7.1 Introduction

7.1.1 Herbal Formulation

Herbal formulations, also known as phytomedicines, have been an integral part of traditional medicine systems around the world for centuries. These formulations leverage the therapeutic properties of various plant parts, including leaves, roots, flowers, and bark, to treat a wide range of ailments. The resurgence of interest in natural and holistic health approaches has led to a renewed focus on herbal formulations in modern healthcare.

7.1.2 Historical Context

Herbal medicine has a rich history, with roots tracing back to ancient civilizations such as those of Egypt, China, India, and Greece. Traditional systems of medicine, including Ayurveda, Traditional Chinese Medicine (TCM), and Unani, have long employed complex herbal formulations to promote health and treat diseases. These formulations were developed through meticulous observation, experimentation, and documentation over generations.

7.1.3 Modern Relevance

In contemporary healthcare, herbal formulations are valued for their holistic approach to health, targeting the root causes of ailments rather than merely addressing symptoms. The growing demand for natural and organic products has further propelled the popularity of herbal formulations. Additionally, the World Health Organization (WHO) recognizes the significance of traditional medicine and promotes the integration of safe and effective herbal remedies into conventional healthcare systems.

7.1.4 Advantages of Herbal Formulations

1. **Natural Origin**: Herbal formulations are derived from natural sources, making them appealing to those seeking alternatives to synthetic drugs.
2. **Safety Profile**: When used appropriately, many herbal formulations have fewer side effects compared to conventional pharmaceuticals.
3. **Synergistic Effects**: The combination of multiple herbs in a formulation can produce synergistic effects, enhancing therapeutic outcomes.
4. **Cultural Acceptance**: Herbal formulations are often culturally accepted and trusted due to their long history of use.
5. **Economic Benefits**: Herbal medicines can be more affordable and accessible, particularly in developing countries.

7.1.5 Challenges and Considerations

Despite their benefits, herbal formulations face several challenges:

- **Standardization**: Ensuring consistent quality and potency of herbal products is a significant challenge due to variations in plant sources, harvesting, and processing methods.
  - **Scientific Validation**: There is a need for rigorous scientific research to validate the efficacy and safety of many herbal formulations.
  - **Regulation**: The regulatory frameworks for herbal medicines vary widely between countries, affecting their market approval and acceptance.

7.1.6 Components of drug development

A typical drug development process from herbal medicine broadly includes the following aspects:

1. Isolation of bioactive ingredients and synthetic modifications of it.
2. Evaluation of safety and efficacy.
3. Regulatory approval of the therapeutic agent in case of new drug.
4. Clinical Trials.

The drug should be subjected to drug standardization followed by biological activity, preclinical studies and safety studies etc. The standardization protocols of various single and compounds formulations are appended at appropriate places.

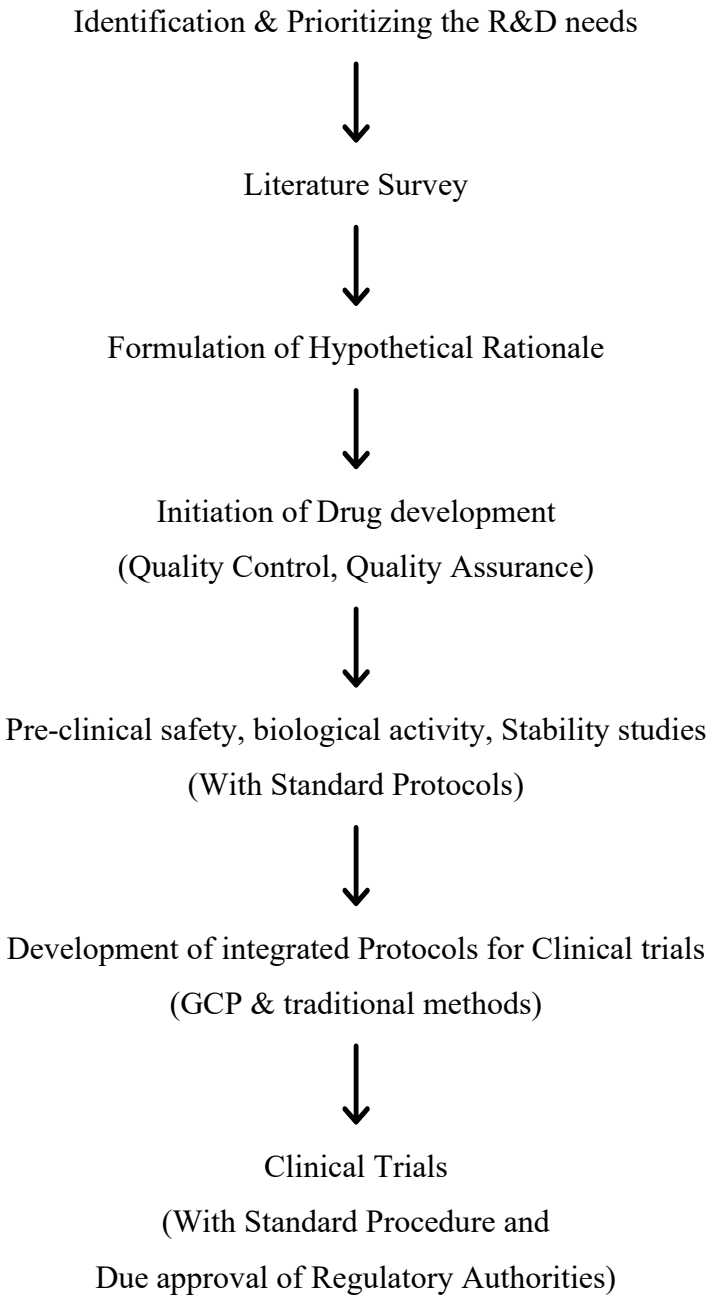


Figure 7.1: Components of drug development [1]

7.1.7 Physiology of the skin:

The skin is made up of several layers. The topmost layer is the epidermis, and underneath it is the dermis. The dermis houses a network of blood vessels, hair follicles, sweat glands, and sebaceous glands. Beneath the dermis is the subcutaneous layer, which is composed of fatty tissue. Hair bulbs extend down into this fatty tissue.

The layers of the epidermis include:

- Stratum Germinativum (the Growing Layer)
- Malpighian Layer (the Pigment Layer)
- Stratum Spinosum (the Prickly Cell Layer)
- Stratum Granulosum (the Granular Layer)
- Stratum Lucidum
- Stratum Corneum (the Horny Layer)

