



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Review Article

Formulation and Evaluation of Emulgel from Carica Papaya Seeds Extract

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ARTICLE INFO

Published: 25 Apr 2026

Keywords:

Emulgel, Ethanolic extract, Soxhlet extraction, Hydrophobic drugs, 2,3,4-trihydroxytoluene and (glyceryl-1-2',3',4'-trihydroxybenzoyl)- 2,3-dioleate.

DOI:

10.5281/zenodo.19759239

ABSTRACT

Herbal or plant-based remedies have been used to prevent and cure illnesses since ancient time and many more elements of these natural origins are still to be discovered. Emulgel is a new technology in revolutionary drug delivery system that allows for controlled release of emulsion and gel for topical application.[51] When an emulsion is mixed into a gel, it becomes more stable. Papaya seeds extract shows antifungal, anthelmintic, antimicrobial, and many more activities that has been used to treat various type of infections. Emulgel is the promising drug delivery system for the delivery of hydrophobic drugs. Emulgel, an interesting topical drug delivery system and has dual release control system, i.e., gel and emulsion. Emulgel has several merits like greaseless, easily spreadable, easily removable, emollient and transparency. Extracts from different papaya tissues have been shown to be bioactive. Aqueous extracts of leaves and seeds are known to have antifungal, anthelmintic, antimicrobial activity against the infectious microorganism. 2,3,4-trihydroxytoluene and (glyceryl-1-2',3',4'-trihydroxybenzoyl)- 2,3-dioleate are the main phytoconstituents shows antifungal activity. The phytochemical assay of ethanoic extract of papaya seed shows the presence of alkaloids, flavonoids, steroids, polyphenols, tannins and saponins. From this, we can conclude that ethanoic extract of papaya seeds has a good potential against various infections as mentioned above.[1] The bio-efficacy of Carica papaya is predominantly to its active phytoconstituents- papain, an enzyme contained in the fruit and stem latex, vitamins, such as vitamin- C and B complex, minerals such as- calcium, phosphorus, and iron, polysaccharides, alkaloids, saponins, flavonoids, and phenolic acid. In this paper, we discussed about the various therapeutic activities of Carica papaya seeds extract and its crucial role in the formulation of “Emulgel- a novel approach to formulation development.” [49]

INTRODUCTION

ANATOMY OF SKIN:

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



The skin is the largest organ in covering the body. It acts as a barrier, preventing the penetration of foreign molecules into the body and the loss of water from the body.^[50] The skin is made up of three distinct layers: the outermost being the epidermis, followed by the dermis and hypodermis (includes subcutaneous fat). The stratum corneum forms the outermost hydrophobic layer of the epidermis, with 10–30 μm thickness, and acts as a prominent barrier for skin permeation. The barrier property of the stratum corneum is due to its extreme lipid component and corneocytes, which are filled with keratin filaments and filaggrin. The corneocytes are embedded in the dense structure of the multilamellar lipid, comprised of lipid-like sterols, phospholipids, and glycosphingolipids (ceramides). The corneocytes embedded in the lipid matrix are well-described as a brick and mortar model.^[1,2]

EPIDERMIS:

It is a stratified squamous epithelium layer, which is composed primarily of two types of cells: dendritic and keratinocytes cells.^[3]

It is the outermost layer of skin. Stratum corneum layer forms permeability barrier to external environment. Stratum corneum contain water

around 20%. The moisture required for Stratum corneum is about 10% to maintain flexibility and softness. It consists of ceramides and neutral lipids such as steroid, free fatty acid and triglycerides. The epidermis acts like armor to protect body from harm, including ultraviolet (UV) radiation, pathogens (bacteria, viruses, fungi and parasites) and chemicals. The epidermis is the most superficial layer of skin and behave like a primary barrier of protection from the invasion of substances into the body.^[4]

Epidermis Epithelium tissues is made up of 5 layers:

1. Layers of Epidermis

- Stratum corneum
- Stratum lucidum
- Stratum granulosum
- Stratum spinosum and
- Stratum germinativum

