

# Design of Curcumin Suppositories for Effective Treatment of Vaginal Candidiasis using Cow Ghee as a Suppository Base

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## Abstract

**Aim:** The objective of the present investigation was to design curcumin suppositories for the effective treatment of vaginal candidiasis to eliminate side effects that are caused by existing antifungal drugs. **Materials and Methods:** Curcumin has promising antifungal activity in comparison with the existing azole antifungal drugs. Curcumin suppositories were prepared by fusion method with cow ghee (CG) and combinations of CG and polyethylene glycol 6000 in different ratios. The suppositories were evaluated for their visual, physicochemical, and *in vitro* release characteristics as well as *in vitro* antifungal activity. **Results and Discussion:** Formulation F6 showed all the results within the pharmacopeial and in-house specifications. The antifungal activity of the F6 formulation has demonstrated a significant effect against *Candida albicans*. **Conclusion:** The study indicates the possible and effective use of curcumin suppositories for vaginal candidiasis as a promising approach for natural antifungal treatment.

**Key words:** Candidiasis, cow ghee, curcumin, polyethylene glycol, suppositories

## INTRODUCTION

Curcumin is most abundant polyphenol present in the dietary spice turmeric. It is obtained by extraction from the powdered rhizomes of *Curcuma longa* belonging to family Zingiberaceae.<sup>[1]</sup> It is generally used as food preservative, food color, and spice.<sup>[2,3]</sup> It is chemically 1,7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, and 5-dione; diferuloylmethane, a yellow bioactive pigment, is the major component of turmeric [Figure 1].<sup>[4]</sup> Turmeric is an ancient color spice of Asia, as the main source of curcumin is traditionally used for many remedies.<sup>[5]</sup> Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, antifungal, and antimalarial activities.<sup>[6,7]</sup> Vaginal candidiasis is a very common condition caused by *Candida albicans* that affects up to 75% of women at least once in their life.<sup>[8]</sup> Curcumin shows antifungal activity against the strain of *C. albicans*.<sup>[9]</sup>

A suppository is a medicated solid dosage form intended for use in the rectum, vagina, and urethra. Rectal and urethral suppositories

are usually employed as vehicles that melt or soften at body temperature, whereas vaginal suppositories are called as pessaries and made as compressed tablets that disintegrate in the body fluids. Suppositories are administered for their local or systemic action. They have several advantages over oral dosage forms.<sup>[10]</sup> History of suppositories shows that variety of substances has been employed as suppository bases. Their use was governed by the factor of their availability rather than scientific approach. They play an important role in release of drug from suppository and therefore affect the bioavailability of drug.<sup>[11]</sup>

Most of the suppository bases have definite disadvantage of producing hypersensitivity or immunogenic responses in certain individuals. Still today, animal fats were not very

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commonly used as pharmaceutical excipients. However, cow ghee (CG) or clarified butter have relatively high melting point. It is an important component of our daily diet and absolutely free from the hypersensitivity and other reactions. It meets all these conditions as it has softening ability, well suited with all drug types even from olden days, easily melts, and in case of proper storage, it can be used for longer period even many years by reason of its antiaging property.<sup>[12]</sup> Very less literature was available where CG was used as suppository base.<sup>[13-15]</sup>

Hence, a humble attempt has been made to investigate ability of using CG to behave as novel suppository base using fusion method.

## MATERIALS AND METHODS

### Materials

Curcumin was purchased from Feather Touch Industries, Mumbai, India. CG (Patanjali) was procured from Local Market Akola, India. Potassium dihydrogen phosphate, butylated hydroxytoluene, and ethylenediaminetetraacetic acid disodium salt were purchased from Modern Chemical Corporation, Mumbai, India. All other materials and chemicals are of laboratory and analytical grade.