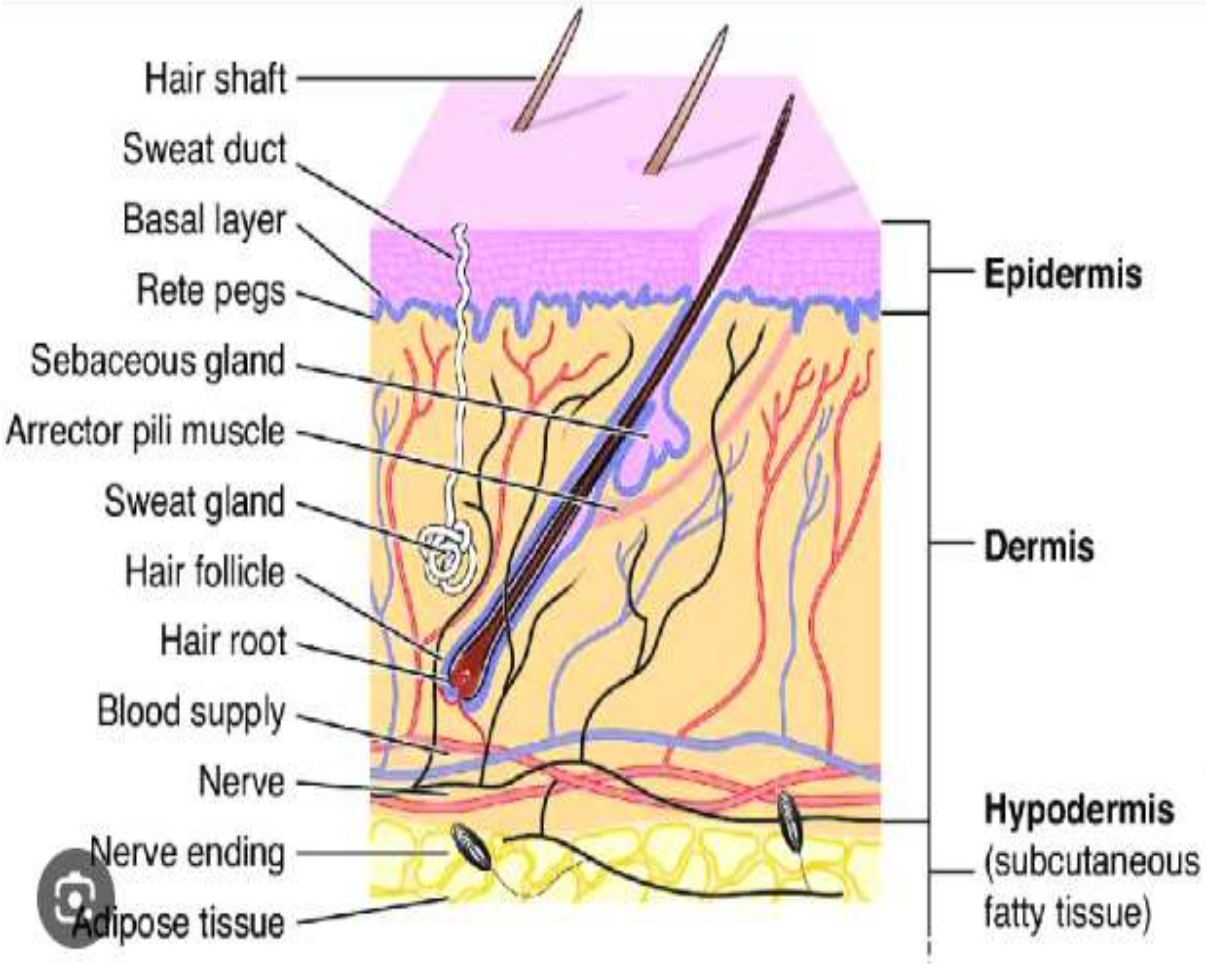


Figure 7.2 Cross section of skin

7.1.8 Functions of the skin:

- Retaining body fluids and protecting tissues.
- Acting as a barrier against external factors like chemicals, light, heat, cold, and radiation.
- Sensing stimuli such as pressure, temperature, and pain.
- Producing biochemical substances.
- Managing metabolism and eliminating waste products.
- Regulating body temperature.
- Controlling blood pressure.
- Preventing the entry of harmful foreign substances and radiation.
- Providing cushioning against physical impacts.
- Aiding in interspecies identification and/or sexual attraction.

Table 7.1Classification of topical preparations [6]

Class	Examples
Liquid preparations	Liniment, lotions, paints, topical solution
Semi solid preparations	Creams, pastes, gels, ointments
Solid preparations	Topical Powders, poultices
Miscellaneous preparations	Topical aerosol, transdermal drug delivery system, rubbing alcohols, tapes and gauze

7.1.9 Advantages of Local drug administration [7]

1. Avoidance of first pass metabolism.
2. Convenient and easy to apply.
3. Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time etc.
4. Achievement of efficacy with lower total daily dosage of drug by continuous drug input.
5. Avoids fluctuation in drug levels, inter- and intrapatient variations.
6. Ability to easily terminate the medications, when needed.
7. A relatively large area of application in comparison with buccal or nasal cavity
8. Ability to deliver drug more selectively to a specific site.
9. Avoidance of gastro-intestinal incompatibility.

10. Providing utilization of drugs with short biological half-life, narrow therapeutic window.
11. Improving physiological and pharmacological response.
12. Improve patient compliance.
13. Provide suitability for self-medication

7.1.10 Disadvantages of Local drug administration [8,9]

1. They are very sticky causing uneasiness to the patient when applied.
2. Moreover, they also have lesser spreading coefficient and need to apply with rubbing.
3. They also exhibit stability problems.
4. Skin irritation or contact dermatitis may result from the drug and/or excipients.
5. Some drugs have poor permeability through the skin.
6. There is a possibility of allergic reactions.
7. This method is suitable only for drugs that need very low plasma concentrations for effectiveness.
8. Enzymes in the epidermis may degrade the drugs.
9. Drugs with larger particle sizes are difficult to absorb through the skin
10. Due to all these factors with the major group of preparations, the use of Topical spray has expanded both in cosmetics and in pharmaceutical preparations.

7.1.11 Limitations of Local drug administration [10]

1. Powders are slippery and caution during application should be taken since, inhalation of powders can cause irritation of airways and lungs.
2. Plasters are heavy weighted and are to be kept for longer duration of time.
3. In semi-solids, great variation is found in composition, pH, and tolerance.
4. Sticky in nature.
5. Applied with special spatulas or gloves.
6. Formulations are messy and occlusive.
7. Rancidification of oils may occur.
8. Physical stability, sedimentation and compaction of sediment causes problems in suspension.
9. Concentration variation of emulsifier may cause breaking of emulsion.
10. Careless rubbing the formulation may cause inconvenience.

7.1.12 Introduction to Polyherbal Gel

Herbal medicine has been a cornerstone of traditional healthcare systems worldwide, offering a natural and holistic approach to treating various ailments. The use of plants and their extracts for medicinal purposes dates back thousands of years and continues to be relevant in modern healthcare due to their therapeutic properties and minimal side effects. In recent years, there has been a growing interest in incorporating herbal ingredients into topical formulations, such as gels, to leverage their medicinal benefits for skin-related issues and other localized conditions.

Topical gels are semi-solid systems that provide a convenient and effective means of delivering active ingredients directly to the site of action. They are preferred over other topical formulations for several reasons, including ease of application, enhanced penetration of active compounds, and the ability to provide a cooling and soothing effect. When formulated with herbal extracts, these gels can offer a range of therapeutic benefits, such as anti-inflammatory, antimicrobial, antioxidant, and wound-healing properties.

❖ **Advantages of Herbal Topical Gels**

1. **Enhanced Bioavailability**: The gel matrix facilitates the efficient delivery of herbal active compounds to the targeted area, improving their bioavailability and therapeutic efficacy.
2. **Minimal Systemic Absorption**: Topical application of herbal gels ensures that the active ingredients exert their effects locally, reducing the risk of systemic side effects.
3. **Natural and Safe**: Herbal ingredients are generally well-tolerated and have a long history of safe use. They are less likely to cause adverse reactions compared to synthetic compounds.
4. **Versatility**: Herbal gels can be formulated to address a variety of skin conditions, including inflammation, infections, wounds, and burns, making them versatile and multifunctional.
5. **Consumer Preference**: There is a growing consumer preference for natural and organic products, driven by the desire for safer and more sustainable healthcare options. Herbal gels align well with this trend.

❖ **Formulation Considerations**

The development of an effective herbal topical gel involves several formulation considerations:

1. **Selection of Gelling Agents**: Gelling agents such as Carbopol, Hydroxypropyl methylcellulose (HPMC), and Xanthan gum are used to provide the desired viscosity and stability to the gel.
2. **Compatibility of Ingredients**: Ensuring the compatibility of herbal extracts with the gel base and other excipients is crucial for stability and efficacy.
3. **Preservation and Shelf Life**: Natural preservatives and antioxidants may be incorporated to enhance the shelf life and prevent microbial contamination.
4. **Sensory Properties**: The texture, spreadability, and absorption rate of the gel are important for user satisfaction and compliance.

❖ **Benefits of Herbal Gel Formulations**

1. **Enhanced Delivery**: Gels provide an excellent medium for the controlled release of active herbal ingredients. The semi-solid consistency allows for easy application and adherence to the skin, ensuring prolonged contact and absorption.
2. **Localized Treatment**: Herbal gels enable targeted therapy, delivering active compounds directly to the affected area. This localized action is particularly beneficial for conditions like inflammation, wounds, and skin disorders.
3. **Reduced Side Effects**: By focusing the treatment on the site of application, herbal gels minimize systemic absorption, thereby reducing the risk of side effects and toxicity.
4. **Patient Compliance**: The non-greasy, soothing texture of gels is more acceptable to patients compared to other topical formulations like ointments and creams. They are easy to apply and provide a cooling effect, enhancing user comfort.

❖ **Scientific Basis and Mechanisms**

The efficacy of herbal gel formulations is rooted in both the pharmacological properties of the herbal extracts and the physicochemical characteristics of the gel base. The active compounds in herbs, such as alkaloids, flavonoids, tannins, and essential oils, exhibit a wide range of therapeutic activities including anti-inflammatory, antimicrobial, antioxidant, and analgesic effects. When these extracts are incorporated into a gel matrix, the bioavailability and stability of the active compounds are often enhanced.

❖ **Formulation Considerations**

Developing an effective herbal gel requires careful consideration of several factors:

1. **Selection of Herbal Extracts**: The choice of herbs is critical and is based on their traditional use, scientific evidence of efficacy, and compatibility with other formulation components.
2. **Gel Base Composition**: Common gelling agents like Carbopol, hydroxypropyl methylcellulose (HPMC), and xanthan gum are selected based on their ability to form a stable, homogenous gel that can release the herbal actives in a controlled manner.
3. **Stability and Compatibility**: Ensuring the stability of the herbal actives within the gel matrix is essential. This includes assessing the pH, viscosity, and potential interactions between the herbal compounds and the gelling agents.
4. **Quality Control and Standardization**: Standardizing the concentration of active ingredients and ensuring batch-to-batch consistency is vital for the therapeutic efficacy and safety of the formulation.

