ORIGINAL ARTICLE

Development of a liquid crystalline gel based on Amazonian butter extracted from ucuúba seeds (*Virola surinamensis* (Rol. ex Robbt.) Warb.) for vaginosis treatment

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ABSTRACT

This study focuses on developing a liquid crystalline gel based on ucuúba butter from the Amazon. This gel, which offers distinct technological advantages for vaginal sustained drug release systems, was characterized by polarized light microscopy (PLM), pH measurements, water uptake assay, drug release profile and kinetic study (using the Excel Add-in and DDSolver). The unloaded and drug-loaded liquid crystalline gel containing 10% metronidazole exhibited a hexagonal mesophase structurally favorable for sustained drug release, with a pH value of 6,4 ± 0.024. The water uptake profile showed a fast water uptake in up to 1 h, approximately 16%, with the maximum amount of water, 40%, in 8 h. After water uptake, the liquid crystalline gel undergoes self-assembly to a viscous cubic phase, avoiding rapid erosion in an aqueous medium. *In vitro*, the release of metronidazole was slow, with 52% of the drug released in 10 h. The best fit was the Higuchi kinetic model with the highest MSC (3,94) and R2 (0,98) values, indicating that the drug release was diffusion-controlled. Korsmeyer's model showed that formulation exhibited anomalous transport (non-Fickian). Given the limited number of options for topical vaginal treatment, this liquid crystalline gel based on *Virola surinamensis* (Rol. ex Robbt.) Warb.) butter could potentially find applicability to reduce the frequency of administration and improve local concentrations of metronidazole on vaginal mucous.

Keywords: Liquid crystalline gel; Amazonian fat; Sustained drug release; *Virola surinamensis*, DD solver.

INTRODUCTION

.Antibiotics are the primary treatment for vaginal infections, often taken orally or applied topically (Superti and De Seta 2020). While they effectively eliminate pathogens, they can also trigger adverse reactions and disrupt the beneficial vaginal microbiota. This microbiota is essential for maintaining pH balance, which is crucial for defending against infections and preventing the development of resistant pathogenic bacteria (Jodar et al. 2023).

Despite the wide availability of antibiotics, microorganisms have developed virulence factors that enable them to resist the action of these drugs when invading the human body (Antunes et al. 2013). The excessive and inappropriate use of oral antibiotics is the primary factor contributing to the emergence of resistant bacteria. This has become a critical global public health problem, resulting in high costs for health systems, prolonged hospital stays, and increased exposure of patients to drugs with more significant risks of adverse reactions. (Karnwal et al. 2023).

Amphiphilic lipids, such as monoolein, are commonly used to create liquid crystals (Kulkarni et al. 2011). Employing lipid matrices made of

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Amazonian butter offers potential technological advantages (Américo et al. 2020; Da Silva et al. 2021). These matrices can produce delivery systems that are biocompatible, biodegradable, and have physicochemical properties suitable for application to the vaginal mucosa. (Lee et al. 2021; Feitosa et al. 2021; Sharma et al. 2024). This research is important not only for the field of drug delivery but also for promoting the sustainable use of natural resources in the Amazon. It adds value to raw materials and contributes to the regional and socioeconomic development of the North region. Additionally, it emphasizes the importance of environmental consciousness in our approach to drug delivery.

Conventional topical treatments, carried out using traditional vaginal creams and gels, are known for their low effectiveness due to their short residence time in place, which leads to prolonged use (Jodar et al. 2023). Therefore, the liquid crystalline gel based on ucuúba butter is a potential candidate for vaginal administration. Its complex release matrix (liquid crystalline mesophase) may enable longer time retention into the vaginal cavity and continuous drug release.

This technological strategy could optimize treatment adherence, safety, and therapeutic efficacy, offering a promising future for vaginosis treatment. Therefore, this study aimed to develop and evaluate a liquid crystalline gel using butter extracted from ucuúba seeds for vaginal administration of metronidazole.