### Methods

#### Calibration of molds

Before preparation of suppositories, the molds were calibrated because they may vary in their capacity. The suppository base was melted alone and filled into the mold. It was allowed to solidify. Suppositories were removed and mean weight was taken as true capacity of the mold.<sup>[16]</sup>

#### Preparation of suppositories

The suppositories were prepared using fusion method. CG, curcumin, polyethylene glycol (PEG) 6000, and all other ingredients were accurately weighed [Table 1]. PEG 6000 was melted in china dish and CG was added to it with continuous stirring to prevent excessive heating of CG or CG was melted (when used alone as a base in suppositories). Finely sifted required quantity of curcumin, and other ingredients were added geometrically to above base or bases with continuous stirring to avoid lump formation. Care was taken to prevent bubble formation in the final mixture. The molds were lubricated with light liquid paraffin, and the final mixture was poured into the molds. The molds were kept at refrigerated conditions for solidification. The suppositories were removed from molds after solidification and packed properly.<sup>[17]</sup>

## Evaluation of suppositories

### Visual examination

Color, odor, and surface characteristics of the suppositories were relatively easy to assess. Suppositories were checked for absence of exudation, fat blooming, fissuring, pitting, sedimentation and migration of active ingredients. Suppositories were observed as an intact unit and also by splitting them longitudinally.<sup>[18]</sup>

### Color

The intensity, nature, and homogeneity of suppositories color were verified.

### Odor

Verification in the odor can prevent confusion when similar suppositories are being processed. A change in the odor is indication of a degradation process.

### Shape

The shape of the suppositories was checked for the consistency, irrespective of whether the suppositories may oblique or torpedo shaped.

### Surface condition

The suppositories were checked for brilliance, dullness, mottling, cracks, dark regions, axial cavities, bursts, air bubbles, holes, etc.

### Weight variation test

Randomly 20 suppositories were selected from each formulation and weighed. The average weight of suppositories was calculated. All suppositories were weighed individually and variation from the average was determined. Not more than two suppositories should deviate by more than 5% and non-deviate by 10% of the individual weights from average weight.<sup>[19]</sup>

### Melting point and melting range

The melting point was determined by measuring the time taken by the entire suppository to melt when immersed in phosphate buffer pH 6.8 in constant temperature bath maintained at  $37 \pm 0.5^\circ\text{C}$ . Micromelting range test was

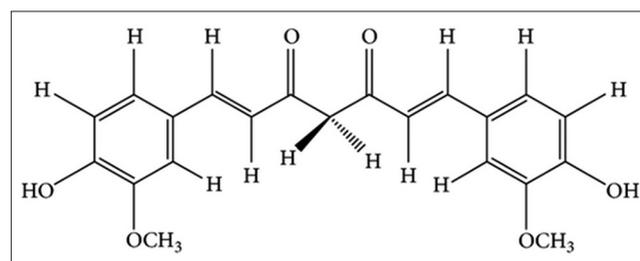


Figure 1: Structure of curcumin

carried out using capillary tube of 10 cm length which the formulation was filled up to 1 cm height and dipped in water bath. The temperature was increased slowly and the temperature at which the mass liquefies was noted.<sup>[20]</sup>

#### Hardness test

Hardness test was carried out to determine the tensile strength and ability of the suppositories to withstand the hazards of packing and transportation using Monsanto hardness tester (Veeco Scientific, Model HT-1).<sup>[21]</sup>

#### Liquefaction time

Liquefaction time was measured using a pipette having a broad opening on one side and a narrow opening on the other. Suppository was pushed inside in such a way that the broad end side of suppository to reach to the narrow end of pipette. Accurately measured 5 ml of phosphate buffer pH 6.8 was placed inside the pipette and maintained at  $37 \pm 0.5^\circ\text{C}$ . A thin iron rod of 30 g was placed on the top of the suppository and the time at which the iron rod just inserts into the suppository was recorded as liquefaction time. This indicates the time taken by the formulation to liquefy in vagina.<sup>[21]</sup>

#### Disintegration test

The disintegration time of the suppositories was determined using disintegration test apparatus. The time taken for the disintegration of entire suppositories was recorded. Phosphate buffer pH 6.8 maintained at  $37 \pm 0.5^\circ\text{C}$  was used as disintegration medium.<sup>[22]</sup>