❖ **Applications and Therapeutic Potential**

Herbal gels are widely used in the treatment of a variety of conditions, particularly those involving inflammation, pain, and skin disorders. For instance, gels formulated with anti-inflammatory herbs like *Adhatoda vasica*, *Calotropis procera*, and *Rosa indica* shown promising results in reducing inflammation and promoting healing in conditions such as arthritis, muscle sprains, and wounds.

7.1.13 Polyherbal Spray

❖ **Background and Significances**

The use of herbal medicine, a practice with deep historical roots, continues to thrive in modern healthcare due to its natural origins and therapeutic benefits. Herbal remedies are often preferred for their holistic approach and minimal side effects compared to synthetic pharmaceuticals. Among various forms of herbal formulations, sprays have gained popularity for their convenience, ease of application, and effective delivery of active compounds. This introduction explores the concept, benefits, and formulation considerations of herbal sprays, highlighting their role in contemporary medicine. Herbal spray formulations have gained significant attention in the field of natural and alternative medicine, combining the therapeutic benefits of herbal extracts with the convenience and efficacy of spray delivery systems. These formulations provide a novel approach to

administering herbal remedies, offering advantages such as ease of application, rapid onset of action, and targeted delivery. Herbal sprays are particularly effective for topical applications and for conditions requiring localized treatment.

❖ **Concept of Herbal Sprays**

Herbal sprays are liquid formulations designed to deliver therapeutic herbal extracts directly to the affected area through a fine mist. They offer a convenient and non-invasive method of application, making them suitable for treating a variety of conditions, including skin irritations, respiratory issues, and oral health problems. The fine mist ensures even distribution of the active ingredients, enhancing their absorption and efficacy.

❖ **Advantages of Herbal Sprays**

1. **Convenience and Ease of Use**: Sprays are easy to apply, especially on hard-to-reach areas. They offer a mess-free alternative to creams and ointments.
2. **Rapid Absorption**: The fine mist allows for quick absorption of active ingredients through the skin or mucous membranes, providing fast relief.
3. **Targeted Delivery**: Herbal sprays deliver active compounds directly to the site of action, ensuring localized treatment and minimizing systemic exposure.
4. **Minimal Contamination**: Unlike creams or gels that require direct contact, sprays reduce the risk of contamination as they do not require touching the applicator tip to the affected area.
5. **Versatility**: Herbal sprays can be formulated for various therapeutic purposes, including skin care, respiratory health, wound healing, and oral hygiene.
6. **Enhanced Stability**: The formulation and packaging of herbal sprays can protect sensitive herbal compounds from degradation due to environmental factors such as light, air, and moisture.
7. **Convenience and Portability**: Sprays are portable and convenient, making them suitable for use in various settings, including at home, at work, or while traveling.

❖ **Scientific Basis and Mechanisms**

The effectiveness of herbal spray formulations is attributed to both the pharmacological properties of the herbal extracts and the technology of the spray delivery system. Active compounds in herbs, such as flavonoids, terpenoids, alkaloids, and essential oils, exhibit a wide range of therapeutic activities, including anti-inflammatory, antimicrobial, antioxidant, and

analgesic effects. The spray mechanism enhances the bioavailability of these compounds by creating fine droplets that can penetrate the skin or mucosal barriers efficiently.

❖ **Formulation Considerations**

Developing an effective herbal spray involves several critical considerations:

- **Selection of Herbal Extracts**: The choice of herbs is based on their therapeutic properties, historical use, and evidence from scientific research. The extracts should be compatible with the spray formulation and stable over the product's shelf life.
- **Solvent and Base Selection**: The solvent or base used in the spray must be compatible with the herbal extracts and suitable for the intended route of administration. Common solvents include water, ethanol, and glycerine.
- **Preservatives and Stabilizers**: To ensure the stability and safety of the herbal spray, appropriate preservatives and stabilizers may be added to prevent microbial growth and degradation of active compounds.
- **Packaging**: The choice of packaging is crucial for maintaining the integrity and efficacy of the herbal spray. The packaging should protect the formulation from light, air, and contamination while providing a convenient and effective delivery mechanism.

❖ **Applications and Therapeutic Potential**

Herbal sprays are used in various therapeutic applications, particularly for conditions requiring topical treatment. They are effective in managing skin disorders, wounds, burns, pain, and inflammation. For instance, sprays containing herbal extracts with anti-inflammatory and analgesic properties are used to relieve muscle pain and joint stiffness. Additionally, herbal sprays are used in aromatherapy and for the treatment of respiratory conditions, where the fine mist can deliver active compounds directly to the respiratory tract.

7.1.14 Optimization by Experimental Design

With progressive research for development of newer drug delivery systems, it has become apparent to assess the factors influencing formulation and drug release in a short time. It is very important to develop and optimize a formulation with a few experiments and at a low cost in order to overcome the rapidly increasing cost of experiments. Optimization of formulations using statistical experimental designs is a powerful and efficient tool in the development of

pharmaceutical dosage forms. The experimental design allows for studying various processing parameters influencing the selected responses with the lowest number of experiments, thereby reducing the time required in the development work.

Design of Experiments (DoE) is the main component of the statistical toolbox to deploy Quality by Design in both research and industrial settings. While the first DoEs were applied by Fisher in the field of agriculture, their use entered the chemical process industries during the 1950s, as these were affected by the key paper of Box and Wilson on the experimental attainment of optimum conditions.[11]

Experimental design can be regarded as a process by which certain factors are selected and deliberately varied in a controlled manner to obtain their effects on a response of interest, often followed by the analysis of the experimental results. According to the number of the factors to be investigated at a time, the experimental design can be classified into two categories: one-factor-at-a-time design (single-factor design) and factorial design (multiple-factor design).[12]

The pharmaceutical industry is heavily focused on processes and quality, so it would seem natural for it to embrace these paradigms soon after their introduction. However, Quality by Design (QbD) was only proposed by regulatory bodies like the FDA and EMA at the start of the new millennium. They emphasized that "Quality cannot be tested into products; instead, it must be built in by design." [13]

7.1.15 Box-Behnken designs (BBD)

The Box-Behnken design is a type of independent quadratic design that does not include an embedded factorial or fractional factorial design. In this design, the treatment combinations are positioned at the midpoints of the edges of the process space and at the center point. These designs are either rotatable or nearly rotatable and require three levels for each factor. Compared to central composite designs, Box-Behnken designs have limited capability for orthogonal blocking. For optimizing the gel formulations, the Box-Behnken Design was selected because it integrates a 2k factorial with an incomplete blocking design. The optimization was carried out using the Software Design Expert Version 10.0.3.0, incorporating three factors (x) at three levels (low, medium, and high). The experimental data were interpreted using a response surface quadratic model, which establishes the relationship between the factors (x) and the responses (y), as represented by Equation (1).

$y = f(x1 + x2 + x3) + \varepsilon$ ……..Equation (1)

These are a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs. [14]

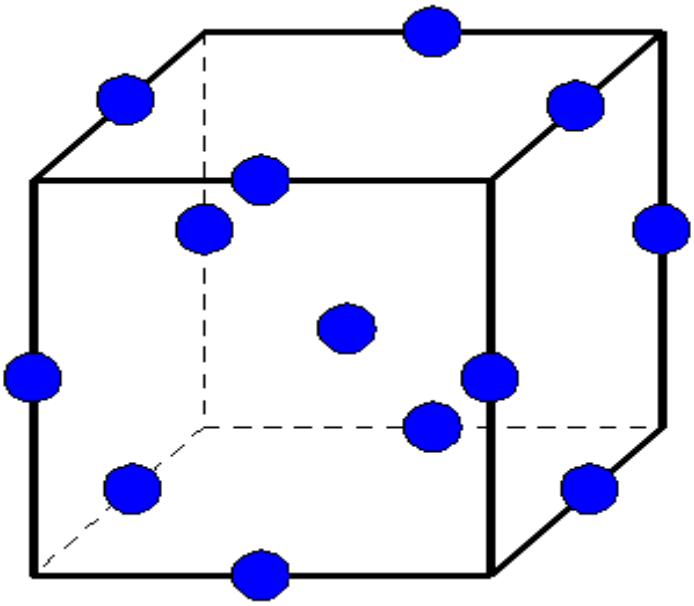


Figure 7.3 A Box-Behnken Design for Three Factors

7.2 Materials and Methods

7.2.1 Development of Polyherbal formulations

7.2.1.1 Preparation of Polyherbal gel-

The gel was prepared using the dried extract of *Adhatoda vasica*, *Rosa indica* and Calotropis procera. The gel was prepared using Carbapol-934, Polyethylene glycol, Methyl paraben, Propyl paraben, Tri-ethanolamine and distilled water in a quantity sufficient to prepare 100 g of gel. Water required for these formulations was divided in to two parts. In one part the exact amount of extracts were dissolved and in other part, Carbapol-934 was dissolved and to this solution Methyl paraben and Propyl paraben were added. Both of these solutions were mixed in a beaker and Tri-ethanolamine was added to the mixture dropwise to obtain the gel consistency.

