



DEVELOPMENT AND EVALUATION OF EMULGELS FOR TREATMENT OF VIRAL BORN SKIN DISEASE WART

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ABSTRACT

Purpose: The purpose of the study was to develop and evaluated emulgels for treatment of viral born disease wart.

Methods: The 2³ factorial designs were used to prepare emulgels. The effect of three factors, concentration of turmeric oil, glycerol and tween-80 were selected. The Thuja oil was used as drug, carbopol-934P as gelling polymer, glycerol as humectant, tween-80 as surfactant and turmeric oil as permeation enhancer. The drug and excipients were mixed in mortar pestle. The prepared batches of emulgel were characterized for appearance, drug content uniformity, homogeneity, pH, skin irritation, spreadability, in-vitro drug release, scanning electron microscopy and antiviral activity on humans. The BIT-soft 1.12 was used to determine the kinetics of drug release.

Results: The emulgel batches showed the white and cream colored, homogenous mixture with no aggregates. The FTIR studies showed drug excipient compatibility. The pH of various batches of emulgel was 6.1-6.7 and formulations showed no skin irritation. The spreadability was found to be 3.5±0.11-4.3±0.12 g.cm/sec. The drug content was found to be 98.95-99.85%. The percentage drug release was found to be 57.65±0.15-68.65±0.62 up to 150 minutes. Batch-F was selected best on the basis of maximum drug release. In-vivo study on human volunteers showed the 1 mm inhibition of wart after 2 weeks of application. The kinetics of drug release showed Korsmeyer-Peppas equation as a best-fit model with r² value 0.9971 and k value 1.4123 that indicates anomalous transport.

Conclusion: It was concluded from result that batch-F of emulgel was best to inhibit the wart.

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INTRODUCTION

The targeting of drug/ formulation to target site results in best benefits and drop the need for oral administration of the medicament and decrease dose size.^{1,2} Among various topical formulations like solutions, creams, ointment, sprays, gel etc., the gel as drug delivery system have some better advantages like it stuck to surface where it is applied and can release the drug for prolonged time period while the oil may be flush off from application site and ointments may stain the cloths or clear the applied medicament from application site. Emulgel is a concept developed by combing emulsion and gel. Emulgel has benefits over gel and emulsion alone.^{3,4}

The warts are non-cancerous growths of the skin caused by a DNA virus; human papillomavirus (HPV). This infection occurs in the superficial layers of the epidermis, causing keratinocytes proliferation and hyperkeratosis.

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These have a hard surface and spread by direct skin-to-skin contact or autoinoculation. The incubation period can be as long as twelve months. Warts are particularly common in school-aged children, eczema (defective skin barrier) and people who are immune suppressed with medications such as azathioprine or cyclosporine or with human immunodeficiency virus (HIV) infection.^{5,6}

The Thuja oil is obtained from leaves of *Thuja standishii* (Family-Cupressaceae) is used in the treatment of wart. It is an evergreen coniferous, 20-35 meter tall plant. The fresh leaves contains 65% α/β -thujone, 8% isothujone, 8% fenchone, 5% δ -sabinene, 2% α -pinen, camphene, as the main monoterpenes and carvotanacetone, organol, organes, myrcen and camphone, camphen, bornyl acetate, terpinenolas other monoterpenes. The hydrodistilled oil contains α -pinene, δ -3-carene, α -cedrol, caryophyllene, α -humulene, limonene, α -terpinolene and α -terpinyl acetate. It's oil is used as emmenagogue, stimulant, anti-rheumatic, diuretic, rubefacient, detoxifier, astringent, bactericidal, antifungal, viricidal, tonic, febrifuge, expectorant, vermifuge, insect repellent and in the treatment of menstrual pain, chronic and acute infections of the upper respiratory system, angina, pharyngitis and bronchitis,

insomnia, gout, cold, dandruff and eczema, congestion, uterine carcinomas, intestinal parasites, cystitis, fever, headache and stomach pain. Its oil pacifies kapha and pitta dosha and increase vatadosha according to ayurveda.^{7,8}

MATERIALS AND METHODS

The Thuja oil and curcumin oil was extracted from fresh leaves of plant *Thuja standishii* and dried rhizome of *Curcuma longa* respectively. The herbal parts were procured in the month of December at Roorkee. The carbopol and HPMC were procured from SD Fine Chemicals. The potassium dihydrogenphosphate, absolute ethanol and tween-80 and other chemicals were procured from Rankem Pvt. Ltd., Delhi.