2. Cells of the Epidermis

- Keratinocytes
- Melanocytes
- Langerhans' cells
- Merkel's cell

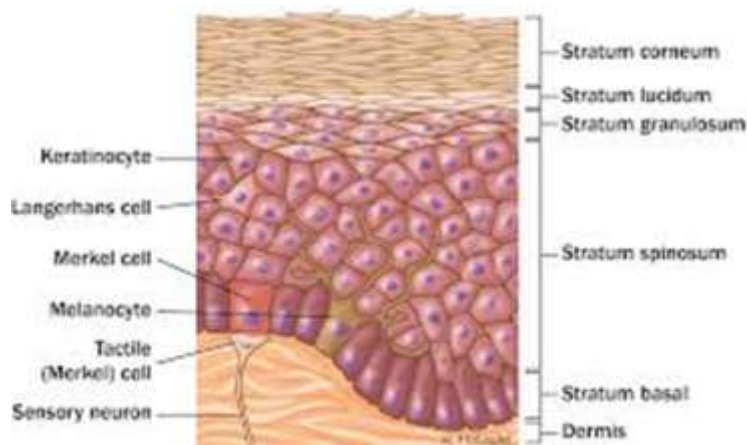


Fig.1. Layers of skin

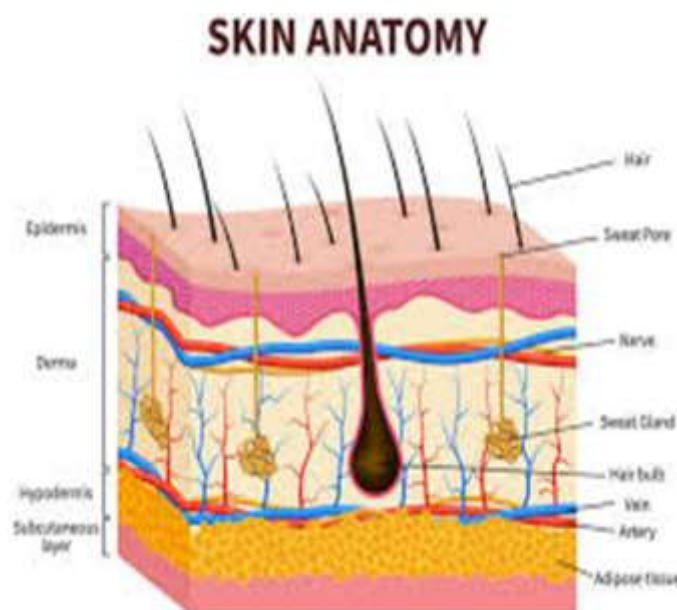


Fig.2. Anatomy of skin [5]

DERMIS :

The dermis is the layer of skin just 3-5 mm below the epidermis and is made up of a matrix of connective tissue, containing blood vessels, lymph and nerves. The skin's blood supply has an essential function in regulating body temperature. It also delivers nutrients and oxygen to the skin, and removes toxins and waste. The capillaries reach within 0.2 mm of the skin surface and facilitate the escape of most of the molecules to penetrate the skin barrier. Thus, the blood supply maintains very low topical concentrations, and the difference in concentrations across the epidermis provides the essential driving force for topical penetration. For topical drug delivery system, this layer is generally considered to be mainly composed of water and thus providing a minimal barrier to the use of most polar drugs, although the skin barrier may be important while using highly lipophilic molecules .^[6]

Once a drug molecule passes the stratum corneum, it passes the deeper epidermal tissues and enters into the dermis. It is mainly made of fibrous tissues and is 1-2 mm thick. The dermis has a rich supply of blood vessels from where the drug gets

absorbed into the general circulation. Sebaceous glands, sweat glands, and hair follicles rise to the surface of the skin from the dermis and subcutaneous layer where they originate. The skin surface of human is recognized to contain an average of 10-70 hair follicles and 200-250 sweat glands on every centimetre square of the skin area. ^[7]

The dermis consists of the following sublayers,

- Papillary layer
- Reticular layer

HYPODERMIS:

The dermis or subcutaneous fatty tissue supports the dermis and epidermis. It serves as a fat storage area. This layer provides temperature regulation, nutritional support, and mechanical protection. It carries major blood vessels and nerves to the skin and may contain sensory pressure organs. In order for a drug to be delivered through the skin, it must penetrate all three layers and reach the circulatory system. ^[8]

