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Preparation and characterization of vaginal suppository of semisynthetic derivatives of ergot alkaloids cabergoline

Pouria Rahmanian-Devin [a,b], Mohammad Reza Fadaei [b], Mohammad Mashreghi [d], Vahid Reza Askari [c,e,f,*]

[a] Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] International UNESCO Center for Health-Related Basic Sciences and Human Nutrition, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[e] Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[f] Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Background: There is evidence that vaginal cabergoline can help to prevent ovarian hyperstimulation syndrome. Therefore, the vaginal suppository may be a good choice because it can be administered directly into the vagina and has no adverse effects on the stomach. In this regard we developed a cabergoline suppository as an alternative to cabergoline tablets. Design-Expert was used to determine the most suitable concentrations of PEG 6000/400, and Tween 80 to obtain a stable suppository. Specific ratios of PEG6000/400 and Tween 80 were entered as factors, and release, melting time, and hardness were evaluated as responses. In addition, the final formulation was evaluated for weight changes, pH, drug content, degradation time, deformation time, *in vitro* drug release, DSC analysis, infrared spectroscopy, and stability properties.

Results: The suppositories were all smooth and white. They all had a weight that averaged less than 5 %. The formulations showed a pH between 6 and 6.5. The active ingredient content ranged between 79.666 ± 8.54 % and 99.67 ± 6.55 %. Suppository stiffness was between 2.74 ± 0.04 and 4.20 ± 0.03. The decomposition time of the suppositories varied between 11.25 ± 0.15 to 20.19 ± 0.08 min. The deformation time was between 26.11 ± 0.06 to 38.59 ± 0.47 min. Cabergoline content was released over 45 min from formulations of F10 (~46 %), F2 (~64 %), F6 (~69 %), F4 (~79 %), F1 (~88 %), and F7 (~93 %). However, other formulations released more than 95 % within 45 min.

Conclusions: All variables except melting time significantly affected our responses. *In vitro* studies have indicated that the optimized cabergoline formula could be an excellent alternative to cabergoline oral formulations.

1. Background

Cabergoline (Dostinex®) has the chemical formula C26H37N502, a derivative of ergot (Schiff, 2006). Cabergoline acts as a dopamine agonist and mimics the effects of dopamine by binding to dopamine receptors. Like hypothalamic androgen dopamine, it directly inhibits prolactin secretion from pituitary lactotroph cells and, by lowering serum prolactin levels, causes the hypothalamic-pituitary axis to function normally, resulting in ovulatory function (Speroff and Fritz, 2005). A study by Alvarez *et al.* showed that cabergoline administration prevents ovarian hyperstimulation syndrome (OHSS), along with conventional therapies, and has no adverse effects (Alvarez, 2007). Several

Abbreviations: OHSS, Ovarian hyperstimulation syndrome; FSH, Follicle-stimulating hormone; VEGF, Vascular endothelial growth factor; IVF, *In vitro* fertilization; hCG, Human chorionic gonadotropin; CCD, Central composite design; PEG, Polyethylene glycole; FTIR, Fourier transform infrared; DSC, Differential scanning calorimetric; CUR, Curcumin; PLA, Polylactide acid; BCS, Biopharmaceutics classification system.

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* Corresponding author at: Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; International UNESCO Center for Health-Related Basic Sciences and Human Nutrition, Mashhad University of Medical Sciences, Mashhad 9177948564, Iran.

E-mail addresses: pouryar2@gmail.com (P. Rahmanian-Devin), fadaei.mrf@yahoo.com (M.R. Fadaei), askariv@mums.ac.ir, vahidrezaaskary@gmail.com (V.R. Askari).

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studies indicated that cabergoline also could selectively bind to dopamine D2 receptors in lactotroph cells, providing long-term inhibits serum prolactin levels (Eguchi, 1995), including pituitary adenoma (Eguchi, 1995; Miyagi, 1996).

Furthermore, cabergoline is also used in the treatment of other disorders and diseases such as Parkinson's, acromegaly, Cushing's syndrome, and Nelson's syndrome (Mancini et al., 2008; Pivonello, 2009) and is involved in the treatment of follicle-stimulating hormone (FSH) -secreting tumors such as pituitary tumors (Leese et al., 1997). Serum FSH levels are significantly reduced after treatment with cabergoline. Furthermore, compared to other drugs in the family, cabergoline effectively and long-term reduces serum prolactin levels and pituitary tumor weight (Schiff, 2006; Eguchi, 1995; Miyagi, 1996; Colao et al., 2000; Baeza Pertegaz et al., 2002). In addition, it had an influential role in preventing ovarian hyperstimulation syndrome by inhibiting the secretion of vascular endothelial growth factor (VEGF)(Ferrero, 2015). There is an emerging prevalence of ovarian hyperstimulation syndrome, and complications related to this syndrome include ovarian enlargement and fragility, extracellular fluid accumulation and intravascular volume reduction, abdominal pain, ascites, and hydrothorax (Tang, 2012). This complication affects 20 to 30 percent of *in vitro* fertilization (IVF) patients in the United States (Papanikolaou, 2006). The presence of more than 11–13 mm follicles during an IVF cycle signifies that the ovaries are overproducing. Stimulation of VEGF secretion by human chorionic gonadotropin (hCG) injection increases its level in granulosa cells, which increases the permeability of arteries in this area, followed by fluid leaking into the interstitial space and causing symptoms such as decreased intracellular volume of vascular, abdominal pain, ascites, and hydrothorax are all symptoms(Toffle, 2011).Cabergoline is influential in preventing ovarian hyperstimulation syndrome by inhibiting VEGF secretion. Various studies have shown that cabergoline can effectively treat and prevent this syndrome(Ferrero, 2015; Tang, 2012; Amir, 2015; Leitao, 2014; Kilic, 2015; Carizza, 2008). However, some of the adverse effects of cabergoline lead to patients' dissatisfaction and their treatment interruption, including nausea and vomiting, hypotension, postural hypotension, palpitations, and exacerbation of depression and anxiety(Sweetman, 2005). Various studies have shown that cabergoline is useful in the treatment and prevention of this syndrome (Leitao, 2014; Carizza, 2008; Kılıç, 2015; Soares, 2008). It has been observed in various studies that cabergoline, as a dopamine agonist, prevents the increase in vascular permeability by inhibiting the phosphorylation of VEGFR2 (Alvarez, 2007; Álvarez, 2007; Papaleo, 2001; Ata et al., 2009). The best treatment for ovarian hyperstimulation syndrome is prevention. It is divided into mild, moderate and severe types. Mild conditions do not require treatment, and moderate conditions must be observed, and severe conditions require timely measures. Hematocrit, electrolytes, and liver and kidney function should be monitored(Chen, 2011). Usually, these patients receive heparin to prevent venous thrombosis. The current trend for the treatment of patients is intravenous injection of fluids and electrolytes, albumin, aspiration of ascites fluid through the abdomen, and oral administration of cabergoline(Aboulghar, 2010). Several studies have shown that dopamine can regulate the VEGF/VEGFR2 pathway as a dopamine agonist in *in vivo* and *in vitro* models, so cabergoline suppositories can control the size of follicles through local effects and systemic absorption(Cristina, 2005; Sinha, 2009; Ferrero, 2015). In addition to these, cabergoline has gastrointestinal side effects, sometimes these side effects cause the patient to stop the treatment, when it is taken vaginally, it is absorbed into the systemic circulation of the body, without showing any digestive side effects(Motta, 1996). In a study conducted by Motta T et al. (Motta, 1996), in two cases of patients who could not take cabergoline orally, they observed that taking one 0.5 mg cabergoline tablet weekly through the vagina can bring the prolactin level to normal. Cabergoline is also absorbed systemically from the vagina.

