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*Research Article*

# An Efficient Herbal Approach for Treating Fungal Infection in Cervical Cancer Patients by Developing and Optimizing a Vaginal Suppository

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*Aim*. The study is aimed at developing curcumin suppositories as a promising approach for natural antifungal management of vaginal candidiasis in cervical cancer patients to eradicate side effects produced by current antifungal drugs. The objective of the study was to optimize the suppositories using optimal (custom) design employing Design-Expert 13 software to recognize the concentration of polyethylene glycols (PEG) and Poloxamer 407 and obtain a stable suppository. *Methodology*. Combinations of PEG 1500 (10%–40%), PEG 6000 (40%–60%), and Poloxamer 407 (5%–30%) were entered as factors, and the responses evaluated were hardness, deformation time, and % drug release. In addition, the formulation was also evaluated for visual examination, weight variation, pH determination, drug content, hardness test, disintegration time, melting zone, deformation time, in vitro drug release, antifungal activity, and stability tests. *Results*. Suppositories were devoid of holes and cracks, with a characteristic odor and a dark yellowish-orange color. All formulations passed the weight variation test. Formulations exhibited pH ranging from 5.5 to 6.5. Drug content was observed to be $98.65 \pm 0.041\%$ –$99.85 \pm 0.041\%$. The hardness of the formulation was between 2.9 and 4.2 kg/cm$^2$. The disintegration time ranged from 11 $\pm 0.052$ min to $20 \pm 0.011$ min. The melting point was between $41 \pm 0.31$°C and $58 \pm 0.62$°C. Deformation time ranged from $10 \pm 0.45$ to $35 \pm 0.52$ min. Most of the formulations resulted in 90% of drug release at 40 min, and the zone of inhibition noted was $19.6 \pm 0.4$ mm. All the selected factors have a significant effect on the response chosen for the study. *Conclusion*. The optimized curcumin vaginal suppository formulation can be an efficient herbal treatment devoid of side effects to treat vaginal candidiasis in cervical cancer patients.

## 1. Introduction

Cancer of the cervix is a frequently occurring cancer with the second-highest cancer mortality worldwide among women [1]. Fungal infections in patients who have cancer are a major cause of disease and mortality [2]. Prescribing antifungal medications can reduce the chances of a patient acquiring a fungal infection [3].

Suppositories are solid formulations with different shapes and masses adjusted to rectal, vaginal, or urethral administration. They mostly dissolve, melt, or liquefy at body temperature, and they can be formulated to obtain systemic or local action. The bases usually utilized are cocoa butter, glycerinated gelatin, hydrogenated vegetal oils, and a mixture of polyethylene glycols of diverse molecular weights [4, 5]. Vaginal suppositories are commonly used to handle urogenital infections and other local diseases. The vaginal route is efficacious in allowing the efficient transport of some drugs such as progesterone and azoles to the uterus while alleviating systemic side effects [6, 7].

Vaginal candidiasis is produced by *Candida albicans* and affects up to 75% of women at least once in their life [8, 9]. Fluconazole is an inexpensive and well-tolerated medication that is easily dispensed orally and is the commonly used antifungal drug. However, in the last decade, fluconazole resistance has been observed in women with vaginal candidiasis. Furthermore, it is important to be aware that the extreme use and overuse of such topical agents have had other adverse effects such as edema, irritability of the skin and, perhaps, the chronic vulvar pain condition (vulvodynia) [10]. The antifungal drugs prescribed for the treatment of vaginal candidiasis are metronidazole, clotrimazole, fluconazole, ketoconazole, and itraconazole, among others, and they show various side effects. Curcumin, a rich polyphenol from rhizomes of *Curcuma longa* belonging to the family *Zingiberaceae*, has shown a greater effect on *Candida albicans* among the *Candida* species studied [11, 12]. It was reported that curcumin is 2.5-fold more potent than fluconazole at inhibiting the adhesion of *Candida albicans*. Curcumin, along with its well-known anticancer and anti-inflammatory activities, also exhibits antifungal activity in that it can diminish the adhesion of *Candida albicans*. Formulations developed using curcumin can be used as an herbal vaginal treatment for candidiasis free from side effects in cervical cancer patients [13–16].

A multivariate formulation optimization strategy is important to recognize the effect of factors on formulation quality. It helps select factors to develop an optimized formulation. An optimization strategy can be developed using well-known statistical analysis tools such as mixture design [17, 18]. The present study is aimed at developing curcumin suppositories as a promising approach for natural antifungal management of vaginal candidiasis to eradicate side effects produced by current antifungal drugs. Suppositories were optimized using mixture design to identify the proportions of polyethylene glycol (PEG) and Poloxamer 407 that would yield a fully formed suppository that would remain solid and stable at room temperature. The factors used for developing the suppository formulations from mixture design were Poloxamer 407 (5%–30%), PEG 1500 (10%–40%), and PEG 6000 and evaluated responses are hardness, deformation time, and % drug release. Other characteristics of the suppository that were evaluated were pH, disintegration time, melting time, antifungal activity, and stability studies [19–21].

## 2. Materials and Methods

*2.1. Materials.* Curcumin was purchased from Loba Chemie (purity 98%), Mumbai, India. PEG 1500 and PEG 6000 were obtained from Sigma Aldrich Chemie. Poloxamer 407 (Kolliphore P407) was obtained from SD Fine Chemical. All other chemicals and reagents used were of analytical grade.

*2.2. Methods*

*2.2.1. Formulation and Optimization of Curcumin Vaginal Suppository.* The design methodology used considered material attributes to optimize the formulation of the curcumin vaginal suppository quality. The expected responses from the formulations were ease of administration, faster release of drug from the base, and meeting quality control requirements as per the United States Pharmacopeia (USP). Critical quality attributes of the optimum formulation were that formulation must be solid at room temperature and should melt or disintegrate within 5–10 min after administration. The fusion molding method was adopted to formulate suppositories using stainless steel suppository molds.