PHYSIOLOGY OF SKIN

The topical preparations are meant to be applied to the skin. Hence, a basic knowledge of the skin and its physiology function are very important for designing topical dosage form. The skin of an average adult body covers a surface area approximately 2m² and receives about one-third of the blood circulating through the body. An average human skin surface is known to contain, on the average 40–70 hair follicles, and 200–300 sweat ducts on every square centimetre of the skin. The pH of the skin varies from 4 to 5.6. Sweat and fatty acid secreted from sebum influence the pH of the skin surface. The skin can be considered to have four distinct layers of tissue. [9]

Non-viable epidermis: Stratum corneum is the outermost layer of skin, which is the actual Physical barrier to the most substance that comes in contact with the skin. The stratum Corneum is 10–20 cell layer thick over most of the body. Each cell is a flat, plate like structure 34–44 µm long, 25–36 µm wide, and 0.5–0.20 µm thick with a surface area of 750–1200 µm² Stocked up to each other in brick-like fashion. Stratum corneum consists of lipid (5–15%) Including phospholipids, glycosphingolipid, cholesterol sulphate, and a neutral lipid, protein (75–85%) which is mainly keratanin. [10]

Viable epidermis: This layer of the skin resides between the stratum corneum and dermis and has a thickness ranging from 50 to 100 µm. The structures of the cells in the viable epidermis are physicochemically similar to other living tissues. Cells are held together by Tonofibrils. The density of this region is not much different than water. The water content is about 90%. **Dermis** Just beneath the viable epidermis is the dermis. It is structural fibrin, and very few cells are like it can be found histological in normal tissue. Dermis thickness ranges from 2000 to 3000 µm and consists of a matrix of loose connective tissue composed of fibrous protein embedded in an amorphose ground substance. [11]

Subcutaneous connective tissue: The Subcutaneous tissue or hypodermis is not actually considered a true part of the structured connective tissue which is composed of loose textured, white, fibrous connective tissue containing blood and lymph vessels, secretory pores of the sweat gland, and cutaneous nerves. Most investigators consider drug is permeating through the skin enter the circulatory system before reaching the hypodermis [11].

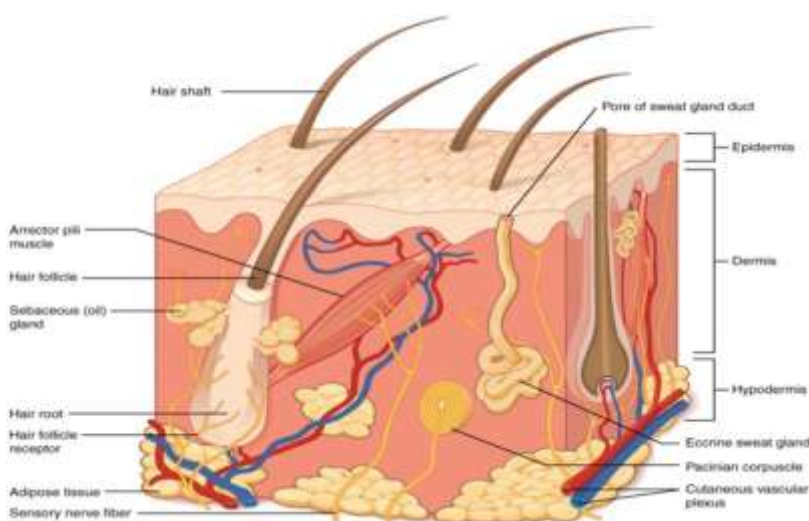


Fig.3. Physiology of skin

PENETRATION THROUGH SKIN:

Once the product is applied on the skin, a complex interaction occurs between the formulation, the active compounds, and the skin itself. The penetration of the active compound in the skin follows Fick's first law of diffusion, which describes the transfer rate of solute as a function of the concentration of the various ingredients, the size of the treatment surface area, and the permeability of the skin. However, the skin's permeability can be influenced by many factors, such as the drying, moisturizing, or occluding effects of the excipients in the formulation, which in combination, can modulate the release of the product at the treatment site. $J = -D \cdot \{dC/dx\}$ Where, J is Flux D is the diffusion coefficient of the drug dC/dx is the concentration gradients.^[12]

pH of skin: Normal range of pH of skin is 5.4 to 5.9 Formulation with high or low pH can harm the skin. Therefore, the moderate pH value is suitable for the topical delivery. The degree of ionization at particular pH also plays an important role.^[13]

