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Development and Evaluation of Nanostructured Lipid Carriers for Enhanced Oral Bioavailability of Progesterone

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ABSTRACT

Progesterone is an essential steroidal hormone that cannot be ignored in the reproductive health management; however, its aqueous interpretation has been found to be deplorable, accompanied by strong expression of initial metabolism and, accordingly, its bioavailability is low. Based on this the current study set out to design and evaluate progesterone packed nano-lipid carriers (NLCs) with an aim of enhancing oral bioavailability. Such studies as preformulation (physicochemical characterization, solubility, and partition coefficient) confirmed the lipophilic nature of progesterone, thus arguing in favor of a lipid-based delivery method. The FTIR and DSC induced drug-excipient compatibility assessments showed no any considerable interaction and complimentary signify a stable formulae matrix. The production of the NLCs was carried out through a hot homogenization process and ultrasonication, and optimization of formulation variables was done through Design of Experiments (DoE) technique. Optimal formulation that had been obtained portrayed a particle size of nanoscale, low polydispersity index and high entrapment efficiency, which is reflective of efficient drug loading and homogenous distribution of these lipid particles within the lipid matrix. The shape of morphological analysis proved to be spherical and stable of the nanoparticles. Drug release studies In-vitro studies showed a biphasic release profile, consisting of an early burst release followed by sustained release, therefore, giving the free drug its superior cumulative drug release. The last step to the stability was done and it was found that the optimized formulation was physically and chemically stable in several storage conditions. Comprehensively, the nano-lipid carrier system thus developed showed a significant enhancement in the physicochemical properties and release pattern of progesterone and consequently highlighted its applicability as a viable oral delivery system.

Keywords: Progesterone; Nano-Lipid Carriers (NLCs); Oral Bioavailability; Lipid-Based Drug Delivery; Controlled Drug Release; Formulation Optimization; Nanoformulation; Lipophilic Drugs; Pharmaceutical Nanotechnology.

1.0. Introduction

Progesterone is one of the key steroid hormones and has a cardinal impact on reproductive physiology, including the sustenance of the luteal phase, decreased preterm birth exertion, and hormone replacement therapy (Ferguson et al., 2012). However, its oral bioavailability is severely affected, due to its Class II aqueous solubility, high hepatic first-pass metabolism (with your systemic exposure dropping below 10% frequently 25-50% when measured by LC -MS) and rapid presystolic breakdown in the gastrointestinal tract (Thierry van Dessel et al., 1996). This leads to supratherapeutic doses of 200-600 mg/kg/day of micronized preparations used in oil vehicles to achieve measurable plasma levels, but even these dosages provide highly variating pharmacokinetics, non-optimal systemic levels and reduced therapeutic efficacies compared to parenteral or intravaginal route (Goletiani et al., 2007) (Ghaedi et al., 2022).

The progesterone belongs to the number of key steroid hormones and has a cardinal effect on the reproductive physiology, including the maintenance of the luteal period, the reduction of preterm birth cases, and the situation of hormone replacement therapy (Valenta et al., 2001). The oral bioavailability of it, however, is significantly impaired, due to the Class II aqueous solubility, and the large hepatic first-pass clearance, which leads to systemic exposure that can often be less than 10 per cent with LC-MS determinations indicating occasionally up to 25-50 per cent (Ball et al., 1992). Furthermore, a quick rate of degradation in the gastrointestinal system also reduces the pharmacological footprint (Pakharenko, 2020) (Chwalisz et al., 2005). Accordingly, therapeutic doses of

micronized preparations (200 to 600 mg kg1) given daily by oil vehicles are super therapeutic, but even at these high levels their pharmacokinetics are subject to large variation with highly irregular systemic concentrations and reduced efficacy of therapy (Maheshwari et al., 2024).

2.0. Objectives

1. To study the physicochemical properties of progesterone, including solubility, lipophilicity, and compatibility with selected lipid excipients.
2. To optimize the formulation for desired physicochemical performance.
3. To perform drug–excipient compatibility studies using FTIR and DSC.

3.0. Materials and Methods

3.1. Materials

Progesterone (analytical grade) was also obtained in the Pharmacy Laboratory of ITM University, Gwalior, and it was used as the model drug in the current investigation. The solid lipids, glyceryl monostearate (GMS) and stearic acid, were used but the liquid lipids, medium-chain triglycerides (MCT oil), were used in the development of nano-lipid carriers (NLCs). Span 20 and tween 80 (polysorbate 80) were added as the co-surfactant and surfactant, respectively, to stabilise the dispersion and increase the rate of emulsification. As organic solvents, analytic-grade methanol, ethanol, and chloroform were used, which can be obtained through normal commercial vendors? The solvent system used during the experimental procedures was double filtered water. Fourier Transform Infrared (FTIR) spectroscopy was done with potassium bromide (KBr, FTIR grade), whereas Differentiated Scanning Calorimetry (DSC) analyses were done with standard aluminium pans.

The rest of the chemicals and reagents used in this investigation were of analytical or HPLC grade, and used in pure form without undergoing any other purification.

Table 3.1. List of Materials Used with Approximate Quantities

S. No.	Material	Category	Approximate Quantity Used
1	Progesterone	Drug	10–50 mg per batch
2	Glyceryl Monostearate (GMS)	Solid lipid	100–500 mg
3	Stearic Acid	Solid lipid	100–500 mg
4	Medium Chain Triglycerides (MCT oil)	Liquid lipid	50–200 mg
5	Tween 80	Surfactant	0.5–2% w/v
6	Span 20	Co-surfactant	0.5–1% w/v
7	Methanol	Solvent	50–100 mL
8	Ethanol	Solvent	50–100 mL
9	Chloroform	Solvent	20–50 mL
10	Potassium Bromide (KBr)	FTIR reagent	~100–200 mg
11	Distilled Water	Solvent	q.s. (up to 100 mL per batch)

3.2. Drug Identification and Preliminary Characterization

The identification and preliminary characterization of the drug progesterone were conducted in a systematic manner in order to justify its identity, its purity and explain its suitability to be included in nano-lipid carriers. These analyses cannot be done away with in determining the minimum physicochemical characteristics required prior to the formulation development.

3.2.1. Organoleptic Properties

Progesterone was tested on his appearances, color and smell. It was revealed that the compound was a white to off-white crystalline formation with no noticeable odor and therefore met the set standards as described in the literature of modern pharmacopoeia.

3.2.2. Melting Point Determination

The determination of the purity of progesterone by the capillary method was done with the help of the melting point. A small amount of the drug was placed in a closed capillary tube and then placed in a digital melting point apparatus. The obtained melting point was observed to be within the range (126-131 °C) as reported hence confirming that the sample was pure.