Therefore, if we use the suppository form to deliver the drug to the vaginal area, in addition to the elimination of drug inactivation and gastrointestinal complications, due to the lack of first hepatic passage, a lower dose of the drug will probably be required, and we will see fewer systemic complications. Unfortunately, no vaginal formulation of cabergoline has entered the market to date. Therefore, the present study optimized the cabergoline suppository formula and selected the best formulation.

2. Methods

2.1. Materials and chemicals

Cabergoline was purchased from Pharmco, Indore CO, India. Polyethylene glycol (PEG) 400, PEG 6000, polysorbate 80 (Tween 80), pure methanol, and all other chemicals and solvents were obtained from Sigma-Aldrich, Germany, and were analytical reagent grade.

2.2. Study outlook

In this study, the test design method is used to achieve the optimal formulation regarding material properties and select the best formulation in terms of response. The formula's desired responses were faster drug delivery from the base and meeting quality control requirements (melting point and hardness). Our formulation should be solid at room temperature (22–24 ℃) and release the drug in less than 30 min after use. The fusion molding method for preparing suppositories was done using stainless steel suppository mold. PEG 6000, PEG 400, and Tween 80 were used to develop the suppositories. The effect of formulation factors on product characteristics was also examined statistically using Design Expert v.11 software. The optimal formulation was selected from the values of PEGs and Tween 80 concentrations that remain solid and stable at room temperature. The factors used to obtain the optimal design formulation were PEG 6000/PEG 400 (1.55–11.45), Tween 80 (1 %–2%), and the evaluated responses were 1. hardness, 2. melting point, and 3. percentage of drug release.

2.3. Experimental design

A central composite design (CCD) was used to optimize cabergoline suppositories. Regarding to our pervious experiments and pretests we reached to two critical variables in fabrication of this type of suppository. We used RSM to find the optimized formulation and evaluate the effect of each factor on suppositories properties. As we had two numeric factors, we couldn't use design such as box Behnken, which need at least three numeric factors. In addition, we want to optimize and find out the true level ranges of our factors that we reached them with experiment. The axial point in CCD helps us to have better estimation of curvature in our model. Based on our experiment the coded minimum and coded maximum (the minimum and maximums that we used for design in software) for PEG ratio was 3 and 10 respectively. In regard to use CCD design the alpha value is also being considered. In CCD design the minimum and maximums are our coded alpha-value. When alpha in over 1 (here the value of alpha is 1.45), means the axial point are outside the cube. These axial points are useful to show better view of our model surfaces. The ratios of PEG6000 to PEG400 (A) and Tween 80 percentage (B) were selected as independent variables (factors), whereas release (%), melting time (minute), and hardness were obtained as dependent variables(response). Design expert software (Version 11.1.1.0) was used for this experimental design. The software generated 11 experiments with 3 center points. The coded and decoded independent variables are shown in Table 1 and Table 2..

2.4. Preparation of suppositories

The suppositories were prepared by the molding method. First, pour a certain amount of PEG 6000 and PEG 400 into a human, then add tween 80 to it and put it on a heater at 65 ℃ to melt, then add 0.5 mg of

Table 1
Experimental design table, independent variables for preparing cabergoline vaginal suppository.

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High
A	PEG6000/PEG400	ratio	Numeric	1.55	11.45	−1 ↔ 3.00	+1 ↔ 10.00
B	Tween80	%	Numeric	0.7929	2.21	−1 ↔ 1.00	+1 ↔ 2.00

Table 2
Experimental design tablet, variables response.

Response	Name	Units
R1	Release	%
R2	Melting time	min
R3	Hardness	kg/cm^2

cabergoline powder and mix it manually with a mixer. Next, dip the molds inside in paraffin so that the suppositories come out of the mold more easily. Next, pour the melted mixture into the mold, then cool the mold to room temperature. Finally, we put the suppositories in a plastic can and kept them at a temperature of 20–25 ℃ (Christ, 2020; Kauss, 2013).

2.5. Physical and chemical properties evaluations

An ocular examination was performed as a physical evaluation. Color, melting point, odor, and the presence of bubbles or cracks were examined. A change in the smell of the suppository is a sign of the degradation process. The suppository shape is approved for greater compatibility (Christ, 2020; Sudke, 2017).

2.6. Variety of weights

According to the 2011 British Pharmacopoeia, 12 suppositories were selected from each formulation, and their average weight was measured at 24 h (Christ, 2020; Sudke, 2017).

2.7. Determining the pH

The pH of the formulated suppositories was measured using a digital pH meter (Metrohm 780 pH Meter). First, the suppositories were opened in hot water, and then the liquid was passed through a paper filter. Finally, the pH of the liquid was measured at 37 ± 0.5° C(Christ, 2020; Sudke, 2017).

2.8. Content of the drug

We randomly selected three suppositories from each formulation. Place each suppository in a 100 ml flask. Then add 5 ml of pure methanol and shake the flask for 15 min. Using a phosphate buffer with a pH of 4.5, make a volume of 100 ml. Then, we determine the absorption and concentration using a UV-spectrophotometer (Shimadzu 1601, Japan) at a wavelength of 282 nm. At various time intervals, aliquots of 2 ml were withdrawn and diluted further with methanol. Each time, the volume of aliquots was changed with new dialyzing media. Using a UV–visible spectrophotometer at 282 nm and methanol as a blank(Sharma et al., 2009).

2.9. Hardness

In this test, three suppositories were randomly selected, and their hardness was measured using a mechanical strength/breaking apparatus (Erweka-apparatus, Germany, model: SBT). Briefly, a hardness test was performed for three suppositories using a hardness tester at 25 °C. The weight required to break the suppository was taken as a measure of its hardness. This test was performed to evaluate the suppositories' strength and determine if the prepared formulation could withstand the risks of packaging and shipping (Baviskar et al., 2013; Sah and Saini, 2008).

2.10. Disintegration time

Disintegration time was assessed using apparatus (Model: TDI2, ElectroPharmed, Iran) to determine the time of the collapse of suppositories. The complete decomposition time of the suppository at 37.5 ℃ in an acetate buffer (305 ml of 0.2 M acetic acid, 195 ml of 0.2 M sodium acetate, and 500 ml distilled water, pH 4.5)was recorded as the decaying medium (Sudke, 2017).