ROUTE OF DRUG PENETRATION THROUGH SKIN:

At the skin, molecules contact cellular debris, microorganism, sebum, and other materials, which negligibly affect the permeation. The penetrant has three potential pathways to the viable tissue through hair follicles with associated sebaceous glands, via sweat ducts, or across continuous stratum corneum between these appendages. The route usually contributes negligibly to steady state drug flux. This pathway may be important for ions and large polar molecules that struggle to cross intact stratum corneum. Appendages may be providing shunts, important at short times prior to steady state diffusion. Additionally, polymers and colloidal particles can target the follicle. The intact stratum corneum thus provides the main barrier; its

'Brick and Mortar' structure is analogous to a wall. The coenocytes of hydrated keratin comprise of 'Bricks', imbedded in 'Mortar', composed of multiple lipid bilayers of ceramides, fatty acids, cholesterol and cholesterol esters. These bilayers form regions of semi crystalline, gel and liquid crystals domains. Most molecules penetrate through skin via this intercellular micro route and therefore many enhancing techniques aims to disrupt or bypass elegant molecular architecture. Viable layers may metabolise a drug, or activates a prodrug. The dermal papillary layers is so reach in capillaries that most penetrant clear within minutes. Usually, deeper derma regions do not significantly influence absorption, although they may bind e.g. testosterone, inhibiting its systematic removal.

AN OVERVIEW OF EMULGEL:

Topical drug delivery system is the dosage form which is administered on the skin and other routes of drug delivery get failed or for skin disorders. The topical drug delivery system has the advantage of negotiating the first pass metabolism. It also helps to avoid the risk and inconvenience of i.v. route therapy. ^[15] Topical formulations are prepared in different consistency such as solid, semisolid, and liquid. The topical delivery system is failed in the administration of hydrophobic drug. In each formulation with the active ingredients many excipients are used. Sometimes more than one formulation can be combined to enhance the drug delivery; emulgel is such type of combination. It is the combination of emulsion and gel.^[15,16] Emulgel is prepared both in oil- in- water and water- in oil type emulsion mixed with gel. Oil- in- water type is used for lipophilic drugs and water- in- oil type is used for hydrophobic drugs' delivery ^[15,17]. The emulgel have many advantages like thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, bio-



friendly, pleasing appearance, transparent and cosmetically acceptable, which also have a good skin penetration and long shelf- life. [15,18]

The emulsion and gel preparations have their own properties. But the gels show some limitations as

hydrophobic drug delivery. This limitation is overcoming by emulgel. By the use of gelling agent classical emulsion can be converted into emulgel. [19]

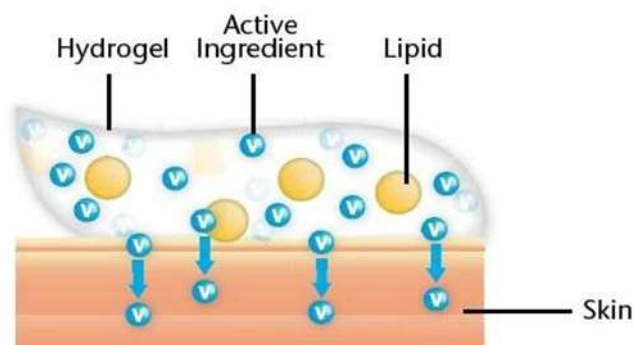


Fig.4. Structure of Emulgel

ADVANTAGES AND DISADVANTAGES OF EMULGEL:

ADVANTAGES :

- Incorporation of hydrophobic drugs
- Better loading capacity
- Better stability
- Controlled release
- No intensive sonication
- Avoiding first pass metabolism
- Avoiding gastrointestinal incompatibility
- More selective for a specific site
- Improved patient compliance
- Convenient and easy to apply

DISADVANTAGES :

- Skin irritation on contact dermatitis
- The possibility of allergenic reactions
- The poor permeability of some drugs through the skin
- Drugs of large particle size are not easy to absorb through the skin
- The occurrence of the bubble during formulation of emulgel.