The suppositories were formulated using different concentrations of PEG 1500, PEG 6000, and Poloxamer 407. The effect of formulation factors on product characteristics was evaluated statistically using Design Expert v. 13 software.

An optimum mixture design was selected to identify the proportions of PEGs and Poloxamer 407 that would yield a fully formed suppository that would remain solid and stable at room temperature. The factors employed for obtaining the suppository formulations from mixture design were PEG 1500 (10%–40%), PEG 6000, and Poloxamer 407 (5%–30%), and the evaluated responses were (a) hardness, (b) deformation time, and (c) % drug release.

*2.2.2. Preparation of Suppository*

*(1) Calibration of Mold.* Before suppository preparation, the mold used for the preparation must be calibrated as they may vary in their capacity. The base was melted without the drug and transferred to the mold. The weight was measured to determine the true capacity of mold. The displacement value (the number of grams of medicament that displaces one gram of the base) of curcumin for every formulation was also determined.

*(2) Curcumin Suppository Preparation.* Suppositories were prepared by the molding method [22, 23]. To obtain suspended curcumin, vaginal suppository PEGs were melted at 65°C using a water bath, and curcumin was dispersed under manual stirring for 2 to 3 min. For all suppositories, after final mixing, the blends were cooled to a temperature of 55°C–60°C and poured into a suppository mold. They

were then solidified at 24°C (room temperature). The samples were stored in individual aluminum blister packs at room temperature (20°C–25°C) for further analysis. The composition of independent variables for the preparation of the curcumin vaginal suppository is shown in Table 1. The formulation chart for the preparation of vaginal suppository is shown in Table 2.

### 2.3. Evaluation

*2.3.1. Characterization of Vaginal Suppositories. Physicochemical Properties.* The physical evaluation was made by a visual examination of the formulation. Color, odor, and characteristics for the presence of bubbles or cracks were reported. The intensity, nature, and color homogeneity of the prepared suppository formulation was observed and recorded. A change in the odor of the suppository is an indication of the degradation process. The shape of the suppositories is verified for consistency [24, 25].

*2.3.2. Weight Variation.* Weight variation for the prepared curcumin suppository formulations was measured as per British Pharmacopoeia (2011). Twenty suppositories of each formulation were weighed to determine the average weight ($n = 20$). Not more than two suppositories varied from the average weight by more than 5%, and no suppository differed from the average weight by more than 10% [26].

*2.3.3. Determination of pH.* The pH of the formulated suppositories was measured using a digital pH meter (Jenway 3510). The suppositories were digested in warm water then filtered, and the pH of the filtrate was measured by immersing the electrode at 37 ± 0.5°C and recording the reading.

*2.3.4. Drug Content.* Estimation of drug content was carried out by randomly selecting three suppositories from each batch. Each suppository was dissolved using 5 ml methanol in a 100 ml volumetric flask by shaking the flask for 15 min. The volume was made up to 100 ml with pH 4.5 phosphate buffer. The solution was then filtered and analyzed after suitable dilutions for curcumin content at 417 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan).

*2.3.5. Hardness Test.* The resistance offered by the suppository to break or collapse is referred to as hardness [26]. The hardness test was done for ten suppositories using an Erweka hardness tester (Pharma Test, Germany) at room temperature (25 ± 0.5°C). The weight required for the suppository to break or fracture is taken as a measure of its hardness. The test was performed to assess the tensile strength of the suppositories to determine whether the prepared formulation could withstand the hazards of packing and transporting [27, 28].

*2.3.6. Disintegration Time.* Disintegration test apparatus (Model: ZT41, Erwika, Germany) was employed to determine the disintegration time. The time taken for the complete disintegration of the entire suppository was recorded at 37 ± 0.5°C using a phosphate buffer at pH 4.5 as the disintegration medium [25].

Table 1: Composition of independent variables for the preparation of the curcumin vaginal suppository.

| Variables | Actual values (%) | | Coded values | |
|---|---|---|---|---|
| | Lowest | Highest | Lowest | Highest |
| A: PEG 1500 | 10 | 40 | −1 | +1 |
| B: PEG 6000 | 40 | 60 | −1 | +1 |
| C: Poloxamer 407 | 5 | 30 | −1 | +1 |

Table 2: Mixture design for formulation designed using Stat-Ease Design-Expert® Software (version 13.0.2.0).

| Formulations | PEG 1500 (%) | PEG 6000 (%) | Poloxamer 407 (%) | Curcumin (%) |
|---|---|---|---|---|
| F1 | 40 | 43.1993 | 16.8007 | 10 |
| F2 | 33.4196 | 50.1743 | 16.4061 | 10 |
| F3 | 36.2578 | 40 | 23.7422 | 10 |
| F4 | 22.662 | 47.338 | 30 | 10 |
| F5 | 35 | 60 | 5 | 10 |
| F6 | 25.2137 | 56.5428 | 18.2435 | 10 |
| F7 | 17.2018 | 59.9957 | 22.8025 | 10 |
| F8 | 29.151 | 40.849 | 30 | 10 |
| F9 | 40 | 49.1694 | 10.8306 | 10 |
| F10 | 10 | 60 | 30 | 10 |
| F11 | 35 | 60 | 5 | 10 |
| F12 | 25.2137 | 56.5428 | 18.2435 | 10 |
| F13 | 25.2137 | 56.5428 | 18.2435 | 10 |
| F14 | 40 | 49.1694 | 10.8306 | 10 |
| F15 | 28.7641 | 47.7178 | 23.5181 | 10 |
| F16 | 22.662 | 47.338 | 30 | 10 |

*2.3.7. Measurement of the Melting Zone.* The melting test was conducted according to method reported earlier [23]. The melting range can be determined by filling the formulation to about 1 cm height in capillary tubes of 10 cm length and dipping them in beaker containing water. The beaker was placed on a hot plate, and the temperature was slowly raised. As soon as the mass present in the capillary liquefied, the temperature was recorded as the melting zone.