#### Swelling index

The swelling index of the curcumin suppositories was determined using five suppositories from each formulation. Suppositories were weighed ( $W_1$ ) and placed in stainless steel basket with 200 mesh aperture. Accurately measured 5 ml of simulated vaginal fluid (SVF) as media was

added to each beaker. Stainless steel baskets containing suppositories were placed in beakers and stored in an incubator at  $37 \pm 0.5^\circ\text{C}$  for 2 h. After 2 h, beakers were taken out from incubator and excess media were removed. The swollen suppositories were weighed ( $W_2$ ). Experiment was repeated 3 times and the average  $W_1$  and  $W_2$  were reported, and the swelling index was determined from the following formula:<sup>[23]</sup>

$$\text{Swelling Index} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

#### Dissolution test

USP Type I basket apparatus (Electrolab, TDT-08L) containing 900 ml of SVF of pH 7.4 in each dissolution vessel was maintained at  $37 \pm 0.5^\circ\text{C}$ . When the temperature was reached to the mark, one suppository was added in each vessel. The basket speed was maintained at 100 rpm for 60 min. About 1 ml of the sample from each vessel was withdrawn at predetermined time, and fresh equivalent volume of diTssolution medium was the replaced to maintain the constant volume. The samples were filtered through  $0.45 \mu$  membrane filter, diluted suitably and analyzed at 428 nm using a ultraviolet (UV)-visible spectrophotometer (Shimadzu, UV 1601).<sup>[22]</sup>

#### Drug content

Randomly selected suppository from each formulation was taken and melted in a volumetric flask of 100 ml capacity. About 25 ml of methanol was added to volumetric flask and final volume was made with SVF pH 7.4. The solution was continuously sonicated for 5 min in bath sonicator. The solution was filtered using  $0.45 \mu$  membrane filter. The solutions were subjected to UV-visible spectrophotometrically at 428 nm wavelength.<sup>[22]</sup>

#### Antifungal activity

The curcumin suppositories were tested against *C. albicans* j1023 using agar cup plate method. Cups of 10 mm diameter were

**Table 1:** Formulation of curcumin suppositories

Ingredients	Formulation								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Curcumin	250	250	250	250	250	250	250	250	250
CG	450	400	350	300	250	225	200	150	100
PEG 6000	0	50	100	150	200	225	250	300	350
Propylparaben	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Methylparaben	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Disodium EDTA	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
BHT	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
L-ascorbic acid	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7

All weights were taken in mg

CG: Cow ghee, PEG: Polyethylene glycol, EDTA: Ethylenediaminetetraacetic acid, BHT: Butylated hydroxytoluene

made aseptically in agar media after being inoculated with tested fungal suspension by spreading on agar surface. The optimized formulation was placed in each cup by aseptic transfer and stored in incubator for 48 h. The zone of inhibition of each cup was observed and radius of zone of inhibition was measured.<sup>[23]</sup>

### Stability study

Suppositories were wrapped in the aluminum foil and kept in stressed condition by six cycles of freeze (2–8°C) and thaw (25°C) process. Suppositories were also kept in accelerated condition temperature (30°C) for 45 days. Suppositories were evaluated for visual appearance, drug content, and antimicrobial activity.<sup>[24]</sup>

## RESULTS AND DISCUSSION

### Calibration of molds

#### Determination of calibration factor

- Mold = Stainless steel (20/20)
- Total weight of suppositories = 2.60 g
- Average weight of 1 suppository = 0.65 g
- Calibration factor = 0.65.

#### Determination of displacement value

- a. Weight of 4 suppositories with only base = 2.60 g
- b. Weight of 4 suppositories with base and drug = 2.97 g
- c. Weight of base (64%) =  $64/100 \times 2.97 = 1.90$  g
- d. Weight of drug in suppository (32%) =  $32/100 \times 2.97 = 0.95$  g
- e. Weight of base displaced by drug =  $2.60 - 1.90 = 0.7$  g. If 0.7 g base is displaced by 0.95 g drug, then 1 g drug displaced as  $0.95/0.7 = 1.36$ .