3.2.3. Solubility Studies

Progesterone was tested in tubes over solvents as distilled water, methanol, ethanol among a range of lipid excipients in a series of solvency studies. A large amount of the drug was added to each solvent to which the combination was stirred over a period of 24 hrs, then it was filtered and the concentration analyzed subsequently. Results proved the conclusion that the progesterone exhibits a significant reduced solubility in the aqueous solution, with the organic solvents and lipid layers having significantly greater solubility, which supports its lipophilic nature (Maheshwari et al., 2024).

3.2.4. Partition Coefficient (Log P)

We have used the classic n-octanol/water biphasic system in order to determine the partition coefficient of progesterone. The solvent phases were also pre-saturated to remove unintended changes in concentration and the drug was added to the equilibrated mixture. After attaining equilibrium, the progesterone concentrations at the octanol and aqueous phase were measured carefully. The obtained high partition coefficient is conclusive evidence of the strong lipophilicity of the drug, hence confirming its candidature as the lipid-based drug delivery formulations.

Table 3.2. Organoleptic Properties of Progesterone

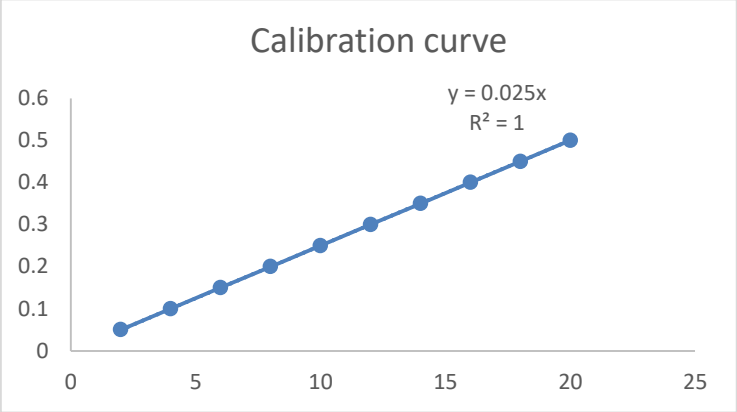
Parameter	**Observation**
Appearance	White to off-white crystalline powder
Odor	Odorless
Nature	Crystalline

3.2.5. UV–Visible Spectroscopic Analysis

I made a standard solution of progesterone in methanol and performed UV-visible spectrophotometry using the value of 200-400nm range to determine the λmax. The compound was found to be having a specific absorption peak in the 240 250nm range which was used later as determining the content of the compound quantitatively.

3.2.6. Calibration Curve of Progesterone

Calibration curve was developed by making standard solutions of progesterone in methanol with varying levels of concentration. Absorbance was recorded at the lmax determined and a linear correlation between concentration and absorbance was obtained ensuring that the law of Beer-Lambert was obeyed.



3.3. Solubility Studies of Progesterone

Progesterone was also evaluated in a systematic manner on the solvents of the different solvents and lipid excipients to help them choose the appropriate components on the development of the nano-lipid carriers. The progesterone was put in excess in a row of solvents, namely distilled water, methanol, ethanol, and commonly used lipid materials like glycerl monostearate, stearic acid, and medium-chain triglycerides. A mechanical shaker was used to mix the mixtures in the continuous manner of 24 hours at ambient temperature in order to achieve equilibrium (Lee et al., 2007). After equilibration, the samples were centrifuged and with a membrane filter to filter any undissolved drug. The spectrophotometric measurement of the concentration of the dissolved progesterone was then obtained at the characteristic wavelength.

The solubility of the progesterone was also found to be very low in the aqueous medium, and this revealed that the drug was also hydrophobic, although the drug showed very high solubility in organic solvents and lipid excipients. In the lipids analyses, greater solubility in the selected solid and liquid lipids was an indication that the lipids are suitable to be used in the formulation of nano-lipid carriers. These results were very important in the rational choice of lipid constituents and in improvement of drug loading capacity and general efficiency of the formulation.

Table 3.3. Solubility Profile of Progesterone

S. No.	Solvent/Lipid	Solubility
1	Distilled Water	Practically insoluble
2	Methanol	Freely soluble

3	Ethanol	Soluble
4	Glycerol monostearate	Soluble
5	Stearic Acid	Moderately soluble
6	MCT Oil	Highly soluble

3.4. Lipophilicity Coefficient of progesterone

The lipophilicity of progesterone was measured by calculating the partition coefficient (log P) of water biphasic system. Before the experiment the equal amounts of n-octanol and distilled water were mixed and saturated with each other by forcefully swirling the mixture. A 0.3 l aliquot of progesterone was added to the biphasic mixture and left in the same position in continuous agitation in a mechanical shaker over a period of 24h at ambient temperature so as to have the two phases to have attained an equilibrium (Sasaki et al., 2014).

After balancing the apparatus was left standing so as to allow full phase separation. A fine filter was used to filter the aqueous phase and it was carefully filtered to eliminate any particulate matter. The amount of progesterone in this aqueous phase was determined using a UV- Visible spectrophotometer at the right frequency. The concentration of the reagent at the organic phase (n -octanol) was then determined by the difference between the initial concentration of the compound added into the system and the concentration of the reagent in the aqueous phase.

The partition coefficient (P) was calculated using the equation:

$$P = \frac{C_{octanol}}{C_{water}}$$

The high partition coefficient established with progesterone is a clear indication of its extreme lipophilicity and therefore supports its use in lipid-based delivery systems, such as nano-lipid carriers. This physicochemical feature comes into play to aid in enhancement of drug loading capacity in lipid matrices and later increase oral bioavailability.

3.5. Drug–Excipient Compatibility Studies

Progesterone compatibility with the specified lipid excipients was studied to examine possible physicochemical reactions. These studies are subjectively important to ensure the stability, effectiveness, and systems of the formulation during manufacturing and storage. The best methodologies that were used to analyze compatibility were Fourier Transform Infrared (FTIR) spectroscopy and the Differential Scanning Calorimetry (DSC).

3.5.1. FTIR Analysis

FTIR spectroscopy was used to explain how progesterone could interact with each of the excipients of interest, by observing the changes in the typical functional group peaks. Pure progesterone samples, each excipient, and their respective physical mixtures were carefully made through triturating with potassium bromide (KBr, FTIR grade) after which they were pressed to a thin, translucent pellet using a hydraulic press. The pellets that resulted were exposed to spectral acquisition in the 4000-400 cm^{-1} range using a calibrated FTIR spectrophotometer. The obtained spectra of both pure progesterone and those obtained on physical mixtures were compared to identify any significant changes, loss or new ancillary peaks.