*2.3.8. Measurement of Deformation Time (Liquefaction Time).* The test gives information on the suppository behavior when exposed to a temperature of 37°C. Generally, suppositories will undergo liquefaction within 30 min. A simple apparatus fabricated in the laboratory was used. The stop cock of a glass burette was removed. The burette, which has one wide end and one narrow end, was used for the study. The burette was then immersed in water maintained at body temperature (37°C) so that the narrow end of the burette was in the water. The suppository was then placed into the broad end of the burette and carefully pushed toward the narrow end of the burette by pushing it carefully to avoid breaking. A glass rod was placed above the suppository. When the suppository melts completely, the glass rod reaches the narrow end of the burette. The time taken for this process is the liquefaction time [29].

*2.3.9. In Vitro Drug Release Studies.* The percentage drug release of the prepared curcumin vaginal suppositories was estimated using a USP basket-type dissolution apparatus (EDT-08Lx, Electro Lab, Mumbai, India). Each curcumin vaginal suppository was placed in the basket and fitted to the apparatus. A phosphate buffer of pH 4.5 with 1.5% of sodium lauryl sulfate (100 ml) was used as a dissolution medium, the temperature was maintained at 37°C, and the basket speed was fixed to 50 rpm. At 10, 20, 30, 40, and 60 min, 3 ml of sample was retracted and replaced with an equal volume of fresh dissolution media (phosphate buffer of pH 4.5 with 1.5% of SLS) following every sampling to maintain a consistent volume during the study. The concentration of curcumin in each filtered sample was estimated after suitable dilutions using a UV visible spectrophotometer at $\lambda_{max}$ 417 nm. Studies were carried out in triplicate.

The release mechanism was determined by fitting the release data in empirical or semiempirical models such as zero-order, first-order, and Higuchi diffusion models and Pepas model. They are depicted by the following equations.

$$\text{Zero} - \text{order model} : \frac{Mt}{M} = kt, \tag{1}$$

$$\text{First} - \text{order model} : Ln\left(1 - \frac{Mt}{M}\right) = -kt, \tag{2}$$

$$\text{Higuchi model} : \frac{Mt}{M} = k_h t^{0.5}, \tag{3}$$

$$\text{Pepas model} : \frac{Mt}{M} = kt^n. \tag{4}$$

*2.3.10. In Vitro Anticandidal Activity.* The cup-plate method was employed to determine *in vitro* antifungal activity using Sabouraud's agar culture medium against clinical isolates of *C. albicans* j1023 (procured from the JSS Medical College, Mysuru). The optimized suppository was melted and transferred into a well of an agar Petri dish which was previously streaked with *C. albicans* j1023. Distilled water was used as a control. The Petri plate was covered and incubated for 40 h using a Bio-Oxygen Demand (BOD) incubator maintaining a temperature of 32°C (LHC-78-Labhospmake, India). The zone of inhibition (ZOI) was determined after the incubation period.

*2.3.11. Stability Studies of Curcumin Suppositories.* Optimized curcumin suppositories were subjected to stability studies. Studies were carried out in triplicate. Suppositories were weighed, properly wrapped in aluminum foil, and placed in a stability chamber at room temperature ($25 \pm 2$°C) for 30 days. The suppositories were then evaluated for their drug content estimation at $\lambda_{max}$ 417 nm using a UV spectrophotometer (UV-1800, Shimadzu, Kyoto Japan). Physical characteristics, such as color and surface texture changes, were evaluated visually at 0, 15, and 30 days. The formulation is stable if no distinct changes in physical characteristics are observed, and more than 90% of the initial drug concentration is maintained.

## 3. Results and Discussions

Cervical cancer is the most frequent cancer observed, with the second-highest mortality rate, among women. In patients who have cancer, fungal infections are a major cause of disease and mortality. Prescribing antifungal medications can reduce the chances of acquiring a fungal infection. The chosen herbal medication for the present research, curcumin, is known to be an efficient candidate with antifungal and anticancer activity. Curcumin is extensively used in the anticancer research field. The developed curcumin vaginal suppository can be a herbal treatment free from side effects to prevent candidiasis in cervical cancer patients, and it may also aid in treating cancer.

*3.1. Physical Evaluation.* Curcumin suppositories were inspected individually for any cracks, the presence of holes and air bubbles, color, and odor. All the prepared formulations were devoid of holes, air bubbles, or cracks, and a smooth surface was observed after cutting the suppository longitudinally. Due to the presence of curcumin, the developed suppositories were dark yellowish-orange color with a characteristic odor. The presence of PEG gave a smooth and shiny appearance to suppositories due to its plasticizing effect, which is advantageous in reducing vaginal irritation during its administration [30].

*3.2. Weight Variation Test.* The results of the weight variations test for the prepared curcumin suppositories are shown in Table 3. The average weight for all the formulations was within the British Pharmacopeia limit. No more than two formulations vary from the average percentage weight by more than 5%, representing a well-calibrated mold.

*3.3. Determination of pH.* The pH value of the prepared suppository formulation was in the range of 5.5 to 6.5, close to vaginal physiological pH (4–5). As a result, these formulations will not cause vaginal irritation when administered.

*3.4. Drug Content.* The drug content was determined as per USP. All the prepared curcumin vaginal suppository formulations were observed to be within the pharmacopeial limit (as shown in Table 3).

