

# Formulation and *in-vitro* evaluation of emulsion loaded topical gel for the enhancement of diffusion through the skin for the treatment of skin irritation

Ram Babu SHARMA<sup>3\*</sup> , Chandresh KUMARI<sup>1</sup> , Arpita KAPILA<sup>1\*</sup> , Kundan Singh BORA<sup>2</sup>   
Abhishek SHARMA<sup>2\*</sup> 

<sup>1</sup> School of Pharmaceutical Sciences, Bahra Univeristy, Wagnaghat, Solan, Himachal Pradesh, India

<sup>2</sup> University Institute of Pharma Science, Chandigarh University, Gharuan, Mohali, Panjab, India.

<sup>3</sup> Himalayan Institute of Pharmacy Kala-mb, Himachal Pradesh.

\* Corresponding Author. E-mail: sharmaram77@gmail.com (R.B.S.); Tel. +91-701 837 99 26.

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**ABSTRACT:** The purpose of the current study is to formulate and develop an emulsion gel (oil-in -water) which removes all obstacles related to its diffusion, stability and irritation of ascorbic acid. Pursued this study with preformulation and determined melting point ( $190.3\pm 0.577$ ), solubility, preferably soluble in water and 7.4 Phosphate buffer solution. Drug identification studies were performed by UV spectrophotometer and FTIR. The  $\lambda_{max}$  of ascorbic was found at 260nm while during FTIR studies, all important peaks were observed, showed the authenticity of the drug. Drug and the excipient were found compatible. Nine formulations of emulsion were formulated by the dispersion method and they were evaluated for particle size diameter (138.71 nm), zeta potential (-37.2mV), optical microscopy, drug content ( $99.211\pm 0.202$ ) which was found within limit, of drug was, the globule size discovered by TEM and observed spherical in shape. Among all nine formulations, F5 showed its best results and it was used for further emulsion gel preparation. The Carbopol 934 p polymer was used with varies concentration from 0.5 to 2% for gel preparation and it was added into F5 formulation and noted that, while 1% polymer concentration was used, it has given the best release ( $99.778\pm 0.073$  in 6 hours), stability and viscosity.

**KEYWORDS:** Emulsion gel; stability; skin irritation; FTIR; TEM studies; drug release.

## 1. INTRODUCTION

Ascorbic acid (Vit. C) has a lot of physiological effects on the skin, like lowering melanin, increasing collagen production, lipids in the stratum corneum barrier, and reducing free radical oxidation, all of which are linked to the substance's well-known antioxidant properties [1-3]. It also improves the appearance of the skin by eliminating the anisotropic pattern on the surface and repairing wrinkles. In such research, it has been found that vitamin C helps in the synthesis of collagen fibres, which results in the removal of aging and dark spots from the skin surface. It also helps in increasing skin hydration, which makes the skin glow forever [4-5].

Therefore, vitamin C plays a significant role in skin ageing and should be examined as a component of topical formulations. Ascorbic acid formulation is an extremely difficult task since it is a highly unstable chemical that is freely soluble in water and undergoes oxidation when exposed to light and temperature, especially in an aerobic environment. In this way, the decomposition of ascorbic acid can take place in an alkaline medium spontaneously. it changes to the irreversible inactive compound 2,3-diketo-L-gulonic acid. To address this issue, fatty acid esters such as ascorbyl-palmitate and water-soluble esters such as magnesium ascorbyl phosphate are being used [6]. It has also been observed that ascorbic even loses its potency rapidly at room temperature and in dark conditions as well [7-9].

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Although, due to its oxidizing properties, it cannot be stored for an extended period of time. Aerobic oxidation, heat breakdown, and light all destroy vitamin C. The majority of vitamin C is chemically made up of dehydro ascorbic acid (DHAA). Emulsions (O/W) have been appealing and outstanding stable delivery mechanisms for active ingredient release on the skin for a long time. As a result, an emulsion (O/W) gel will be recommended as a good delivery method for ascorbic acid in our current investigation. The goal of this study is to develop and characterize an ascorbic acid emulsion gel formulation that increases ascorbic acid stability and reduces skin irritation [10-12].

## 2. RESULT AND DISCUSSION

The melting point of ascorbic acid was found to be in the range of  $190.30 \pm 577$ , which is equal to its pure form. Therefore, it shows that the drug is actually ascorbic acid and free from any impurities. The solubility of the drug in various solvents was tested in order to find the components that would be used in formulation development. The drug was tested using a UV Spectrophotometer set to 260 nm. After getting results, it was found that ascorbic acid is highly soluble in water, 7.4 phosphate buffer, ethanol and methanol [13]. The Ascorbic acid partition coefficient in n-octanol: water was determined to be  $-1.965 \pm 0.001$ . This indicates that the drug's slightly more hydrophilic side [14]. The result of the UV spectrum of ascorbic acid is shown in Fig. 1, which is scanned at 260 nm. The result indicates that the drug is pure and authentic. Ascorbic acid calibration curve with the regression equation  $Y = 0.0496x + 0.0474$  and an  $R^2$  value of 0.9994, indicating good linearity. The FTIR spectra of Ascorbic acid is shown in the Fig. 2.