Factors Affecting Topical Absorption of Drug :

(i) Physiological Factors:

1. Skin thickness
2. Lipid content.
3. Density of hair follicles.
4. Density of sweat glands.
5. Skin pH.
6. Blood flow.
7. Hydration of skin.
8. Inflammation of skin

(ii) Physiochemical Factors:

1. Partition coefficient.
2. Molecular weight (less than 400 Dalton)
3. Degree of ionization (only unionized drugs gets absorbed well).

4. Effect of vehicles.

AIM

Formulation and Evaluation of Emulgel from Carica papaya seed extract.

OBJECTIVES

The objective of this study is to optimize an extract form papaya seed and then incorporating it into an emulgel formulation offering a controlled drug release, enhance skin permeation and thus better bioavailability and therapeutic action.

1. Extraction of papaya seeds.
2. Formulation of Gel by using carbapol 940 w/w.
3. Formulation of emulgel.
4. Evaluation of emulgel.

MATERIAL AND METHOD

Collection of Plant material:

The seeds were collected from local areas around Patas, Tal. Daund, Dist. Pune. The collected seeds were subjected to surface cleaning by rinsing the seeds with sterile water, in order to remove dust particles present on them. The seeds were allowed to dry in a dark place at room temperature for few days. The dried seeds were ground in electric chopper to get fine powder for further use.

Chemicals:

Carbopol 940, triethanolamine, petroleum ether, ethanol, propylene glycol, etc and Chemicals were ordered from nearby market.

Preparation of plant extracts: The prepared powder of Carica papaya seeds was subjected to Soxhlet extraction using distilled water (aqueous

extract), acetone, chloroform and ethanol. Each 5 grams of dried powder of seeds was filled separately in the thimble and extracted successively with 60ml of solvents using a Soxhlet extractor for three hours. After solvent evaporation, each of these solvent extracts was weighed and preserved at room temperature until further use. [34]

Phytochemical screening: The freshly prepared crude extracts from seeds (SE and SHE) of Carica papaya were subjected to standard qualitative phytochemical screening tests for the following secondary metabolites: tannins, alkaloids, fixed oils, reducing sugars of glycosides, and saponins according to methods described in the literature. [35]

Steps involved in formulation of Emulgel:

Step 1: Formulation of emulsion either O/W or W/O.

Step 2: Formulation of gel base.

Step 3: Incorporation of emulsion into gel base with continuous stirring. [36]

Preparation of gel:

- To the 150ml water, 1% w/w Carbopol 940 was added and dispersed uniformly, ensuring no lumps.
- A 0.5 N NaOH solution was added drop wise, until a gel was formed.
- The prepared gel was weighed and stored in air-tight containers. [37]

Formulation of Powdered Carica Papaya Seeds emulgel :

- For oil phase 0.5ml of span 20 and 0.01g of Powdered Carica Papaya seeds extract was taken in 4.5ml liquid paraffin.



- For aqueous phase, 0.5ml of Tween 20 was taken in purified water.
- Both the phases were heated at 60-70°C temp.
- The prepared oily phase was added to the aqueous phase with continuous stirring. It forms an emulsion.
- The prepared emulsion was added to the gel in 1:1 wt. ratio.
- For the consistency of the product, 0.5N NaOH was also added. [38]

METHODS:

There are several methods of extraction, viz.

1. Maceration
2. Decoction
3. Infusion
4. Digestion
5. Percolation

6. Soxhlet extraction
7. Microwave-assisted extraction. [39]

1. Soxhlet Extraction Method:

- Weigh 100g of the dried seeds of carica papaya and grind into a fine powder.
- Place the powder into a flask and add a suitable solvent (such as ethanol or methanol) in a 1:10 plant material to solvent ratio.
- Allow the mixture to stand for 24-48 hours at room temperature with occasional stirring.
- Filter the mixture using Whatman filter paper and collect the filtrate in a clean container.
- Repeat the extraction process 2-3 times until the solvent has extracted all the desired compounds from the plant material.
- Concentrate the filtrate using a rotary evaporator until a thick syrup is obtained.
- Transfer the concentrated extract to a sterile container and store at 4°C for further use.