Therefore, displacement value of drug = 1.36.

### Evaluation of suppositories

#### Visual appearance

The suppositories obtained by fusion method were oviform-shaped, yellow–orange-colored and with characteristic odor suppositories. These suppositories were devoid of any physical flaws. The average weight of the suppositories was found to be 0.8 g, and the average length was recorded as 2 cm [Table 2].

#### Weight variation test

All the prepared suppositories except F1 were showed their results within the pharmacopeial limits for the weight variation test [Table 2].

#### Melting point and melting range

Melting point of suppository formulations was ranging from 34°C to 42°C. Melting point of F6 suppository formulation

was found to be 37°C. Melting range of suppository formulations was ranging from 33–35°C to 41–43°C. Melting range of F6 suppository formulation was found to be between 36°C and 38°C [Table 2].

#### Hardness test

Hardness of suppository formulations was ranging from 0 to 4.2 kg/cm<sup>2</sup>. Hardness of F6 suppository formulation was found to be 2.6 kg/cm<sup>2</sup>. Hardness of the suppository was found to be low in CG suppository as compared to PEG 6000–CG suppositories. However, with increase in the PEG concentration in the formulation, higher values for hardness were obtained. However, the PEG 6000 increases the hardness of suppository formulations from 0.5 to 4.2 kg/cm<sup>2</sup>. The F9 batch hardness shows maximum 4.2 kg/cm<sup>2</sup> as shown in Table 2.

#### Liquefaction time

Liquefaction time of suppository formulations was ranging from 1:17 to 14:33 (min:s). Liquefaction time of F6 suppository formulation was found to be 9:26 (min:s). The liquefaction time of suppository formulations increases with increase in the amount of PEG in the suppository formulation. The liquefaction time of suppository formulation containing CG was found to be 1:17 (min: s) as shown in Table 2.

#### Disintegration test

Conventionally, the disintegration time of suppository is preferred to be 20–23 min or less. The investigated suppository showed that disintegration time of F6 was <23 min which was well within the acceptability range. The disintegration time of the suppository formulations was ranging from 18 min to 26 min. [Table 2].

#### Swelling index

Swelling behavior of suppository as a function of time is illustrated in formulation. This property of suppository has direct influence on percent release of drug. Swelling index study was performed to study and compare the hydration characteristics of suppository polymer and percent release of drug for the optimized batch (F6) which was maximum. The swelling index of suppository formulations was ranging from 69.91% to 99.54% [Table 2].

#### Dissolution test

*In vitro* dissolution of curcumin suppositories was carried out in SVF pH 7.4 as a dissolution media. *In vitro* dissolution of curcumin suppositories is shown in Figure 2. The formulation (F6) showed the total drug release at the end of 60 min.

#### Drug content

The individual drug content of each suppository was found to be within the range of 85–115%. Thus, the requirements for content uniformity were meet as per USP [Table 2].

### Antifungal study

*In vitro* antifungal activity performed by cup plate method showed an average zone of inhibition of the curcumin pure drug against *C. albicans* j1023 was  $20.1 \pm 0.48$  mm, whereas curcumin suppositories showed enhanced *in vitro* antifungal activity having average zone of inhibition  $32.1 \pm 0.4$  mm. The developed curcumin suppositories were found to be effective.

### Stability study

The accelerated stability studies were carried for developed optimized formulation (F6) analyzed for appearance, drug content, and antimicrobial activity after 45 days [Table 3].