2.11. Measurement of deformation time (liquefaction time)

This test provides information on the behavior of the suppository at 37° C. It demonstrates how long it takes for the suppository to melt at 37° C completely. To perform this test, we designed a simple device, the schematic of which is presented in Table 3. In short, we first made a glass board with a diameter equal to the suppository. Then we removed the screw cutter. The burette has a narrow end and a wide end. We immersed the burette in water at body temperature (37° C) from a narrow end. Carefully put the suppository into the burette from the wide end until the narrow end. Finally, we placed a glass rod on the suppository and measured how long it took for the glass rod to reach the narrow end of the port. The time taken for this process is the time of liquefaction (Havaldar, 2017).

2.12. In-vitro drug release studies

The percentage of cabergoline release from suppositories was performed in accordance with USP 38 using the basket dissolution device (Model: TD06, ElectroPharmed, Iran). First, we put the suppository in the basket and installed it on the machine. The dissolution medium was a phosphate buffer with a pH of 4.5 and 0.1 % Tween 80, and the temperature was kept at 37° C with the basket speed set to 50 rpm. At 10, 20, 30, 40, and 60 min, 3 ml of sample was collected and replaced with an equal volume of fresh dissolution medium (pH = 4.5 phosphate buffer with 0.1 % Tween 80) after each sampling to maintain a constant volume. During the study, the concentration of cabergoline in each filtered sample, after reaching a volume of 5 ml with pure methanol, the appropriate dilutions were estimated using a visible UV spectrophotometer at λmax (282 nm). Studies were performed in three replications (Fig. 1) (Christ, 2020; Sudke, 2017).

2.13. Diffusion mechanism

The diffusion mechanism was determined by fitting the diffusion data to experimental or quasi-experimental models such as zero-order, first-order, and Higuchi diffusion and Pepas models(Christ, 2020; Sudke, 2017).

2.14. Fourier transform infrared(FTIR)

Cabergoline, PEG400, and 6000 spectra and suppositories were analyzed using FTIR Spectrometer (PerkinElmer-Spectrum 100 N FTNIR). The samples were ground and mixed with 1 % by weight potassium bromide and then pressed at a pressure of 8 tons for 5 min (hydraulic press). Single-beam spectra were recorded after an average of 20 scans with a resolution of 0.5 cm^{-1} between 4000 and 400 cm^{-1}.

Table 3
Parameters of the prepared cabergoline vaginal suppository formulations.

Formulations	Weight variation (%) * $n = 20$	Disintegration time (min) $n = 3$	Drug content (%)** $n = 3$	Deformation time	Hardness ** $n = 3$	pH
F1	1.791 ± 0.49689	17.41 ± 1.35031	87.666 ± 4.527525	37.44 ± 0.98	3.606 ± 0.42755	6.4
F2	1.704 ± 0.39552	19.51 ± 2.57735	85.333 ± 8.486715	37.913 ± 4.2445	4.026 ± 0.98224	6.1
F3	1.742 ± 0.48816	17.12 ± 3.0504	99.166 ± 6.040833	35.12 ± 5.333	3.478 ± 0.50895	6.5
F4	1.721 ± 0.30089	18.24 ± 4.20139	87.666 ± 7.516611	38.49 ± 1.435	3.766 ± 0.50332	6.2
F5	1.742 ± 0.48073	11.25 ± 2.5	99.666 ± 1.527525	26.11 ± 2.602	2.438 ± 0.64086	6.4
F6	1.767 ± 0.44501	19.35 ± 3.65574	85.333 ± 5.516611	37.18 ± 4.041	3.891 ± 0.63011	6.5
F7	1.667 ± 0.20526	17.35 ± 1.77675	92.833 ± 8.258306	37.26 ± 3.7637	3.636 ± 0.16371	6.5
F8	1.706 ± 0.40104	17.42 ± 6.9609	92.333 ± 10.527525	37.5 ± 4.066	3.654 ± 0.50846	6.3
F9	1.771 ± 0.55501	15.18 ± 4.7858	96.166 ± 7.40833	31.22 ± 3.063	2.888 ± 0.25027	6.1
F10	1.739 ± 0.49275	20.19 ± 5.81854	79.666 ± 8.54701	38.59 ± 4.712	4.208 ± 0.30447	6
F11	1.791 ± 0.49689	14.56 ± 4.06325	99.9 ± 6.55744	30.21 ± 2.322	2.740 ± 0.4549	6.4

*All values with mean ± SD (n = 12); **all values with mean ± SD (n = 3).

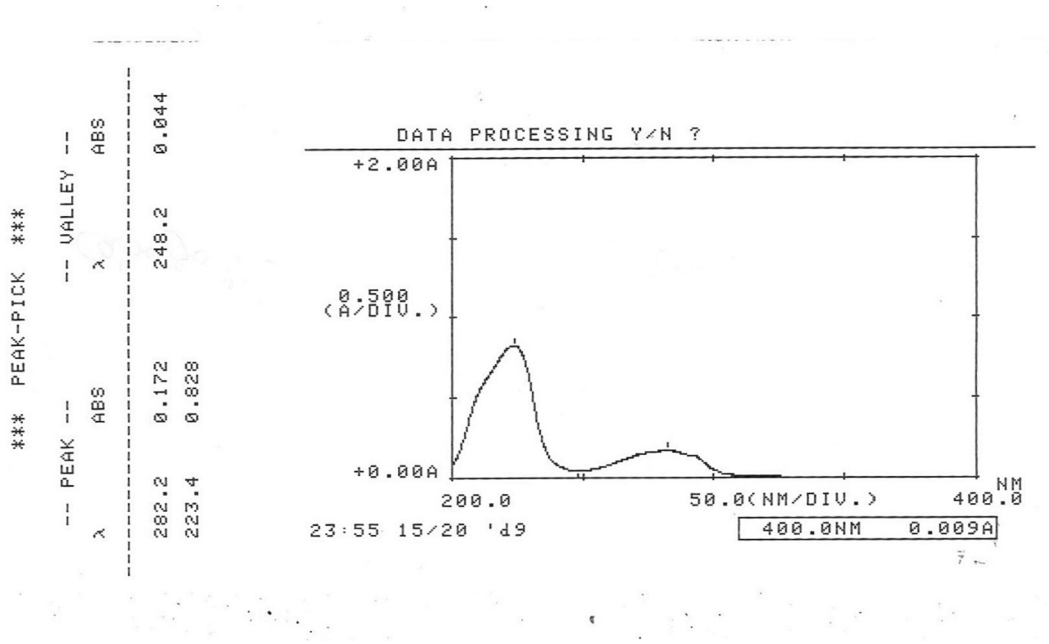

Fig. 1. The λ_{MAX} of cabergoline in the mixture of water and methanol (3:5).

These spectra were then corrected against the background spectrum of the atmosphere(Christ, 2020; Sudke, 2017).