The most common peaks of progesterone such as the C=O stretch just below 1700 cm^{-1} , the C 5H stretching region between 2800-3000 cm^{-1}, and the supporting functional group vibration were examined meticulously. The absence of any noteworthy red or blue shifts, and the emergence of no new absorptions in the physical mixtures all testify to the fact that no substantial chemical interaction has occurred between progesterone and the excipients selected, and thus prove that the two are compatible.

3.5.2. DSC Analysis

Differential Scanning Calorimetry (DSC) study was conducted to strictly evaluate the thermal properties and solubility of progesterone in the selected lipid excipients. Pure progesterone in the accurate weight, the individual lipid excipients and their physical combinations were loaded into the standardized aluminum pans and hermetically capped. A sealed pan with no water in it was used as an erosion base reference.

A purge with nitrogen through a temperature sweep of 25 C to 300 degree C was performed on each sample at a controlled heating rate, usually 10°C/min. The obtained thermograms were analyzed in terms of changes in melting transition temperature, enthalpy transformation and other thermal occurrences. The pure progesterone exhibited a sharp endothermic peak at the melting point, which is typical of the crystalline structure. With the juxtaposition of the thermograms of the physical mixtures and those of the unadulterated drug, retention of the characteristic progesterone melting peak with insignificant shift points are attributed to a lack of consequential drug–excipient interactions.

3.6. Selection of Lipid and Surfactant Excipients

Choice of appropriate lipid and surfactant excipients steps is a critical phase in the development of nano lipid carriers (NLCs), which has a direct effect on drug loading capacity, colloidal stability and general formulation functionality. The selection criteria used in this investigation were mainly based on the solubility profile of progesterone, compatibility studies done in parallel and the ability of the excipients to form stable nanoscale dispersions.

The reason behind the use of solid lipids, namely, glycerol monostearate (GMS) and stearic acid, was based on their demonstrated biocompatibility, long history of application in lipid-based drug delivery systems, and their capacity to form a rigid shell when encapsulating a drug. Inclusion of liquid lipids was done so as to create structural defects in the solid plane, increasing drug loading capacity and reducing the premature expulsion of drugs on storage. The co-crystallization of solid and liquid lipids is a step that is characteristic of the NLC technology, and significantly increases the encapsulation capacity (efficiency) and selective drug release rate.

Surfactants were chosen based on their emulsification effectiveness and on their hydrophilic-hydrophobic partitioning expressed in their hydrophilic-lipophilic balance (HLB) and on their safety. To stabilize the oil-in-water dispersion and reduce interfacial tension, tween 80 (polysorbate 80) which is a non-ionic surfactant with a high HLB value would be used. The co surfactant was used to increase the interfacial stability and nano-sized particle formation, taken as Span 20.

3.7. Preparation of Progesterone-Loaded Nano-Lipid Carriers

The progesterone containing nano lipid carriers (NLCs) were prepared using the hot homogenization regime followed by ultrasonication technique widely used in preparing lipid based nanocarriers. The solid lipids, glyceryl monostearate and/or stearic acid, were melted with the liquid lipid (medium-chain triglycerides) at a temperature marginally higher than the melting point of the solid phase, approximately 5-10 degree C resulting in a homogeneous lipid layer. This molten mixture was then added to progesterone and the mix then stirred until the drug was completely dissolved.

At the same time, the aqueous solution containing the selected surfactant, Tween 80, and the co-surfactant, span 20, was made by adding both surfactants to the twice-distilled water, and by heating the solution to the identical temperature as the lipid phase, which was done to avoid premature solidification. The resultant aqueous heated phase was then gradually added to the molten lipid phase under constant stirring and this led to formation of a coarse pre-emulsion.

The resulting pre-emulsion was subjected to high-speed homogenization at a range of 1000 -15000 rpm which was predetermined and ended up taking a predefined time of time, thereby realizing a substantial decrease in droplet size. This was then followed by the ultrasonication of the probe a few minutes later which enabled the further reduction of the particle size as well as enabling the particle to come together to form a homogeneous nano dispersion.

Hot nano emulsion, which was produced as a result of these processes was then left to cool down to ambient temperature, which caused recrystallization of lipid phase and creation of stable progesterone loaded nano lipid carriers. Lastly, the ready NLC dispersion was archived in airtight beakers in appropriate conditions in order to characterize and assess it later.

3.8. Optimization of Formulation Variables Using DoE

To optimise the formulation variables of progesterone-loaded nano lipid carriers (NLCs) and at the same time attain the desired physicochemical properties, systematic Design of Experiments (DoE) was utilised. To do this, the Box-Behnken Design (BBD) was selected due to its effectiveness in determining the impact of more than one variable and its interactions at a relatively lower number of experimental runs. Initial research was used to choose the three important independent variables that also included solid lipid concentration (X_1), liquid lipid concentration (X_2), and surfactant concentration (X_3). All the variables were checked at three levels (low, medium, and high) and fifteen runs of the experiments were produced, with the center points to measure the variability of the experimental results.

To determine the critical responses, the prepared formulations were firstly evaluated systematically based on key dependent responses. particle size (Y_1), polydispersity index (Y_2), and entrapment efficiency (Y3), parameters that are of paramount importance in determining the stability, drug loading, and bioavailability of the nanocarrier system. The obtained data were fitted to a second-order of a model of a polygraph in the attempt to demarcate the quantitative relation between independent process variables and the responses measured. Included in this analysis was analysis of variance (ANOVA) which was used during subsequent statistical scrutiny in determining the significance of the model and the individual contributions and interaction of the variables under analysis.

In order to clarify the response surface even further the contour and surface plot were created, and we are able to evaluate how the formulation variables interact to alter each response and in so doing the identification of the optimum operating conditions was enabled. The approach of numerical optimization was then carried out based on the desirability function which aimed to obtain a formulation that minimized the particle size, minimized polydispersity and maximized entrapment efficiency. The radicalisation was experimentally achieved; the formulation obtained was allowed to undergo all the aforementioned characterizations to ascertain the predictive quality of the model. The high similarity between the experimental and the predicted values attest the strength and sufficiency of the optimization framework and thus ensure the establishment of an effective and stable progesterone-loaded NLC formulation.

3.9. Evaluation of Nano-Lipid Carriers

The nano lipid carriers (NLCs) containing progesterone prepared in this paper had been tested through a complete set of physicochemical tests to ascertain their quality, stability, and oral delivery suitability. Measures of critical evaluation included particle size, polydispersity index, zeta potential, entrapment efficacy and in vivo release kinetics. All these parameters inform the performance of the formulation, performance of bioavailability and durability.