Extraction of Thuja oil form fresh leaves of *Thuja standishii*

Accurately weighed 450 g of fresh leaves of *Thuja standishii* were placed in a round-bottomed flask and Clevenger apparatus was placed. The run the assembly for 5 h to isolate the oil.^{9,10}

Characterization of Thuja oil

Pycnometer was used to determine density of Thuja oil.¹¹ The Fourier transform infrared spectroscopy of Thuja oil was performed using FTIR (FTIR 8400S, CE, Software Irresolution). Perfectly dried potassium bromide KBr powder (10 mg) was compressed into thin disc by KBr press at pressure of 15,000 Psi. Sample was placed on disc. Charged disc was placed on IR window of spectrometer to determine various bonds and group present in sample.¹²

Extraction of curcumin oil form dried rhizome of *Curcuma longa*

Accurately weighed 300 g of dried curcumin powder was placed in a round-bottomed flask and Clevenger apparatus was placed. The run the assembly for 5 h to isolate the oil.^{9,10}

Characterization of curcumin oil

Pycnometer was used to determine the density of curcumin oil.¹¹ The FTIR was performed using the procedure as previously discussed.¹²

Pre-formulation studies

The UV-visible spectrophotometer was used to prepare calibration curve and FTIR was used to confirm the types of bonds and the functional group present in the oil.

UV-calibration curve of Thuja oil in petroleum ether

The stock solution was prepare by dissolving 100 mg drug in 100 ml petroleum ether and scanned between 190-400 nm to determine λ_{\max} of Thuja oil. The dilutions were prepared (1-7 $\mu\text{g/ml}$) and calibration plot was plotted.¹²

Fourier transform infrared spectroscopy and compatibility study

FTIR study was performed to determine the compatibility and interaction between the drug and the excipients. The FTIR spectra of pure Thuja oil, excipients and formulation were obtained using potassium bromide disc method by recording samples in the range of 2000 cm^{-1} and 400 cm^{-1} using IR spectrometer (Shimadzu, IR Affinity-1).¹²

Preparation of emulgel

The composition of emulgel to prepare various batches was designed as given Table No.1. The three factors were selected (Turmeric oil, glycerol and tween-80) and their levels were set to apply 2^3 factorial design as given in Table No.2. The 8 batches of emulgel with and additional 9th batch of emulgel was prepared using the middle values of factors as given in Table No.3. The accurately weighed quantity of carbopol was taken in a beaker and water was added to hydrate. Mixture was allowed to mix continuous using a mechanical stirrer for 2 h. After hydration and swelling of carbopol, glycerine, tween-80 and oils were mixed for 1 h using a mechanical stirrer to prepare emulgel.¹⁵

Table No 1 The composition of emulgel

| Formula | Quantity |
|-------------------|----------------|
| Thuja oil (ml) | 1 |
| Turmeric oil (ml) | X ₁ |
| Glycerol (ml) | X ₂ |
| Tween-80 (ml) | X ₃ |
| Carbopol 934P (g) | 1 |
| Water (18 ml) | q.s. |

Table No 2 The 2^3 Factorial design to prepare emulgel

| Factor | Symbol | Levels | | |
|-------------------|----------------|----------|---------|-----------|
| | | Low (-1) | Mid (0) | High (+1) |
| Turmeric oil (ml) | X ₁ | 0 | 1 drop | 2 drop |
| Glycerol (ml) | X ₂ | 5 | 6 | 7 |
| Tween-80 (ml) | X ₃ | 0 | 1 drop | 2 drop |

Table No 3 Combinations of factors (Batches)

| Combinations of factor | X ₁ | X ₂ | X ₃ |
|------------------------|----------------|----------------|----------------|
| A | - | - | - |
| B | + | - | - |
| C | - | + | - |
| D | + | + | - |
| E | - | - | + |
| F | + | - | + |
| G | - | + | + |
| H | + | + | + |
| I | 0 | 0 | 0 |

Evaluation of emulgel

The formulated batches of emulgel were evaluated for the following parameters:

Color

Among prepared batches of emulgel the batches contained curcumin oil were cream in color and rest batches were white in color.^{16,17}