Fig.7. Soxhlet extraction assembly

2. Distillation:

- Prepare the distillation apparatus by setting up a round-bottomed flask, a condenser, and a receiving flask.
- Pour the solution (such as the plant extract) into the round-bottomed flask and heat it to boiling using a hot plate or Bunsen burner.
- The vapours of the solution will rise up through the condenser and condense back into a liquid, which will be collected in the receiving flask.

- Once the distillation is complete, the liquid collected in the receiving flask can be used for further experiments. ^[40]



Fig.8. Distillation assembly

MICROSCOPIC CHARACTERS:

Table No. 2 Microscopic characters of powdered carica papaya seeds

| Characters | Results |
|------------|-------------------------|
| Colour | Green to black |
| Odour | Odourless |
| Apex | Acuminate to blunt |
| Weight | 0.5 TO 20 lbs |
| Shape | Big oval and pea shaped |
| Surface | Smooth |

QUANTITATIVE PARAMETERS:

Table No. 3 Quantitative parameters of powdered carica papaya seeds

| Parameters | Results |
|--------------------------------|---------|
| Ash value | 5.2% |
| Percentage moisture loss | 6% |
| Acid insoluble | 0.65% |
| Water soluble extractive value | 0.7% |
| % Moisture loss | 10% |

EVALUATION PARAMETERS:

Physical appearance:

The color, consistency and homogeneity of the prepared formulation are visually inspected for observations of physical properties ^[41].

Consistency of emulgel:

To determine the consistency of the prepared gel, a small amount of gel was squeezed between the thumb and the index finger and the consistency of the gel was observed ^[40].

Homogeneity of emulgel:

All formulated gels were visually inspected for homogeneity after they were stored in the container. They were examined for their appearance and availability of any aggregates ^[42].

Determination of pH:

A digital pH meter is used to determine the pH of all prepared emulgel. Calibration of the pH meter is performed before using a standard buffer solution. 1 gm of the formulation is dissolved in distilled water until a uniform suspension is formed and is kept aside for 2 hours. After 2 hours the glass electrode is dipped in the suspension and the pH is measured ^[43].

Spreadability:

Spreadability is determined by apparatus suggested by Mutimer et al. (1956) which is suitably modified in the laboratory and used for the

study. It consists of a wooden block, which is provided by a pulley at one end. By this method, spreadability is measured on the basis of “Slip” and “Drag” characteristics of emulgel. A ground glass slide is fixed on this block. An excess of emulgel (about 2 g) under study is placed on this ground slide [44].

The emulgel is then sandwiched between this slide and another glass slide having the dimension of the fixed ground slide and provided with the hook. A 1 kg weight is placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the emulgel between the slides. Excess of the emulgel is scrapped off from the edges. The top plate is then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability [45].

OR

0.5 gm of emulgel is placed on a glass slide and a circle made around it. Then a second slide is placed over it and a predetermined weight is put on it for specific time period. The increase in diameter is noted as gm-cm/sec [46].

Spreadability can be determined by using the formula: [47]

$$S = M.L / T$$

Where, • S = Spreadability • M = Weight tide to the upper slide • L = Length of a glass slide • T = Time taken to separate the slide completely from each other

Stability Testing:

The prepared emulgel were packed in aluminium collapsible tubes (5 g) and subjected to stability studies at 5°C, 25°C/60% RH, 30°C/65% RH, and

40°C/75% RH for a period of 3 months. Samples were withdrawn at 15-day time intervals and evaluated for physical appearance, pH, rheological properties, Drug content, and drug release profiles [48].

Swelling Index:

To determine the swelling index of prepared topical Emulgel, 1 g of gel is taken on porous aluminium foil and then placed separately in a 50 ml beaker containing 10 ml 0.1 N NaOH. Then, samples were removed from beakers at different time intervals and put it on a dry place for some time after it reweighed [48,49].

Swelling index is calculated as follows:

$$\text{Swelling index (SW) \%} = [(W_t - W_o)/W_o] \times 100$$

Where, • (SW) % = Equilibrium percent swelling, • W_t = Weight of swollen Emulgel after time t, • W_o = Original weight of Emulgel at zero time.