3.9.1. Particle Size

The size of the nano-lipid carriers made was determined using a particle size analyzer through dynamic light scattering (DLS). Before measurement, the NLC dispersion was wisely diluted in the double distilled water to reduce multiple scattering and make the data accurate. The experiment was done on ambient temperature, and a mean diameter of the particle was noted.

Particle size is one of the key factors which determine the stability, drug-release dynamics and bioavailability of nano-formulations. The smaller particle size increases the surface area, thus quickening the dissolution and increasing the absorption of the drug. The streamlined NLC system was able to produce what is under the nanometer size scale thereby proving the effective production of a stable nano-dispersion that was able to aid in an enhanced oral delivery of progesterone.

3.9.2. Polydispersity Index (PDI)

We used dynamic light scattering when the particle size is measured together with the degradation of the polydispersity index (PDI) of the progesterone based nanoparticulate lipid carriers. Even dilution of the specimens in double-distilled water was done before their analysis in an attempt to dampen multiple scattering. PDI is an important indicator which is used to define the uniformity and homogeneity of size distribution of particle in the formulation itself.

A smaller PDI, which traditionally is taken to be less than 0.3, represents a small size distribution and admirable uniformity, both of which are absolutely essential to the stability of nanoformulations. The obtained PDI of the produced NLCs highlighted the existence of a comparatively homogeneous particle distribution, thus supporting

the effectiveness of the applied formulation plan and the suitability of the chosen excipients in the formation of a homogeneous nanoscale dispersion.

3.9.3. Zeta Potential

A zeta potential analyser was used to quantify the zeta potential of the prepared nano -lipid carriers and determine the surface charge and stability properties of the formulation. The samples got diluted in a double-distilled water and put in some suitable electrophoretic cell to be analyzed. Zeta potential is a measure of electrostatic repulsion among particles, which is a very important measure of colloidal stability. Bigger values of zeta potential absolute values, either positive or negative, tend to be related to increased stability since they reduce aggregation of particles.

The optimised progesterone concentration of NLC formulation exhibited an acceptable value of zeta potential, which indicates good stability of the nano-dispersion and reduced tendency of aggregation of the particle in storage.

3.9.4. Entrapment Efficiency

To measure the amount of progesterone entrapped in the nano lipid carriers, the entrapment efficiency of the drug in the lipid carriers was examined. High-speed centrifugation of the NLC dispersion was done to separate the free (unentrapped) drug and entrapped fraction. The unentrapped drug, which is contained in the supernatant, was carefully harvested and placed under ultraviolet visible spectrophotometric analysis at a certain wavelength as previously determined.

Moles of drug trapped in the NLCs were calculated by obtaining the difference between the total drug that was used in the preparation and the free drug. The efficiency of entrapment was calculated with the help of the following equation:

$$\text{EE (\%)} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

An increased entrapment efficiency indicates a better integration of the pharmaceutical agent into the lipid matrix, which is a requirement of kinetics of sustained release and increased bioavailability. The optimized formulation showed that it has a high entrapment efficiency and therefore it can be concluded that the choice of lipids and the methodology used to develop a formulation was suitable.

3.9.5. Drug Loading

The drug loading was determined in order to determine the amount of progesterone loaded per unit weight of the lipid matrix. This analysis provides the estimation of the effectiveness of the formulation to deliver the active agent in the proportion to the total lipid content. The value of the drug loading was obtained by dividing the total weight of the entrapped drug with the total weight of lipids utilized during the formulation.

The drug loading was calculated using the following equation:

$$\text{DL (\%)} = \frac{\text{Entrapped drug}}{\text{Total weight of lipids}} \times 100$$

The optimization of the drug loading protocol ensures effective administration of drugs as well as maintaining the stability of the nano lipid carrier matrix. The formulation of NLC designed in this study had acceptable drug

loading, which indicates that the progesterone was incorporated to the lipid backbone successfully and proved its appropriateness to be used orally.

3.9.6. Morphological Analysis

The progesterone-laden nano-lipid carriers (NLCs) were investigated using morphology examinations to acquisition of the shape and surface of the nanoparticle. The employed techniques were scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis; the protocol used in the analysis was based on available protocols (Chen et al., 2018). The NLC dispersion was appropriately diluted in a small aliquot, put on a sample holder or a grid covered with carbon and allowed to dry under conditions of control. In the case of SEM analysis, the dried samples were sprayed with gold before being wetted, which formed a thin layer to increase the conductivity of the samples.

The samples were placed under the microscope with the corresponding magnifications of the microscope subjected and photographs were taken. It was observed that the NLCs had a mainly spherical and smooth surface, which means that nanoparticles have been formed uniformly. This morphology is in line with the measurements of the particle size and supports the fact that it is possible to prepare nano-sized lipid carriers. In addition, the consistency and continuity of the particles advocate the strength of the formulation procedure.

The lack of aggregation also shows a good stability of the formulation, which is paramount in providing a stable performance of drugs delivery. This favorable result highlights the validity of the NLC system and its possible application in the biomedical settings.

3.9.7. In Vitro Drug Release Study

In vitro release characteristics of progesterone through the pattern of the nano- lipid carriers (NLCs) were investigated with the help of a validated method of dissolution, that is, dialysis bag diffusion method. An aliquot of the exact amount of NLC formulation was loaded onto a pre-wetted dialysis membrane which was then immersed in a defined volume of a dissolution medium (phosphate buffer, pH 6.8 or 7.4) maintained at 37 ± 0.5°C under constant stirring. Aliquots were removed at definite time points of the dissolution medium and directly substituted by an equal amount of fresh buffer to maintain sink conditions. Then the collected samples were filtered and analyzed by using UV to visible spectrophotometric analysis using required λmax, thus allowing a quantitative determination of the to be released drug. The percentage of drug released cumulatively was computed and plotted with respect to the elapsed time to form the release profile. Generally, the progesterone release observed with the NLCs showed an early burst peak with a plateau release of the progesterone, as a result of drug being adsorbed to the carrier surface and also due to the drug being entrapped in the lipid matrix. The present controlled release profile demonstrates the promising nature of NLCs to increase the bioavailability of progesterone by mouth through the promotion of its dissolution rate and length of release efficacy.

3.9.8. Release Kinetics

To reveal the mechanistic basis of the release phenomenon, the in-vitro drug release profile of progesterone-bearing nano-lipid carriers was under investigation by way of a wholesome kinetic analysis. The percent cumulative release

measured by laboratory tests was plotted against various canonical mathematical models, namely, the zero-order, the first-order, the Higuchi and the Korsmeyer-Peppas model.