Drug content uniformity

Accurately weighted 100 mg of prepared emulgel was taken in 100 ml volumetric flask and phosphate buffer solution of pH 6.8 was added. The volumetric flask containing emulgel solution was shaken for 2 h on a mechanical shaker in order to get complete mixing of the drug. This solution was filtered and estimated spectrophotometrically at 260 nm using phosphate buffer (pH 6.8) as blank.^{18,19}

Homogeneity

All developed emulgel bathes were tested for homogeneity by visual inspection after the emulgels have been set in the container. They were tested for their appearance and presence of any aggregates.^{20,21}

pH

Accurately weighed 1 g emulgel was dissolved in 100 ml of distilled water and stored at 4°C for 2 h to prevent volatile oil content. The measurement of pH of each formulation was carried out using digital pH meter (Labline) at 25°C in triplicate and the average values were tabulated.^{22,23}

Skin irritation

The human volunteers were tested for skin irritation. For each emulgel, five volunteers were selected and 1 g of formulated emulgel was applied on an area of 2 inch² on hands. The volunteers were observed for irritation or lesions.^{24,25}

Spreadability

Spreadability of formulations was determined by wooden block and glass slide apparatus. Accurately weighed 1 g emulgel was placed in between wooden block and glass slide. The 1000 g weight was placed on slides for 5 min to squeeze the sample to a uniform thickness. Weights about 20 g were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slides. A shorter interval signifies better spreadability. The time (seconds) required to take apart the two slides, was taken as a measure of spreadability. Spreadability was then calculated by using the formula.^{26,27}

$$S = M.L/T$$

Where,

S = Spreadability

M = Weight tide to upper slide

L = Length of glass slide

T = Time taken to separate the slide completely from each other.

In-vitro drug release

Phosphate buffer of pH 6.8 was used for *in-vitro* release study using the skin of albino mice in Franz diffusion cell. The emulgel sample was placed in donor compartment and placed on mice skin pre-placed on receptor compartment of the diffusion cell. The receptor compartment contained phosphate buffer (15 ml) of pH 6.8. The temperature of diffusion medium was thermostatically controlled at 37 ± 1 °C by surrounding water in a jacket and the medium was stirred by a magnetic stirrer at 50 rpm. The sample were withdrawn at predetermined intervals (15, 30, 45, 60, 90, 105, 120, 135 and 150 min) and replaced by equal volume of fresh phosphate buffer of pH 6.8 to maintain sink conditions. The absorbance of withdrawn samples was determined at 260 nm by UV-visible spectrophotometer (Shimadzu 1700S) using phosphate buffer of pH 6.8 as blank.^{28,29}

Inhibition of wart

The best selected emulgel batch-F was applied on the wart for two weeks to determine the inhibition of wart.³⁰

Stability study

The emulgel batch-F were studied for stability at 25 ± 2 °C/60 ± 5% RH, 37 ± 2 °C/65 ± 5% RH, 45 ± 2 °C/75 ± 5% RH for 6 months in screw-capped amber colored glass bottles. The emulgel was evaluated for color change and percent drug content after 1, 3 and 6 months. The initial drug content was considered as 100%.^{31,32}

RESULTS AND DISCUSSION

The yield of Thuja oil was 0.7%. The density of Thuja oil was 0.946 g/ml at 25 °C. The FTIR showed the presence of C-H (619.54, 887 cm⁻¹), germinal methyl (1111.22 cm⁻¹), germinal methyl (1382.17 cm⁻¹), -CH₃(1443.24 cm⁻¹), -CH₂- (1443.24 cm⁻¹), C-C (1599.84 cm⁻¹) and C=O-R (1727.12 cm⁻¹) groups as presented in Fig1.