Quality by Design (QbD) Approach for Formulation Development

Before embarking on the development of any formulation, it is crucial to identify key variables that may influence the product's characteristics. These include formulation variables, process variables, and environmental variables.

Optimization of Formulation by Box-Behnken Design (BBD)

Traditional optimization methods, such as varying one variable at a time, are complex and only measure the impact of a single variable on the experimental outcome rather than considering all variables simultaneously. The Box-Behnken Design (BBD) is employed for formulation optimization due to several advantages:

- **Three-Level Design:** BBD is a 3-level design that combines two-level factorial designs with incomplete block designs, making it robust for various experimental setups.
- **Cubic Design:** This design is characterized by a set of points that lie at the midpoints of each edge, along with a replicate center point within the multidimensional cube.
- **Efficiency:** BBD requires fewer experimental runs compared to a full factorial design with 3 factors and 3 levels, as well as the Central Composite Design (CCD).
- **Predictive Accuracy:** BBD is known for its excellent predictability within the spherical design space, making it ideal for investigating quadratic response surfaces, especially when extreme level predictions are not necessary.

The specific values of independent and dependent variables (response parameters) selected for the BBD are presented in Table 7.2.

Table 7.2 Selected values of variables for BBD

Variables	**Levels**		
Independent variables	**Low**	**Medium**	**High**
Carbopol-934 %(X1)	0.5	1	1.5
TEA %(X2)	0.4	0.5	0.6
Extract %(X3)	1	2	3
Dependent variables			
Viscosity (cp)(Y1)			
Spreadability (g.cm/s) (Y2)			
pH (Y3)			

The selection of critical formulation variables was guided by the findings from the preliminary investigation. A Box-Behnken Design (BBD) matrix was created using Stat-Ease Design-Expert Software version 13.0, resulting in a total of 15 experimental runs. Each batch of the Polyherbal Gel was prepared in accordance with this design matrix, with all other process variables held constant to ensure consistency. The viscosity, spreadability, and pH of the formulated gels were chosen as the critical quality attributes (CQAs) and used as response parameters for the study.

1)Viscosity

Viscosity of gel was determined using Brookfield viscometer (S-62, model DV-1) at 25 ℃ with a spindle speed of the viscometer rotated at 12 rpm.

2) Spreadability

Spreadability was assessed using an apparatus that included a wooden block equipped with a pulley at one end. This method evaluates spreadability based on the slip and drag characteristics of the gels. Approximately 2 grams of the gel being tested was placed on a ground glass slide. The gel was then sandwiched between this slide and another glass slide of the same size, which was fitted with a hook. A 1 kg weight was placed on top of the slides for 5 minutes to eliminate air bubbles and create a uniform gel film between the slides. Excess gel was removed from the edges. The top slide was then pulled with a force of 80 g using a string attached to the hook, and the time (in seconds) it took for the top slide to move a distance of 7.5 cm was recorded. A shorter time indicated better spreadability. Spreadability was calculated using the appropriate formula.

$S = M \times L/T$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the upper slide from the ground slide.[15]

3) pH measurement

The pH of the gel was determined using a digital pH meter by fully immersing the glass electrode into the gel to ensure complete coverage. The measurement was conducted three times, and the average of the three readings was documented.

7.2.1.2 Preparation of Optimised batch of Polyherbal gel

The gel was prepared using the dried extract of *Adhatoda vasica*, *Rosa indica* and Calotropis procera. The gel was prepared using Carbapol-934 (1%), Polyethylene glycol, Methyl paraben, Propyl paraben, Tri-ethanolamine and distilled water in a quantity sufficient to prepare 100 g of gel. Water required for these formulations was divided in to two parts. In one part the exact amount of extracts were dissolved and in other part. The concentration of the extract (3%) was selected based on the result of Design of experiment (Figure 7.24 Overlay Plot of Design of Experiment) and to achieve better therapeutic activity within safe range. Carbapol-934 was dissolved and to this solution Methyl paraben and Propyl paraben were added. Both of these solutions were mixed in a beaker and Tri-ethanolamine was added to the mixture dropwise to obtain the gel consistency.

Table 7.3 Formula of Optimized batch of Polyherbal Gel

Name of Ingredients	Quantity of Ingredient
Carbopol 934	1%
Adhatoda vasica extract	1%
Rosa indica extract	1%
Calotropis procera extract	1%
Polyethylene glycol 400	5 gm
Triethanolamine	0.4 %
Methyl paraben	0.1%
Propyl paraben	0.05%
Water	Quantity sufficient to 100 gm

7.2.2 Evaluation of Optimised Batch of Polyherbal gel

1)Appearance and Homogeneity

Physical appearance and homogeneity of the prepared gels were evaluated by visual perception.

2) pH measurement – As mentioned in 7.2.1.1 (3)

3) Viscosity - As mentioned in 7.2.1.1 (1)

4) Spreadability -- As mentioned in 7.2.1.1 (2)

5)Extrudability

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between

two glass slides and were clamped. 500 g was placed over the slides, and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair).[16]

6)Stability study

The main objective of the stability testing is to provide evidence on how the quality of the finished product varies with time under the influence of temperature and humidity. The stability study for the prepared topical herbal formulation was done as per ICH guidelines in a stability chamber for a period of six months. The selected topical formulations consisting of Polyherbal Gel was loaded in a humidity chamber (Floor standing model, Remi Pvt Ltd, CHM-6PLUS), at 40°C ± 2°C/75% RH ± 5% RH. Samples were withdrawn at first, second, third and six months and evaluated for appearance, pH, viscosity and spreadability. [17,18]

7.2.3 Preparation of Optimized Polyherbal Spray-

Ethyl alcohol 95% and distilled water were selected as solvents for formulating of spray due to the good solubility of all three extracts. First dissolve weighed quantities of all three extracts in 10 ml of distilled water. Shake it well for 5 -10 minutes for proper mixing of the extracts. Then add 90 ml of ethanol to the solution. Mix thoroughly then filter the solution and fill it in the spray bottle. The concentration of the extract (3%) was selected based on the result of Design of experiment and to achieve better therapeutic activity within safe range.

Table 7.4 Formula of Optimized Polyherbal Spray

Name of Ingredients	Quantity of Ingredient
Distilled water	10 ml
Adhatoda vasica extract	1%
Rosa indica extract	1 %
Calotropis procera extract	1 %
Ethanol	Quantity sufficient to 100 ml

7.2.4 Evaluation parameters of the developed polyherbal Spray

1. pH: pH meter was calibrated using two buffers (pH 4 and pH 7) for calibration. The tip of the probe after rinsing with water was dipped in to samples. The meter was allowed to equilibrate and then pH was noted. pH measurement of the Spray was carried out by dipping

the glass electrode completely into the spray to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded.

2. Clarity of solution: The clarity of solution was done by naked eye by analyzing the settling down of suspending particles in the formulation solution.

3. Delivery rate of Polyherbal spray:

The delivery rate of spray was evaluated according to procedure stated in USP. Six containers were used. Each valve was actuated for 5 seconds at a temperature of 25 °C. The test was repeated three times for each container. The average delivery rate was calculated, in grams per second.

4. Spray pattern: The spray pattern of an optimized formulation was done by spraying from a mechanical spray bottle from a distance of 15 cm on a paper.

5. Area covered by each spray: Area covered by each spray was calculated by using formula, $A = \pi r^2$.

6. Leakage from container: The leakage test was conducted according to the method in USP. Nine containers were selected and the date and time were recorded to the nearest half hour. Each container was weighed to the nearest mg and recorded as W1. The containers were allowed to stand in an upright position at a temperature of 25.0 ± 2.0 ºC for not less than 3 days, before the second weight was recorded as W2. The leakage rate, in mg per year, of each container was calculated using formula: (365) (24/T) (W1 – W2), where T is the test period, in hours.

7. Stability-. The stability study for the prepared topical herbal formulation was done as per ICH guidelines in a stability chamber for a period of six months. The selected topical formulation consisting of Polyherbal Spray was loaded in a humidity chamber (Floor standing model, Remi Pvt Ltd, CHM-6PLUS) at 40°C ± 2°C/75% RH ± 5% RH. For Spray formulation the parameters evaluated were pH. Clarity of solution, Delivery rate and Leakage from container.