MATERIAL AND METHODS

Material

Ucuúba butter and Palm Kernel Oil were acquired from Amazon Oil (Ananindeua, PA, Brazil), Procetyl® was purchased from Mapric (Campinas, SP, Brazil), and Metronidazole was supplied by All Chemistry do Brasil Ltda (Sao Paulo, SP, Brazil). All solvents and chemicals used in this study for analyses were analytical grade.

Preparation of liquid crystalline gel

Using magnetic stirring, the unloaded and drug-loaded liquid crystalline gel was prepared by mixing ucuúba butter, palm kernel oil, surfactant, water, and metronidazole (MDZ). The mixture was stirred at 250 rpm and maintained at a constant temperature of 40 ± 1 °C. After homogenization, the components were left at room temperature (25 ± 1 °C) for 24 h before further evaluation (Nunes et al. 2016). The pH values of the liquid crystalline gel were measured in triplicate using a digital pH meter (MS Tecnopon).

Polarized Light Microscopy (PLM)

After 24 h from preparation, the unloaded and drug-loaded liquid crystalline gel was placed on a glass slide, covered with a coverslip, and analyzed under polarized light microscopy in a Leica DM4 P (Barra Funda, São Paulo, Brazil) microscope to identify the birefringence behavior of the mesomorphic phase (Abraham et al. 2020).

Water uptake

The analysis was performed gravimetrically using a 15-mm cylindrical plastic device (0.45 m pore size) at one of its ends containing the test sample. Samples from each formulation (160 mg) were placed into the device, weighed (M1), and immersed in 10 ml of phosphate buffer (0.1 *M*, pH 7.0) at room temperature. At predetermined incubation times (i.e., 0.25, 0.5, 1, 2, 6, and 8 h), each device was removed from the buffer, blotted dry, and reweighed M2 (Perioli et al. 2008).

The percentage increase in mass as a result of water uptake (Wu) was calculated using Equation 1 (Nunes et al. 2016). All experiments were performed at least in triplicate.

$$Wu\ (\%\ w/w) = (M2-M1)/M2 \times 100$$

Method validation of metronidazole

The metronidazole analytical method was developed and validated according to the ANVISA guidelines (Brazil 2017) using a spectrophotometer (LGI Scientific LGI-VS-721N (São Paulo, SP, Brazil). The validation parameters obtained included linearity, selectivity, precision (intraday and inter-day), accuracy, limit of detection (LOD), and limit of quantification (LOQ). The UV detection was accessed at 330 nm. The stock solution was prepared with 500 µg/ml of metronidazole in ethanol or sodium acetate buffer pH 4.5 in 1, 2, 5, 10, 15, and 20 µg/ml concentrations. To analyze the selectivity of a liquid crystalline gel, ethanolic solutions were prepared without the drug to evaluate possible interference. For accuracy, three standard concentrations of the drug were prepared (5, 10, and 15 µg/ml), and the result was expressed as a percentage (%) of metronidazole recovery. All samples were performed in triplicates.

In vitro drug release and kinetic study

Metronidazole's in vitro release study was carried out using the dialysis method. Triplicates of the sample of each formulation (1 g) will be placed in a dialysis bag (12,000-14,000 Da, Sigma, USA). The dialysis membranes were incubated in 200 ml of release medium of sodium acetate buffer solution pH 4.5 at 37.0 °C, with continuous agitation. Aliquots

(5 ml) of the release medium were collected at time intervals of 0.25; 0.5; 1; 1.5; 2; 2.5; 3; 4; 6; 8; 10, and 24h. The release medium was replaced with each collection, and quantification was carried out in a spectrophotometer at a wavelength of 330 nm. The result was expressed as a percentage (%) of the amount of drug released from the formulation. (Bansal et al. 2018).

The DDSolver program was employed in the kinetics study to model dissolution data. This software leverages nonlinear optimization methods and encompasses forty dissolution models (Zuo et al. 2014). DDSolver offered a range of statistical criteria and specifically considered the adjusted coefficient of determination (R^2 adjusted) and the Model Selection Criterion (MSC) to assess the adequacy of model fitting. The drug release mechanism for the liquid crystalline gel was examined using the release exponent "n" with the Korsmeyer-Peppas model (1983).