*3.5. Hardness Test.* It is important to determine the hardness of a suppository to know whether the prepared suppository will endure mechanical force deprived of cracking. The hardness value must be between 1.8 kg and 2 kg of pressure as per the pharmacopeia [30]. Due to the presence of PEG 1500 and 6000 in the formulations, the suppositories showed enhanced resistance to breaking. The hardness value obtained was between 2.9 kg/cm$^2$ and 4.2 kg/cm$^2$. The obtained results showed that the prepared vaginal suppositories could withstand pressure during handling and shipping. Formulations such as F7, F9, F10, F11, and F14, which have a smaller amount of PEG 1500 and PEG 6000 than the other formulations, have a lower hardness value (as shown in Table 3). This may be due to the higher molecular weight of PEGs. The suppository will retain its shape during handling, shipping, and insertion if the hardness is optimum

Table 3: Evaluation parameters of the prepared curcumin vaginal suppository formulations.

| Formulations | Melting range (°C)** | Weight variation (%)* $n = 20$ | Disintegration time (min) $n = 3$ | Drug content (%)** $n = 3$ | pH |
|---|---|---|---|---|---|
| F1 | 58 ± 0.31 | 2.5 ± 0.041 | 20 ± 0.066 | 99.55 ± 0.041 | 6.4 |
| F2 | 56 ± 0.63 | 2.4 ± 0.033 | 19 ± 0.024 | 99.75 ± 0.022 | 6.3 |
| F3 | 49.5 ± 0.4 | 2.3 ± 0.056 | 16 ± 0.037 | 99.35 ± 0.011 | 5.6 |
| F4 | 47.5 ± 0.44 | 2.2 ± 0.031 | 12 ± 0.087 | 99.45 ± 0.011 | 5.6 |
| F5 | 41.5 ± 0.2 | 2.5 ± 0.072 | 14 ± 0.037 | 99.15 ± 0.028 | 6.4 |
| F6 | 61 ± 0.57 | 2.4 ± 0.022 | 17 ± 0.042 | 99.85 ± 0.041 | 6.5 |
| F7 | 50 ± 0.4 | 2.2 ± 0.023 | 15 ± 0.031 | 99.45 ± 0.022 | 6.4 |
| F8 | 54.5 ± 0.5 | 1.5 ± 0.031 | 16 ± 0.038 | 98.65 ± 0.041 | 6.5 |
| F9 | 41 ± 0.62 | 2.4 ± 0.046 | 20 ± 0.044 | 98.95 ± 0.081 | 5.6 |
| F10 | 27 ± 0.53 | 2.4 ± 0.032 | 14 ± 0.072 | 99.55 ± 0.044 | 5.9 |
| F11 | 56.5 ± 0.3 | 2.5 ± 0.021 | 20 ± 0.011 | 98.95 ± 0.031 | 6.5 |
| F12 | 60 ± 0.62 | 2.5 ± 0.031 | 18 ± 0.046 | 99.75 ± 0.071 | 6.2 |
| F13 | 61 ± 0.3 | 2 ± 0.044 | 18 ± 0.024 | 99.45 ± 0.049 | 6.5 |
| F14 | 41.5 ± 0.24 | 2.5 ± 0.045 | 18 ± 0.072 | 99.83 ± 0.034 | 6.5 |
| F15 | 44 ± 0.42 | 2 ± 0.065 | 14 ± 0.094 | 99.35 ± 0.046 | 5.8 |
| F16 | 55.5 ± 0.31 | 2.2 ± 0.015 | 11 ± 0.052 | 99.25 ± 0.031 | 6.5 |

*All values with mean ± SD ($n = 20$); **all values with mean ± SD ($n = 3$).

up to 4 kg/cm$^2$. Lower hardness values lead to rapid melting before insertion [25, 29].

*3.6. Disintegration Time.* The test determines the time required for the prepared suppository to soften or disintegrate when placed in an immersion fluid. As per British Pharmacopoeia, the time to disintegrate for a suppository must not exceed 60 min. The prepared vaginal suppository formulations showed a disintegration time between 11 ± 0.052 min and 20 ± 0.011 min. The results were obtained to comply with requirements for the disintegration test as per BP.

*3.7. Measurement of the Melting Zone.* Due to the presence of higher molecular weight PEG 1500 and 6000 in the formulations, all the suppositories exhibit higher melting point ranging from 41 ± 0.31°C to 58 ± 0.62°C; hence, the prepared suppositories release the drug by slowly dissolving instead of melting. Similar results were also observed by Mahjabeen et al. Drug release may also be influenced by the difference in melting point of the bases [31].

*3.8. Measurement of Deformation Time (Liquefaction Time).* The time taken by suppository to withstand normal body temperature (37°C) and allow convenient handling and drug release after insertion is called liquefaction time. The prepared formulations showed liquefaction time between 10 ± 0.45 min and 35 ± 0.52 min.

*3.9. In Vitro Drug Release Studies.* Curcumin is a hydrophobic drug, and therefore, hydrophilic bases were chosen to prepare suppositories as the hydrophobic drug exhibits a greater tendency to diffuse out of hydrophilic bases [32, 33]. Polyethylene glycol exhibits greater water-absorbing properties that, in turn, enable dissolution medium penetration into the base following moistening and drug desorption. The percentage of drug release of curcumin suppositories is depicted in Figure 1. The percentage drug release of the formulation is shown in Figure 1. All the formulations showed variation in drug release. F1 and F2 released nearly the same amount of drug, at 60% in 30 min. F3 and F11 showed around 95% of drug release in 40 min, whereas F4, F5, F13, and F14 resulted in 90% of drug release at 40 min. F6 and F15 showed 100% drug release at 40 min. F7, F8, F9, F10, F11, F12, and F16 showed drug release in the range of 54% to 100% in 40 min.