2.15. Differential scanning calorimetric (DSC) analysis

Differential Scanning Calorimetric analysis was performed using a DSC (Switzerland-METTLER TOLEDO DSC82) with STARe software. The protocol was included a heating rate of 5 °C/min in the range of 30–200 °C for the thermogram or 2 °C/min in the range of 30–200 °C for the determination of the melting point (n = 3). Accurate specimens weighing 5 mg were sealed in aluminum pans and perforated with a pin. An empty perforated aluminum pan was used as a reference. For comparison, the theoretical melting point was calculated as the mean of individual melting points considering the relative content(Christ, 2020; Sudke, 2017). In fact, the test shows us that cabergoline combines with other formulation components and is placed in an amorphous or crystalline form among the excipients.

2.16. Cabergoline suppository stability studies

Optimized cabergoline suppositories were subjected to stability studies. Studies were performed in three replications. First, the suppositories were weighed, adequately wrapped in aluminum foil, and stored for 90 days in a stable chamber at room temperature (25 ± 2° C and 40 % humidity), and an accelerated degradation study (40° C and 75 % relative humidity) was carried out. The suppositories were then evaluated to estimate the drug content at 282 nm λ max using a UV spectrophotometer (1800 UV, Shimadzu, Kyoto, Japan). Physical properties, such as color and surface texture changes, were visually assessed on days 0, 30, 60, and 90. If no marked difference in physical properties is observed, the formulation is stable, and more than 90 % of the initial drug concentration should be maintained (Christ, 2020; Sudke, 2017).

2.17. Statistical analysis of data

All data were analyzed using GraphPad Prism V.8 and Student's *t*-test. This difference was considered significant at $P < 0.05$.

3. Results

Physical evaluation of cabergoline suppositories was evaluated separately for any cracks, holes, air bubbles, asymmetrical structure, color, and odor. All prepared suppositories had a smooth and polished surface without holes, cracks, or air bubbles. The color of the suppositories was also white.

3.1. Weight change

The results of the weight change test for the cabergoline suppository are shown in Table 3. No more than two suppositories should weigh more than 5 % of the average weight, and none of the suppositories should weigh more than 10 % more than the average weight. All suppositories weighed less than 5 % on average.

3.2. Suppository pH measurement

The pH of the prepared suppositories is in the range of 6 to 6.5, close to the physiological pH of the vagina (Table 3).

3.3. Content uniformity testing

Drug content was observed between 79.666 ± 8.54 % and 99.67 ± 6.55 %. Drug content was determined based on USP-30. The content of all suppositories was between 85 % and 115 % (Table 3).

3.4. Hardness test

Suppository stiffness was observed between 2.74 ± 0.04 and 4.2 ± 0.03 kg/cm^2 (Table 3). According to USP30, the hardness of the suppositories must be at least about 2.8–2 kg/cm^2 to easily withstand the mechanical pressures applied to them. Our results showed that the lowest hardness is Formula F5 (about 2.4 kg/cm^2), and the highest hardness is Formula F10 (about 4.2 kg/cm^2).

3.5. Disintegration test

The decomposition time of the suppositories varied between 11.25 ± 0.15 to 20.19 ± 0.08 min (Table 3). This test shows the time it takes for the suppositories to open when placed in a similar environment to the vagina. Our results showed that formulation F5 (11.25 min) had the shortest decay time, and formulation F10 (20.19 min) had the highest decay time.

3.6. In vitro drug release study

The percentage of drug release from suppository formulations is shown in Fig. 2. All formulations demonstrated distinct release characteristics. Formulations of F10 (~46 %), F2 (~64 %), F6 (~69 %), F4 (~79 %), F1 (~88 %), and F7 (~93 %) of the drug over a period of 45 min have been released. Other formulations release more than 95 % of the drug within 45 min. (F8 95 %, F9 96 %, F3 97 %, F11 99 %).Formulations F5 and F3 released more than 50 % of the drug in the first 10 min of the test. Formulations F11 and F9 released more than 50 % of the drug in 20 min. The F7 formulation released more than 50 % of the drug in 30 min. All formulations release more than 45 % of the drug in 45 min. According to the results, the dissolution test follows the Pepas model Table 5, Table 6, and Fig. 5. Therefore, the equation is Pepas model: $\frac{Mt}{M} = kt^n$; where Mt/M describes the amount of cabergoline released at the time (t), where the release rate constant is denoted as k. n indicates the release mechanism.

3.7. Experimental design

Variables used for optimization were X1 (PEG 6000/PEG 400 ratio) and X2 (Tween 80 %). The effect of formulation factors on release (%), melting time (minute), and hardness was investigated in a total of 11 tests. Table 4 summarises the results of all experiments. The values of y1 (release), y2 (melting time), and y3 (hardness) responses range from 45.67 to 99.9 %, 26.12 to 38.59 min, and 2.44 to 4.21, respectively. Models were designed for each response individually, which will be discussed in the following. A p-value under 0.05 was used to measure significance (Singh et al., 2018).

Table 4
Design of experiment; optimization of the proportion of PEG 400, PEG 6000, and tween 80 to prepare cabergoline vaginal suppositories.

Runs	Factor 1	Factor2	Response 1	Response 2	Response 3
	A: PEG6000/ PEG400 (ratio)	B: Tween80 (%)	Release (%)	Melting time (min)	Hardness
1	6.50	1.50	87.67	37.44	3.61
2	10.00	1.00	65.33	37.91	4.03
3	6.50	2.21	99.17	35.12	3.48
4	6.50	0.79	77.67	38.49	3.77
5	1.55	1.50	99.67	26.12	2.44
6	10.00	2.00	70.33	37.18	3.89
7	6.50	1.50	92.83	37.26	3.64
8	6.50	1.50	92.33	37.50	3.65
9	3.00	1.00	96.17	31.22	2.89
10	11.45	1.50	45.67	38.59	4.21
11	3.00	2.00	99.90	30.21	2.74