Data analysis

All experiments were performed as technical and biological triplicates. Data were analyzed using variance analysis and GraphPad Prism® Software, version 7.02 (The United States 2017). The significance level was set at $p < 0.05$. Excel Add-in DDSolver version 1.0 was used to fit the release profiles to kinetic models.

RESULTS AND DISCUSSION

Preparation of liquid crystalline gel

Amphiphilic molecules form lyotropic liquid crystals; that is, they have a non-polar and a polar part and can form different types of phases, such as lamellar, hexagonal, and cubic, depending on the amount of water used, the temperature, and the nature of the components of the formulation (Petrilli 2013). The formulation used to obtain the vaginal gel was based on ucuúba butter (25%), palm kernel oil (25%), surfactant (40%), and water (10%) (Figure 1). The percentage of 10% water was chosen because this percentage allows for adequate viscosity for good spreadability in the oral cavity, as the use of small amounts of water makes it possible to obtain more fluid formulations, in addition to enabling the phase transition (Da Silva et al. 2021). As well, the pH value (6.4 ±0.024) of the liquid crystalline gel was suitable for vaginal administration and is within physiological limits (Karavana et al. 2012; Pozharani et al. 2023).

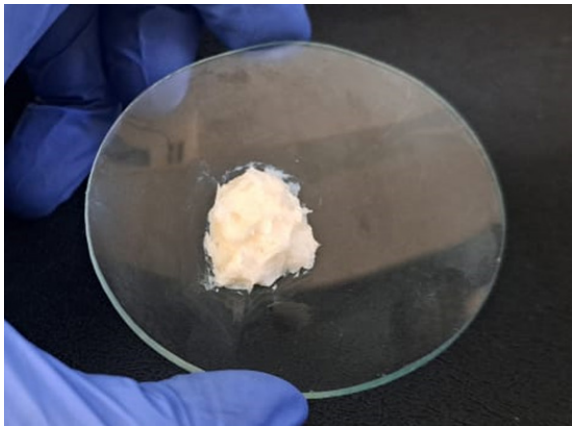

Figure 1. Liquid-crystalline gel based on *Virola surinamensis* butter.

Polarized light microscopy (PLM)

Due to their unique physicochemical properties, lyotropic liquid crystal systems are attracting increasing interest from the pharmaceutical industry. Several studies have demonstrated the usefulness of these systems for drug release (Kaambo et al. 2018). Liquid crystal mesophases of the hexagonal and cubic types can be resistant to body liquids and are challenging to erode, which increases the residence time of the formulations at the site of application, ensuring the release of the drug (Chen 2014; Baez-Santos et al. 2016).

Liquid crystals have three types of mesophases: lamellar phase, characterized by anisotropic Maltese cross-shaped structures; hexagonal phase, which are micelles arranged in a hexagon shape with a striated structure; and cubic phase, with an isotropic structure that does not deviate from the plane of polarized light, showing only a dark field (Carvalho et al. 2013). The unloaded and drug-loaded liquid crystalline gel exhibited a striated structure characterized by a hexagonal mesophase (Figure 2).