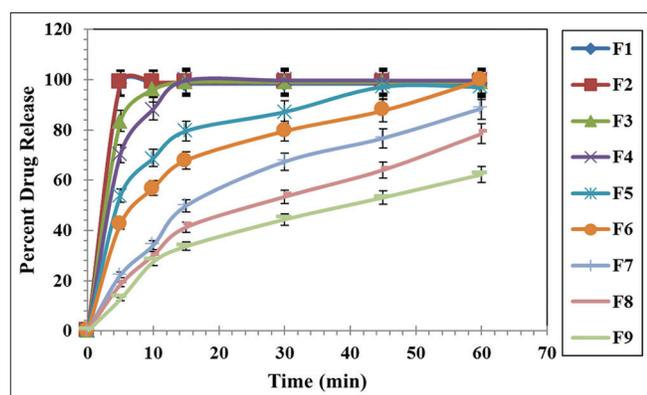


Figure 2: *In vitro* dissolution of curcumin suppositories

## CONCLUSIONS

The suppositories of curcumin prepared by fusion method using CG and PEG 6000 were yellow–orange in color, smooth, and glossy in appearance. The CG alone was unable to form the suppositories of desired physical properties (F1), and hence, PEG 6000 was successfully employed as adjuvant. Among the formulations, as CG concentration decreases and PEG 6000 concentration increases, the suppositories show higher melting point, melting range, melting time, average weight, softening time, and hardness. PEG 6000 plays a vital role in drug releases from the suppositories in controlled manner. All the formulations were evaluated for various parametric and non-parametric tests. The drug content, disintegration time, average weight, and hardness of most of formulations were in the official limit which indicates uniform distribution of drug in the formulations except F1 formulation.

From all nine formulations, the F6 was selected as optimized formulation by observing the physicochemical properties and results of pharmacopeial tests. F6 formulation also shows excellent *in vitro* antifungal activity against *C. albicans* j1023 strain using agar cup plate method. The zone of inhibition indicates sufficient antifungal activity.

Stability studies reveal no significant differences on day of preparation and after 45 days when optimized formulation

Table 2: Physicochemical evaluation of curcumin suppositories

Parameter	Formulation								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Color	Yellow	Yellow	Yellow	Orange	Orange	Orange	Orange	Orange	Orange
Length (cm)*	2	2	2	2.1	2.1	2	2.1	2	2
Average weight (g) ‡	0.71	0.73	0.74	0.77	0.79	0.80	0.81	0.84	0.88
Melting point (°C)*	34	35	35	36	36	37	38	40	42
Melting range (°C)*	33–35	34–36	34–36	35–36	35–36	36–38	38–39	39–41	41–43
Melting time (min: s)*	2:0	2:40	3:35	4:58	5:21	8:05	10:45	12:33	15:17
Hardness (kg/cm <sup>2</sup> )*	-	0.5	0.8	1.2	1.9	2.6	2.9	3.7	4.2
Liquefaction time (min: s)*	1:17	1:56	4:45	7:15	8:58	9:26	11:51	12:11	14:33
Disintegration time (min)*	18±1.56	20±1.58	21±1.55	21±1.56	22±1.55	22±1.57	23±1.58	24±1.22	26±1.31
Swelling index (%) †	75.19±2.22	85.60±1.31	83.34±1.44	56.98±1.75	89.44±0.56	99.5±0.67	77.38±2.71	72.75±2.16	69.91±1.92
Drug content (%)*	98.22±1.32	98.87±1.68	99.45±0.94	99.73±2.11	97.05±3.64	99.92±0.67	98.37±2.78	98.66±0.78	99.67±3.45

Where, \*, †, and ‡ values indicate mean±SD for sample size  $n=3$ ,  $n=5$ , and  $n=20$ , respectively. SD: Standard deviation

**Table 3: Stability study**

Study conditions	Appearance	Drug content*	Zone of inhibition*
Freeze and thaw 6 cycles	No change in physical appearance	99.72±0.53	31.02±0.02
Accelerated condition (30°C)	No change in physical appearance	99.58±0.17	31.08±0.03

Where \*Values indicates mean±SD for sample size (n=3). SD: Standard deviation

was exposed to freeze-thaw cycle and accelerated stability conditions. Further, *in vivo* study is needed before commercialization of product.

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