7.2.4.1 Assay of developed Polyherbal Gel and Polyherbal Spray

1) Quantification of Vasicine in developed Polyherbal Gel and Spray

- **Preparation of Standard Solution:** Weigh accurately 1 mg of Standard Vasicine in 1 mL volumetric flask. To it add 0.5 mL of Methanol and sonicate till the standard gets dissolved completely. Then, make up the volume up to 1 mL with Methanol. Use the standard solution thus obtained for HPTLC fingerprinting.

- **Preparation of Test Solution (Extract and Gel):** Weigh accurately 1 g of sample in a 250 mL reflux flask. To it add 10 mL of Methanol and reflux on water bath for 60 minutes. On completion of time, remove the reflux flask from the water bath and allow to cool. Filter with the help of Whatman filter paper no. 1 in a 10 mL volumetric flask. Then, make up the volume up to 10 mL with Methanol. Use the Test Solution thus obtained for HPTLC fingerprinting.
- **Preparation of Test Solution (Spray):** Weigh accurately 1 g of sample in a 10 mL volumetric flask. To it add 5 mL of Methanol and mix well for 1 minute on a cyclo mixer. Then, make up the volume up to 10 mL with Methanol. Use the Test Solution thus obtained for HPTLC fingerprinting.

Table 7.5 HPTLC Chromatographic Conditions for Vasicine quantification

Chromatographic Conditions:	
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F_{254} on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Standard Application Volume	10.0 µL
Sample Application Volume	10.0 µL
Distance Between Tracks	13.3 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Dioxane: Toluene: Methanol: Ammonia (5: 2: 2: 1 v/v)
Visualization	@ 254 nm
Quantification	@ 254 nm
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100 ± 5^0C for 3 minutes

2) Quantification of Rutin in developed Polyherbal Gel and Spray

- **Preparation of Standard Solution**: Weigh accurately 2 mg of Standard Rutin in 1 mL volumetric flask. To it add 1 mL of Methanol and sonicate till the standard gets

dissolved completely. Then, make up the volume up to 1 mL with Methanol. Use the standard solution thus obtained for HPTLC fingerprinting.

- **Preparation of Test Solution (Extract and Gel)**: Weigh accurately 1 g of sample in a 250 mL reflux flask. To it add 10 mL of Methanol and reflux on water bath for 60 minutes. On completion of time, remove the reflux flask from the water bath and allow to cool. Filter with the help of Whatman filter paper no. 1 in a 10 mL volumetric flask Then, make up the volume up to 10 mL with Methanol. Use the Test Solution thus obtained for HPTLC fingerprinting.
- **Preparation of Test Solution (Spray):** Weigh accurately 1 g of sample in a 10 mL volumetric flask. To it add 5 mL of Methanol and mix well for 1 minute on a cyclo mixer. Then, make up the volume up to 10 mL with Methanol. Use the Test Solution thus obtained for HPTLC.

Table 7.6 HPTLC Chromatographic Conditions for Rutin quantification

Chromatographic Conditions:	
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Standard Application Volume	10.0 µL
Sample Application Volume	10.0 µL
Distance Between Tracks	13.3 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene: Ethyl acetate: Formic acid: Methanol
Visualization	@ 254 nm
Quantification	@ 257 nm
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100 ± 5^0C for 3 minutes

3) Quantification of Gallic acid in developed Polyherbal Gel and Spray

- **Preparation of Standard Solution**: Weigh accurately 2 mg of Standard Gallic acid in 2 mL volumetric flask. To it add 1 mL of Methanol and sonicate till the standard gets dissolved completely. Then, make up the volume up to 2 mL with Methanol. Use the standard solution thus obtained for HPTLC fingerprinting.
- **Preparation of Test Solution (Extract and Gel)**: Weigh accurately 1 g of sample in a 250 mL reflux flask. To it add 10 mL of Methanol and reflux on water bath for 60 minutes. On completion of time, remove the reflux flask from the water bath and allow to cool. Filter with the help of Whatman filter paper no. 1 in a 10 mL volumetric flask. Then, make up the volume up to 10 mL with Methanol. Use the Test Solution thus obtained for HPTLC fingerprinting.
- **Preparation of Test Solution (Spray)**: Weigh accurately 1 g of sample in a 10 mL volumetric flask. To it add 5 mL of Methanol and mix well for 1 minute on a cyclo mixer. Then, make up the volume up to 10 mL with Methanol. Use the Test Solution thus obtained for HPTLC fingerprinting.

Table 7.7 HPTLC Chromatographic Conditions for Gallic acid quantification

Chromatographic Conditions:	
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Standard Application Volume	10.0 µL
Sample Application Volume	10.0 µL (Extract and Spray); 20.0 µL (Gel)
Distance Between Tracks	13.3 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes

Mobile Phase (MP)	Toluene: Ethyl acetate: Formic acid (10: 7: 1 v/v)
Visualization	@ 254 nm
Quantification	@ 278 nm
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100 ± 50C for 3 minutes

7.3 Results and discussion

7.3.1 Formulation optimization by Box-Behnken Design

Based on the preliminary investigation, three critical material attributes (CMAs) were identified, and their relationship with the critical quality attributes (CQAs) was thoroughly examined using the Box-Behnken Design. A randomized matrix comprising 15 experimental runs was generated by the Design-Expert software, and the details of these runs are presented in Table no.7.8.

Table no. 7.8 Randomized BBD design matrix generated by Design-Expert software

	Independent Variable			**Dependent Variable**		
Run	**A: Carbopol**	**B: TEA**	**C: Extract**	**Viscosity**	**Spreadability**	**pH**
	%	%	%	cps	g.cm/s	mV
1	0.5	0.5	1	4983	6.64	5.48
2	1.5	0.5	3	6237	5.19	5.82
3	0.5	0.6	2	5568	6.32	6.02
4	0.5	0.5	3	5704	6.21	5.59
5	1	0.4	1	5038	5.78	5.55
6	1.5	0.5	1	6109	5.39	5.89
7	1	0.6	1	5267	5.63	6.39
8	1	0.5	2	5160	5.49	5.73
9	1	0.5	2	5099	5.51	5.76
10	1	0.6	3	5600	5.45	6.41
11	0.5	0.4	2	5523	6.53	5.41
12	1	0.5	2	5143	5.61	5.79
13	1.5	0.4	2	6234	5.23	5.59

14	1.5	0.6	2	6308	5.31	6.59
15	1	0.4	3	5628	5.42	5.52

7.3.2 Effect analysis of critical variables on responses

7.3.2.1 Influence of investigated parameters on Viscosity

A) Statistical Analysis for Viscosity

Table 7.9 The statistical analysis of the design for Viscosity

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0338	0.0063	0.4038	0.1870	
2FI	0.8855	0.0045	0.2405	-0.5116	
Quadratic	**< 0.0001**	**0.3142**	**0.9918**	**0.9620**	**Suggested**
Cubic	0.3142		0.9954		**Aliased**

As shown in Table7.9, the best model to fit the experimental results of Viscosity in Polyherbal Gel is the quadratic model and was chosen for further evaluation.

B) ANOVA Analysis for Viscosity

The ANOVA for Viscosity is given in Table 7.10

Table 7.10 ANOVA for Response Surface Quadratic Model for Viscosity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.028E+06	9	3.364E+05	188.69	< 0.0001	significant
A-Carbopol	1.209E+06	1	1.209E+06	678.08	< 0.0001	
B-TEA	12800.00	1	12800.00	7.18	0.0439	
C-Extract	3.925E+05	1	3.925E+05	220.13	< 0.0001	
AB	210.25	1	210.25	0.1179	0.7453	
AC	87912.25	1	87912.25	49.31	0.0009	
BC	16512.25	1	16512.25	9.26	0.0286	
A^2	1.219E+06	1	1.219E+06	683.78	< 0.0001	
B^2	1.471E+05	1	1.471E+05	82.52	0.0003	
C^2	9092.83	1	9092.83	5.10	0.0735	
Residual	8915.00	5	1783.00			
Lack of Fit	6933.00	3	2311.00	2.33	0.3142	not significant
Pure Error	1982.00	2	991.00			

Cor Total	3.037E+06	14				

Factor coding is **Coded**. Sum of squares is **Type III – Partial**. The **Model F-value** of 188.69 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values below 0.0500 suggest that the model terms are statistically significant. In this instance, the significant model terms are A, B, C, AC, BC, A^2, and B^2. Conversely, terms with P-values above 0.1000 are considered not significant. If there are numerous insignificant terms (excluding those necessary to maintain hierarchy), simplifying the model could enhance its effectiveness.

The Lack of Fit F-value of 2.33 indicates that the lack of fit is not significant compared to the pure error, with a 31.42% probability that such a large Lack of Fit F-value could arise due to random variation. A non-significant lack of fit is desirable, as it signifies that the model is adequately fitting the data.