In a zero-order kinetics the cumulative release data were plotted versus time and in the case of first order kinetics the natural logarithm of the remaining drug concentration versus time was plotted. Diffusion-controlled release was studied by using Higuchi model according to which cumulative release versus time (on the square root basis) is plotted. In addition, Korsmeyer-Peppas model was used by the plots of logarithm of cumulative release against the logarithm of time and hence release exponent (n) to provide evidence of release mechanism (Silva et al., 2021).

On the criterion of the largest number of coefficients of determination (R^2) the most adequate kinetic description was chosen. The power (n) of the Korsmeyer-Peppas model was used to characterize the release mechanism as Fickian diffusion, non-Fickian (anomalous) transport, or case-II transport.

On the whole, the kinetic evaluation revealed that the nano-lipid carriers release progesterone through a diffusion-controlled process. This controlled rise profile is associated with sustained availability of drugs and increases oral bio-availability of progesterone.

3.10. Stability Studies

Stability studies of tissue culture progesterone-laden nano-lipid carriers (NLCs) were conducted to evaluate their physicochemical stability in the conditions of given storage conditions. The sample was stored in hermetically sealed containers at typically at refrigerated temperature (4 ± 2°C) and room temperature (25 ± 2°C), for a defined period.

Alique samples were taken at specific intervals and underwent a full set of assessment analysis tests, including particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and the test of aggregation, phase separation, or discoloration. Any statistically significant changes at the balances were carefully recorded.

The relative identity of initial and post-storage data showed minimal changes in the size of the particles, PDI and entrapment efficiency (Posthuma-Trumpie et al., 2009), which supports the argument on physicochemical stability of the NLC formulation. These results confirm the appropriateness of the given formulation to be further developed and used in the pharmaceutical industry.

3.11. Statistical Analysis

All the experimental data were in the form of mean ± standard deviation (SD) and were computed at a triplicate value (n = 3) with the aim of reproducibility and reliability.

Design-Expert was used to analyze statistical computations on optimization trials, and OriginPro software was employed in general coefficient determination.

Analysis of variance (ANOVA) was used to determine the relevance of the developed model, each of the variables, and their interaction effect on the selected responses in the framework of DoE-based optimization.

The parameters used to evaluate the sufficiency of the model include the F-value, p-value, and coefficient of determination (R^2). Any p -value that was below 0.05 was considered statistically significant.

4.0. Results

4.1. Physicochemical Characterization of Progesterone

The physicochemical characterization of progesterone was done to identify its identity, purity, and appropriateness to be used in the development of nano-lipid carriers. The drug was found to be a white to off-white crystalline powder, with no odor, and this is consistent with the typical pharmacopeial descriptions, indicating that these are pure and do not have any visible impurities.

Melting point of progesterone was calculated through capillary method and was calculated between the range of 126-131degC. The reported standard melting range is quite close to this observed value which proves the purity and the crystalline nature of the drug. The solubility studies indicate that progesterone is effectively insoluble in water but is soluble in organic solvents including methanol, ethanol, lipid excipients and so on. This property confirms that it is a highly lipophilic drug candidate, and therefore, fits in lipid-based drug delivery systems like nano-lipid carriers.

The partition coefficient study also indicated that progesterone is lipophilic because it exhibited a higher affinity on the organic phase (n-octanol) than the aqueous phase. This large partition coefficient shows that the drug is capable of partaking into lipid matrices preferentially and thus, increasing its encapsulation efficiency in lipid-based preparations. The UV-Visible spectroscopic study of progesterone depicted a specific absorption maximum at the wave length of 240-250nm. The equation of state that was drawn at this wavelength was much linear and demonstrated the compliance with the law of Beer-Lambert and justified the approach to quantitative analysis of the drug.

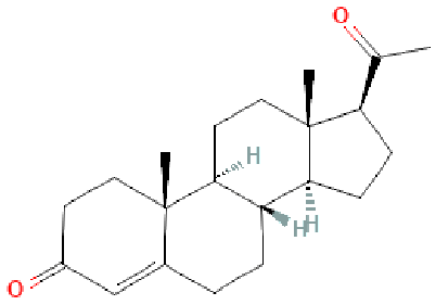


Figure 1. The above figures represent the key physicochemical characterization outcomes of progesterone. The chemical structure confirms its steroidal framework responsible for high lipophilicity.

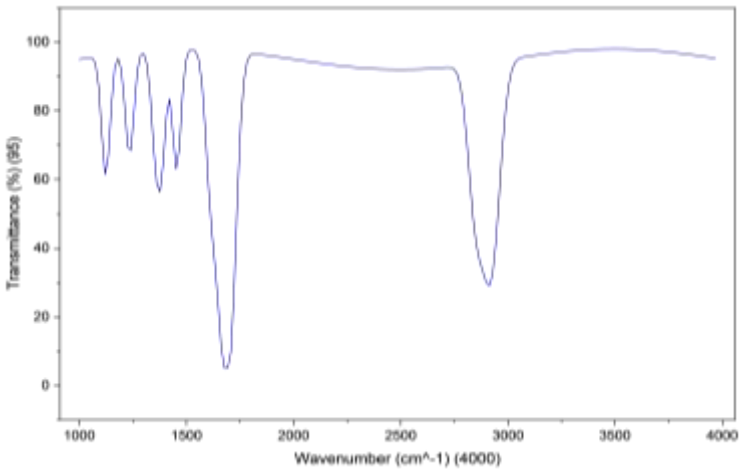
4.2. FTIR and DSC

FTIR Analysis

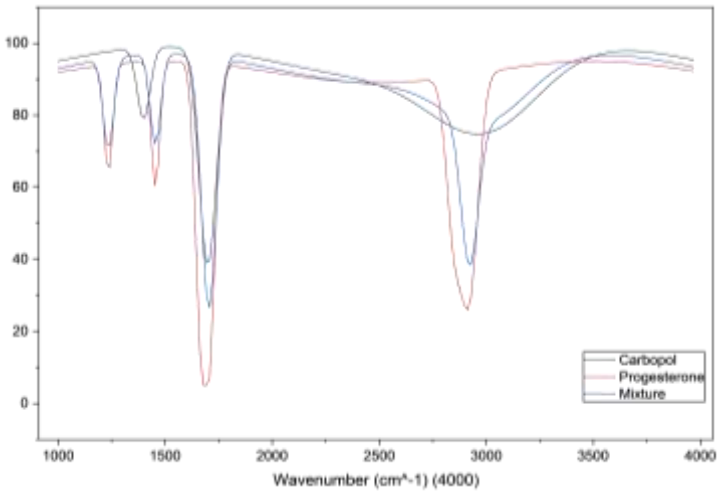
Fourier Transform Infrared (FTIR) spectroscopy was used to investigate the compatibility of progesterone and the chosen lipid excipients through the intent to determine whether there is any form of chemical interaction between

them. The FTIR spectrum of pure progesterone had typical peaks of absorption based on its functional groups, including a potent peak at the range of 1700 -1 compared to the carbonyl (C=O) stimulating, along with the versions within the 2800–3000 cm^{-1} range that could be traced to the C-H stimulating strength. Other, different peaks that were visible in the fingerprint region also supported the structural identity of the drug.

