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Article

CHITOSAN/GELATIN AND PROGESTERONE OVULES FOR PRETERM BIRTH PREVENTION

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Premature birth accounts for nearly half of perinatal deaths worldwide, making its prevention a major public health priority. Although vaginal administration of natural progesterone is an effective prophylactic strategy, its daily use is often associated with irritation and low patient adherence. In this context, biodegradable, biocompatible, and mucoadhesive polymers such as chitosan and gelatin emerge as promising materials for controlled drug delivery systems. This study aimed to develop and characterize progesterone-loaded ovules based on chitosan and gelatin for the prevention of premature birth. The formulations were produced by lyophilization and evaluated in terms of morphology, chemical structure, thermal stability, swelling behavior, biodegradation, and cytotoxicity. Fourier transform infrared spectroscopy (FTIR) analysis indicated successful incorporation of progesterone into the polymeric matrix. Swelling and biodegradation assays provided insight into the degradation profile of the system, suggesting that the matrix may support modulated progesterone diffusion. Thermal analysis (TGA) showed that pure chitosan presented greater thermal stability, while the addition of gelatin and progesterone slightly reduced this property. Cytotoxicity results confirmed the biocompatibility of the developed formulations. Overall, the findings suggest that these ovules are a promising platform for progesterone delivery and may contribute to innovative biomaterial-based approaches for the prevention of preterm birth.

Keywords: chitosan; biomaterials; progesterone; vaginal drug delivery; preterm birth.

INTRODUCTION

Preterm birth represents a global perinatal health challenge, being responsible for neonatal complications and socioeconomic burdens. It is defined as birth occurring between 20-22 weeks and 36 weeks and 6 days of gestation. Before this period, it is considered a miscarriage, and after, a full-term pregnancy.[1]

Preterm newborns have an increased risk of short- and long-term complications, including neurodevelopmental impairments, behavioral problems, childhood asthma, diabetes, cardiovascular diseases, and depression in adulthood. Additionally, preterm birth is associated with high economic costs and adverse psychosocial and emotional impacts on families.[2]

Prematurity is the leading cause of perinatal morbidity and mortality, and its prevalence varies according to population characteristics but has generally increased in recent years despite advances in medicine. Globally, the rate of prematurity is estimated to range from 5 to 18%, resulting in nearly 15 million preterm births annually. In Brazil, according to the World Health Organization (WHO), 11.7% of all births occur before 37 weeks of gestation.[3]

Several strategies have been employed to reduce prematurity rates, among which prophylactic use of progesterone stands out. This hormone exerts physiological effects such as uterine muscle relaxation, inhibition of oxytocin action, and immunosuppressive and anti-inflammatory properties.[4] Patients with a singleton pregnancy and a history of spontaneous preterm birth should receive progesterone supplementation starting between 16 and 24 weeks of gestation to reduce the risk of recurrence.[5] Vaginal progesterone is also effective in asymptomatic women with a singleton pregnancy and a short cervix (< 25 mm), regardless of prior preterm birth history.[6]

Natural progesterone is typically administered via the vaginal route and presents few systemic side effects; however, it requires daily administration over prolonged periods during pregnancy, and vaginal irritation may cause discomfort.[7] The discussion regarding the development of a mucoadhesive ovule with potential for controlled drug delivery can be enhanced by considering the various intravaginal dosage forms currently available on the market. These formulations illustrate the diversity of delivery systems and highlight the potential advantages of prolonged local drug administration. Among the existing options are vaginal capsules, which provide gradual release of the active ingredient, and gels and creams, which facilitate application and improve mucosal absorption, although they generally require frequent administration;[8] vaginal rings, which provide prolonged hormone release and can be temporarily removed during sexual intercourse; and medicated intrauterine devices (IUDs), which offer long-term hormonal or mechanical contraception through medical insertion.[9] Despite their advantages, these systems still present limitations such as repeated administration, discomfort, or invasiveness.

In this scenario, the proposed chitosan/gelatin ovule was designed to combine mucoadhesion, biodegradability, and local retention capacity in a single system, aiming to reduce discomfort associated with repeated vaginal administration and potentially improve patient adherence during prolonged progesterone therapy. Unlike intrauterine devices or vaginal rings, the proposed formulation does not require

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invasive placement or removal procedures, which may contribute to greater patient comfort and accessibility.

The ovule format was selected due to its suitability for vaginal administration, ease of application, and ability to adapt to the vaginal cavity after hydration. Furthermore, ovules can promote prolonged contact between the formulation and the vaginal mucosa, favoring local retention and potentially improving progesterone delivery effectiveness. The porous structure obtained by lyophilization may also facilitate fluid absorption and matrix swelling, characteristics considered advantageous for mucoadhesive vaginal delivery systems.

Chitosan is a biopolymer derived from chitin, consisting of a cationic polysaccharide composed of linear binary units of β-(1→4)-2-acetamido-2-deoxy-D-glucose. The use of chitosan and its derivatives in pharmaceutical technology has become increasingly promising, as these materials form non-toxic structures and enhance therapeutic efficiency.[10] In light of the above, the aim of this study was to develop ovules based on chitosan, gelatin, and progesterone for the prevention of preterm birth.

EXPERIMENTAL

Materials

Medium molecular weight, medical-grade chitosan was supplied by the Laboratory for Evaluation and Development of Biomaterials of the Northeast (CERTBIO) in powder form, with a degree of deacetylation between 85 and 90%, according to the supplier (SisGen Registration: AEBD12D). Gelatin, lysozyme, and phosphate buffer solution (pH 7.2) were purchased from Sigma-Aldrich®. Acetic acid (CH_3COOH), lactic acid, and sodium hydroxide (NaOH) were obtained from Vetec®. The hormone progesterone was acquired from Eurofarma. Each ovule contained approximately 20 mg of progesterone, based on the dosage commonly employed for vaginal progesterone therapy during pregnancy.