$Mt/M$ describes the amount of curcumin released at time $t$, where the release rate constant is denoted as $k$. $Kh$ is the Higuchi rate constant, and $n$ denotes the release mechanism. The $K$ value is directly proportional to drug release. An $n$ value of 1 denotes a zero-order release. For non-Fickian release, the $n$ value will be $0.5 < n < 1$, and an $n$ equal to 0.5 indicates Fickian diffusion. The results of the release study showed an $n$ value between 0.566 and 0.862, indicating the release mechanism as non-Fickian (Higuchi).

*3.10. In Vitro Anticandidal Activity.* An array of members of *Candida* genus forms normal bioflora of vaginal cavity of which the *Candida albicans* is comparatively prevalent and responsible for the vaginal candidiasis. It has been established in literature through diverse research reports that every woman experience episodes of vaginal candidiasis at least once in their lifetime. Among the range of *Candida* species studied, curcumin has a greater effect on *C. albicans* and, therefore, was selected for the study as an active principle constituent. Curcumin is a potential candidate for persistent antifungal activity thought to be the result of causing

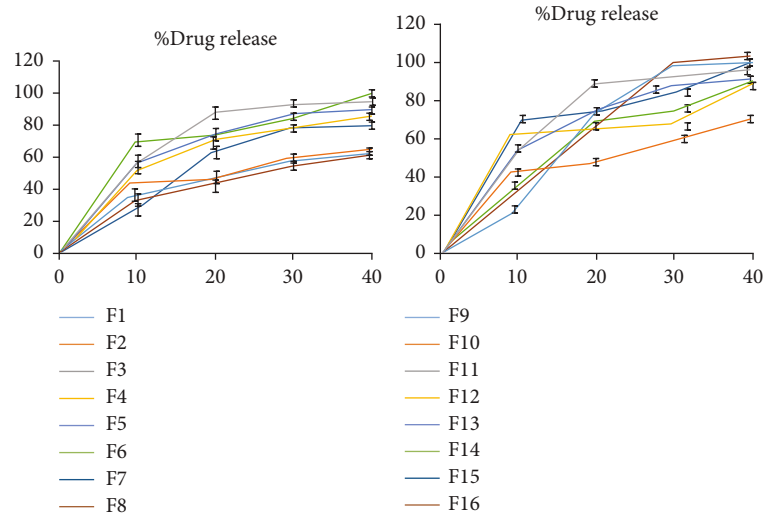


Figure 1: Percentage drug release of the prepared suppository formulations.

programmed cell death in *C. albicans* cells due to the generation of reactive oxygen species (ROS), leading to deviations in properties related to the cell membrane, such as ergosterol biosynthesis, protein secretion, and ATPase activity. The antifungal activity was assessed by measuring the ZOI of prepared formulations and comparing the results with the control by cup-plate method. Curcumin suppositories showed a ZOI of 19.6 ± 0.4 mm, whereas no ZOI was observed with the control.

*3.11. Stability Studies of Curcumin Suppositories.* Curcumin suppositories prepared using PEG will remain stable at room temperature, unlike cocoa butter suppositories; hence, the stability study was carried out at room temperature alone. The formulations were wrapped in aluminum foil to avoid adsorption or absorption of humidity. The optimized formulation was analyzed for curcumin content and physical characteristics. No distinct physical changes were noted during the study period. Results of stability studies are depicted in Table 4. The percentage drug content for the formulation at 0, 15, and 30 days was analyzed as 99.6% ± 0.22, 99.4% ± 0.67, and 98.9% ± 0.87, respectively, indicating the chemical stability of curcumin in the optimized formulation.

## 4. Experimental Design

The selected variables to prepare suppository formulations from mixture design were PEG A: 1500 (10%–40%), B: PEG 6000 (40%–60%), and C: Poloxamer 407 (5%–30%). The evaluated responses were (a) suppository formation and (b) deformation time. A total of 16 formulations were obtained from the software. These formulations were prepared with different concentrations of suppository bases, and responses were recorded as shown in Table 5. All the formulations resulted in a solid suppository devoid of fracture or cracks and retained their integrity, indicating the combination of bases used to prepare the suppository does not interfere with its integrity.

Table 4: Results obtained for curcumin vaginal suppository stability study.

| Optimized suppository formulation | | | |
|---|---|---|---|
| Color intensity | Times (days) | Aspect | Assay (%) $n = 3$ |
| | O | NC | 99.6% ± 0.22 |
| Yellow color | 15 | NC | 99.4% ± 0.67 |
| | 30 | NC | 98.9% ± 0.87 |

NC: not changed; (+), (++), and (+++) refer to color intensity. All values with mean ± SD ($n = 3$).

*4.1. Effect of Factors on Hardness.* The results show that the selected independent variables significantly impact the hardness of the suppository, given the disparity in hardness among the formulations. Equation (5) represents the effect of a factor on the hardness of the suppository. A positive value on the equation means deformation time, and the factors are directly proportional to each other. A negative value in the equation signifies they are inversely proportional to each other. The interaction between factors and hardness is shown as a 3D response surface plot in Figure 2 and a counterplot in Figure 3. A special quadratic model was suggested by the software for response hardness, showing the significant $p$ value (0.0003), $F$ value (20.99), and $R^2$ (0.9600). From the polynomial equation obtained, it was noted that factors A, B, and C have a positive effect on hardness, whereas factors AB and AC together showed a negative effect on the hardness. A formulation with a deformation time near 10 min can be an optimized formulation as deformation time is causally related to drug release.

$$\begin{aligned}\text{Hardness}\left(\text{kg/cm}^2\right) = {} & +4.65\,A + 38.71\,B + 10.71C - 63.14\,AB \\ & - 17.52\,AC - 80.98BC + 316.3\overset{C}{ABC} \\ & - 60.9\overset{C}{AB}C + 21.18\,AB\overset{C}{C}.\end{aligned} \tag{5}$$