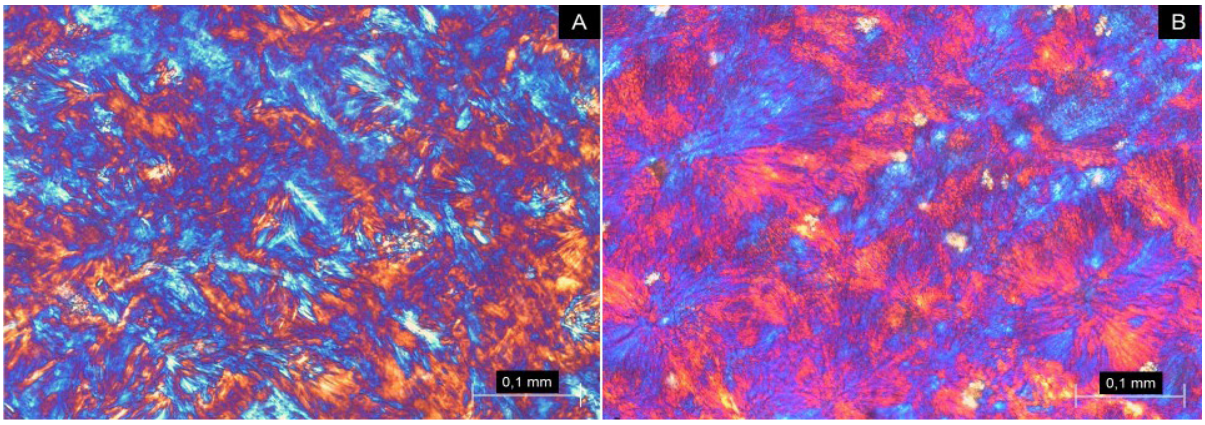

Figure 2. Photomicrographs by polarized light microscope of liquid crystalline gel. (A) Unloaded and (B) drug-loaded (10x).

Water uptake

Water uptake studies are essential for developing the liquid-crystalline drug delivery system because they are decisive for the mucoadhesion and modulation of drug release of the semisolid formulation (Calixto et al. 2018).

Figure 3 shows the water uptake profile for the formulation based on ucuúba butter. In the first hour of the study, it's possible to observe that the formulation quickly captured a large amount of water, approximately 16%. After that, the capture occurred gradually until, at the end of the test, the formulation captured the maximum amount of water, 40%. This uptake profile is suitable for drug release as the amount of water captured influences the drug release rate from the formulation, which can lead to the transition to the cubic phase (Nunes et al. 2016).

The rapid uptake of water during the first hour of the study and a constant uptake speed after that are suitable profiles for mucoadhesive formulations. This allows the liquid crystal to self-assemble to a more viscous phase and avoid erosion by organic fluids, providing longer residence time at the site of action (Karavana et al. 2012).

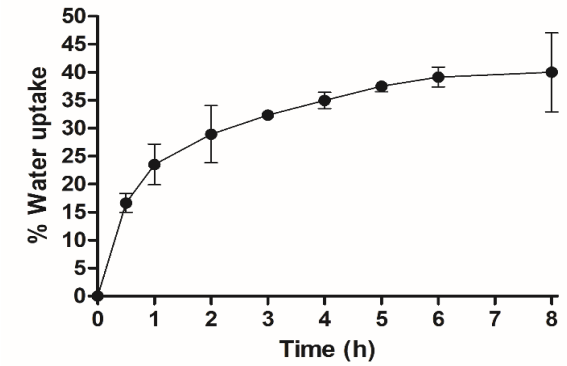

Figure 3. Percentage of water uptake of the drug formulation as a function of time (hours) in excess sodium acetate buffer (pH 4.5). The data presented the mean SD, n = 2.8.

Method validation of metronidazole

Linearity

The calibration curve for validating the drug release study was carried out with acetate buffer (pH 4.5) to simulate the release medium. The analytical curve of metronidazole in acetate buffer shows linearity from 1 to 20 µg/ml with an R^2 of 0.9989 and the straight-line equation represented by y=0.0439x+0.0024, where y represents the drug's absorbance at 330 nm and x represents the concentration of metronidazole in µg/ml (Figure 3).

The analytical curve of metronidazole in ethanol shows linearity between concentrations of 2 to 20 µg/ml with an R^2 of 0.9928 and the straight-line equation represented by y=0.0304x+0.0112, where y corresponds to the absorbance at 330 nm and ex corresponds to drug concentration.