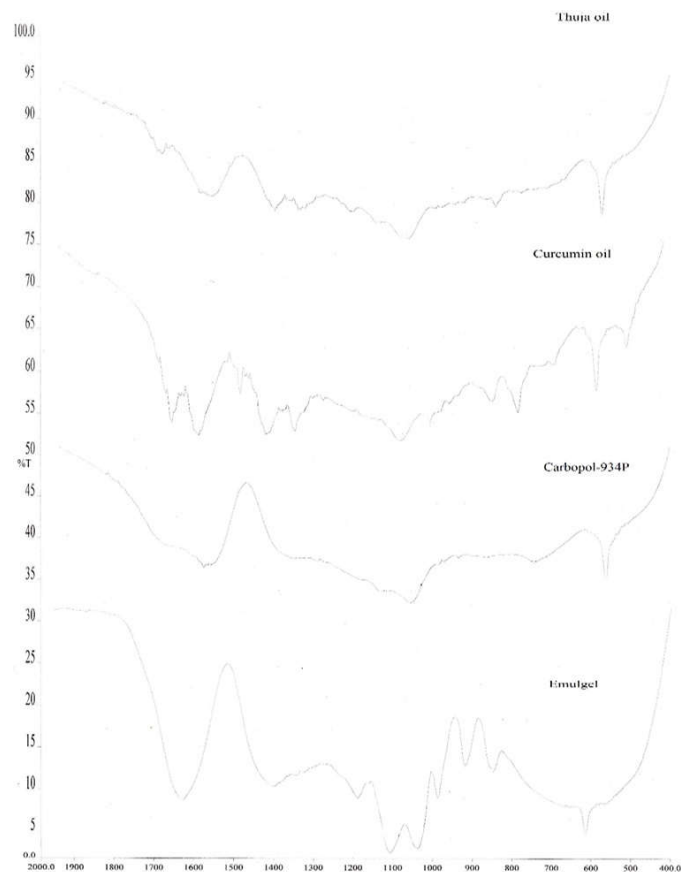


Fig 1 FTIR of Thuja oil, Curcumin oil, Carbopol-934P and Emulgel

The yield of curcumin oil was 0.5%. The density of curcumin oil was 0.88 g/ml at 25 °C. The FTIR showed the presence of C-H (542.11 cm⁻¹), C-H (619.59 cm⁻¹), C-H (816.32 cm⁻¹), C-H (882.50 cm⁻¹), C-C (1038.10 cm⁻¹), C=O (1112.46 cm⁻¹), germinal methyl (1377.39 cm⁻¹), -CH₃(1449.40 cm⁻¹), -CH₂- (1449.40 cm⁻¹), C-C (1513.96 cm⁻¹), C-C (1619.15 cm⁻¹) and C=C (1686.50 cm⁻¹) groups as presented in Fig1.

The calibration curve of Thuja oil in petroleum ether was prepared by measuring absorbance at 260 nm using UV-visible spectrophotometer as showed in Table No. 4 and Fig 2.

Table No 4 UV-Visible calibration data of Thuja oil in petroleum ether at 260 nm.

| Concentration (µg/ml) | Absorbance |
|-----------------------|------------|
| 1 | 0.129 |
| 2 | 0.255 |
| 3 | 0.376 |
| 4 | 0.512 |
| 5 | 0.638 |
| 6 | 0.771 |
| 7 | 0.901 |