Methods

Preparation of chitosan

A 2% (m/v) chitosan solution was prepared by dissolving 2 g of chitosan in a final volume of 100 mL of a 1% (v/v) acetic acid solution under mechanical stirring (230 rpm) at room temperature (approximately 25 °C) for about 2 h. The 2% (m/v) concentration was selected based on preliminary experiments carried out by the research group, in which different chitosan concentrations were evaluated regarding physicochemical stability, swelling behavior, and biodegradation profile.

Preparation of chitosan/gelatin solution

To obtain the chitosan/gelatin solution, gelatin was incorporated at different concentrations (5, 10, and 15% m/m) into the 2% (m/v) chitosan solution under constant mechanical stirring for 30 min until a homogeneous system was achieved. The resulting solutions were frozen in an ultra-freezer at approximately –80 °C for 48 h and subsequently lyophilized for 72 h. After lyophilization, the samples were neutralized using a 1 mol L^{-1} sodium hydroxide solution for 10 min. The samples were then characterized by Fourier transform infrared spectroscopy (FTIR), swelling degree, and biodegradation analyses to determine the optimal gelatin concentration in the chitosan solution for subsequent drug incorporation.

Preparation of chitosan/gelatin/drug solution

After obtaining the solutions, progesterone was added based on the drug concentration required for a 7-day treatment. The solutions (5 mL) were then cast into ovule-shaped molds. The ovules presented approximate dimensions of 2.5 cm in length and 2.12 cm in diameter after lyophilization. The samples were frozen in an ultra-freezer at approximately –80 °C for 48 h and subsequently lyophilized for 72 h. After lyophilization, the samples were neutralized using a 1 mol L^{-1} sodium hydroxide solution for 10 min.

Subsequently, the samples were washed with distilled water to remove residual sodium hydroxide until reaching a pH of approximately 7.4. The samples were then frozen again in an ultra-freezer at approximately –80 °C for 48 h, followed by lyophilization for 72 h. Finally, the samples were coded and characterized. Table 1 presents the sample coding.

Table 1. Composition and coding of samples

Sample coding/composition	
OQ	chitosan ovule with a concentration of 2% chitosan
OQG	chitosan ovule with 2% chitosan, 15% gelatin
OQGP	chitosan ovule with 2% chitosan, 15% gelatin, and progesterone

All percentages are given as % (m/m).

Characterizations

Physical-chemical analysis

Surface morphology, pore size, and pore distribution were evaluated using a Shimadzu SuperScan SS500 Scanning Electron Microscope (SEM). Samples were mounted on aluminum stubs with carbon tape and gold-sputter-coated with a thin gold layer (ca. 10 nm) prior to analysis. Imaging was performed at 500× magnification under an acceleration voltage of 10-15 kV.

FTIR spectra were obtained using a PerkinElmer Spectrum 400 in the 4000-650 cm^{-1} range, with a resolution of 4 cm^{-1} and 32 scans *per* sample. Lyophilized powdered samples were analyzed using an ATR (attenuated total reflectance) accessory equipped with a diamond crystal, enabling the identification of characteristic functional groups of chitosan, gelatin, and progesterone and the detection of possible intermolecular interactions.

Thermogravimetric analysis (TGA) of the samples was performed using a Perkin Elmer Pyris 1 TGA instrument, with approximately 5.0 mg of material. The samples were weighed on a precision scale (± 0.1 mg). The material was heated at a rate of 10 °C min^{-1} under a synthetic air atmosphere with a flow rate of 50 mL min^{-1}, using an alumina crucible. TG curves were recorded from 30 to 500 °C to verify any possible mass loss as a function of temperature.

The swelling capacity of the systems was evaluated by measuring their ability to absorb liquid and expand. Samples were weighed (W_i) and immersed separately in physiological saline solution (0.9% NaCl) and lactic acid solution (pH 4.5). Immersion was conducted at 37 °C for 1 h, a period previously established in preliminary tests as sufficient for the samples to reach swelling equilibrium without compromising their structural integrity. Therefore, only the equilibrium swelling point was considered for comparative analysis among the formulations. After immersion, the samples were surface-dried with absorbent paper and weighed again (W_f). The swelling degree was calculated as Equation 1:

$$SD(\%) = \frac{W_f - W_i}{W_i} \times 100 \quad (1)$$

All swelling experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard error (SE).

Biodegradation was assessed over 28 days. Samples were

incubated at 37 °C in phosphate buffer solution (pH 7.2), distilled water, lactic acid solution (pH 4.5), and phosphate buffer with lysozyme (1.5 μg mL^{-1}) to simulate enzymatic degradation. Mass loss was determined at 7, 14, 21, and 28 days using Equation 2:

$$\text{Mass Loss}(\%) = \frac{W_0 - W_t}{W_0} \times 100 \quad (2)$$

where W_0 is the initial mass and W_t is the mass at time t.

Biological assays

The *in vitro* cytotoxicity of the ovules was evaluated using the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to assess fibroblast cell viability, in accordance with ISO 10993-5:2009.[11] The L929 cell line, obtained from the Cell Bank of Rio de Janeiro, was used. The evaluated parameters included the percentage of cell death and the IC_{50} (the concentration of the product that inhibits 50% of cell growth). Cells were incubated with the extracts for 24 h prior to MTT evaluation.