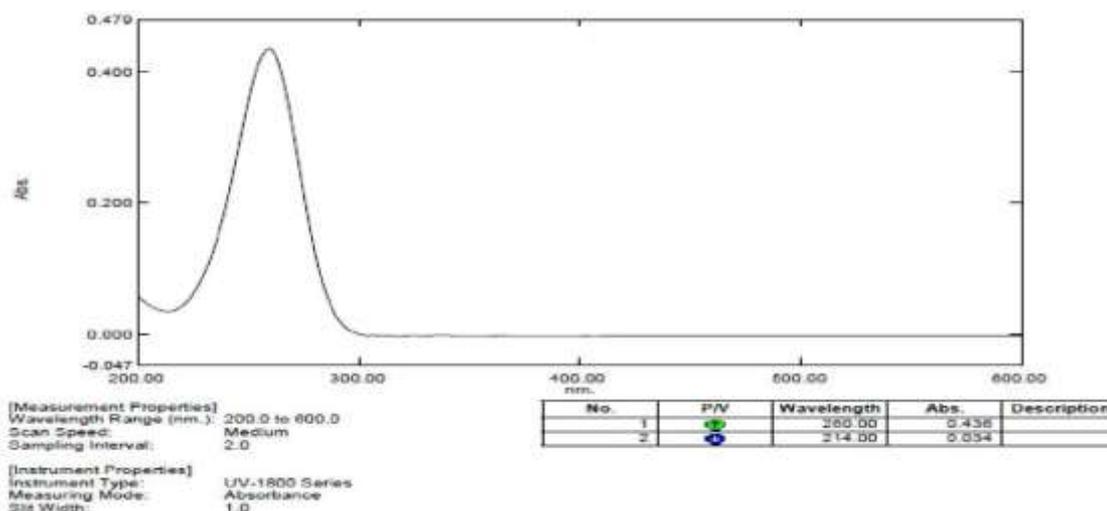


Figure 1.  $\lambda_{max}$  of pure drug ascorbic acid.

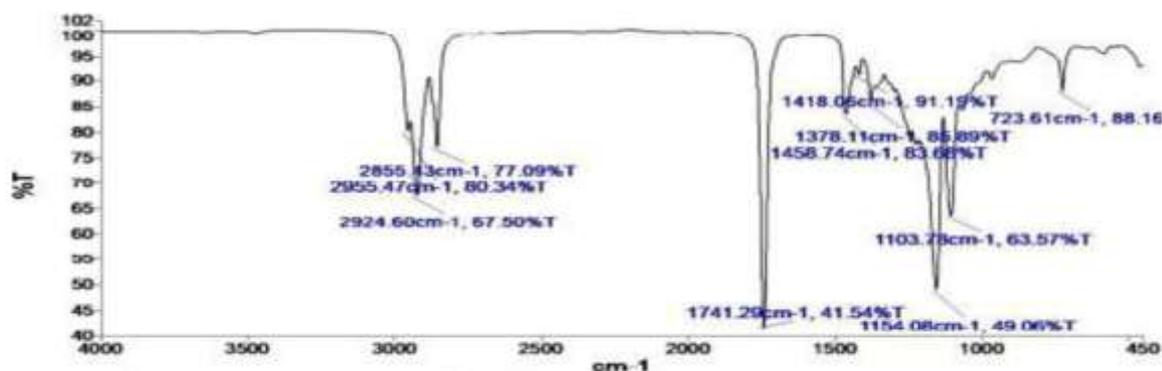


Figure 2. FTIR of pure drug ascorbic acid

The principal IR absorption peaks of Ascorbic acid at 3548.77 cm<sup>-1</sup> (O-H str.), 1750.47 cm<sup>-1</sup> (C=O str.), 1651.24cm<sup>-1</sup>(C=C str), 1103.68 cm<sup>-1</sup> (C-O-C str.) were all observed in the spectra of Ascorbic acid. This observation confirmed the purity and authenticity of the Ascorbic acid. The drug excipient compatibility also exhibits that there are no changes in major peaks, thus the drug is completely compatible with excipient, shown in Fig. 3.