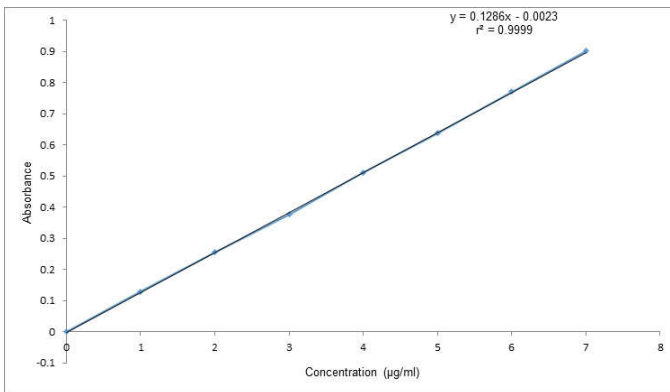


Fig 2 UV-Visible calibration curve of Thuja oil in petroleum ether at 260 nm

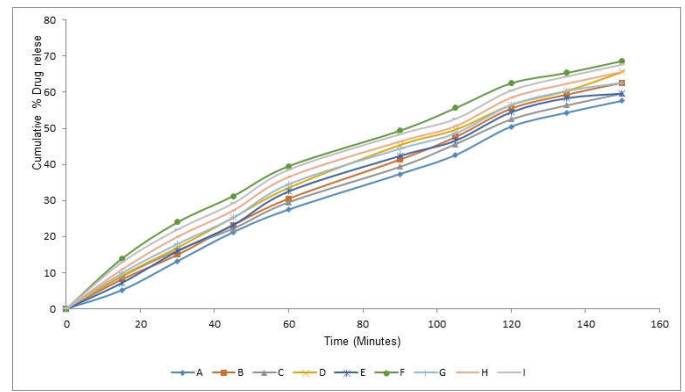


Fig 3 In-vitro drug release study

FTIR study was performed to determine the compatibility and interaction between the drug and the excipients. The FTIR spectra of pure Thuja oil, excipients and formulation were obtained using potassium bromide disc method by recording samples in the range of 2000 cm⁻¹ and 400 cm⁻¹ using IR spectroscopy (Shimadzu, IR Affinity-1) as presented in Fig 1. The color, drug content uniformity, homogeneity (appearance and aggregates), pH, skin irritation test, consistency and spreadability were measured and tabulated in Table No.5.

Stub was coated using of argon and gold in coating chamber using a high-vacuum evaporator (Polaron SEM coating system). After coating the plated stub was place in the scanning electron microscopy chamber. Sample was analyzed using Scanning Electron Microscopy (CARL ZEISS AG-EVO[®]40 Series) using Thermo Ultra Dry SDD EDS detector at 20 kV.

Table No 5 Results of emulgel batches evaluation

| Batch | A | B | C | D | E | F | G | H | I |
|--------------------------|-----------|------------|------------|------------|------------|------------|------------|------------|------------|
| Color | White | Cream | White | Cream | White | Cream | White | Cream | Cream |
| Drug content (%) | 99.85±0.5 | 98.95±0.14 | 99.52±0.63 | 99.63±0.21 | 99.46±0.44 | 98.18±0.25 | 99.84±0.22 | 99.36±0.15 | 98.82±0.53 |
| Homogeneity | Clear | Clear | Clear | Clear | Clear | Clear | Clear | Clear | Clear |
| Aggregates | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| pH | 6.1±0.1 | 6.2±0.1 | 6.1±0.1 | 6.2±0.1 | 6.1±0.1 | 6.2±0.1 | 6.1±0.1 | 6.2±0.1 | 6.2±0.1 |
| Skin irritation | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| Consistency | Viscous | Viscous | Viscous | Viscous | Viscous | Viscous | Viscous | Viscous | Viscous |
| Spreadability (g.cm/sec) | 4.2±0.12 | 3.8±0.62 | 3.9±0.42 | 4.3±0.12 | 3.6±0.28 | 3.5±0.11 | 3.7±0.12 | 3.9±0.27 | 4.1±0.21 |

(n=3)

The *in-vitro* drug release showed the drug release varied between 57.65±0.15 - 68.65±0.62% up to 150 m as presented in Table No.6 and Fig 3. The emulgel batch-F was selected best on the basis of maximum drug release up to 68.65±0.62% in 150 minutes.

The emulgel batch-F showed the inhibition of wart was 1 mm after 2 weeks of application of emulgel thrice a day as showed in Fig 5.

Table No 6 Drug permeation study/ *In-vitro* drug release study in 6.8 pH solution at 260 nm

| Time (Minutes) | Cumulative % Drug release | | | | | | | | | |
|----------------|---------------------------|------------|------------|------------|------------|------------|------------|------------|------------|--|
| | A | B | C | D | E | F | G | H | I | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 15 | 5.17±0.12 | 8.17±0.10 | 8.98±0.12 | 9.17±0.11 | 7.17±0.15 | 13.89±0.11 | 9.89±0.16 | 10.89±0.12 | 12.89±0.11 | |
| 30 | 13.22±0.15 | 14.98±0.23 | 16.22±0.42 | 16.98±0.13 | 15.98±0.26 | 23.98±0.14 | 17.98±0.13 | 19.98±0.52 | 21.98±0.52 | |
| 45 | 21.23±0.15 | 23.23±0.53 | 22.23±0.12 | 25.23±0.11 | 23.23±0.13 | 31.23±0.21 | 25.23±0.63 | 27.23±0.22 | 29.23±0.33 | |
| 60 | 27.53±0.21 | 30.53±0.62 | 29.53±0.25 | 33.53±0.23 | 32.53±0.20 | 39.53±0.13 | 34.53±0.13 | 36.53±0.13 | 38.53±0.13 | |
| 90 | 37.35±0.14 | 41.35±0.15 | 39.35±0.15 | 45.35±0.13 | 42.35±0.31 | 49.35±0.21 | 44.35±0.23 | 46.35±0.62 | 48.35±0.14 | |
| 105 | 42.62±0.15 | 47.62±0.36 | 45.62±0.27 | 49.62±0.24 | 46.62±0.13 | 55.62±0.33 | 48.62±0.53 | 50.62±0.13 | 52.62±0.52 | |
| 120 | 50.48±0.12 | 55.48±0.17 | 52.48±0.11 | 56.48±0.22 | 54.48±0.42 | 62.48±0.13 | 56.48±0.15 | 58.48±0.52 | 60.48±0.12 | |
| 135 | 54.36±0.11 | 59.36±0.25 | 56.36±0.21 | 60.36±0.13 | 58.36±0.14 | 65.36±0.41 | 60.36±0.13 | 62.36±0.24 | 64.36±0.13 | |
| 150 | 57.65±0.15 | 62.65±0.11 | 59.65±0.13 | 65.65±0.23 | 59.65±0.11 | 68.65±0.62 | 62.65±0.62 | 65.65±0.13 | 67.65±0.51 | |