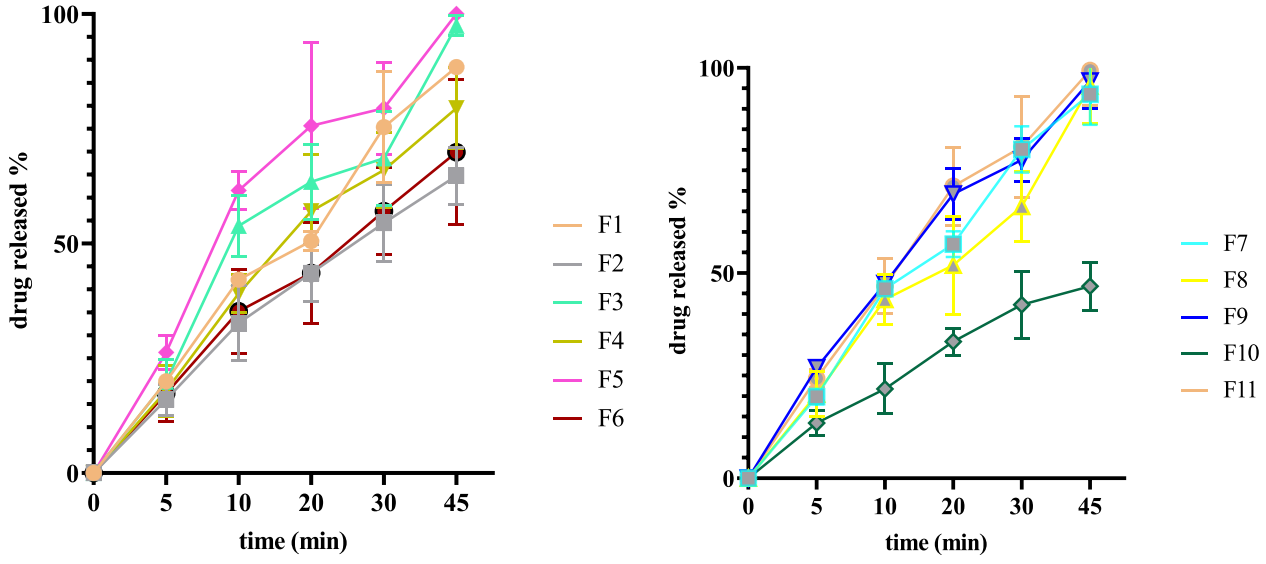

Fig. 2. Percentage of drug release from the prepared suppository formulations.

Table 5
Percentage drug release of the prepared suppository formulation F12.

Time (Min)	
0	0
5	21.12
10	41.54
20	52.15
30	64.13
45	97.18

Table 6
The release mechanism of cabergoline vaginal suppository (1).

	Zero-order	First-order	Hiquchi	Pepas model
R2	0.9066	0.9510	0.9570	0.9809

All 3D surface response graphs are given in Figs. 3 and 4. The proposed formulation of Design Expert software is called F12 and its results can be seen in Table 5.

3.8. Release percentage

The minimum release (45.67 %) was related to run10 with a maximum PEG6000/PEG400 ratio, and the maximum release (99.9) was related to run 11 with a minimum PEG6000/PEG400 ratio and maximum Tween 80 %. Desired model equation showed in equation Fig. 3. This model was significant, and its lack of fit was insignificant, which means our data fit well with actual data. The model showed that cabergoline release from suppositories depends on both PEG 6000/PEG 400 ratio and Tween 80 percent. With increasing PEG 6000, we observed a decrease in cabergoline release, which showed a significant effect on the model. In addition, this effect of high molecular weight PEG on release percentage was shown in a study by Moradkhan Nezhad *et al.,* and their study indicated that an increase in PEG molecular weight to 6000 lowered the release of curcumin from nanofibers of PLA/CUR/PEG (Moradkhannejhad, 2018). High molecular weight PEGs (like PEG 6000) are less hydrophilic than low molecular weight PEGs (like PEG 400); hence by increasing the PEG 6000 ratio, dissolution of the suppository's matrix decreased. On the other hand, because of the hydrophobic properties of cabergoline molecules, they tend to have more hydrophobic interaction with PEG 6000 and then could not release from the suppositories quickly. By increasing in Tween80 %, the release of cabergoline was increased but based on the coded equation, the effect of Tween 80 was lower than PEG 6000/PEG400 ratio. Final Equation in Terms of Coded Factors:

Release = +90.32—17.10A + 4.89B −8.35A^2 (Fig. 3).

3.9. Melting time

The minimum melting time was related to run 5 with a minimum amount of PEG 6000. It was expected because of the higher melting temperature of PEG 6000 to PEG 400. The provided model was significant, but its lack of fit was not significant, which means some observations will be out of model predictions.

3.10. Hardness

As expected, the maximum hardness related to formulation with a high ratio of PEG 6000 and experimental design also proved the relation between PEG 6000/PEG 400 ratio and hardness. Tween 80 % in the formulation also had a significant effect on hardness; however, its effect was lower than the effect of the PEG 6000 ratio in the formulation. The final coded equation of hardness is: Hardness = +3.61 + 0.5989A −0.0862B −0.1684A^2 (Fig. 4). A formulation with 1.5 % Tween 80 and a PEG6000/PEG400 ratio of 6.5 was recognized as the best formulation using Design Expert results.

3.11. Fourier transform infrared spectroscopy (FTIR) of formulations

The following results were obtained from the FTIR examination of cabergoline powder and suppository. A prominent absorption peak in the region of 3398 cm^{-1} is seen in Fig. 6, which can be attributed to the tensile state of the indole and N–H amide groups of the cabergoline molecule. The peak observed at 1583 cm^{-1} can be attributed to the carbonyl and urea groups on carbon 8(Perkampus and Bellamy, 1975). The peak at 1477 cm^{-1} shows C–H degraded in the aromatic ring. The peaks of 1287 cm^{-1} and 1116 cm^{-1} can be attributed to the tensile vibrations of C-O and C-N, respectively. Strong peaks at 1036 cm^{-1} and 891 cm-1 are attributed to N-O-C and C-N-C vibrations, respectively. The sharp peaks of 769 cm^{-1} and 659 cm^{-1} are due to the presence of –CH2 vibrations and -N–H deformation in the cabergoline molecule. Comparing the pure FTIR spectrum of pure cabergoline and the cabergoline suppository shows changes within their spectra. The intensity and

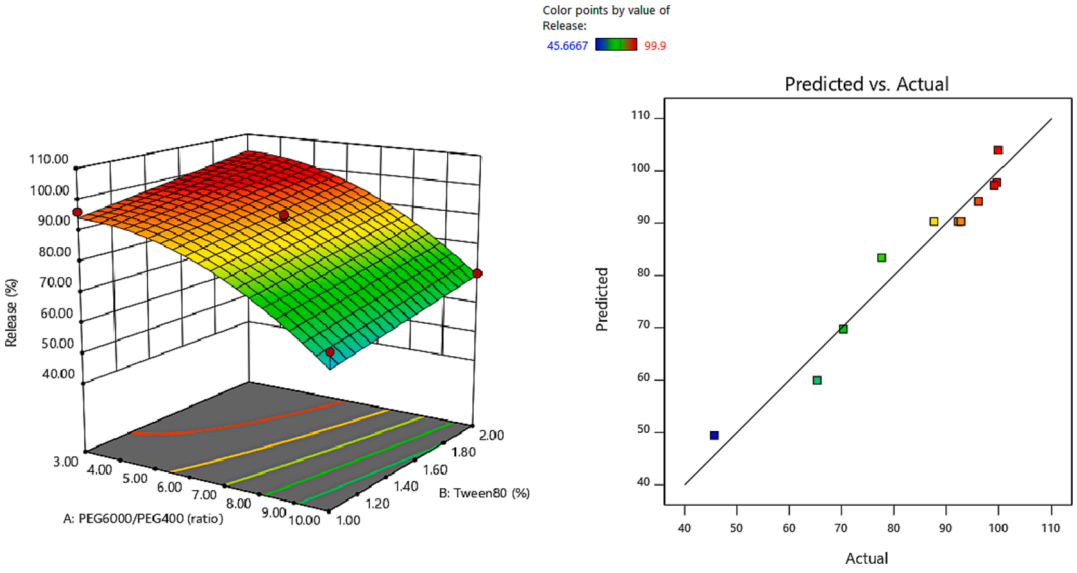

Fig. 3. 3D response surface plot showing the effect of factors on % drug release.