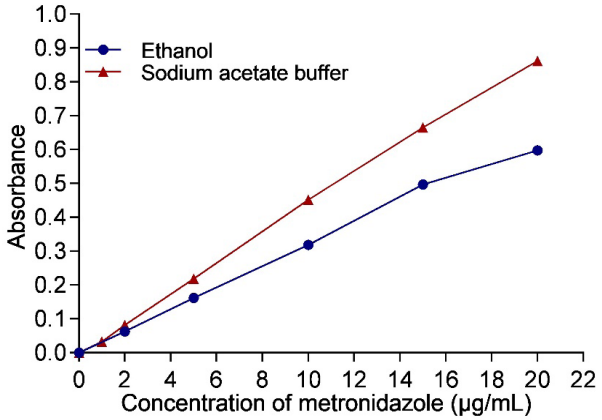

Figure 4. Analytical curve of metronidazole in ethanol and sodium acetate buffer pH 4.5 and ethanol.

Limit of detection (LD) and Limit of quantitation (LQ)

The LD and LQ were 0.162 and 0.541 µg/ml for the metronidazole in acetate buffer, respectively. The LD and LQ were 0.065 and 0.218 µg/ml for the metronidazole analytical curve in ethanol, respectively.

Selectivity

The method did not show any interferents in the liquid crystalline matrix for quantification of metronidazole.

Precision

Inter-day precision demonstrated RSD of 3.222% on day 1 and 2.314% on day 2 in ethanol and RSD of 0.881% on day 1 and 0.893% on day 2 (Table 1). Intra-day precision in ethanol and sodium acetate buffer demonstrated RSD of 2.314 and 0.531%, respectively (Table 2). The %RSD values for precision were less than 5, indicating that the method was sufficiently precise according to the guidelines.

Accuracy

The recovery values obtained ranged from 96,32 to 101,5%, and the percent relative standard deviation was less than 5, indicating that the method is accurate. The results are summarized in (Table 3). The recovery percentage as the accuracy parameter also met the acceptance criteria since its values were in the range of 80–115% (Anvisa 2017).

***In vitro* drug release and kinetic study**

Liquid crystals are utilized in the technology drug delivery field because they offer rate-controlled drug delivery at the site administration (Ghate et al. 2016). In addition, the properties of the situ self-assembled into a viscous mesophase also provide stability against disintegration in biological fluids (Pedreiro et al. 2016).

Figure 5 illustrates the release profile from the liquid-crystalline gel based on ucuúba butter. After a comprehensive 24-hour study, the analysis of the aliquots removed from the release medium at pre-determined periods conclusively reveals that the liquid-crystalline gel achieved a 12% drug release within the first hour.

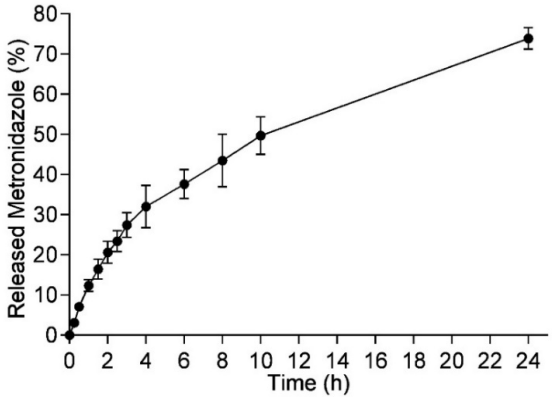

Figure 5. *In vitro* release profile of metronidazole in sodium acetate buffer pH 4.5 at 37.0 °C from the formulation containing metronidazole. The data presented a standard deviation of n =2.30.

Table 1. Metronidazole inter-day precision.

	Day 1			Day 2		
	Mean (%)	**RSD (%)**	**SD ± SD**	**Mean (%)**	**RSD (%)**	**± SD**
Ethanol	103.846	3.222	0,334	104.338	2.314	0,241
Sodium Acetate Buffer	105.527	0.881	0.929	106.970	0.893	0.955

Table 2. Metronidazole intra-day precision.

	Mean	RSD (%)	± SD
Ethanol	104.33%	2.314	0,241
Sodium Acetate Buffer	103.85%	0.531	0.552

Table 3. Accuracy and recovery of the metronidazole quantification method.