The MTT assay is a quantitative, sensitive, and reliable colorimetric method that measures cell viability, proliferation, and metabolic activity. It is based on the ability of mitochondrial dehydrogenase enzymes present in viable cells to convert the yellow, water-soluble MTT substrate into a purple-colored product, resulting from the formation of water-insoluble formazan crystals. These crystals can then be quantified by measuring absorbance using a spectrophotometer. The amount of formazan produced is directly proportional to the number of viable cells. Cytotoxicity assays were performed in triplicate (n = 3), and the results are presented as mean ± SE.

RESULTS AND DISCUSSION

Characterization of progesterone-loaded ovules

Macroscopic images of the ovules (Figure 1), obtained by the lyophilization method, revealed well-defined structures with an oval shape and homogeneous surface, indicative of an efficient lyophilization process. The white coloration and slightly opaque appearance are associated with the formation of a porous matrix typical of lyophilized materials, resulting from ice sublimation during the process.

This morphology suggested a uniform distribution of the formulation components, particularly chitosan and progesterone,

Figure 1. *Macroscopic image of the ovules*

indicating the stability of the dispersion prior to freezing and the preservation of structural integrity during lyophilization, as reported in similar studies involving chitosan-based hydrogels.[12]

Visual inspection also showed that the ovules exhibited good physical integrity, with no evidence of collapse or fissures, indicating appropriate lyophilization parameters and effective control of temperature and pressure during the freezing and primary drying stages. This compact yet slightly spongy appearance is desirable for vaginal delivery systems, as it promotes rehydration and gradual dissolution in the biological environment, which may favor sustained drug diffusion.

Furthermore, the uniform shape and cohesive texture reflect precise and reproducible molding, which is essential to ensure batch-to-batch standardization. The final appearance of the ovules, characterized by a smooth luster and continuous surface, further confirms the efficiency of the lyophilization process in producing a stable and visually homogeneous system. The structure obtained through this method also provides additional advantages, such as enhanced thermal stability and preservation of progesterone bioactivity, minimizing potential chemical or physical degradation.

Thus, the macroscopic images demonstrate that lyophilization is an effective method for producing chitosan/progesterone ovules, resulting in systems with appropriate appearance, morphological integrity, and potential for safe and efficient therapeutic application.

Scanning electron microscopy (SEM) images (Figure 2) revealed significant differences in the internal morphology of pure chitosan ovules (OQ), chitosan/gelatin ovules (OQG), and chitosan/gelatin/progesterone ovules (OQGP), reflecting the impact of compositional

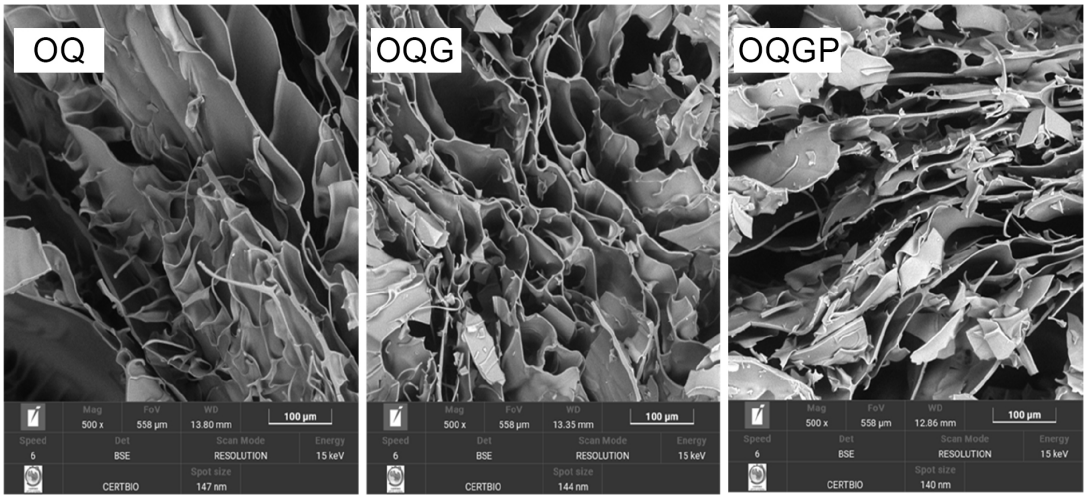

Figure 2. *SEM images of the internal structure of the samples at 500× magnification*

modifications on the final structure after lyophilization. The OQ sample exhibited a rough, lamellar surface with thick walls, characteristics typical of freeze-dried chitosan matrices, as reported by Ayensu *et al*.,[13] and Ma and Prabhu.[14] This morphology indicates a continuous and cohesive polymeric network, which is essential for the structural integrity of the system.

In the OQG sample, an increase in porosity and irregularity of the internal walls was observed, highlighting the influence of gelatin on matrix formation. The presence of this biopolymer, which exhibits more hydrophilic and thermosensitive behavior, promotes greater pore formation due to its ability to retain water during freezing, which is subsequently sublimated during the lyophilization process. The increased porosity observed in the OQG and OQGP formulations is directly related to the higher swelling capacity and biodegradation rates observed in these systems. A more porous matrix facilitates fluid penetration into the polymeric network, increasing chain relaxation and water uptake. Consequently, this structural organization may favor the diffusion of vaginal fluids throughout the matrix and potentially contribute to a more gradual drug diffusion process. This characteristic has also been reported by Gorgieva and Kokol[15] as advantageous for drug diffusion and fluid absorption, potentially enhancing drug diffusion and local retention.