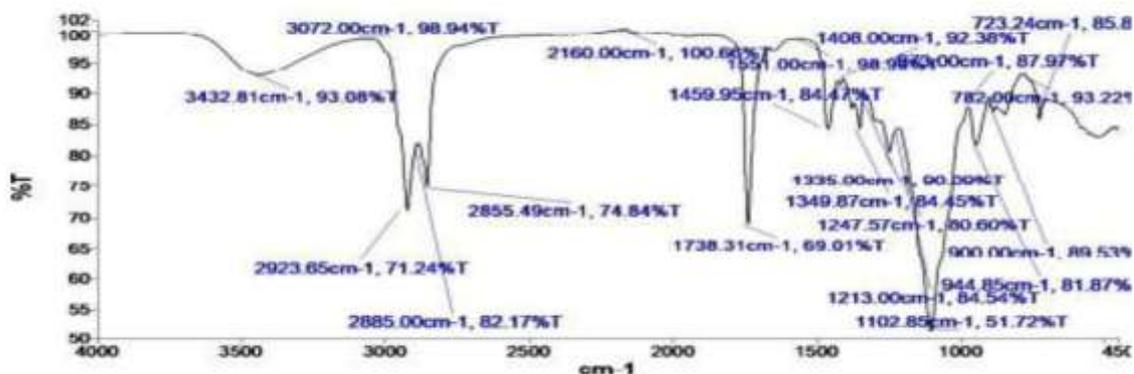


Figure 3. Drug -excipient compatibility studies

### 2.1 Evaluation parameters of emulsions

Prepared F1-F3 ascorbic acid emulsion formulations were evaluated for their appearance and it was found milky white while formulation F4-F9 was slightly yellow in color. Therefore, it has been discussed that while using light liquid paraffin as an oil phase, it gives a white color to the preparation, while using Glyceryl Monooleate, it gives a slightly yellow color. It has also been observed that when the emulsion was kept at different temperatures and storage conditions, like at 40°C (in the oven), 25 °C (in the oven) and 4°C (in the refrigerator), certain changes were observed at 25°C in the F1 to F3 preparations, like a distinct layer was clearly seen in the formulation, which indicates that drug particles were easily settled down at the bottom of the container, which is not a good sign for stability of the emulsion. While in the formulations F6, F7, and F8, creaking of the emulsion was observed. Therefore, formulations F1-F3 and F6-F8 were discarded for further studies.

The uniformity of content of Formulation F4, F5 and F9 was obtained as 95.591±0.116, 99.167±0.420 and 94.954±0.466 respectively. The pH value of formulation F4 was obtained as 8.4 which is more basic in nature while formulation F9 value is 5.6, more acidic in nature. In both the cases it produces skin irritation but formulation F5 has the pH value is 7.2, which is close to neutral value and skin pH as well. Therefore, F5 formulation is best among all formulations and considered for further studies. The all data are shown in Table no 1.

Table 1. Evaluation Parameters of Emulsions

| Parameters/<br>Formulations | Appearance      | Stability at |      |      | Content<br>Uniformity | pH value |
|-----------------------------|-----------------|--------------|------|------|-----------------------|----------|
|                             |                 | 4°C          | 25°C | 40°C |                       |          |
| F1                          | Milky white     | S            | NS   | S    | ~                     | ~        |
| F2                          | Milky white     | S            | NS   | S    | ~                     | ~        |
| F3                          | Milky white     | S            | NS   | S    | ~                     | ~        |
| F4                          | Slightly yellow | S            | S    | S    | 95.591±0.116          | 8.4      |

|    |                 |   |    |   |              |     |
|----|-----------------|---|----|---|--------------|-----|
| F5 | Slightly yellow | S | S  | S | 99.167±0.420 | 7.2 |
| F6 | Slightly yellow | S | NS | S | ~            | ~   |
| F7 |                 | S | NS | S | ~            | ~   |
| F8 |                 | S | NS | S | ~            | ~   |
| F9 |                 | S | S  | S | 94.954±0.466 | 5.6 |

- S= Stable Formulation NS= Nonstable formulation

The particle size and zeta potential of ascorbic acid in the F5 formulation was determined to be 138.71 nm and -37.2mV respectively, reveals that the particles are present in nano meter and having the uniform in size which reflects the stability of the emulsion and zeta potential indicates the repulsive forces are more strengthened than attractive forces, so that particle are not aggregated to each other resultant do not make any flocs, resultant not settle down quickly. Hence, it represents stability of emulsion. **Fig 4 and 5** represents the particle size and zeta potential of ascorbic acid.