Table 5: Design of experiment: optimization of the proportion of PEG 1500, PEG 6000, and Poloxamer 407 to prepare a curcumin vaginal suppository.

| Formulations | PEG 1500 | PEG 6000 | Poloxamer 407 | Response 1 (hardness) | Response 2 (deformation time) min | Response 3 (% drug release) |
|---|---|---|---|---|---|---|
| F1 | 40 | 43.1993 | 16.8007 | 3.8 ± 0.34 | 35 ± 0.14 | 61.5 ± 0.26 |
| F2 | 33.4196 | 50.1743 | 16.4061 | 3.9 ± 0.22 | 29 ± 0.16 | 91.3 ± 0.16 |
| F3 | 36.2578 | 40 | 23.7422 | 2.9 ± 0.29 | 14 ± 0.14 | 71.5 ± 0.2 |
| F4 | 22.662 | 47.338 | 30 | 2.9 ± 0.16 | 22 ± 0.54 | 100 ± 0.9 |
| F5 | 35 | 60 | 5 | 4.2 ± 0.35 | 32 ± 0.22 | 84.1 ± 0.11 |
| F6 | 25.2137 | 56.5428 | 18.2435 | 4 ± 0.11 | 30 ± 0.63 | 84.8 ± 0.14 |
| F7 | 17.2018 | 59.9957 | 22.8025 | 3.1 ± 0.21 | 30 ± 0.20 | 78.3 ± 0.21 |
| F8 | 29.151 | 40.849 | 30 | 3.8 ± 0.21 | 22 ± 0.09 | 98.1 ± 0.44 |
| F9 | 40 | 49.1694 | 10.8306 | 3.7 ± 0.54 | 11 ± 0.08 | 99.02 ± 0.51 |
| F10 | 10 | 60 | 30 | 3.2 ± 0.62 | 18 ± 0.16 | 58.9 ± 0.28 |
| F11 | 35 | 60 | 5 | 4.2 ± 0.21 | 33 ± 0.42 | 68.4 ± 0.20 |
| F12 | 25.2137 | 56.5428 | 18.2435 | 4 ± 0.27 | 29 ± 0.28 | 87.6 ± 0.21 |
| F13 | 25.2137 | 56.5428 | 18.2435 | 4 ± 0.08 | 29 ± 0.32 | 84.5 ± 0.13 |
| F14 | 40 | 49.1694 | 10.8306 | 3.7 ± 0.09 | 11 ± 0.47 | 84.61 ± 0.18 |
| F15 | 28.7641 | 47.7178 | 23.5181 | 3.7 ± 0.29 | 19 ± 0.8 | 45.8 ± 0.52 |
| F16 | 22.662 | 47.338 | 30 | 2.9 ± 0.20 | 17 ± 0.21 | 90.1 ± 0.21 |

*4.2. Effect of Factors on Deformation Time.* The results show that the selected independent variables have a significant impact on deformation time as deformation time varies among the formulations. Equation (6) represents the impact of factors on deformation time. The interaction between factors and deformation is shown as a 3D response surface plot in Figure 2 and a counterplot in Figure 3. The software suggested the cubic model for response deformation time, showing the significant $p$ value (0.0001), $F$ value (40.66), and $R^2$ (0.9838).

$$\begin{aligned}\text{Deformation time(min)} = {} & -164.64A + 2242.26B + 676.68C \\ & - 3760.87\,AB - 978.02\,AC - 5634.7\,BC + 6743.7\,ABC \\ & + 2041.56\,AB(A - B) + 2041\,AC(A - C) \\ & - 1335.2\,BC(B - C).\end{aligned} \tag{6}$$

From the polynomial equation obtained, it was noted that factors B and C have a positive effect on deformation time, and factor A has a negative effect on deformation time. A formulation with a deformation time near 10 min can be an optimized formulation as deformation time is causally related to drug release.

*4.3. Effect of Factors on % Drug Release.* The factors showed a significant effect on the dependent variable % drug release. The interaction between factors and % drug release is shown as a 3D response surface plot in Figure 2 and as a counterplot in Figure 3. From the polynomial equation shown below (Equation (7)), it was observed that both factors A and B have a negative effect on the response, whereas factor C has a positive effect on the response. A special quadratic model was suggested by the software for response deformation time, showing the significant $p$ value (0.0150), $F$ value (9.09), and $R^2$ (0.8140).

$$\begin{aligned}\%\text{Drug release} : {} & -455.7\,A - 220B + 146.59\,C + 1671.5\,AB \\ & - 58.04\,BC + 561.6\,AC.\end{aligned} \tag{7}$$

*4.4. Optimization.* The impact of different factors on the dependent variables can be assessed by desirability and optimization approach. Constraints were applied to the dependent variables to develop an optimized formula by generating desirability plots (as shown in Figure 4). The fixed constraints fixed were hardness (3.6 kg/cm$^2$), deformation time (25 min), and % drug release (58.5%). These constraints were entered into the software with a desirability factor of 1.000. The recommended concentration of independent variables was factor A: 32.12%, factor B: 43.24%, and factor C: 24.6%. The optimized formula provided by the software was prepared and evaluated for the hardness, deformation time, and % drug release responses. It was checked for stability. The results were compared with the model prediction shown in Table 6. The observed experimental values were close to the model-predicted value. These results were attributed to the validity and reliability of the optimization technique used in the present study that used factorial design.