The OQGP sample exhibited a less dense and more collapsed structure, with partially fragmented lamellae and pores of varying sizes, suggesting that progesterone incorporation altered the balance of cohesive forces within the polymeric matrix. This behavior may be associated with interactions between the functional groups of chitosan (–NH_2 and –OH) and the hydrophobic drug molecules, which interfere with the formation of hydrogen bonds between polymer chains, as reported by Damiri *et al*.[16] These interactions interfere with hydrogen bonding between polymer chains, thereby altering the cohesion of the matrix.

Overall, the morphological analyses confirm that the lyophilization process was effective in producing porous and homogeneous matrices, as also reported by Chang and Xiao,[17] these matrices are suitable for pharmaceutical applications with potential use in drug delivery systems. The structural modifications induced by the addition of gelatin and progesterone highlight the potential for fine-tuning the matrix microarchitecture, enabling the development of bioactive ovules with tailored drug delivery behavior.

In the field of pharmacology and drug research, SEM is a valuable technique for investigating the structure of porous materials used as drug delivery vehicles or as components of potential drug delivery applications.

Figure 3 illustrates the characteristic bands of the chitosan ovule without drug incorporation. A broad band in the 3400-3200 cm^{-1} region corresponds to O–H stretching, associated with the polymeric network of chitosan. The band at 2870 cm^{-1} is attributed to C–H stretching of aliphatic groups.

Bands observed at 1630 and 1580 cm^{-1} are related to vibrations of C=O groups of *N*-substituted amides and to in-plane symmetric bending of NH_2 groups. In the region around 1420 cm^{-1}, angular deformation of CH_2 groups adjacent to carbonyl groups is observed, while the band at 1370 cm^{-1} corresponds to angular deformation of CH_3 groups. Additionally, in the region of 1030 cm^{-1}, vibrations associated with the C–O–C groups of the saccharide structure are identified.[18,19]

Figure 4 illustrates the FTIR spectrum of the chitosan ovule containing gelatin, in which an increase in the intensity of the band around 3300 cm^{-1} is observed, attributed to the stretching vibration of O–H groups. This increase is associated with the greater availability of hydroxyl (–OH) groups in the matrix, resulting from the incorporation of gelatin, which has a structure rich in amino acid residues containing hydroxyl groups.

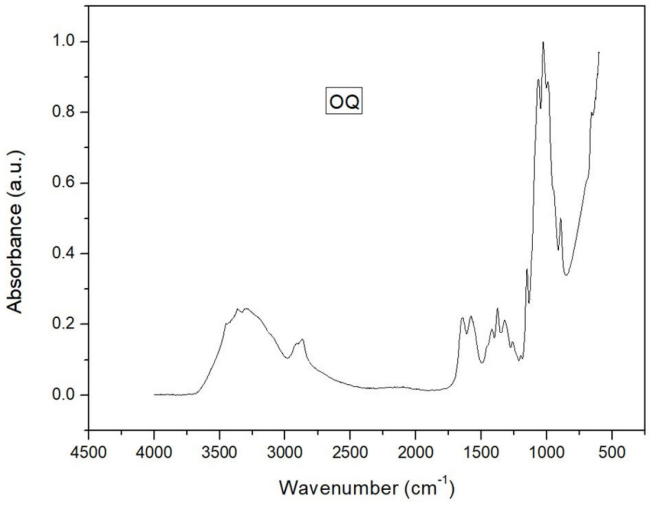

Figure 3. *FTIR (ATR) spectrum of chitosan ovule with a concentration of 2% chitosan*

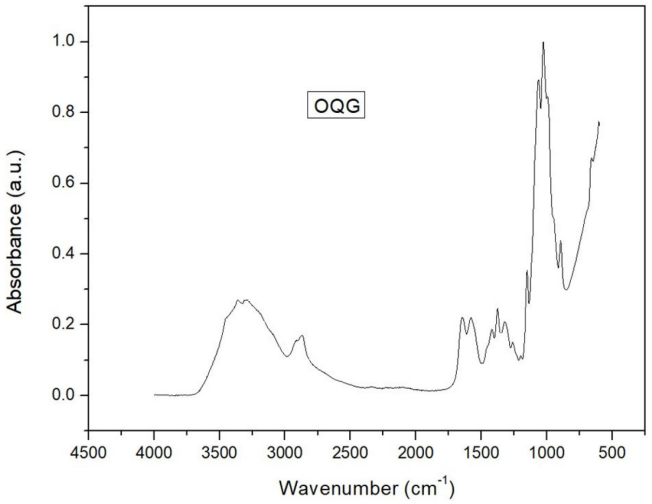

Figure 4. *FTIR (ATR) spectrum of chitosan ovule with 2% chitosan, 15% gelatin*

The intensification and broadening of this band indicate the formation of new hydrogen bonds between chitosan and gelatin chains, as reported by Staroszczyk *et al*.[20] and also by Mahmoud *et al*.,[21] who observed similar behavior in hybrid biopolymer systems. This phenomenon confirms an increase in hydrogen bonding density and in the hydrophilicity of the polymeric matrix, justified by the presence of multiple OH groups derived from gelatin.

Figure 5 shows the FTIR analysis of the chitosan/gelatin/progesterone sample. In general, the spectrum did not exhibit significant changes, only a slight reduction in the bands at 1630 and 1580 cm^{-1}, corresponding to vibrations of C=O groups of *N*-substituted amides and to in-plane symmetric bending of NH_2 groups. This suggested a possible incorporation of the drug and interaction of the drug's COOH group with these characteristic functional groups of chitosan.[22]

Figure 6 illustrates the swelling degree of ovules containing 15% gelatin, with and without the addition of progesterone. The graph presents three groups: OQ (chitosan ovule), OQG (chitosan/gelatin ovule), and OQGP (chitosan/gelatin/progesterone ovule). The swelling results are presented as mean ± standard error obtained from three independent experiments.

