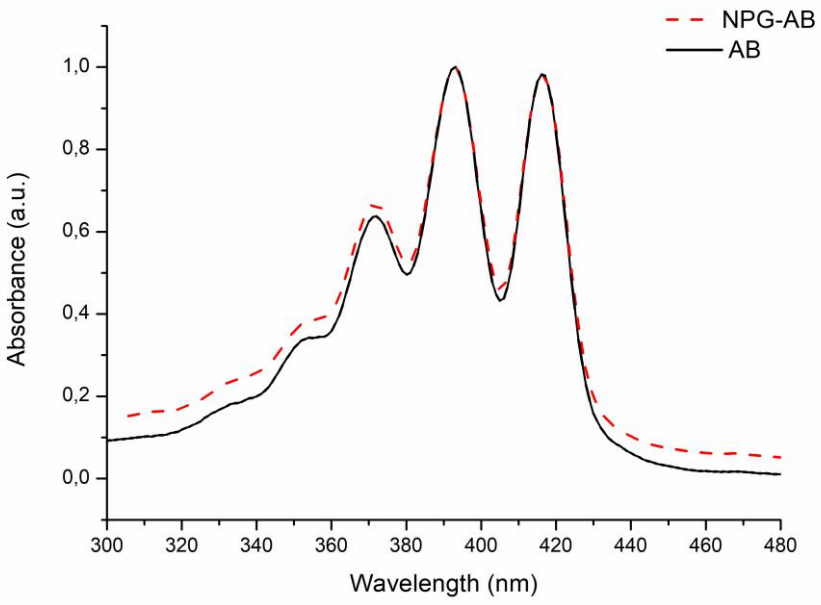
A satisfactory linearity was detected for the spectrophotometric method in the concentration range from 0.5 to 5.0 µmol.L^{-1}. Least squares regression analysis was excellent with correlation coefficient (r^2=0.99827). NPG-AB were initially characterized at steady state by absorbance spectroscopy (Figure 5).

Figure 5: Absorption spectra of AB (2 µmol.L^{-1}) in DMSO: (-) AB standard and (--) AB extracted from gelatin nanoparticles.



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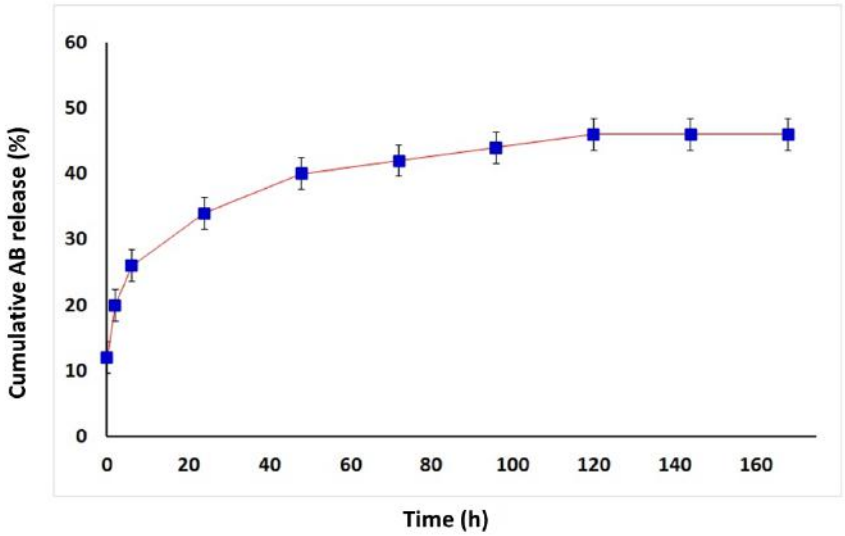
Analyzing the AB spectrum, we observed that there was no change in the physicochemical behavior of the drug, maintaining the absorption spectrum of 419 nm.

The drug was loaded onto the surface of the NPG's by adsorption and the encapsulation efficiency (EE) was 88 %, which was satisfactory.

Saqib et al. (2020) produced polycaprolactone nanoparticles (PCL) nanoparticles for AB loading by nanoprecipitation technique for the treatment of topical leishmaniasis infections (Saqib et al., 2020). The authors determine encapsulation efficiency of 85%.

The in vitro release behavior of AB in the nanoparticulate system was investigated (Figure 6).

Figure 6: The in vitro release profile of AB loaded gelatin nanoparticles. Mean values and their standard deviations (bars), n = 3 determinations.