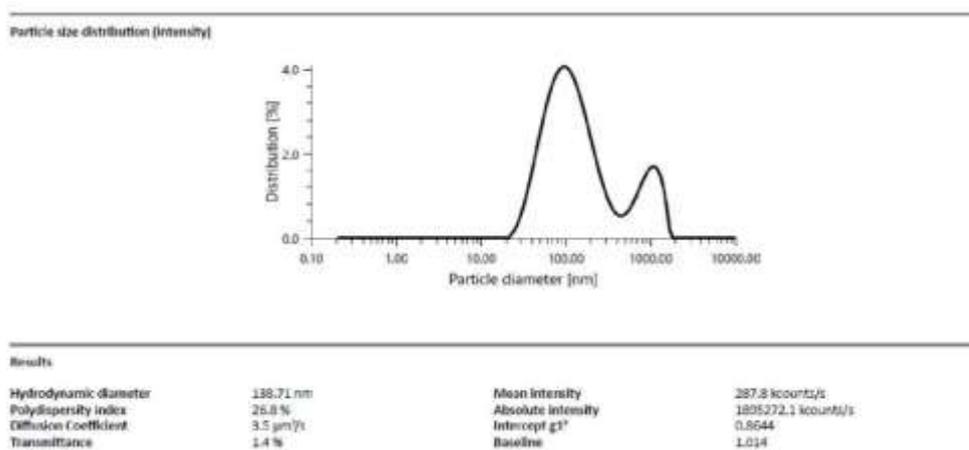


Figure 4. Particle size distribution

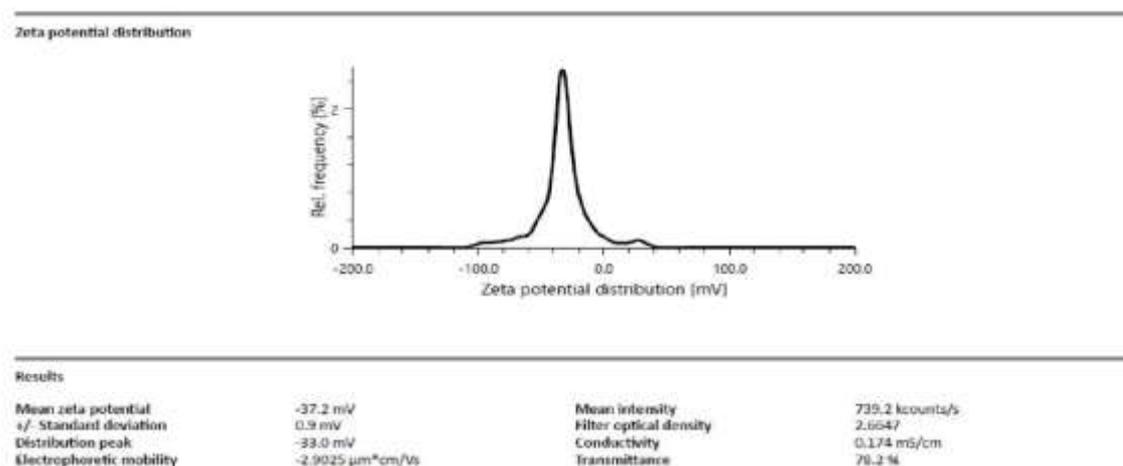


Figure 5. Zeta potential of ascorbic acid emulsion

The optical micrograph of F5 formulation revealed that oils droplets present in uniform shape without any aggregation, and representing the stability of emulsion which is shown in **Fig. 6**.

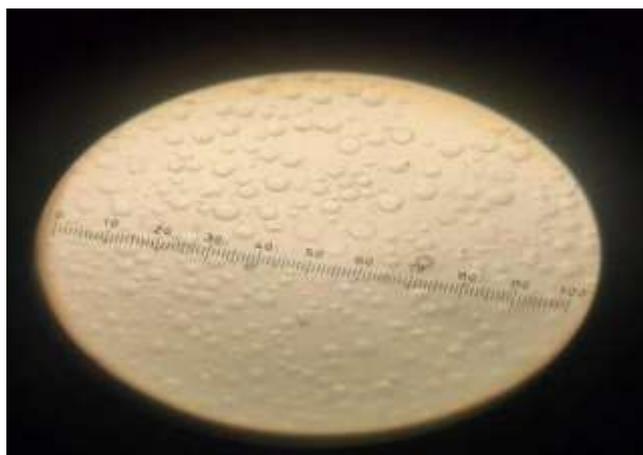


Figure 6. Optical microscopy of oil drops presents in emulsion

## 2.2 Transmission electron microscopy of emulsion

surface morphology of emulsion by TEM, in that way the dark patches in the picture are representing ascorbic acid emulsion and exhibit the size up to 100nm and it also is reflecting the stability of emulsion. The TEM study confirmed that the whole particles are presented in spherical shape, smooth and free from crystals, which is shown in Fig. 7.

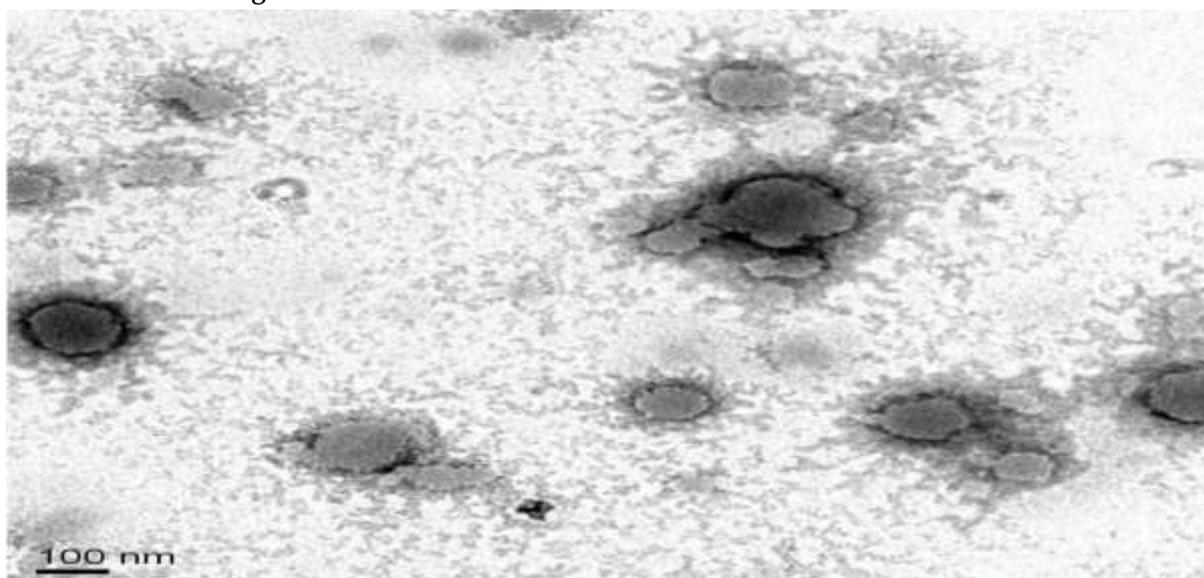


Figure 7. Transmission electron microscopy of emulsion

**2.3 Evaluation of Ascorbic Acid Emulsion Gel:-** Evaluation parameters of gel were carried out which are given as follows:-

### 2.3.1. Appearance

It was done by using visual observation of all gel formulation.