Table 7.11 ANOVA study results for Viscosity

Parameters	**Results of Response**	**Parameters**	**Results of Response**
Std. Dev.	42.23	**R^2**	0.9971
Mean	5573.40	**Adjusted R^2**	0.9918
C.V. %	0.7576	**Predicted R^2**	0.9620
		Adeq Precision	38.9970

The **Predicted R^2** of 0.9620 is in reasonable agreement with the **Adjusted R^2** of 0.9918; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 38.997 indicates an adequate signal. This model can be used to navigate the design space.

C) Mathematical Model for Viscosity

To examine the effect of various factors on Viscosity, contour plots and the 3D plot were referred to along with the value of ANOVA. From Table 7.10, we can observe that with change in the combination of various levels of factors, the final response, i.e., Viscosity,

Table 7.12 Coded equation for Viscosity

Viscosity	=
+5134.00	
+388.75	A
+40.00	B
+221.50	C
+7.25	AB
-148.25	AC
-64.25	BC
+574.63	A^2
+199.63	B^2
+49.62	C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. A detailed examination of the various factors involved provides a clearer understanding of their impact. The equation outlines whether the effects are positive or negative.

From this equation, it is evident that all factors influenced viscosity to some degree. Viscosity, which measures a fluid's thickness, is particularly relevant in gel preparations where high viscosity is desired. The viscosity test results for the gel preparations ranged from 4900 to 6300 cp (Table 7.8). It is important to note that viscosity is inversely proportional to spreadability: as viscosity increases, spreadability decreases. Viscosity increases by increasing the concentrations of Carbopol 934. These results is consistent with the results of the ANOVA analysis, which showed that Carbopol 934 had a significant effect on gel viscosity. Carbopol 934 is a synthetic polymer, which is hygroscopic, slightly acidic and very easily ionized.

Carbopol 934 used as a gelling agent plays an important role in regulating the viscosity of the gel preparations.

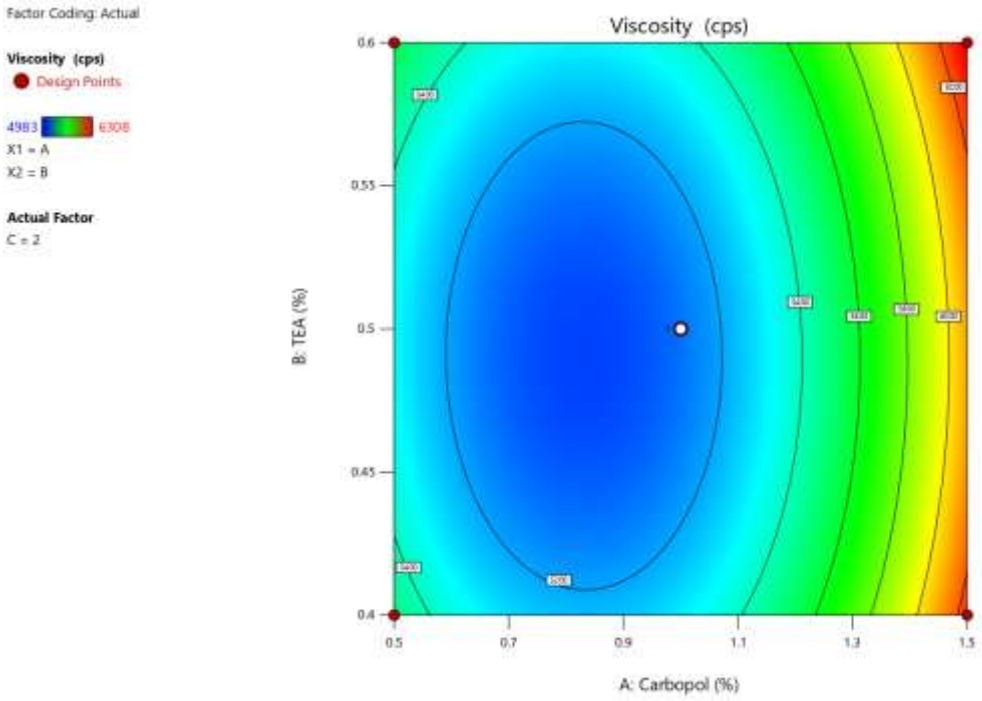


Figure 7.5 Contour plot (2D) showing the combined effect of TEA and Carbopol on Viscosity

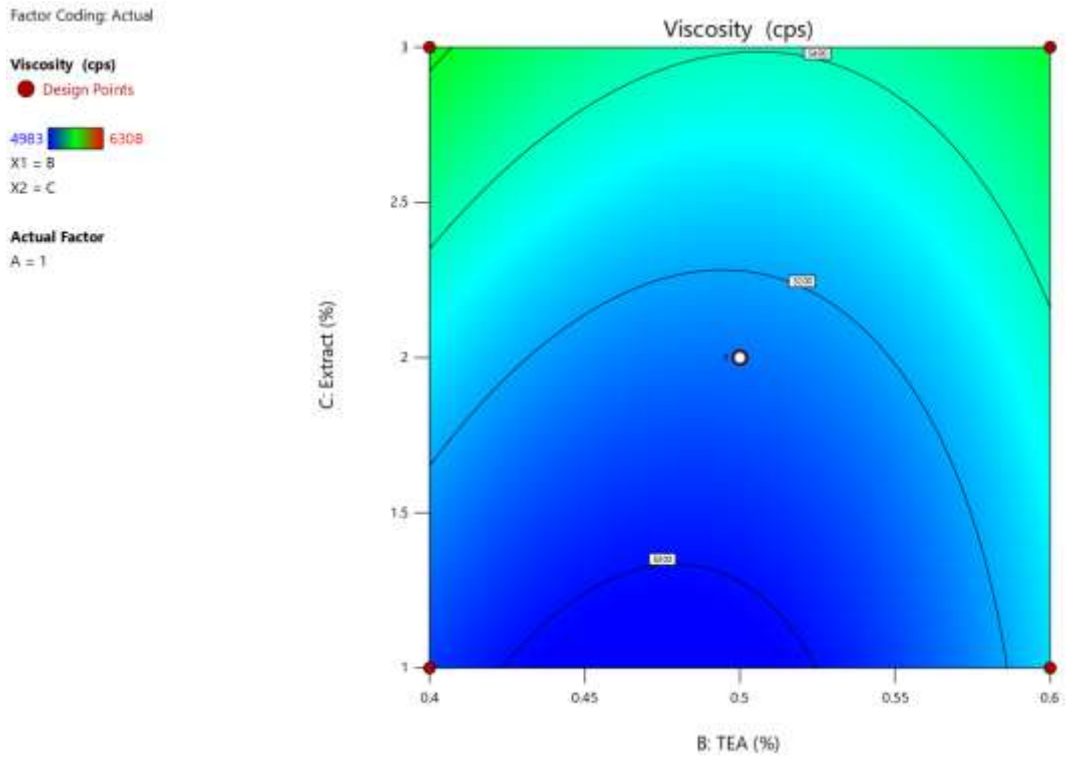


Figure 7.6 Contour plot (2D) showing the combined effect of Extract and TEA on Viscosity

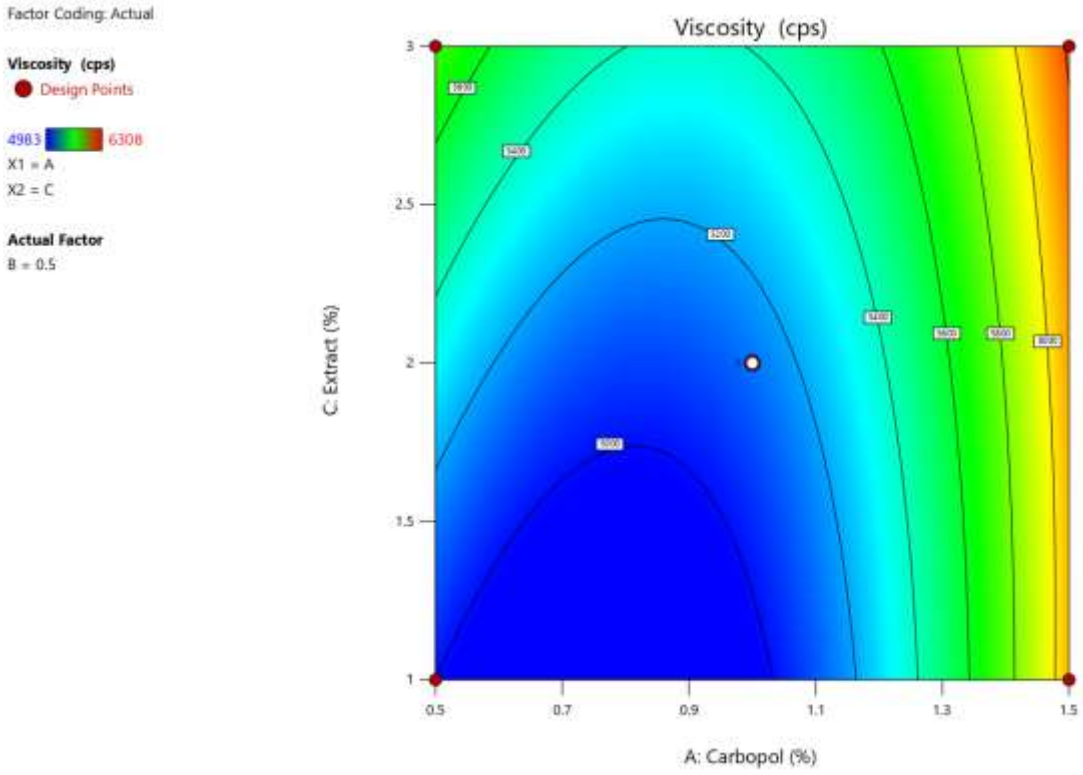


Figure 7.7 Contour plot (2D) showing the combined effect of Extract and Carbopol on Viscosity