It is observed that the OQ group (pure chitosan) exhibits the lowest swelling, with a value around 1200%. Upon the addition of 15% gelatin,

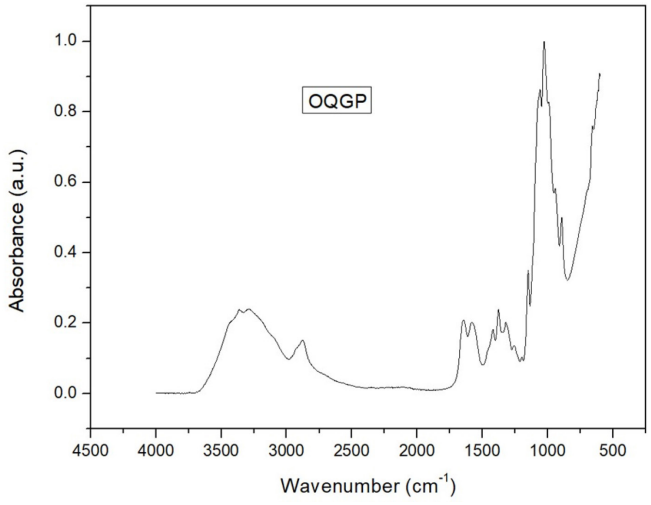

Figure 5. *FTIR (ATR) spectrum of chitosan ovule with 2% chitosan, 15% gelatin, and progesterone*

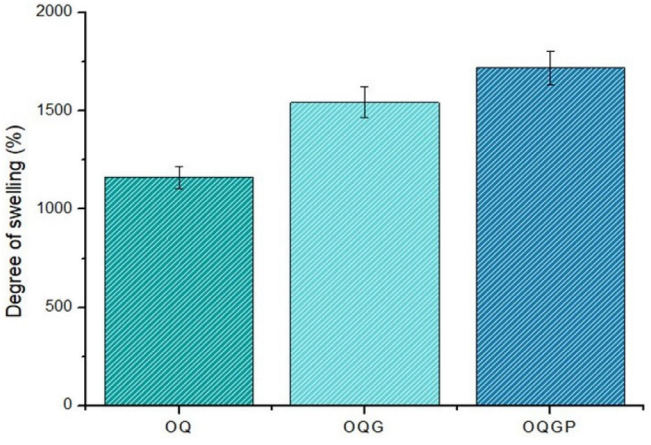

Figure 6. *Swelling degree of the ovules in PBS medium. Data are expressed as mean ± SE (n = 3)*

represented by the OQG group, the swelling increases significantly, reaching values close to 1500%. With the incorporation of progesterone into the chitosan/gelatin mixture (OQGP group), the swelling is slightly higher than that observed for OQG, exceeding 1500%.

These results indicated that gelatin substantially enhances the swelling capacity of chitosan, while the addition of progesterone contributes to a further increase. The hydrophilic nature of gelatin may explain the initial increase, whereas progesterone, as a bioactive compound, may interact with the polymeric matrix in a way that slightly enhances the swelling capacity. From a mechanistic perspective, the increase in swelling behavior can be associated with the higher availability of hydrophilic groups and the reduction in polymer chain packing density caused by gelatin incorporation. This structural modification increases matrix hydration and promotes expansion of the polymeric network. Furthermore, the incorporation of progesterone may have contributed to the formation of microdomains within the matrix, facilitating fluid penetration and increasing free volume within the system. Such characteristics are particularly relevant for vaginal biomaterials, since hydration and matrix relaxation may directly influence drug diffusion and residence time at the application site.

These findings are relevant for the development of potential sustained delivery systems, in which the swelling degree can influence drug release. Understanding these interactions is crucial for optimizing ovule formulations and ensuring appropriate and controlled drug release.

Analysis of the biodegradation results (Table 2) of the samples immersed in PBS (phosphate-buffered saline) and lysozyme/PBS over periods of 7, 14, 21, and 28 days showed that biodegradation was more pronounced in acidic conditions.

The biodegradation results demonstrated that the ovules exhibited behavior dependent on both the polymeric composition and the medium to which they were exposed. In general, degradation was more pronounced in lactic acid medium, followed by lysozyme, and less significant in PBS. This outcome was expected, as lactic acid provides a more acidic and aggressive environment for the polymer structure, promoting hydrolysis of glycosidic bonds in chitosan and peptide bonds in gelatin. In contrast, in PBS, the neutral pH and absence of enzymatic agents limit degradation, allowing mainly swelling and superficial matrix diffusion.

The OQ sample (chitosan ovules) exhibited the lowest degradation rates in all media, which can be attributed to the structural resistance provided by intermolecular interactions in chitosan, such as hydrogen bonding and electrostatic forces. This characteristic imparted a slower degradation rate to the polymer, which is desirable for modulated drug diffusion, as it prolongs residence time and maintains device integrity until the drug is completely released. However, the higher resistance in PBS and the lower sensitivity to lysozyme indicate that enzymatic degradation occurs to a limited extent, depending on the availability of sites susceptible to catalytic attack.