### 2.3.2. Drug content

Drug content of the gels was determined by dissolving an accurately weighed quantity of gel (about 0.1 gm) in about 10 ml of water. The solutions were transferred in micro centrifuge tube. The dispersion was centrifuge for 15 min. at 15000 rpm. After centrifugation the supernatant was collected and drug content of ascorbic acid gel was determined by taking 1ml of this solution in the 10 ml volumetric flask and final volume was made by water. Finally, absorbance of prepared solution was measured at 260nm using UV visible

spectrophotometer. The percentage drug content is calculated [15].

### 2.3.3. pH of Emulsion gel

pH of the gel was measured by a pH meter, in which 0.1g gel was dispersed in 10 ml distilled water and stored it for next 2 hours at constant temperature. After that, at room temperature, pH electrode was inserted in to the gel 10 min prior to measure the pH of emulsion [15].

### 2.3.4. Spreadability

The gel's spreadability was determined using the following method: On a glass plate, 2g gel was placed within a 2 cm pre-marked circle, which was then covered with a second glass plate. For 5 minutes, a weight of 500 g was permitted to rest on the upper glass plate. The spread of the gels resulted in an increase in diameter [16].

### 2.3.5. Viscosity

A viscometer was used to determine the gel's viscosity. For this process, spindle L4 was used which was allowed to rotate at 24 rpm and 25°C. The viscosity was determined as against the speed and the dial reading that corresponded to it was noted. which indicates the viscosity of a gel [16].

### 2.3.6. In-vitro drug release study

In-vitro study of emulsion was performed using Franz's diffusion cell. It consists two chambers, one is donor and another receiver. The donor chamber was filled with fresh phosphate buffer solution, between the two chambers, an appropriate diffusion area was positioned, and below this, a receiver chamber was located. During the experiment, the mixture was continually stirred with a magnetic bar at 100 rpm while simultaneously receiving the solution in the receiving chamber, which was diffusing through the membrane. Now placed 1 g of ascorbic acid gel over the membrane, the fresh solution of 7.4 pH phosphate buffer was filled into the donor chamber, where it diffused slowly through the membrane and received into the receiver chambers, which were continuously stirred. Further, the sample was taken out of the receiver chamber at a specified time interval and the same quantity was replaced by fresh 7.4 phosphate buffer to maintain the sink condition. The sample was analyzed through a double beam UV Spectrophotometer at 260nm. The *in-vitro* release data were used to correlate the drug release kinetics studies like, zero order, or first order, Higuchi's model, or Korsmeyer-Peppas model [15-17].

## 3. RESULT AND DISCUSSION

### 3.1 Physical appearance

The prepared gel formulation F5(P2) containing 1% Carbopol 934P and F5(P3) containing 1.5 % Carbopol)) presented good homogeneity with absence of lumps and selected for further study. The pH value of formulation F5(P2) & F5(P3) Found  $7.410 \pm 0.010$  &  $7.417 \pm 0.012$  respectively, the pH value near to skin pH hence, the formulation is showing good compatibility, and stability and no skin irritation. The spreadability of formulation F5(P2) and F5(P3)) was found to be in the range from  $7.340 \pm 0.010$ ,  $7.460 \pm 0.006$ , which is indicating good spreading ability of gel over the skin surface. The viscosity of formulation F5(P2) and F5(P3)) was found to be in the range of  $8383 \pm 4.93$ ,  $10836 \pm 3.00$  Cp respectively, exhibit a good viscosity. It indicates that the gel will easily pour through the container and will retain on skin for long duration of time. The drug content for both the formulation F5(P2) and F5(P3), was Found  $99.831 \pm 0.202$  percent and  $97.040 \pm 0.202$  percent respectively so that, both the formulations are exhibiting good drug content as per its official standard which are shown in **Table 2**.

**Table 2.** Evaluation parameters of emulsion loaded gel

| Formulation/<br>Observations | Physical<br>Appaerence | pH           | Spreadability | Viscosity<br>(CP) | Drug Content |
|------------------------------|------------------------|--------------|---------------|-------------------|--------------|
| <b>F5(P1)</b>                | Lumps                  | 6.9210±0.010 | 6.740±0.010   | 7486±4.93         | 98.623±0.235 |
| <b>F5 (P2)</b>               | Homogenetity           | 7.410±0.010  | 7.340±0.010   | 8383±4.93         | 99.831±0.202 |
| <b>F5 (P3)</b>               | Lumps and Floccs       | 7.417±0.012  | 7.460±0.006   | 10836±3.00        | 97.040±0.315 |

### 3.2 In-vitro drug release study

The in-vitro drug release data of formulations are given in **Table 3**.