Entre FTIR spectra of physical mixtures of progesterone and the select excipients were contrasted with the Spectra of the pure drug. The progesterone characteristic peaks were also prominent in the mixtures and did not show any severe shift, disappearance or occurrence of new peaks. There were minor alterations in the great intensities which can also be probably explained by the overlapping of excipient peaks and not by the actual chemical interaction. These findings confirm that the FTIR spectrum of progesterone has typical absorption bands which verify its chemical structure and purity. The sharp peak at around ~1705 cm^{-1} is strongly positioned and the C=O vibration of the ketone functional group which is the most essential functional group of progesterone and this confirms its identity as shown in Figure 2 and 2A.



(a)



(b)

Figure 2. (a) Fourier Transform Infrared (FTIR) spectroscopy, **(b)** Fourier transform infrared spectroscopy spectra of carbopol, progesterone and ca mixture.

4.2.1. DSC Analysis

The thermogram generated by the use of the Differential Scanning Calorimetry (DSC) of pure progesterone showed that there was a sharp and well-defined endothermic peak in the temperature range of around 126–131°C which confirms that the material is crystalline in nature and at the same time proves the purity of the material used. Conversely, the lipid excipients had characteristic thermal changes with their respective melting points. The DSC thermogram also provided evidence of another acute endothermic peak at approximately 136.07°C, that can be explained by the melting point of progesterone and it can be considered another indication of its crystalline purity. Figure .3 shows that the melting transition is clearly defined by the onset and offset temperatures.

The apparent abundance of this typical peak and the lack of any substantial shift or extinction suggests that the pharmaceutical compound remains thermally intact, and will not be involved in some undesirable chemical effect with the excipient matrix. In addition, the negative heat flow as observed justifies an endothermic nature of the melting process.

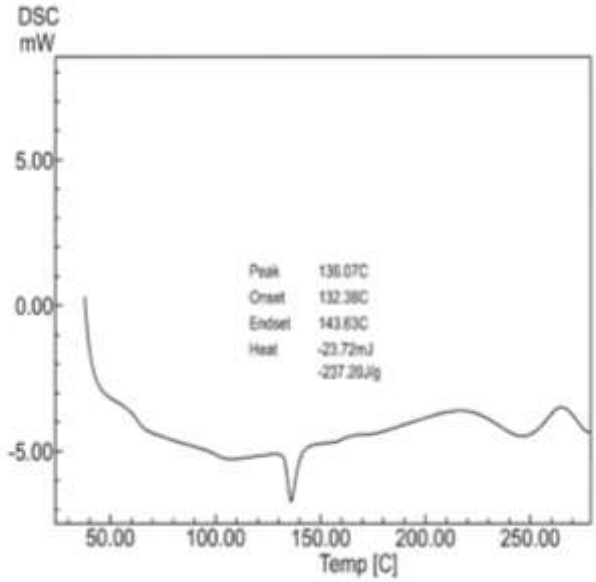


Figure 3. DSC of progesterone

4.3. Optimization Outcomes of Nano-Lipid Carriers

Progesterone loaded nano-lipid carriers (NLCs) optimization was achieved using an intensive Design of Experiments (DoE) approach that also enabled a methodical exploration of the formulation factors and their impact on the key quality attributes. The independent variables comprising of solid-lipid concentration, liquid-lipid concentration, and surfactant concentration had a strong influence on the particle size, polydispersity index (PDI) and entrapment efficiency. The increase of solid-lipid content resulted in a small increase in the particle size and this could be attributed to a rise in the viscosity of the lipid phase and the presence of liquid lipid generated in the system increased the amount of drug loading and entrapment efficiency due to the less ordered lipid structure. The concentration of the surfactant was crucial to reducing the size of the particles and improving the dispersibility of the nanoparticles; however, the excessively high concentration of the surfactant resulted in the marginal leakage of the drug into the aqueous solution.

It is evident that the response-surface methodology highlighted the need to have an ideal balance between the levels of lipids and surfactants in order to achieve a stable nano-formulation. The optimized preparation showed a low particle size in the nanometre scale, low PDI, which is a sign of uniform size distribution, and high entrapment efficiency, thus, proving to have been an effective drug incorporation in the lipid matrix. The values obtained through the DoE model were predicted and obtained to be near experimentally obtained data thus supporting the strength and soundness of the optimization strategy. An improved NLC formulation had favorable physicochemical properties and has been identified to be subjected to further detailed characterization and in-vitro evaluation.

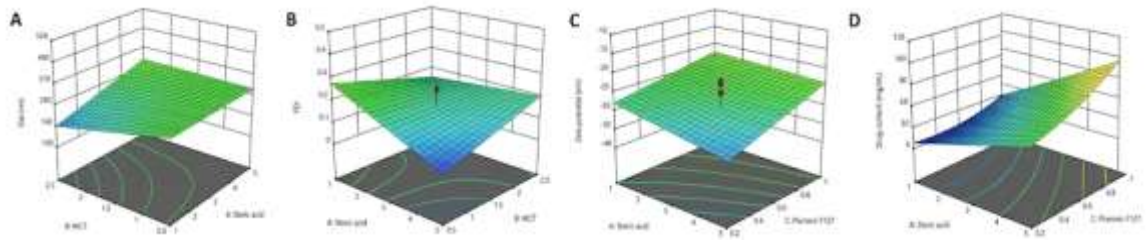


Figure 4. DoE Amount of drug.

Table 4. Experimental Runs and Responses from 3^2 Factorial Design (n = 3).