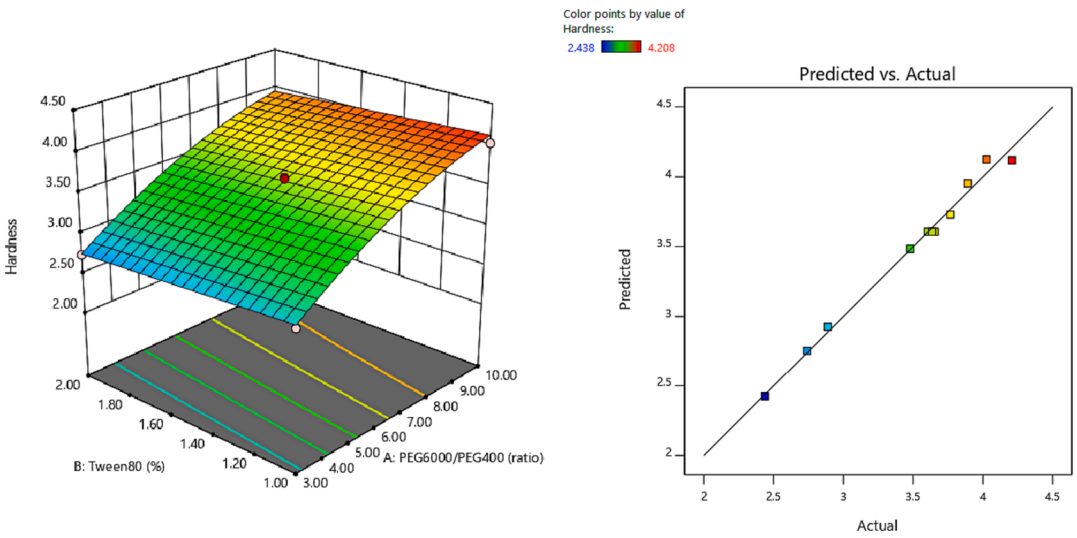

Fig. 4. 3D response surface plot showing the effect of factors on the hardness.

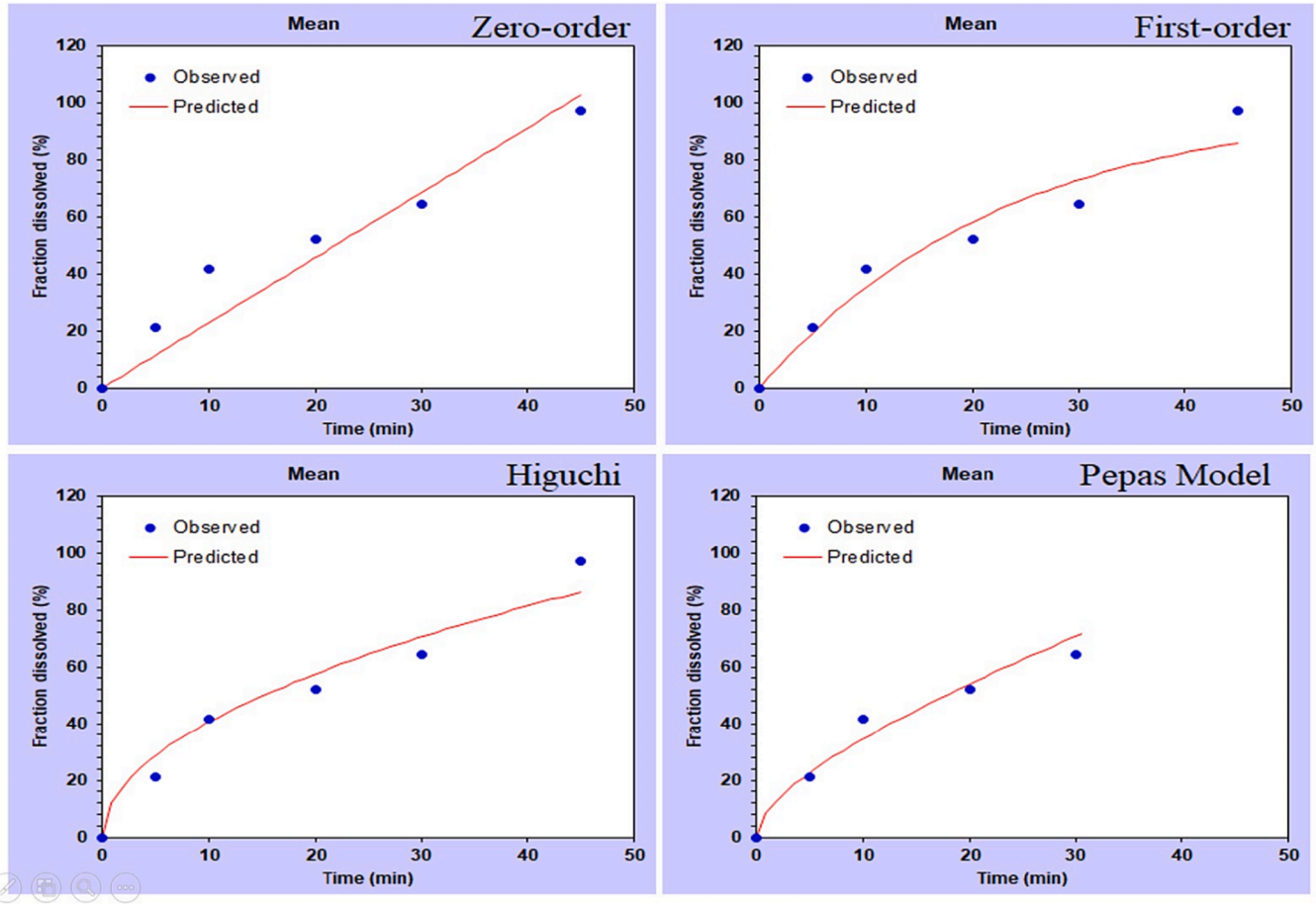

Fig. 5. The release mechanism of cabergoline vaginal suppository.

shape of some bands are very different from those of pure cabergoline (bharathi Balakrishnan, S., G.B. Veerakanellore, and T. Stalin, 2018). The N-O-C absorption band is reduced to the absorption spectrum of 1059 cm^{-1}. We found no peaks related to the -N–H deformation in the suppository formulation, which shows that a new bond has formed on N. The peaks of the N–H tensile bonds and the C–H tensile vibrations in the indole ring decreased to 3379 cm^{-1} and 2883 cm^{-1}, respectively. The C-O-C band peaks are much higher, with an area of 1056 cm^{-1} (Fig. 6).