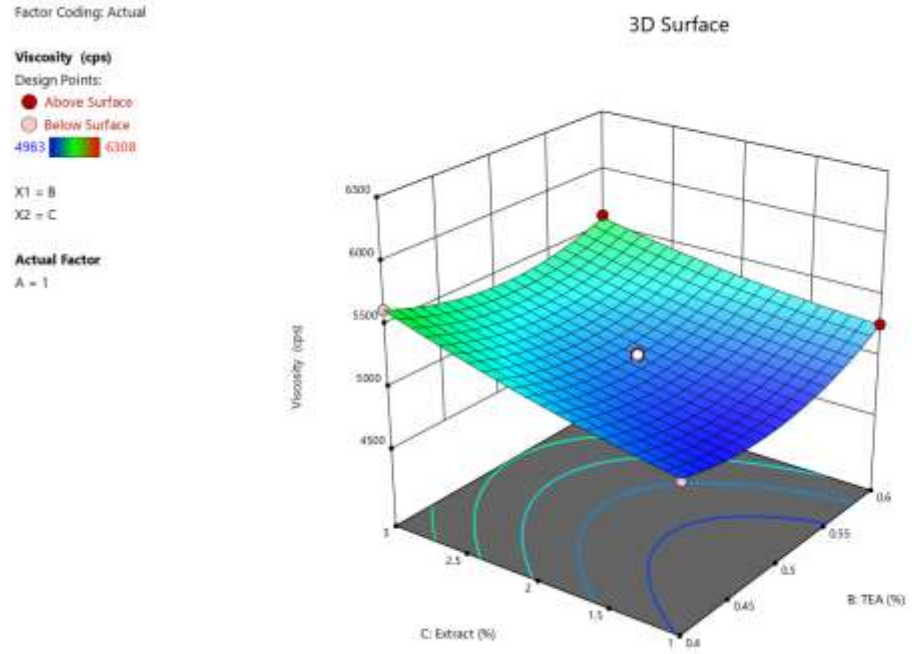


Figure 7.8 Response surface (3D) showing the combined effect of Extract and TEA on Viscosity

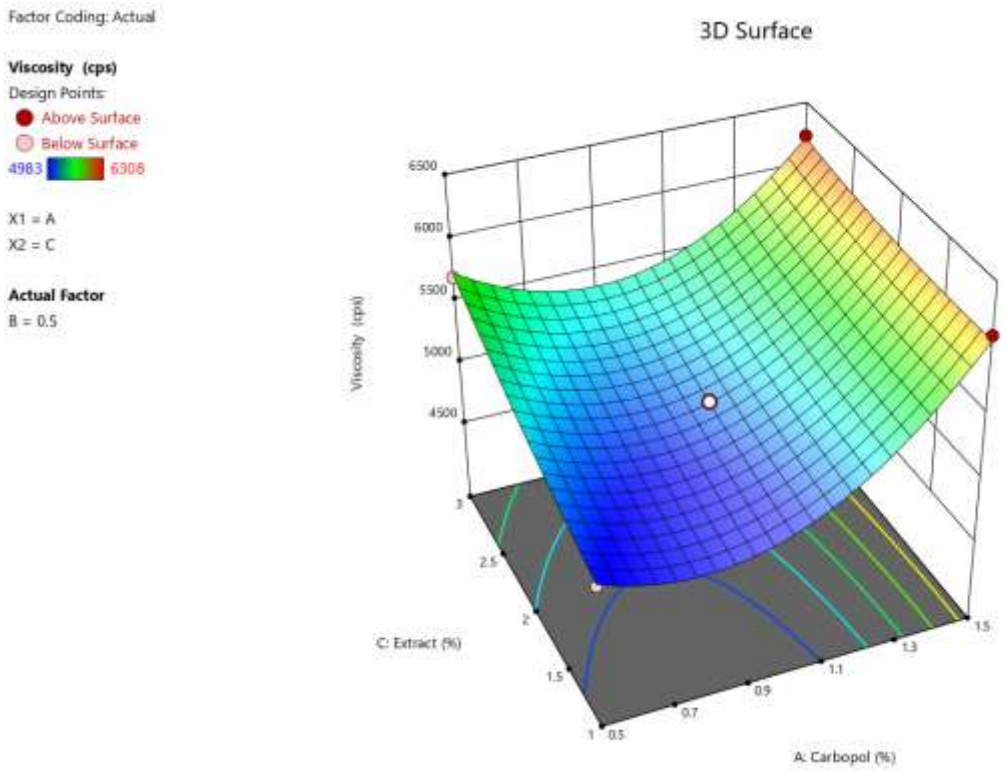


Figure 7.9 Response surface (3D) showing the combined effect of Extract and Carbopol on Viscosity

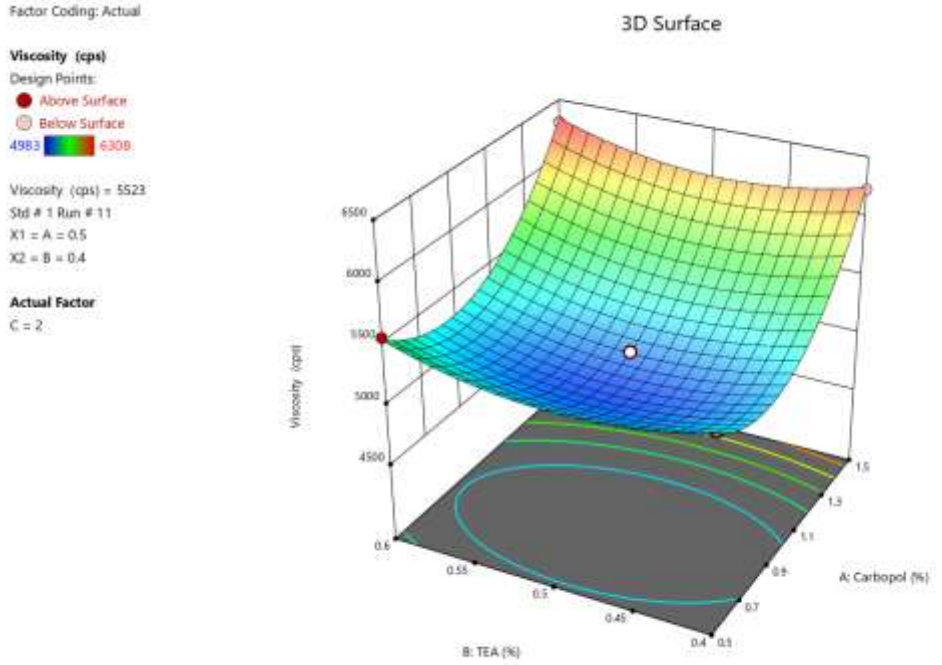


Figure 7.10 Response surface (3D) showing the combined effect of TEA and Carbopol on Viscosity

7.3.2.2 Influence of investigated parameters on Spreadability

A) Statistical Analysis for Spreadability

The statistical analysis of the design mentioned above is as follows:

Table 7.13 Statistical analysis of design for Spreadability

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.0936	0.8470	0.7745	
2FI	0.8029	0.0711	0.8129	0.5652	
Quadratic	**0.0003**	**0.9536**	**0.9917**	**0.9880**	**Suggested**
Cubic	0.9536		0.9818		**Aliased**

As shown in Table 7.13, the best model to fit the experimental results of Spreadability in Gel is the quadratic model and was chosen for further evaluation.