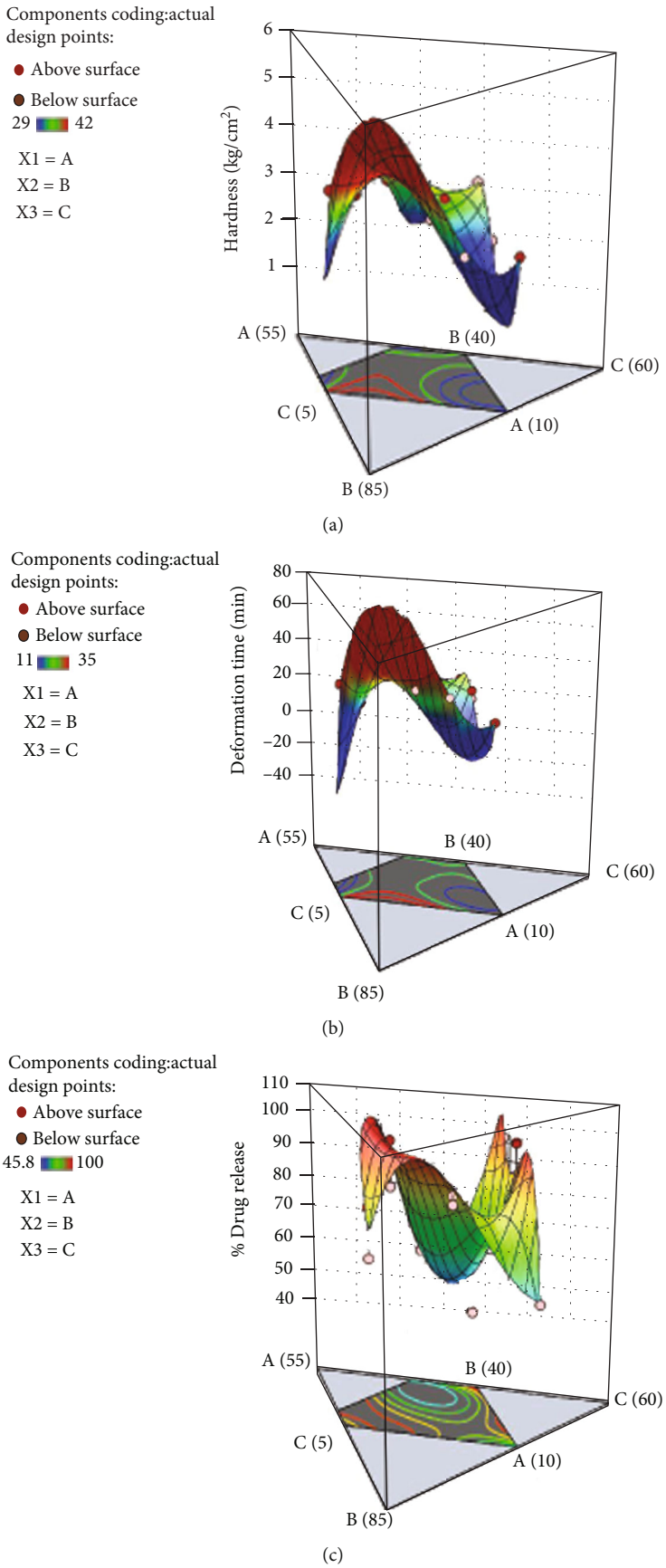


Figure 2: 3D response surface plot showing the effect of factors on hardness, deformation time, and % drug release.