The incorporation of gelatin (OQG) promoted a noticeable increase in degradation rate in all media. This behavior was associated with gelatin, a natural polymer derived from collagen, which has a less crystalline and more hydrophilic structure, facilitating water penetration

Table 2. Percentage mass loss of the samples after 7, 14, 21, and 28 days during the biodegradation assay

Test		Mass loss of samples / % (m/m)			
		7 days	14 days	21 days	28 days
Phosphate buffer	OQ	35.62	41.67	58.13	71.23
	OQG	45.83	61.32	77.36	84.87
	OQGP	46.72	64.21	80.02	87.52
Lysozyme	OQ	37.47	44.07	62.17	78.98
	OQG	47.83	64.71	84.21	92.31
	OQGP	48.97	69.72	89.34	100.00
Lactic acid	OQ	41.38	47.36	64.45	88.52
	OQG	50.25	66.11	86.74	95.67
	OQGP	52.75	72.19	92.27	100.00

OQ: chitosan ovule (2% chitosan); OQG: chitosan ovule (2% chitosan, 15% gelatin); OQGP: chitosan ovule (2% chitosan, 15% gelatin) with progesterone.

and the action of degrading agents. Furthermore, the presence of gelatin tends to reduce the density of interactions between chitosan chains, making the matrix more susceptible to hydrolysis. This characteristic may be advantageous in pharmaceutical applications where faster degradation and more efficient drug release are desired.

The relationship between swelling and biodegradation behavior suggested that the more hydrated matrices became more susceptible to hydrolytic and enzymatic attack. Increased water uptake facilitates polymer chain mobility and accelerates cleavage of glycosidic and peptide bonds, resulting in faster mass loss. Therefore, the structural modifications induced by gelatin incorporation appear to simultaneously affect porosity, swelling capacity, and degradation kinetics, demonstrating the interdependence of these properties in the developed systems.

Ovules containing progesterone (OQGP) exhibited the highest degradation rates, reaching up to 100% in lactic acid and 87.52% in PBS, suggesting that drug incorporation interfered with the polymeric organization, reducing chain cohesion and increasing system permeability. Progesterone, being lipophilic, may create less dense regions and promote heterogeneity within the matrix, facilitating fluid diffusion and structural collapse. Thus, the results indicate that OQGP ovules exhibit degradation behavior that may contribute to modulated drug release, especially in mildly acidic physiological environments such as the vaginal milieu, highlighting the potential of this formulation for safe and effective therapeutic applications.

Taken together, the SEM, swelling, and biodegradation results indicate that the physicochemical behavior of the ovules is strongly dependent on the internal organization of the polymeric matrix. The addition of gelatin increased hydrophilicity and porosity, while progesterone incorporation appeared to reduce polymer chain cohesion, generating a less compact structure. These combined effects may facilitate fluid diffusion, matrix relaxation, and progressive degradation, factors that are commonly associated with modulated drug delivery behavior in polymer-based systems.

Thermogravimetric analysis (TGA) is a thermal analysis technique that allows the evaluation of mass variation of a substance as a function of time or temperature, while the material is subjected to a controlled temperature program. This technique has several applications, including in the pharmaceutical field, where it is used to assess drug thermal stability, analyze decomposition stages, and determine other relevant physicochemical properties.[23]

Thermogravimetric analysis (TG) of the ovules (Figures 7, 8 and 9) revealed the characteristic thermal behavior of chitosan-based polymeric systems and their blends with gelatin and progesterone. In all samples, an initial mass loss is observed between 30 and 150-200 °C, attributed to the elimination of adsorbed water and residual moisture associated with the hydrophilic chains of the biopolymers. This stage accounts for approximately 14.5 to 16.5% of the total mass loss, being slightly higher in the OQG sample, which can be explained by the presence of gelatin, increasing the material's affinity for water due to its more hydrophilic nature.

The second degradation stage, occurring between 200 and 350 °C, corresponds to the thermal decomposition of the main chains of chitosan and gelatin, involving the cleavage of glycosidic bonds,