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The formulation produced a biphasic AB release profile with an initial burst effect in which the AB release varied between 34% at the first sampling time (24 h) for NPG-AB. This rapid release is related to the AB adsorbed on the surface of the nanoparticles (Sheskin et al., 2021) and/or to the release of the drug encapsulated near the surface of the nanoparticles. After this phase, a low constant release of AB up to 45% for NPG-AB, up to 168 h, shows a typical sustained and prolonged drug release that depends on drug diffusion and matrix erosion mechanisms (Geraili, Xing & Mequanint, 2021).

Ghosh et al. (2017) synthesized amphotericin B-loaded poly (L-lactic acid-co-glycolic acid), PLGA nanoparticles for the treatment of visceral leishmaniasis (Ghosh et al., 2017). The authors carried out the study of the cumulative drug release in vitro for 360 h and found that 63.48% ± 1.08% of amphotericin B was released from the formulation.

In vitro assays using the disk diffusion test were able to identify the efficiency of the nanostructured formulation compared to free AB. The results were evaluated by measuring the inhibition halo of the samples (Table 1).

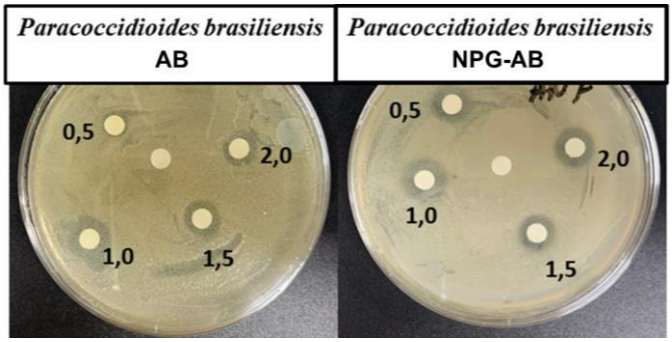
Table 1: Results of Disk Diffusion Test. Relation of the mean diameter of the halo and the concentration of the formulation studied.

	Concentration (mg.mL^{-1})	AB (mm)	NPG-AB (mm)
Paracoccidioides brasiliensis	0.5	7.0	18.0
	1.0	18.0	19.0
	1.5	17.0	17.0
	2.0	17.0	20.0

Source: Authors.

For *P. brasiliensis* yeasts, isolate 18 (Pb18), the antifungal activity of Amphotericin B preparations were similar at concentrations of 1.0 mg.mL^{-1} and 1.5 mg.mL^{-1}. However, nanostructured amphotericin B showed more effective antifungal activity at a concentration of 0.5 mg.mL^{-1} (18 mm halo) when compared to pure Amphotericin B (7 mm halo) which did not reach the considered halo size effective in the literature (Sheskin et al., 2021). At the concentration of 2.0 mg.mL^{-1} nanostructured amphotericin B, it also showed an important difference in the measurement of the halo, figure 7, when compared with pure amphotericin B on yeasts of Pb18, 20 mm and 17 mm, respectively.

Figure 7: Disk diffusion method: Antifungal activity in solid medium against *Paraccocideoides brasiliensis* at different concentrations of nanostructured amphotericin B (NPG-AB) and pure amphotericin B (NPG) on *Paracoccidioides brasiliensis* yeasts (isolate 18).



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The comparison between the data in Table 1 and Figure 7 shows that the halos formed in the assay using nanostructured amphotericin B had a larger diameter compared to the halos formed by Pure Amphotericin B, that is, a concentration of 0.5 mg.mL^{-1} of NPG-AB was more effective compared to the test with free AB. Thus, the toxic effects of the drug will be lower with the use of nanostructured AB.

4. Conclusion

The present work proposed the synthesis of gelatin nanoparticles loaded with amphotericin B for the treatment of paracoccidioidomycoses. The synthesis of the nanoparticles was carried out by the two-step desolvation method to produce spherical and uniformly sized nanoparticles and with encapsulation efficiency of 88%. The controlled-release study demonstrated its constant release over 168 hours. In the disk diffusion test, the concentration of 0.5 mg.mL^{-1} of NPG-AB

proved to be more effective compared to the assay with free AB, that is, the toxic effects of the drug will be lower with the use of nanostructured AB. This study is promising for future applications of gelatin nanoparticles loaded with AB for the treatment of pathologies caused by *Paraccocideoides brasiliensis*. Over the next few years, we hope to enhance the targeting and specificity of novel nanoparticle delivery systems and expand the routes of administration, with the potential patient benefit always.

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