	Mean (mg/g)	**Recovery (%)**	**RSD (%)**	**SD**
5µg/ml	4.816	96.327	1.361	1.311
10 µg/ml	10.151	101.497	1.305	1.325
15 µg/ml	14.958	99.723	0.334	0.333

Table 4. Statistical parameters used to assess the best fit achieved by applying Zero-order, First-order, Higuchi, Korsmeyer, and Peppas models to the drug release profile, using the DDSolver program.

Zero Order		First Order		Higuchi		Peppas	
MSC ± SD	R^2 ± SD	MSC ± SD	R^2 ± SD	MSC ± SD	R^2 ± SD	MSC ± SD	R^2 ± SD
0,79 ± 0,22	0,64 ± 0,07	2,30 ± 0,04	0,92 ± 0,00	3,94 ± 0,13	0,98 ± 0,00	1,89 ± 0,35	0,89 ± 0,03

Afterwards, the drug release rate was slow and constant. At the end of the experiment, the formulation released only 73% of metronidazole, indicating that the system can allow a sustained release of the drug. This corroborates the water uptake profile of the liquid crystalline gel, which made it possible to verify the transition to the viscous cubic phase. This promising development may contribute to reducing the drug release rate.

Studies have consistently demonstrated that the drug release rate decreases as the water content in liquid crystalline formulations increases (Nunes et al. 2016; Calixto et al. 2018). Moreover, Ahmed et al. (2010) have emphasized the significant impact of water content on the drug release profile. They indicated that higher water content leads to increased formulation viscosity, which in turn decreases drug release speed. Chen et al. (2015) showed that an increase in the amount of water in the Phytantriol/ethanol formulation resulted in a slower release profile, in contrast to formulations with minimal water and no capture properties.

The release kinetic study from the liquid crystalline gel based on ucuúba was performed with the efficient and reliable Excel Add-in DDSolver version 1.0 (Zuo et al. 2014). The drug release data were meticulously plotted according to equations for the zero-order, first-order, Higuchi, Korsmeyer, and Peppas models. The best fit was determined based on the highest model selection criterion (MSC) and adjusted coefficient of determination (R2 adj) values (Table 4), ensuring the robustness of the findings (Zhang et al. 2010).

From the result, the liquid crystalline gel followed the Higuchi model (with the highest MSC and R2 values), meaning that the drug release was diffusion-controlled, following Fick's law of diffusion and proportional to the square root of time (Ritger and Peppas 1987). This result is consistent with those reported by Boyd et al. (2006) and Lara et al. (2005) for lipid-based liquid crystalline systems.

The Korsmeyer-Peppas equation was used to calculate the exponent n value to study the drug release transport mechanism. (Korsmeyer et al. 1983; Peppas 1985). The formulation was characterized by an n value of 0.78, signifying that it exhibits anomalous transport (non-Fickian). The observed behavior can be attributed to both diffusion-controlled and relaxation processes associated with the in situ self-assembled swollen matrix containing water-filled cavities, which occur after water uptake. (Wu et al. 2023).

CONCLUSION

Our research findings demonstrate the potential of utilizing Amazonian ucuúba butter and palm kernel oil to develop a liquid crystalline gel. This formulation shows promise as a sustained drug delivery system with suitable properties for vaginal application. Furthermore, the incorporation of 10% metronidazole does not affect the hexagonal phase formation. The water uptake and kinetic drug release profile are appropriate for enabling sustained release of metronidazole.

Additionally, the in situ self-assembly behavior of the liquid crystalline gel has the potential to prolong residence time at the site of action, which is crucial for effective topical vaginal drug delivery. Further comprehensive investigations will be conducted to assess the penetrability and permeability of liquid crystalline gel based on ucuúba butter with drugs with different polarities using the porcine vaginal mucosal model.

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AUTHORS' CONTRIBUTIONS

ACES: Methodology, Formal analysis, Investigation, Data Curation, Validation, Writing - Original Draft; EGO: Conceptualization, Methodology, Investigation, Software; GBS: Conceptualization, Writing - Review & Editing; KMN: Conceptualization, Methodology, Supervision, Resources, Writing - Review & Editing. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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