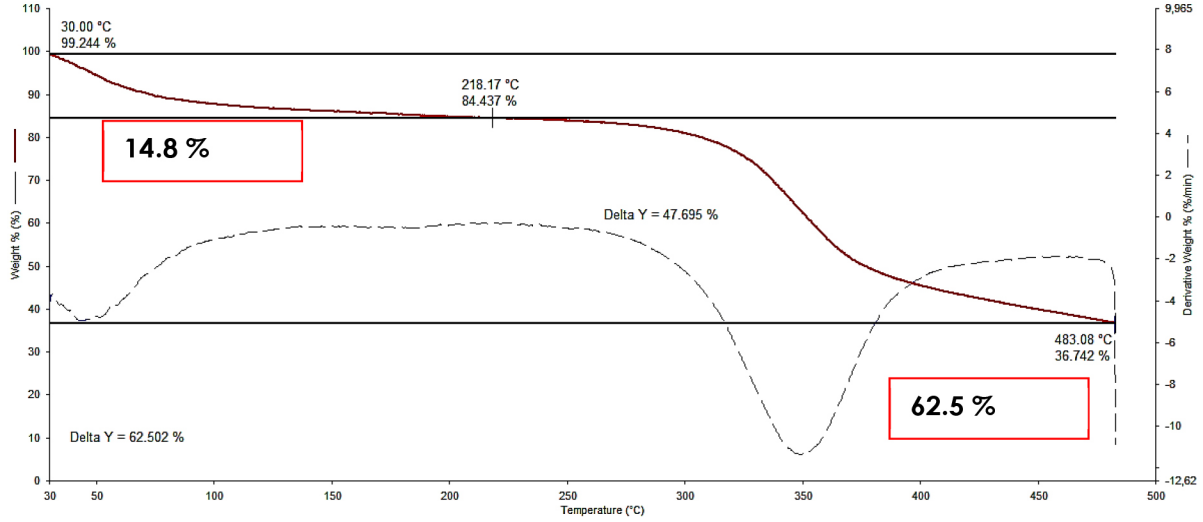

Figure 7. *Thermogravimetric curve for chitosan ovules*

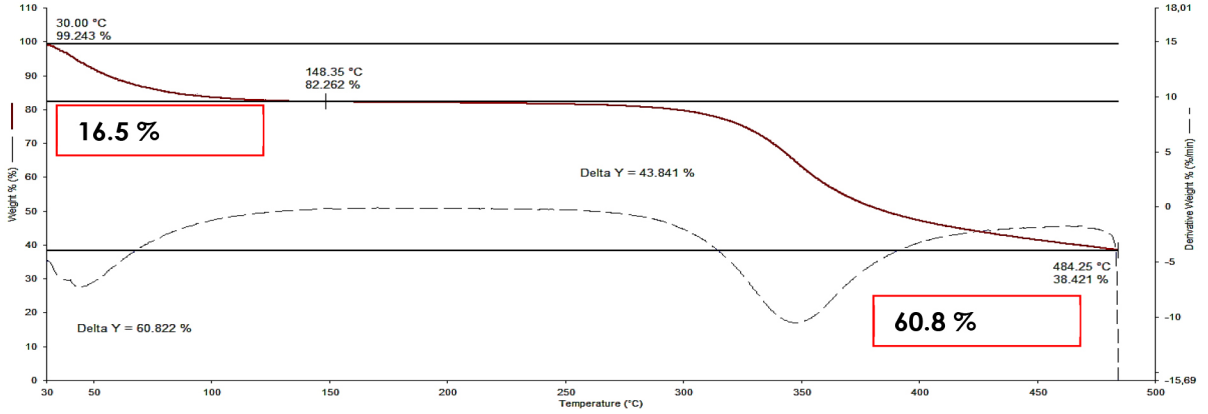

Figure 8. *Thermogravimetric curve for chitosan/gelatin ovules*

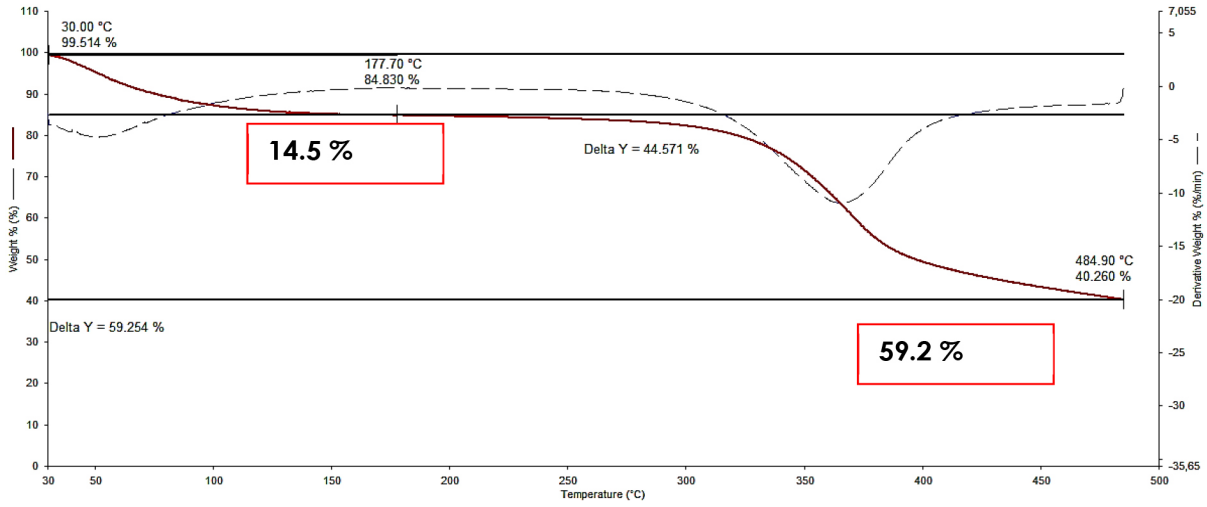

Figure 9. *Thermogravimetric curve for chitosan/gelatin/progesterone ovules*

deacetylation, and breakdown of peptide groups. The OQ sample (chitosan only) exhibited a total mass loss of 62.5%, indicating relatively higher thermal stability, whereas the OQG and OQGP formulations showed mass losses of 60.8 and 59.2%, respectively. This reduction in thermal stability suggested that the addition of gelatin and progesterone alters the organization of the polymeric matrix, decreasing intermolecular interactions and, consequently, the thermal resistance of the system.

The main degradation peak (maximum decomposition temperature) was observed between 177 and 218 °C, with the OQ sample presenting the highest value, reinforcing its greater stability. The decrease in this temperature in the OQG and OQGP samples indicates a plasticizing effect of gelatin and progesterone, which increases chain mobility and reduces the energy required to initiate the degradation process. This behavior is common in multicomponent systems, where the presence of additives or drugs affects the crystallinity and molecular packing density of the primary polymer.

The final residues, obtained after heating up to 500 °C, ranged from 37 to 40%, indicating that part of the material exhibits thermal resistance, possibly associated with the formation of carbonized structures and stable aromatic compounds. Overall, the TG results indicate that the ovules exhibit good thermal stability up to approximately 150 °C, a range higher than that typically observed for hydrated biopolymeric systems, ensuring safety during processing and storage. The slight reduction in stability with the incorporation of gelatin and progesterone is compensated by improved functional properties, such as enhanced biodegradability and potential drug delivery behavior, which are desirable features in pharmaceutical formulations intended for vaginal application. According to Akash and Rehman,[24] thermogravimetric analysis is of great importance, as it provides essential information for the proper storage of membranes and for the selection of the most appropriate sterilization techniques when these materials are intended for use in humans.