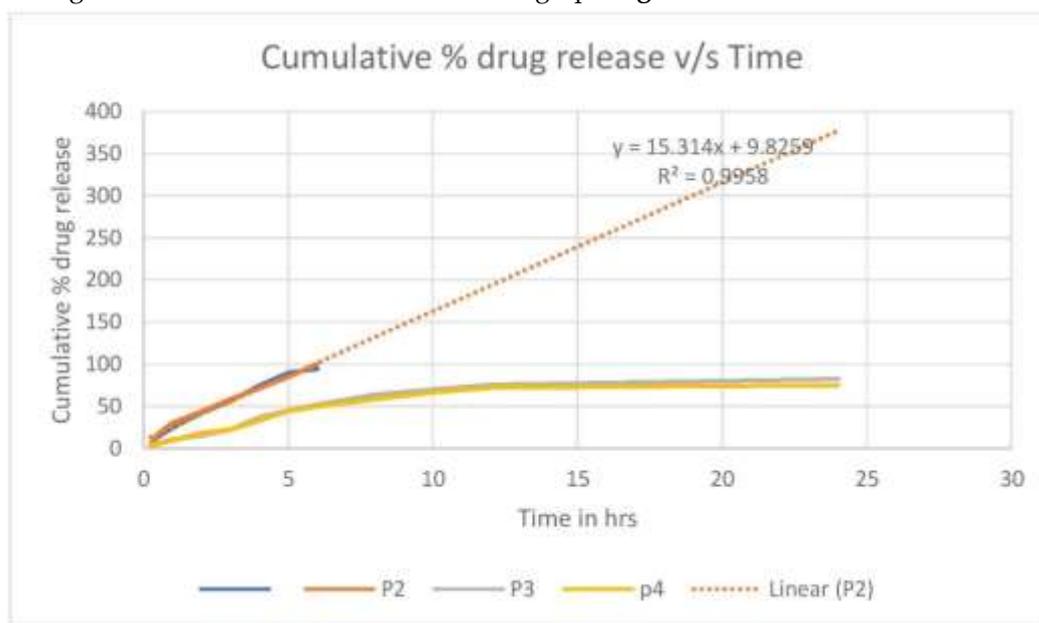
**Table 3:** Cumulative % Drug Release v/s Time

| Time<br>(hrs) | Cumulative<br>% Release of F5(P1) | Cumulative<br>% Release of F5(P2) | Cumulative<br>%Drug Release of F5(P3) |
|---------------|-----------------------------------|-----------------------------------|---------------------------------------|
| 0.25          | 4.764±0.021                       | 6.064±0.032                       | 2.816±0.073                           |
| 0.5           | 14.002±0.036                      | 17.024±0.115                      | 6.268±0.073                           |
| 1             | 24.413±0.076                      | 30.514±0.215                      | 10.663±0.036                          |
| 2             | 42.55±0.055                       | 43.587±0.047                      | 14.961±0.036                          |
| 3             | 56.04±0.554                       | 58.341±0.421                      | 22.510±0.055                          |
| 4             | 74.363±0.554                      | 71.234±0.541                      | 36.643±0.055                          |
| 5             | 89.444±0.554                      | 85.153±0.631                      | 45.040±0.554                          |
| 6             | 94.524±0.755                      | 99.778±0.073                      | 51.911±0.554                          |
| 8             | -                                 | -                                 | 63.903±0.554                          |
| 10            | -                                 | -                                 | 69.685±0.363                          |
| 12            | -                                 | -                                 | 75.677±0.363                          |
| 24            | -                                 | -                                 | 82.302±0.363                          |

**Table 4** depicts that among all the formulations, formulation F5(P2) exhibiting its best releasing pattern where it is released over 99% of drug within 6 hours. While other formulation like, F5(P1) released 94.524±0.755 and after that there is no significant enhancement in its release amount of drug, whereas other formulation like, F5(P3) and F5(P4) exhibiting only 82.302±0.363 and 75.145±0.363 in 24 hours, thus it is indicating very slow and poor release of drug in terms of time taking and amount of drug release.

It has also been observed that the release of drug is not depending on concentration of drug; therefore, it is known as zero order release kinetics. Among all the formulations, F5(P2) is exhibiting excellent release with

linear pattern and release over 99% drug in six hours which is illustrate the zero-order release. Percentage cumulative drug release of all formulation is shown in graph **Fig. 8**.



**Figure 8.** Cumulative % drug release vs time

Other kinetic studies like, first order, Higuchi and Kosmiyar-pappas were also studied according to their release pattern and  $R^2$  value. The graphs were plotted of all release kinetics which revealed that the Higuchi model was exhibiting constant release in a linear manner ( $R^2$ ) which indicating that the drug release from spherical surface. Overall, it can be said that was following zero order and Higuchi model.

In order to compare all three release studies, in each study the value of  $R^2$  is being decided the pattern of drug release. In all cases the value of  $R^2$  is very close to 100% but in zero order and Higuchi model the value of  $R^2$  is 0.9958 and 0.9909 respectively, reflects that the drug is released in both the kinetic model in more linear manner and compared to first order and Kosmeyer-Peppas model i.e., the release is not dependent on concentration of drug and Higuchi model illustrates that the drug is released from spherical shape particle through diffusion mode.

### 3.3. Stability Studies

Accelerated stability investigations for the optimized, chosen F5 (P2) formulation were carried out for a period of three months at a temperature of 40 °C and a relative humidity of 75 percent relative humidity. The gel was maintained in aluminum tubes for around 3 months throughout the stability experiments, and it was evaluated for its physicochemical characteristics, physical appearance, and antifungal activity using the same procedures that were used for the assessment of formed gels, as previously described.

## 4. CONCLUSION

The presented research work exhibited that the preformulation parameters like, solubility, viscosity pH,  $\lambda_{max}$ , FTIR studies, Compatability studies were found as per standard parameters, The method for prepration, ingredients used in the formulation were novel and having good quality of emulsification. The  $\lambda_{max}$  of ascorbic was measured at 260nm which is very closed to its official value, indicates the authenticity of drug. The particle size, zeta potential, transmission electron microscopy (TEM), all studies were supporting the stability of emulsion. The release study of drug has also shown the best result in which formulation F5(P2) , concentration 1%of Carbopol 934p polymer exhibited the best release with  $99.778 \pm 0.073$  in six hour. The  $R^2$  was of zero order and Higuchi model found to be 0.9909 and 0.9726 respectively, illustrate release pattern of drug is zero order and higuchi model. The stability studies of emulsion gel formulation F5(P2) was aslo exhibited very stable on its accelerated parameters. Hence, at the end it can be concluded that the formation

of ascorbic acid emulsion gel very stable and no irritant effect to skin and may have a wide scope at a industrial level scale up in upcoming future.