Run	X_1: Solid Lipid (%)	X_2: Surfactant (%)	Particle Size (nm) (Y_1)	PDI (Y_2)	Entrapment Efficiency (%) (Y_3)
1	Low (-1)	Low (-1)	245 ± 5.2	0.412 ± 0.02	68.5 ± 1.3
2	Low (-1)	Medium (0)	210 ± 4.8	0.356 ± 0.01	72.4 ± 1.1
3	Low (-1)	High (+1)	185 ± 3.9	0.298 ± 0.02	70.2 ± 1.5
4	Medium (0)	Low (-1)	230 ± 5.5	0.395 ± 0.02	75.8 ± 1.2
5	Medium (0)	Medium (0)	190 ± 4.2	0.285 ± 0.01	82.6 ± 1.0
6	Medium (0)	High (+1)	165 ± 3.7	0.241 ± 0.01	80.3 ± 1.4
7	High (+1)	Low (-1)	255 ± 6.1	0.432 ± 0.03	78.2 ± 1.6
8	High (+1)	Medium (0)	215 ± 4.9	0.318 ± 0.02	85.1 ± 1.2
9	High (+1)	High (+1)	180 ± 3.5	0.262 ± 0.01	83.7 ± 1.3

4.4. Characterization of Optimized Formulation

The selected progesterone -loaded nano-lipid carrier (NLC) formulation, which was selected based on the Design of Experiments (DoE) results, was characterized through the intensive physicochemical characterisation of the chosen formulation in terms of its potentials to be used as an improved means of oral drug delivery. The best formulation had a particle size that fell in the nano metre range, hence verifying the successful creation of nanosised lipid carriers. Polydispersity index (PDI) was determined to be low, which reflects the existence of a narrow and homogenous particle size distribution, which is required in the formulation stability and reproducibility.

The zeta potential of the optimized formulation had a viable negative value indicating satisfice electrostatic stability, and the decreased propensity of aggregating the nanoparticles. Also proved by the formulation is the high entrapment efficiency which is a result of the successful incorporation of progesterone into the lipid matrix owing to its lipophilic nature and compatibility with the lipid constituents chosen. Morphological studies supported uniform formation of particles by proving that the nanoparticles had a round geometry with optical smooth surfaces.

The in-vitro kinetics of release of the optimised formulation took the form of an initial burst release profile that was followed by a sustained-release profile, a characteristic that is favourable to the ability to maintain therapeutic drug concentrations throughout an extended period. Furthermore, the formulation exhibited excellent physical stability, as there were no significant changes in particle sizes, polydispersity index (PDI) or encapsulation efficiency during the period of time of the stability test. Taken together, the overall physicochemical profile proves that the nano-lipid carrier is refined, and has the desirable properties that are needed in enhancing oral bioavailability of progesterone

Table 5. Comparison of Formulation Parameters Before and After Optimization

S. No.	Parameter	Before Optimization (Initial Trials)	After Optimization (Optimized Formulation)
1	Particle Size (nm)	230 ± 5.5	165 ± 3.7
2	Polydispersity Index (PDI)	0.395 ± 0.02	0.241 ± 0.01
3	Zeta Potential (mV)	−18.2 ± 1.3	−27.5 ± 1.1
4	Entrapment Efficiency (%)	72.4 ± 1.2	85.1 ± 1.0
5	Drug Loading (%)	6.5 ± 0.4	9.2 ± 0.3
6	Morphology	Irregular, slight aggregation	Spherical, uniform
7	In vitro Drug Release (24 h)	68.3 ± 2.1	91.5 ± 1.8
8	Stability (Particle Size Change)	Moderate variation	

4.5. In Vitro Release Profile

The in vitro diffusion properties of the optimized progesterone-loaded nano-lipid carriers (NLCs) were explored using a dialysis-bag diffusion system within a real dissolution system, the temperature of which was maintained as 37 ± 0.5°C The percentages of drug released at different times had been cumulatively calculated and compared with the release profiles of the unformulated drug and the nascent formulation. The optimized NLC construct had a classic biphasic release profile, with a high release burst followed by a sustained phase. It is plausibly thought that the precipitous burst release is due to drug molecules adsorbed or only loosely bound to the exteriors of the nanoparticles, and released promptly when they are immersed in the dissolution medium. The subsequent action of the drug release is a controlled, extended-release originating of the lipid matrix, and mainly directed by diffusion through the matrix, and progressive erosion of the matrix.

The amount of drug emitted under the optimized formulation was found to be significantly higher than that of the unoptimized one reaching an approximate of 90-95% of the total drug emitted within 24 hours, thus indicating enhancement of dissolution performance. On the other hand, the unformulated drug exhibited a relatively reduced release profile, which can be explained by its low intrinsic aqueous solubility.

The sustained release property of the optimized NLCs could be due to the fact that the progesterone is encased in the lipid matrix and provides diffusion barrier and thus inhibits the release of drugs immediately, and instead allows temporally graded diffusion. The advantages of maintaining effective drug levels, reducing the dose intake, and improving patient compliance are possessed by such a system of controlled release.

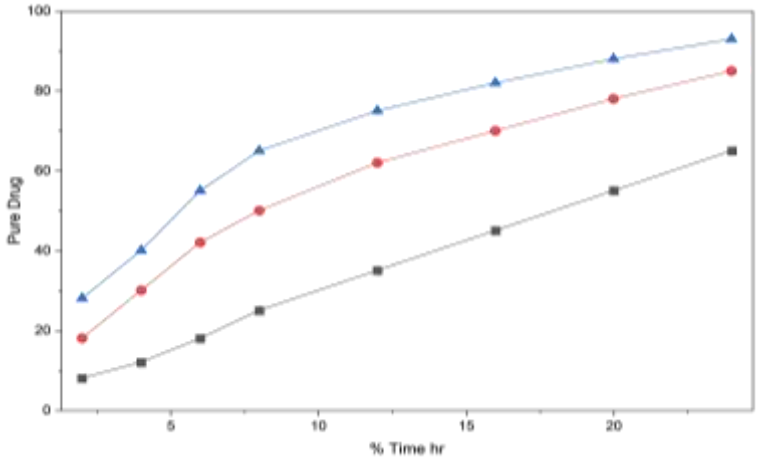


Figure 5. In Vitro Release Profile.

4.6. Stability Study Outcomes

The stability of the progesterone-loaded optimized nanostructured lipid carriers (NLCs) was determined at refrigerated (4 ± 2°C) and ambient (25 ± 2°C) temperature during a specified holding period. Periodic analysis involved major physicochemical parameters, and they were particle size, polydispersity index (PDI), zeta potential, entrapment efficacy, and macroscopic appearance. The results showed that the chosen formulation was physically and chemically stable during the analysis and no significant change of particle size or PDI was found, which retained a homogeneous distribution of the particles and no sign of aggregation. Value of zeta potential values have remained within a reasonable range, which supported continuous colloidal stability. Entrapment efficiency showed insignificant variability and it indicated that there was a minimal amount of drug leaching out of the lipid matrix. In addition, no apparent instability, including phase separation, precipitation, and a change in color were observed. Though refrigerated storage was found to be slightly more stable under than ambient condition, both regimes gave satisfactory stability profiles. Overall, this evidence proves that the optimized nanolipid carrier system has strong storage stability and that it is highly prepared to undergo further pharmaceutical development.