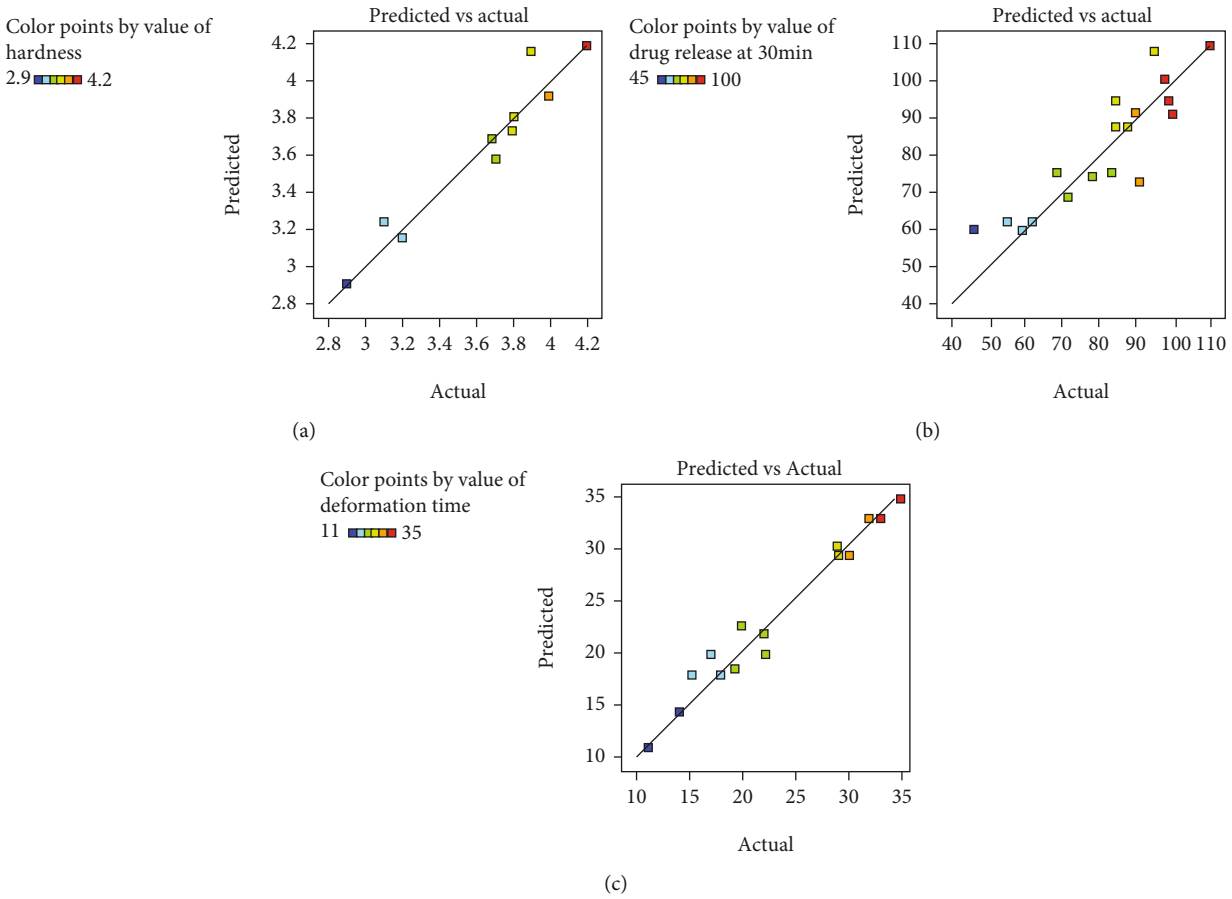


Figure 3: Counterplots showing the effect of factors on hardness, deformation time, and % drug release.

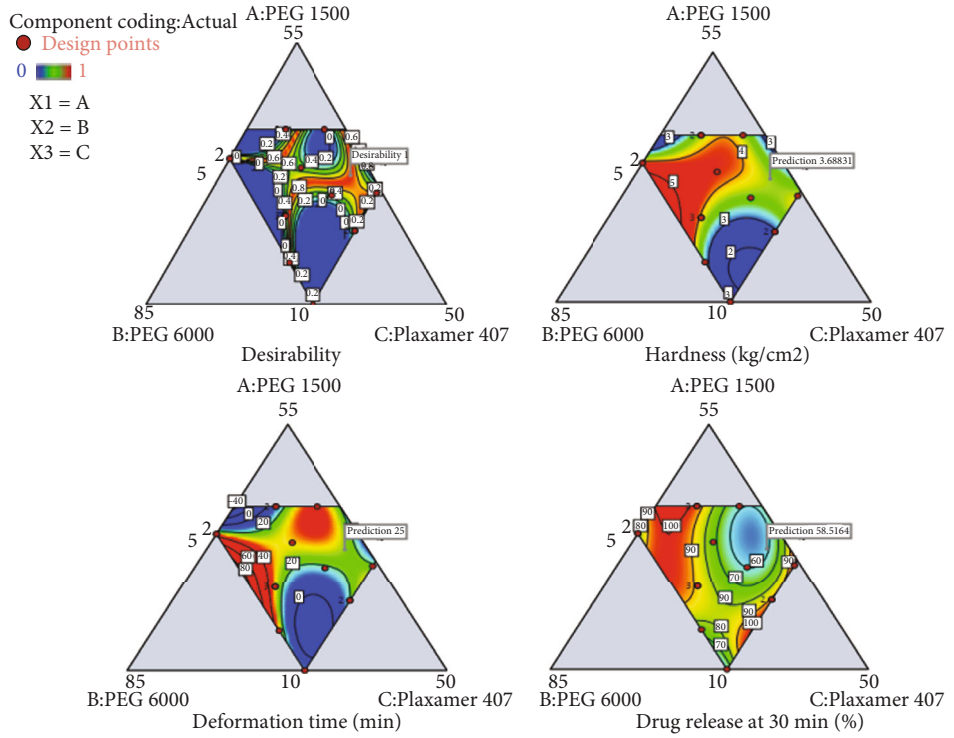


Figure 4: Optimization of curcumin vaginal suppository represented by desirability plot and interaction.

Table 6: Comparison of independent variables' observed value and predicted value given by software for the optimized formulation.

| Factor A (%) | Factor B (%) | Factor C (%) | Optimized formulation independent variables | Predicted value | Observed value | Desirability |
|---|---|---|---|---|---|---|
| | | | Hardness | 3.688 | 3.58 | |
| 32.12 | 43.24 | 24.6 | Deformation time (min) | 25 | 24.5 | 1.000 |
| | | | % drug release (min) | 58.51 | 57.2 | |

## 5. Conclusion

Curcumin vaginal suppositories were successfully developed. The prepared suppositories were devoid of holes and cracks, having a characteristic odor with dark yellowish-orange color due to curcumin. All formulations passed the weight variation test. Formulations exhibited pH ranging from 5.5 to 6.5. Drug content was observed to be $98.65 \pm 0.041\%$ –$99.85 \pm 0.041\%$. The hardness of the formulation was between 2.9 and 4.2 $kg/cm^2$. The disintegration time ranged from $11 \pm 0.052$ to $20 \pm 0.011$ min. The melting point was between $41°C \pm 0.31°C$ and $58°C \pm 0.62°C$. Liquefaction time ranged from 1 : 17 to 14 : 33 min. Most formulations resulted in 90% drug release at 40 min. The zone of inhibition was $19.6 \pm 0.4$ mm. The optimized formulation was stable for 30 days. All the formulations resulted in a solid suppository devoid of fracture or cracks that retained their integrity, indicating the combination of bases used to prepare the suppository does not interfere with its integrity. The selected factors (PEG 1500, PEG6000, and Poloxamer 407) had an impact on selected responses, i.e., hardness, deformation time, and % drug release. All other evaluation parameters also were satisfactory for the optimized formula given by the software, and its observed value was close to predicted values. Formulations developed using curcumin can be used as an herbal vaginal treatment for candidiasis, free from side effects, in cervical cancer patients.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Ethical Approval

Ethical approval is not necessary.

## Consent

Informed consent was obtained from all individual participants included in the study.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Authors' Contributions

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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