## 5. MATERIALS AND METHODS

Reckon Organics Pvt. Ltd. in Gujarat donated ascorbic acid. Glyceryl Monooleate (Capmul GMO-50 EP/NF), Glycerol Tricaprylate (Captex 355 EP/NF/J), and Lauroyl Macroglycerides (Acconon MC8-2 EP/NF) were gifted by Abitec Cooperation. Sodium Hydroxide, Hydrochloric Acid, Methanol, Chloroform, and Acetone were purchased from Fisher Scientific India Pvt. Ltd. Carbopol 934P (Lubrizol Advanced Material Europe BVBA). All the other materials used were of laboratory grade.

The pre formulation studies of powder were carried out and various physio chemical parameters were evaluated in order to established purity and authenticity like, organoleptic melting point partition coefficient etc. The organoleptic property of drug was carried out in terms of determination of appearance, color and odor [18–20].

### 5.1 Melting point determination

The melting point is used for the identification of the drug. It gives an indication of the drug's authenticity, whether it is pure or not. If the sample drug has the same melting point as its standard drug, it indicates the drug is genuine and in pure form. The melting point is determined by a melting point apparatus. The pure drug is taken in a thin-walled capillary tube that is 10-15 mm long and 1 mm in diameter, and the other end of it is sealed so that the drug cannot pass through it. For determination of the melting point, the capillary tube was placed in the melting point apparatus and allowed to heat until the drug was changed into liquid form. That specific temperature was noted as the melting point of the drug. This temperature is compared with that of the standard drug melting point and analyzed [21].

### 5.2 Solubility of Ascorbic Acid

In the determination of solubility, all tubes were thoroughly cleaned, poured 1 ml of solvents like Methanol, Ethanol, Acetone, Chloroform, 0.1N HCl, water, Phosphate buffer pH 7.4, oils (Capmul GMO-50 EP/NF, Capmul MCM-EP/NF, Capmul PG-2L EP/NF, light liquid paraffin), surfactant (Captex 355 EP/NF/J, Tween 80), glycerol, in separate test tubes, then add an excess amount of ascorbic acid to each test tube for quantitative estimation. All test tubes were closed tightly to avoid any kind of environmental reaction. Furthermore, all test tubes were placed over a water bath shaker at normal environmental conditions for 24 h. After proper mixing of the substance, it was centrifuged at 15,000 rpm for 15 minutes. Now, the supernatant liquid has accumulated on the surface, and it was filtered and analyzed by UV/visible spectrophotometrically at 260 nm [22].

### 5.3 pH determination

The pH of the emulsion mixture was measured by a digital pH meter. Dip the glass electrode entirely into the emulsion system to cover the electrode. The measurement was done three times, with the average of the three measurements being recorded [23].

### 5.4 Partition coefficient of drug

It is the proportion of the distribution of unionized drugs to ionized drugs at equilibrium between the organic and aqueous phases. The partition coefficient can be used to determine if a substance is lipophilic or hydrophilic. Drugs with P values larger than 1 are classified as lipophilic, while those with less than 1 are classified as hydrophilic. As shown in **equation (1)**, an organic phase of n-octanol and water is typically used to calculate the partition coefficient.

$$P_{o/w} = \frac{C_{n\text{-octanol}}}{C_{\text{water}}} \dots \dots \dots (1)$$

As a result, the partition coefficient ( $P_{o/w}$ ) is the quotient of two drug concentrations, i.e., in n-octanol ( $C_{n\text{-octanol}}$ ) and water ( $C_{\text{water}}$ ), and is commonly expressed as a logarithm to base 10 ( $\log P$ ). The shake flask method was used in the partition coefficient determination study. An excess amount of ascorbic acid was dissolved in 10 ml of a mixture of two solvents (1:1) and kept aside for 24 hours. The two layers were separated

out, and they were centrifuged for 15 minutes at 15,000 rpm. After proper dilution, the absorbance was estimated by a UV spectrophotometer at 260 nm [24].

## 5.5 Drug Identification

### 5.5.1. UV/Visible Spectrophotometer

**λmax of drug:** - The Drug Partition Coefficient

The max of a drug is determined by a UV/Visible spectrophotometer where the ascorbic acid solution containing 8 g/ml was placed in the UV/visible region. It absorbed the specific wavelength of light that was passing through it and scanned in the range from 200 to 400 nm. An automatic curve is generated between absorbance and wavelength, representing the UV spectrum. The max of ascorbic acid was determined at 260 nm, which is very close to its original 260 nm maximum wavelength [25].

### 5.5.2 FTIR of Ascorbic acid

Any substance or drug FT-IR (Fourier Transform Infrared spectrum) gives knowledge about the basic moiety present in that compound. The structure of the medication and excipients was investigated using FT-IR spectroscopy [26].