3.12. *Differential scanning calorimetric analysis (DSC) of formulations*

In the thermogram of the cabergoline, we found a sharp peak at 111 °C, which also indicates the cabergoline melting point, and the cabergoline enthalpy is −80 mJ. Moreover, the thermogram showed the

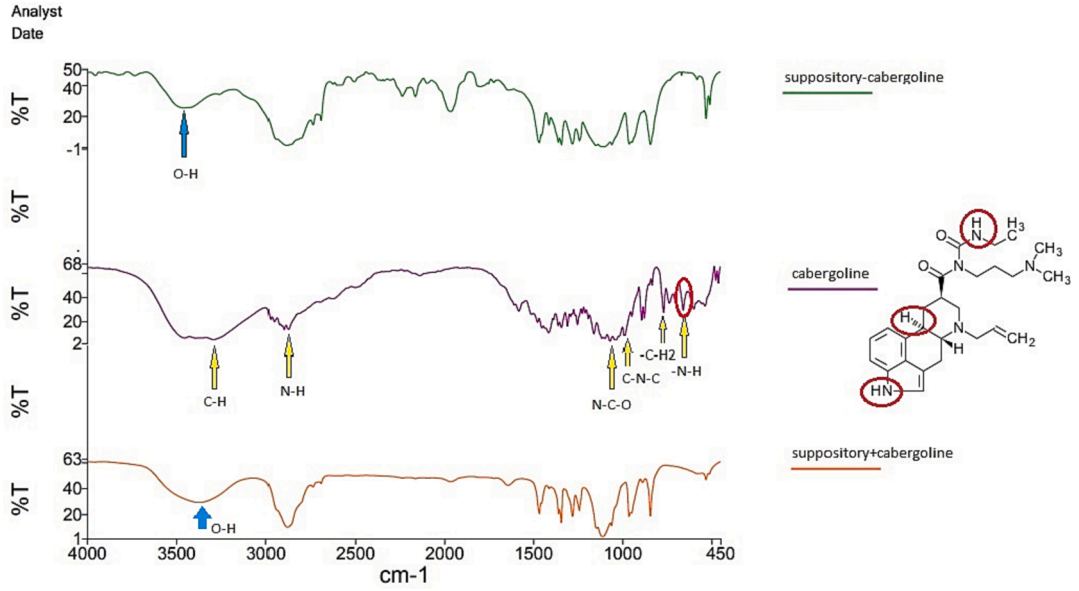

Fig. 6. FTIR spectra of cabergoline vaginal suppository formulations.

graph of the suppository containing PEG 400 and 6000, while with and without the drug. However, the peak guiding the melting point of cabergoline was not seen in the thermogram of suppositories containing cabergoline, which indicates that interaction has occurred between the drug and excipients, and cabergoline was placed in an amorphous form among other excipients. The cabergoline-containing suppository formulation peaks at 56 °C, and the cabergoline-free suppository peaks at 61 °C, representing our suppositories' melting point (Fig. 7).

3.13. Stability test

The stability test was performed under two conditions, room temperature (temperature 25 ℃ and humidity 60 %) and accelerated conditions (temperature 40 ℃ and humidity 75 %) for three months. During this period, suppositories were evaluated for pH, drug content, color change, and drug release percentage at 0, 1, 2, and 3 months for 45 min. The results of this study can be found in Table 7.

4. Discussion

Cabergoline is a drug that has many gastrointestinal adverse effects. These adverse effects have reduced the consumption of cabergoline. Therefore, if a form of medicine is prepared that does not have these side effects, it could be an excellent alternative to the formulation of cod in the market. On the other hand, studies have shown that lower doses of cabergoline are needed when used topically. Therefore, ovarian hyper stimulation syndrome is one of the most critical causes for utilizing a cabergoline vaginal suppository that inhibits this syndrome well.

Cabergoline is a lipid-soluble drug (log P = 2.97), so we used hydrophilic bases such as PEG to prepare the suppository because it releases the drug more quickly (Abou-Taleb et al., 2006). Due to its abundant OH groups, PEG has a high-water absorption property. In fact,

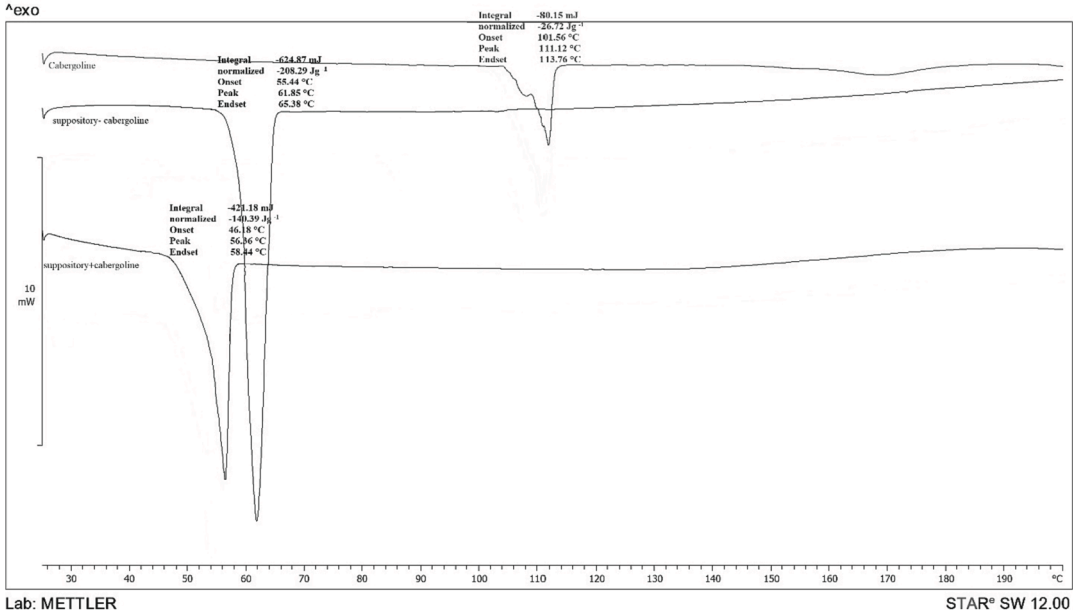

Fig. 7. DSC of cabergoline vaginal suppository formulations.

Table 7
Results obtained for cabergoline vaginal suppository stability study.

		Storage Conditions					
		25 °C/60 %RH			Accelerated40 °C/75 %RH		
Times (Month)	0	1	2	3	1	2	3
pH	6.2	6.2	6.2	6.2	6.2	6.5	6.8
Drug Content (%) ± SD	99 ± 0.14	98 ± 1.6	97.6 ± 2.3	96.8 ± 1.7	97.8 ± 0.8	95.3 ± 1.5	91.8 ± 2.7
Color Change	White	White	White	White	White	White	Yellow
Drug release at 45 min (%)	97 ± 0.45	97 ± 1.55	94 ± 2.6	90.8 ± 0.87	96.3 ± 1.42	89.6 ± 2.44	84.8 ± 3.25

this feature causes water to easily penetrate into the base of the suppository and release cabergoline from the suppository.

Our results showed that the higher PEG 6000/PEG 400 ratio provided a slower drug release. In addition, the higher percentage of PEG 400 in the formulation provided faster drug releases from the suppository. Tween 80 is a non-ionic surfactant due to having a hydrophilic end and a hydrophobic end, so formulations with higher amounts of Tween 80, dissolve the suppository faster in water, and the drug disperses more rapidly in the environment.