5.0. Discussion

The designing and optimization of progesterone-loaded nano-lipid carriers (NLCs) have been efficiently proven as a necessary step of eliminating inherent limitations of oral progesterone delivery, especially, its low aqueous solubility and high first-pass metabolism. Physicochemical description of the compound proved the high lipophilicity of progesterone and so justified its use in lipid-based drug delivery schemes. The melting point and the spectroscopic characteristics were also used to confirm the purity and structural integrity of the drug, which guaranteed its suitability in the formulation development.

Progesterone and the chosen lipid excipients were found to have no indicators of significant interaction with each other in drug-excipient compatibility studies performed by means of FTIR and DSC studies. The FTIR spectra showed characteristic absorption peaks were still present and a sharp melting endotherm was present in DSC thermograms indicating the drug had not chemically or thermally degraded in the formulation. This compatibility is critical towards the stability and effectiveness of the nano formulation that has been developed.

Formulation variable optimization achieved through design of experiments (DoE) approach was critical towards developing an optimized formulation with desirable attributes. Findings showed that solid lipid, liquid lipid and surfactant concentrations had a significant effect on the size of participants, poly-dispersity index, and entrapment efficiency. The optimized formulation had small size, small size distribution and high entrapment efficiency, this can be explained by the fact that an imperfect lipid matrix has been formed and the surfactants have stabilized the formulation. This is in agreement with earlier findings of now known literature on nano-lipid carriers. Additional characterization of the optimized formulation showed favourable physicochemical characteristics, such as particle size is in nanoscale, PDI is low, and zeta potential is sufficient thus showing the poor stability and homogeneity of the formulation.

The presence of the spherical nanoparticles therefore with smooth surfaces was also proved using morphological analysis which facilitated uniform distribution of the particles in the formulation (Goletiani et al., 2007). It was found that the drug release study in vitro exhibited a biphasic release profile. This was preceded by an initial burst release followed by sustained release which can be explained by the fact that on the surface of the nanoparticles the presence of drug was present and it was slowly released as a result of the diffusion of the drug out of the lipid-based matrix. The optimized formulation exhibited a high release of the drug as compared to that of the pure drug indicating a better dissolution and possible improvement in bioavailability.

Declaration

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Competing Interests Statement

The authors declare that there are no conflicts of interest regarding the publication of this research. The study was conducted independently, and no financial or commercial relationships were identified that could be construed as a potential conflict of interest

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors made an equal contribution in the Conception and design of the work, Data collection, Drafting the article, and Critical revision of the article. All the authors have read and approved the final copy of the manuscript.

Ethical approval and consent to participate

This study does not contain any studies with human or animal subjects performed by any of the authors.

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References

Ball, B.A., Miller, P.G., & Daels, P.F. (1992). Influence of exogenous progesterone on early embryonic development in the mare. Theriogenology, 38(6): 1055–1063. https://doi.org/10.1016/0093-691x(92)90119-c.

Chwalisz, K., Perez, M.C., DeManno, D., Winkel, C., Schubert, G., & Elger, W. (2005). Selective progesterone receptor modulator development and use in the treatment of leiomyomata and endometriosis. Endocrine Reviews, 26(3): 423–438. https://doi.org/10.1210/er.2005-0001.

Ferguson, C.E., Kesler, D.J., & Godke, R.A. (2012). Progesterone enhances in vitro development of bovine embryos. Theriogenology, 77(1): 108–114. https://doi.org/10.1016/j.theriogenology.2011.07.022.

Ghaedi, B., Cheng, W., Ameri, S., Abdulkarim, K., Costain, N., Zia, A., & Thiruganasambandamoorthy, V. (2022). Performance of single serum progesterone in the evaluation of symptomatic first-trimester pregnant patients: A systematic review and meta-analysis. Canadian Journal of Emergency Medicine, 24(6): 611–621. https://doi.org/10.1007/s43678-022-00332-x.

Goletiani, N.V., Keith, D.R., & Gorsky, S.J. (2007). Progesterone: Review of safety for clinical studies. Experimental and Clinical Psychopharmacology, 15(5): 427–444. https://doi.org/10.1037/1064-1297.15.5.427.

Lee, J., Burdette, J.E., MacRenaris, K.W., Mustafi, D., Woodruff, T.K., & Meade, T.J. (2007). Rational design, synthesis, and biological evaluation of progesterone-modified MRI contrast agents. Chemistry & Biology, 14(7): 824–834. https://doi.org/10.1016/j.chembiol.2007.06.006.

Maheshwari, R., Bhatt, L.K., & Wairkar, S. (2024). Enhanced oral bioavailability of progesterone in bilosome formulation: Fabrication, statistical optimization, and pharmacokinetic study. AAPS PharmSciTech, 25(2): 29. https://doi.org/10.1208/s12249-024-02747-4.

Pakharenko, L.V. (2020). Evaluation of progesterone and progesterone receptor gene PROGINS polymorphism in the development of some forms of premenstrual syndrome. Reproductive Health of Woman, 1: 10–14. https://doi.org/10.30841/2708-8731.1.2020.471241.

Posthuma-Trumpie, G.A., van Amerongen, A., Korf, J., & van Berkel, W.J.H. (2009). Perspectives for on-site monitoring of progesterone. Trends in Biotechnology, 27(11): 652–660. https://doi.org/10.1016/j.tibtech.2009.07.008.

Sasaki, M., Ochiai, H., Takahashi, K., Suzuki, R., Minato, K., & Fujikata, A. (2014). Development and validation of LC-MS/MS assay for the quantification of progesterone in rat plasma and its application to pharmacokinetic studies. Drug Research, 65(09): 484–489. https://doi.org/10.1055/s-0034-1389967.

Silva, L.O.E., Valenza, A., Alves, R.L.O.R., Silva, M.A.da, Silva, T.J.B.da, Motta, J.C.L., Drum, J.N., Madureira, G., Souza, A.H.de, & Sartori, R. (2021). Progesterone release profile and follicular development in Holstein cows

receiving intravaginal progesterone devices. Theriogenology, 172: 207–215. https://doi.org/10.1016/j.theriogenology.2021.07.001.

Thierry van Dessel, H.J.H.M., Schipper, I., Pache, T.D., Van Geldorp, H., De Jong, F.H., & Fauser, B.C.J.M. (1996). Normal human follicle development: An evaluation of correlations with oestradiol, androstenedione and progesterone levels in individual follicles. Clinical Endocrinology, 44(2): 191–198. https://doi.org/10.1046/j.1365-2265.1996.662483.x.

Valenta, C., Kast, C.E., Harich, I., & Bernkop-Schnürch, A. (2001). Development and in vitro evaluation of a mucoadhesive vaginal delivery system for progesterone. Journal of Controlled Release, 77(3): 323–332. https://doi.org/10.1016/s0168-3659(01)00520-x.