## 5.6. Drug-excipients Compatibility Study by FTIR

FTIR was used to determine the drug-excipient compatibility in a ratio of 1:1. The drug and other excipients were thoroughly blended. In order to determine the spectra of pure drugs and drug-excipients, FTIR was used to scan the samples in the 4000-400 cm<sup>-1</sup> range. The spectra of a pure drug and a drug containing excipients were compared [26].

## 5.7. Preparation of Ascorbic Acid Emulsion

Ascorbic acid was used as a drug, light liquid paraffin and glyceryl monooleate as an oil phase, Tween 80 and tricaprlylate as surfactants, and glycerol lauroyl macroglycerides as a co-surfactant, and water was used to create a binary phase [27]. The nine formulations were prepared as per the given quantity in **Table No. 4**. In a high-speed homogenizer, the surfactant and the water-Lauroyl Macroglycerides phase were mixed for 1 min. at 10,000 rpm to create each emulsion formulation (Remi Instruments, In.). At the same time, the system was emulsified for 5 minutes. At the same pace, the final volume was made up with water [27].

**Table 4. Formulation of Ascorbic Acid Emulsion**

| Sr. No. | Formulation Code | Drug (mg) | Oils                       |                          | Surfactant   |                            | Co-Surfactant |                              | Water (ml) |
|---------|------------------|-----------|----------------------------|--------------------------|--------------|----------------------------|---------------|------------------------------|------------|
|         |                  |           | Light Liquid Paraffin (ml) | Glyceryl Monooleate (ml) | Tween80 (mg) | Glycerol Tricaprylate (mg) | Glycerol (ml) | Lauroyl Macroglycerides (ml) |            |
| 1       | F1               | 100       | 4                          | ~                        | 50           | ~                          | 10.90         | ~                            | 5.10       |
| 2       | F2               | 100       | 5                          | ~                        | 50           | ~                          | 10.90         | ~                            | 4.10       |
| 3       | F3               | 100       | 6                          | ~                        | 50           | ~                          | 10.90         | ~                            | 3.10       |
| 4       | F4               | 100       | ~                          | 2                        | ~            | 50                         | ~             | 10.90                        | 7.10       |
| 5       | F5               | 100       | ~                          | 3                        | ~            | 50                         | ~             | 10.90                        | 6.10       |
| 6       | F6               | 100       | ~                          | 4                        | ~            | 50                         | ~             | 10.90                        | 5.10       |
| 7       | F7               | 100       | ~                          | 5                        | ~            | 50                         | ~             | 10.90                        | 4.10       |
| 8       | F8               | 100       | ~                          | 6                        | ~            | 50                         | ~             | 10.90                        | 3.10       |
| 9       | F9               | 100       | ~                          | 7                        | ~            | 50                         | ~             | 10.90                        | 2.10       |

**5.8 Evaluation of Ascorbic Acid Emulsion (Oil-In-Water):** - The following parameters were evaluated to ensure the quality of emulsion.

### 5.8.1 Visual appearance

To determine the visual appearance such as color, phase separation, and creaming, the emulsion was stored at 37 C 2 for 5 weeks and the results were recorded [27-28].

### 5.8.2 pH of ascorbic acid emulsion

The pH of the emulsion mixture was measured by a digital pH meter. Dip the glass electrode entirely into the emulsion system to cover the electrode. The measurement was done three times, with the average of the three measurements being recorded [29].

### 5.8.3 Drug content of optimized gel formulation

For determination of drug content in the emulsion, it was centrifuged at 15000 rpm for 15 minutes. After completion of this process, the supernatant liquid was separated from the emulsion by filtration very cautiously and diluted from 1 to 10 ml with water in a volumetric flask and analyzed by UV spectrophotometer at 260nm. Each formulation batch was tested in triplicate for drug content [30-31].

### 5.8.4 Microscopic appearance of emulsion

The microscopic appearance of an emulsion was determined by a drop of emulsion (o/w) being placed on a glass slide and covered with a glass cover under a microscope and observing it through highly magnified lenses [32].

### 5.8.5 Determination of particle size and zeta potential

Particle size, diameter, vesicle properties, and zeta potential are important aspects of an emulsion in terms of its stability, and these parameters were determined at room temperature by the Zeta Potential/Particle Sizer analyzer. All formulations were diluted with water to a certain extent as per the needs of the Zeta potential analyzer. The Zeta potential and particle size were determined [33].

### 5.8.6 Transmission electron microscope (TEM)

The morphology of an emulsion is determined by transmission electron microscopy (TEM). A drop of sample was dropped onto a carbon-coated copper grid with a diameter of 400 nm and allowed to dry at room temperature. After that, a phosphotungstic acid solution was used to color it. The sample was examined under the microscope at 80000x magnification with a 100 kV acceleration voltage after it had dried fully [34].

### 5.8.7 Preparation of ascorbic acid emulsion gel

It was evaluated that formulation (F5) is the best formulation among all and selected for further studies for topical gel formulation. Keeping in mind Carbopol 934p (0.5 percent, 1 percent, 1.5 percent, and 2 percent w/ w) was dispersed in distilled water to make the gel formulation. In addition, the dispersed solution was kept aside for a period of 24h to allow for complete swelling of the polymer. Once the swelling was completed, the solution was neutralized with sodium hydroxide solution by adding it dropwise into the gel to maintain the pH of the formulation. In the next step, the Ascorbic acid emulsion formulation was added into preformed gel-base slowly with gentle stirring to get homogenous gel. The developed topical gel was evaluated for various parameters [35-37].

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