(n=3)

The drug release kinetics study showed the mechanism of drug release was anomalous transport that indicates the drug release was disintegration and dissolution based as presented in Table No.7.

Table No 7 The parameters for drug release kinetics of emulgel batch-F

| Best fit model | PeppasKorsmeyer |
|----------------------|---------------------|
| R ² | 0.9971 |
| k | 1.4123 |
| n | 0.6928 |
| k | 1.4123 |
| Mechanism of release | Anomalous Transport |

The scanning electron microscopy was performed to study surface characteristics of dried emulgel as presented in Fig 4. About 100 mg of emulgel was spread over two-sided adhesive carbon tape that was pre-placed on the brass stub.

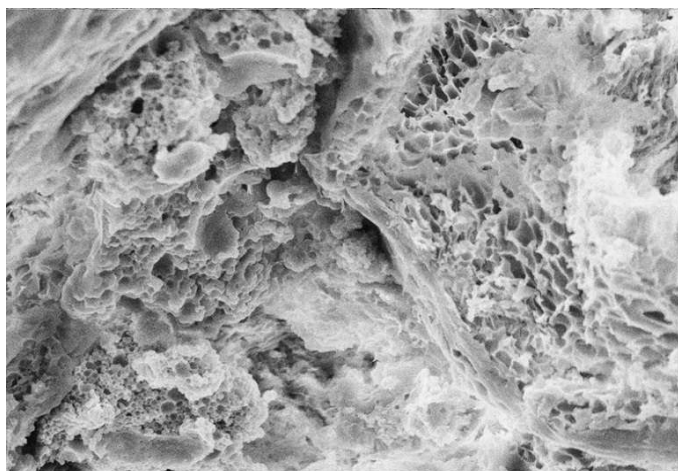


Fig 4 Scanning electron microscopy of emulgel batch-F



Fig 5 Inhibition of Wart using emulgel batch-F

The stability study showed that the storage temperature and relative humidity affected the stability of the emulgel as showed in Table No.8.

Table No 8 Stability study of best batch of emulgel (Batch-F)

| Conditions | Time (Months) | Drug content (%) |
|----------------------|---------------|------------------|
| | | Emulgel |
| 25 ± 2 °C/60 ± 5% RH | 1 | 99.45±0.32 |
| 37 ± 2 °C/65 ± 5% RH | | 99.64±0.22 |
| 45 ± 2 °C/75 ± 5% RH | | 99.62±0.14 |
| 25 ± 2 °C/60 ± 5% RH | 3 | 98.25±0.11 |
| 37 ± 2 °C/65 ± 5% RH | | 98.63±0.15 |
| 45 ± 2 °C/75 ± 5% RH | | 98.53±0.53 |
| 25 ± 2 °C/60 ± 5% RH | 6 | 98.42±0.32 |
| 37 ± 2 °C/65 ± 5% RH | | 98.74±0.71 |
| 45 ± 2 °C/75 ± 5% RH | | 97.23±0.22 |

CONCLUSION

It was found that the emulgel batch-F showed the max drug release in 150 minutes and 1 mm inhibition of wart on human skin.

Conflict of interest statement

We declare that we have no conflict of interest.

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