Biological assays

The cytotoxicity results presented in the Figure 10 demonstrate that all formulations – OQ (chitosan ovules), OQG (chitosan/gelatin ovules), and OQGP (chitosan/gelatin/progesterone ovules) – exhibited cell viability percentages above 90%, indicating the absence of relevant toxic effects on the analyzed cells. Cell viability results are expressed as mean ± standard error from triplicate experiments. These values are above the minimum threshold of 70% established by ISO 10993-5[11] for a material to be considered non-cytotoxic, demonstrating that the developed systems meet international biocompatibility criteria. The viability index observed for the chitosan ovules (OQ) confirms the safe and bioadhesive nature of this polymer, which is recognized for promoting cell adhesion and proliferation without causing damage to the plasma membrane.

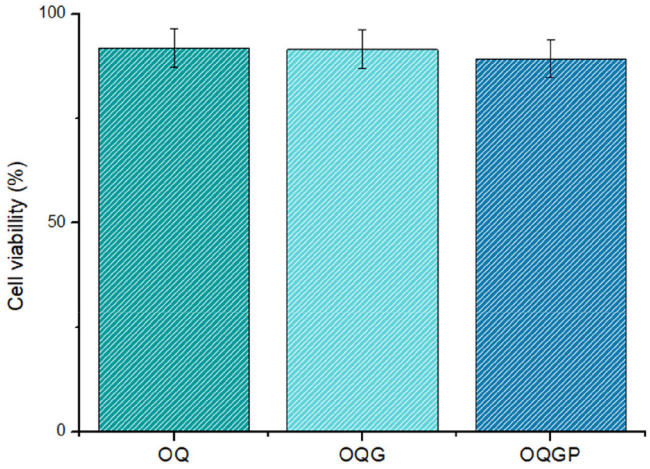

Figure 10. *Cell viability of the ovules. Data are expressed as mean ± SE (n = 3)*

In the OQG formulation, composed of chitosan and gelatin, cell viability remained equally high, demonstrating compatibility between the two polymers and the absence of adverse effects resulting from interactions between their chains. Gelatin, derived from collagen, contributes to the elasticity and hydration of the system and exhibits excellent biological acceptance.

Thus, the combination of these two biopolymers, chitosan and gelatin, reinforces the biocompatible and safe nature of the formulation, ensuring that the obtained polymeric matrix is suitable for direct contact with mucosal tissues, such as the vaginal mucosa, without inducing inflammatory or irritative responses. Studies on mucoadhesive systems based on natural polymers support this observation, such as those reported by Afloarea *et al.*[25] Hassan *et al.*[26] demonstrated that these formulations promote greater adhesion and retention in the vaginal mucosa, in addition to reducing systemic side effects and modulating controlled drug release, thereby enhancing local therapeutic efficacy.

Ovules containing progesterone (OQGP) also exhibited cell viability above 90%, demonstrating that drug incorporation did not

compromise the biocompatibility of the polymeric matrix. This result is crucial, as it ensures that the incorporation process of progesterone, as well as its chemical interactions with chitosan and gelatin, did not generate toxic by-products. Therefore, the cytotoxicity results confirm that all developed formulations meet the requirements of ISO 10993-5:2009.[11]

CONCLUSIONS

The developed methodology proved to be effective for the production of ovules based on chitosan, gelatin, and progesterone, resulting in systems with structural, physicochemical, and biological properties suitable for vaginal application. Among the tested formulations, the combination containing 2% chitosan, 15% gelatin, and progesterone showed the best performance, standing out in terms of stability, mechanical strength, and biodegradability potential. FTIR analyses confirmed the incorporation of progesterone into the polymeric matrix, while SEM images revealed a porous and homogeneous structure obtained by lyophilization, a feature that may support controlled drug delivery.

The results demonstrated a clear relationship between matrix composition, porosity, swelling behavior, and biodegradation profile, indicating that structural modifications induced by gelatin and progesterone incorporation may influence fluid diffusion and the functional performance of the developed ovules.

Overall, the swelling, thermal stability, and biodegradation results indicate that the system is functional, safe, and capable of maintaining its integrity for up to 21 days without exhibiting toxicity. Therefore, the combination of chitosan, gelatin, and progesterone represents an innovative and promising approach for the development of multifunctional biomaterials aimed at preventing preterm birth, constituting a significant advancement in the field of mucoadhesive and drug delivery applications. Although the physicochemical and biodegradation results suggest potential applicability in controlled drug delivery, *in vitro* progesterone release studies are still necessary to confirm the release kinetics and therapeutic performance of the developed ovules.

DATA AVAILABILITY STATEMENT

All data used and analyzed in this study are fully presented within the body of the manuscript.

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AUTHOR CONTRIBUTIONS

F. P. B.: conceptualization, investigation, data curation, writing - original draft; M. E. B. O. M.: formal analysis, methodology, validation; L. S. F.: conceptualization, investigation, data curation, writing - original draft; A. D. S. N. A. L.: resources, software, visualization; J. L. A. S. S.: methodology, validation, formal analysis; A. R. M. L.: formal analysis, methodology, validation; A. A. T.: methodology, validation, formal analysis; W. J. B. S.: supervision, funding acquisition, writing - review and editing; R. M. M.: methodology, validation, formal analysis; S. M. L. S.: supervision, project administration; M. V. L. F.: conceptualization, methodology, supervision, writing - review and editing.

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