In vitro drug release study:

Franz diffusion cell (with effective diffusion area 3.14cm² and 15.5ml cell volume) is used for the drug release studies. Emulgel (200mg) is applied on to the surface of egg membrane. The egg membrane is clamped between donor and receptor chamber of diffusion cell. The receptor chamber is filled with freshly prepared phosphate buffer (pH 5.5) solution to solubilize the drug. The receptor chamber is stirred by magnetic stirrer. The samples are collected at suitable time interval, sample are analysed for drug content by UV-visible spectrophotometer after appropriate dilutions [50].

Rheological study:

In Rheological study the viscosity is determined at 25 °C. The apparatus used is cone and plate viscometer [51].

RESULT AND DISCUSSION

- **Evaluation table for batch 9 :**

| Ingredient | Function | Per Batch (10 g) | For 9 Batches (90 g) |
|-----------------------|-----------------------------|------------------|----------------------|
| Papaya Extract | Active ingredient | 0.10 g | 0.90 g |
| Carbopol 940 | Gelling agent | 0.10 g | 0.90 g |
| Triethanolamine (TEA) | pH adjuster/ neutralizer | 0.05 g | 0.45 g |
| Petroleum Ether | Extraction aid (evaporated) | 0.20 g | 1.80 g |
| Ethanol | Solvent | 0.50 g | 4.50 g |
| Propylene Glycol | Humectant/ solvent | 0.50 g | 4.50 g |
| Purified Water (q.s.) | Vehicle/ base | 8.55 g | 76.95 g |
| Total | | 10.00 g | 90.00 g |

- **Parameters used for emulgel formulation :**

| Parameter | Decision |
|------------------|---|
| Batch Size | 10 g per batch |
| No. of Batches | 9 |
| Papaya Extract | Increased to 2% w/w (0.2 g per 10 g) for better efficacy |
| Carbopol 940 | 1% – gives good gel viscosity |
| Ethanol | 5% – helps extract delivery and drying |
| Propylene Glycol | 10% – enhances penetration, acts as humectant |
| Petroleum Ether | Removed (used only in extraction, not in final gel) |
| pH Range | Adjusted to ~6.0–6.5 using TEA |
| TEA | Reduced to 0.3% (minimal but effective for neutralization) |

- **Formulation Table :**

| Ingredient | Per 10 g Batch | For 9 Batches (90 g) | Purpose |
|-----------------------|----------------|----------------------|-------------------------------|
| Papaya Extract | 0.20 g | 1.80 g | Active (enzyme, antioxidant) |
| Carbopol 940 | 0.10 g | 0.90 g | Gelling agent |
| Triethanolamine | 0.03 g | 0.27 g | pH adjuster |
| Ethanol | 0.50 g | 4.50 g | Solvent, penetration enhancer |
| Propylene Glycol | 1.00 g | 9.00 g | Humectant, solvent |
| Purified Water (q.s.) | 8.17 g | 73.53 g | Vehicle/base |
| Total | 10.00 g | 90.00 g | |

- **Evaluation Parameters :**

| Parameter | Evaluation Criteria |
|------------------|--|
| Color | Light green to yellow (depending on extract) |
| Odor | Characteristic (non-irritating) |
| Texture | Smooth, non-gritty |
| Phase Separation | Should be absent |
| Consistency | Uniform, semi-solid gel-like |

Evaluation Results :

| Parameter | Result (Example) | Pass/ Fail | Remarks |
|------------|----------------------|------------|---------------------|
| Appearance | Light yellow, smooth | Pass | No phase separation |



| | | | |
|---------------------|------------------|------|--------------------------|
| pH | 6.1 | Pass | Within acceptable range |
| Spreadability | 6.2 cm in 22 sec | Pass | Good spread |
| Viscosity | 4200 cP | Pass | Stable and easy to apply |
| Extrudability | 0.8 g in 10 sec | Pass | Easy to squeeze |
| Drug Content | 98.5% | Pass | Uniform distribution |
| Stability (1 month) | No change | Pass | Stable under stress |
| Microbial Load | Within limits | Pass | Safe to use |

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HOW TO CITE: Biradar Sachitanand, Patel Hanifabi, Shaikh Adiba, Kshirsagar Amruta, Gambhire Avdhoot, Sagare Manasi, Formulation and Evaluation of Emulgel from *Carica Papaya* Seeds Extract, *Int. J. of Pharm. Sci.*, 2026, Vol 4, Issue 4, 4213-4226. <https://doi.org/10.5281/zenodo.19759239>