In vitro results showed that the PEG-based cabergoline suppository has sufficient properties for industrial production and could be an option to replace its oral form. The suppositories had a smooth and acceptable appearance. PEG and Tween play a plasticizing role in the structure of the suppositories and make the suppositories soft and flexible. They also make the surface smooth and smooth, thus less irritating to the vaginal wall (Abd Ellah, 2021).

The mean weight for all formulations was in the range of the USP 30. None of the formulations weighed more than 5 % by weight, and none of the suppositories weighed more than 10 % of the weight. Because the suppositories' pH is close to that of the vagina, they do not irritate the vagina. The suppositories need to be a good hardness so they don't break when packed and are easy to use. Determining the hardness of the suppositories is essential to know whether our suppositories in industrial production can withstand mechanical force from various devices without cracking and damage. If the suppository isn't hard enough, it will melt before it gets to the effect site or break while moving (Sudke, 2017; Havaldar, 2017). According to USP 30, the hardness of the suppositories should be at least about 2.2–8 kg/m^2. Because they were made with PEG 6000, our suppositories were very hard to break or damage. The hardness obtained was between 2.4–4.2 kg/m^2. The results showed that all suppositories could withstand pressure during production and handling.

Our study showed that suppository stiffness was directly related to the amount of PEG 6000 in the formulation, and formulas F2 and F10 were stiffer than other formulas because they had a higher amount of PEG 6000. Tween 80 acts as a plasticizer and increases the flexibility and softness of the formula. As a result, formulations F5 and F11 had the highest amount of Tween 80 along with less PEG 6000 than other formulations, and their hardness was less than the rest of the formulations.

Based on the obtained results, the design expert software suggested a formulation for further studies, which was carried out under the name of F12. The software predicted F12 responses that were very close to the actual values (Vaz et al., 2021).

According to USP 30, the decomposition time of the suppository should not exceed 60 min. The prepared vaginal suppository formulation showed a decomposition time between 11.25 ± 0.15 min and 20.19 ± 0.08 min. The results followed USP 30 requirements (Fazeli, et al., 2000).

Observations showed that N–H plays the role of a weak acid here due to its proximity to two C = O and an indole ring and can enter into a hydrogen bond with the O–H group in PEG. The groups next to N–H have a higher electronegativity than N and are very electrophilic, which can absorb electron pairs, so N plays the role of Lewis acid here (Becica and Dobereiner, 2019). This bond increases the solubility and better dispersion of cabergoline in PEG. FTIR results show that the O–H tensile bond has moved (3496 cm^{-1} and 3561 cm^{-1} to 3429 cm^{-1}), which means a hydrogen bond has formed (Kauss, 2013). Since a hydrogen bond has been made, the drug can be released at a faster rate.

As seen in the DSC results, there is no cabergoline melting peak at 110 °C in the suppository formulation, indicating that cabergoline is amorphously trapped between the PEG molecules, and we do not see its melting peak. On suppository thermography, cabergoline and PEG acted as a single entity with a melting point of about 56° C. This indicates the formation of a homogeneous mixture. The cabergoline courier is not seen because the drug is melted, dissolved, and spread out in the carrier, which turns the drug's crystalline form into an amorphous structure. This conversion is thought to increase the solubility and rapid release of the drug (Kauss, 2013). Biopharmaceutics classification system (BCS) for cabergoline is class two (Benet et al., 2011).

cabergoline has low solubility but high permeability; Therefore, PEG as a hydrophilic base for suppositories has many advantages, such as low toxicity, high water solubility, and low cost (Parmar et al., 2011).

In order to investigate the mechanism of drug release from suppositories, release data were analyzed using zero-order kinetics, first-order kinetics, Higuchi kinetics, and Korsmeyer-Pepps model(Kumar, 2013). For zero-order kinetics, the release test data were plotted as the cumulative amount of released drug versus time; for first-order kinetics, the obtained data were plotted as log cumulative percentage versus time. For Higashi kinetics, the cumulative percentage of drug release was plotted against the square root of time. Since in the Korsmeyer-Peppas model, up to 60 % of the initial cumulative release of the drug is valid, in this study, only the first 60 % of the data were selected, and the log cumulative percentage was plotted against time(Dash et al., 2010). The n value was 0.503, which indicates a non-Fickian diffusion mechanism. The drug is almost inside the suppository reservoir(Vaz et al., 2021; Njoku et al., 2020). The stability test results show that stability will be a limiting factor in developing cabergoline suppositories. In the accelerated condition, after three months, we see specific changes in the formulation of the suppositories, but no significant change occurred for the suppositories that were kept at a temperature of 25 °C. In the accelerated condition, the suppositories turned yellow and pasty after three months. Moreover, the suppositories subjected to the accelerated test lost about 7 % of their drug content, while the suppositories kept at room temperature lost about 2 % of their drug content. The drug release profile showed that under accelerated conditions, the amount of drug released after 45 min is much less than when stored at room temperature. In fact, the stability test showed that cabergoline suppositories are sensitive to temperature and humidity. To maintain them for a long time, they must be stored in aluminum molds and refrigerators to be stable for two years in all weather conditions. Conventional vaginal drug delivery formulations have disadvantages such as the ability to load a small amount of drug, short stay in the vagina, and various contaminations (Johal et al., 2016). In order to deal with these disadvantages, new formulations such as bio-adhesives, temperature-sensitive systems, hydrogels, and nanocarriers have been developed (Tanphaichitr, 2016; Donders, 2010). Cabergoline is quickly absorbed to systemic through the mucosa, and after 2–3 h, the highest concentration of cabergoline in plasma is created (Del Dotto and Bonuccelli, 2003). According to the information mentioned above and the small effective dose of cabergoline (0.5 mg per suppository), it can concluded that there is no need to

use new formulations. However, to create a constant concentration of cabergoline in the vaginal environment or to create a formulation that is sensitive to pH and releases the drug in special periods of the monthly cycle when the pH changes, formulations can be optimized for this purpose.

5. Conclusion

Cabergoline is a semisynthetic compound derivate from ergot alkaloids. Oral administration of cabergoline was faced with gastrointestinal problems which cause poor compliance in patients. One of the preferred solutions is to use non-oral dosage forms like suppositories. Vaginal suppositories have ease of use and could hinder gastrointestinal effects of cabergoline. In this study we provide PEG base vaginal suppositories with using *in silico* design experiment. Our formulation showed good stability in room temperature and could release 45 to 99 percent of the cabergoline during 45 min. In addition, the release profile had best fit with *Pepas* model. Other properties like hardness, pH and, melting time were in accepted range. In conclusion we could fabricate vaginal suppositories of cabergoline based on experimental design which could be appropriate for evaluated in animal studies and could be controllable based on in terms of critical variable in producing based on provided design. The main limitation of this study was the lack of use of other suppository bases. In future studies, other types of PEGs, whitecapsyl, cocoa butter, and glycerogelatin base can be used.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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