B) ANOVA Analysis for Spreadability

Table 7.14 The ANOVA for Spreadability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.17	9	0.3527	185.79	< 0.0001	significant
A-Carbopol	2.62	1	2.62	1381.24	< 0.0001	
B-TEA	0.0078	1	0.0078	4.12	0.0983	
C-Extract	0.1711	1	0.1711	90.14	0.0002	
AB	0.0210	1	0.0210	11.08	0.0208	
AC	0.0132	1	0.0132	6.97	0.0460	
BC	0.0081	1	0.0081	4.27	0.0938	
A^2	0.3305	1	0.3305	174.08	< 0.0001	
B^2	0.0005	1	0.0005	0.2647	0.6288	
C^2	0.0017	1	0.0017	0.9131	0.3832	
Residual	0.0095	5	0.0019			
Lack of Fit	0.0012	3	0.0004	0.0988	0.9536	not significant
Pure Error	0.0083	2	0.0041			

Cor Total	3.18	14				

Factor coding is **Coded**. Sum of squares is **Type III – Partial.** The Model F-value of 185.79 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, C, AB, AC, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 0.10 implies the Lack of Fit is not significant relative to the pure error. There is a 95.36% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

The spreadability of gel preparations is defined as the ability of the gel to be spread on the surface of the skin. The greater the scatter diameter, the greater the surface area that can be reached by the gel. Good spreadability can guarantee the distribution of a gel when applied to the skin, good spreadability ranges from 5–7 g.cm/s.

Table 7.15 ANOVA study results for Spreadability

Parameters	**Results of Response**	**Parameters**	**Results of Response**
Std. Dev.	0.0436	**R^2**	0.9970
Mean	5.71	**Adjusted R^2**	0.9917
C.V. %	0.7625	**Predicted R^2**	0.9880
		Adeq Precision	40.4080

The **Predicted R^2** of 0.9880 is in reasonable agreement with the **Adjusted R^2** of 0.9917; i.e. the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 40.408 indicates an adequate signal. This model can be used to navigate the design space.

C) Mathematical Model for Spreadability

To evaluate the effect of various factors on Spreadability, contour plots and 3D plots were referred to along with the value of ANOVA. From Table 7.14, we can observe that with change in the combination of various levels of factors, the final response, i.e., Spreadability confirming

the effect of various factors. Looking closely at different factor involved provide us a better understanding of the extent of the impact. The equation talks about the type of effect that is positive or negative.

Table 7.16 Coded equation for Spreadability

Spreadability	=
+5.54	
-0.5725	A
-0.0313	B
-0.1462	C
+0.0725	AB
+0.0575	AC
+0.0450	BC
+0.2992	A^2
+0.0117	B^2
+0.0217	C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Additionally, from the above equation, the spreadability of gel preparations is defined as the ability of the gel to be spread on the surface of the skin. The greater the scatter diameter, the greater the surface area that can be reached by the gel. Good spreadability can guarantee the distribution of a gel when applied to the skin, good spreadability ranges from 5–7 g.cm/s. All the gel preparations reveal a value between 5.10–6.98 g.cm/s (Table 7.8), which indicates that the gel has a good spreadability.

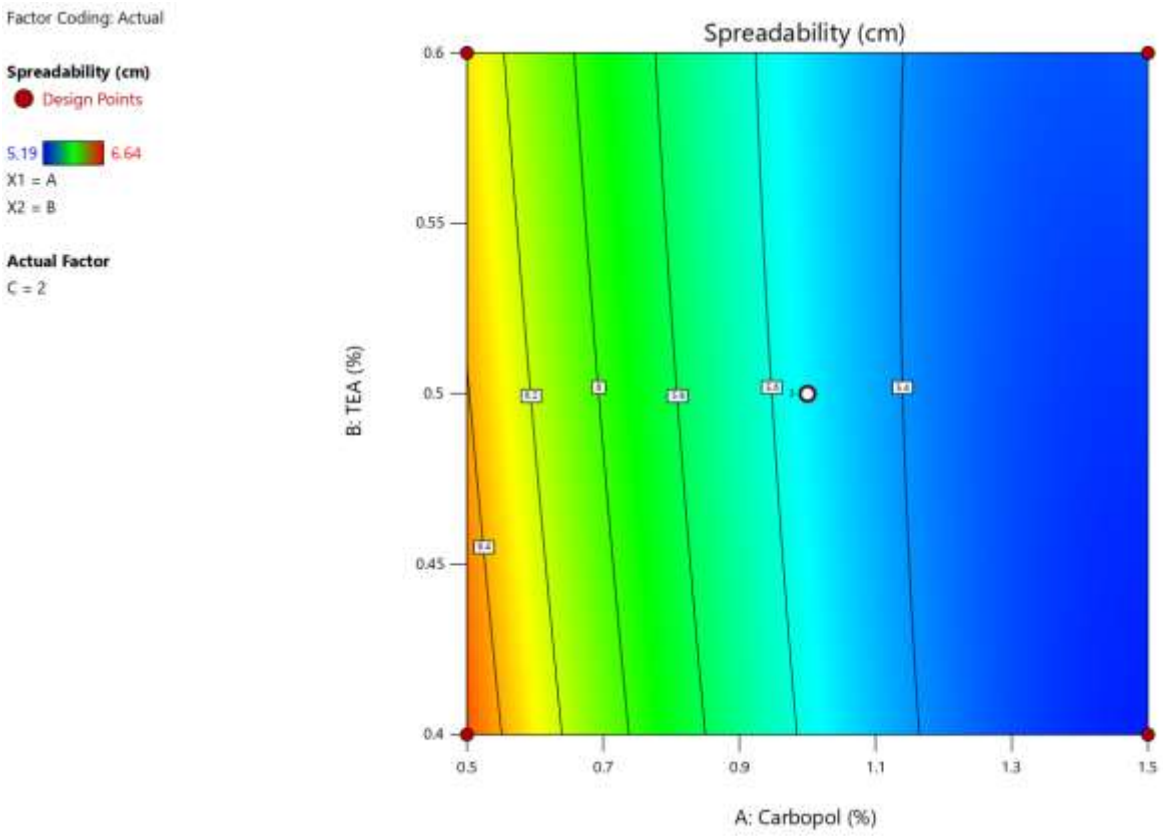


Figure 7.11 Contour plot (2D) showing the combined effect of TEA and Carbopol on Spreadability

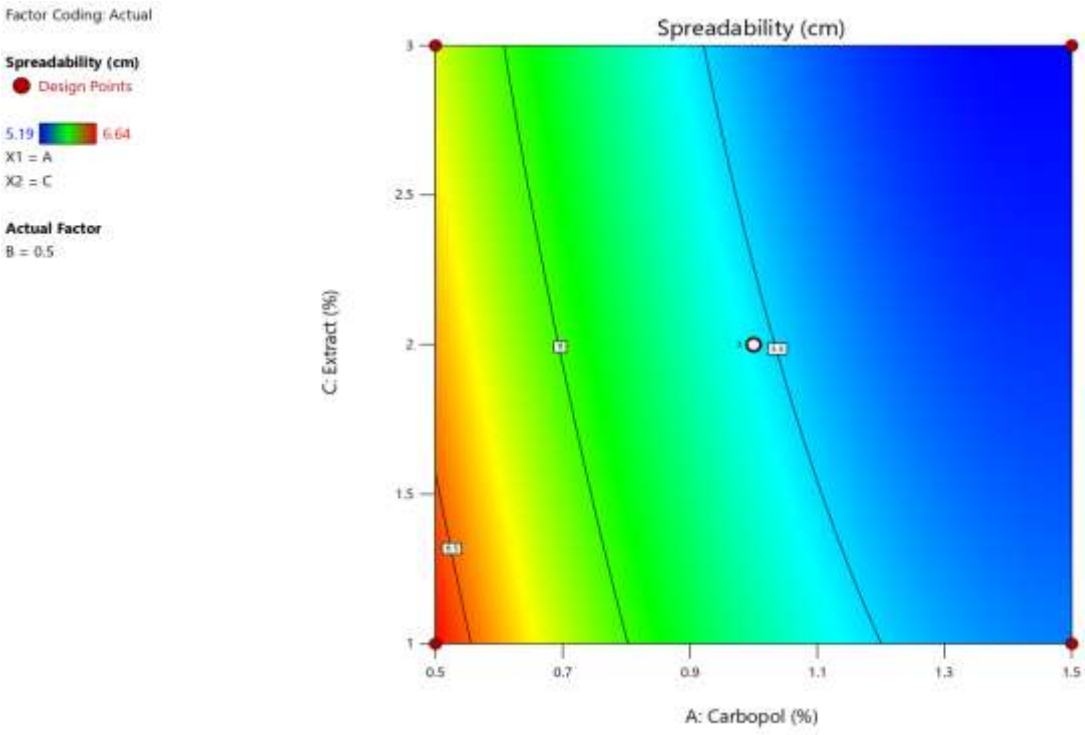


Figure 7.12 Contour plot (2D) showing the combined effect of Carbopol and Extract on Spreadability

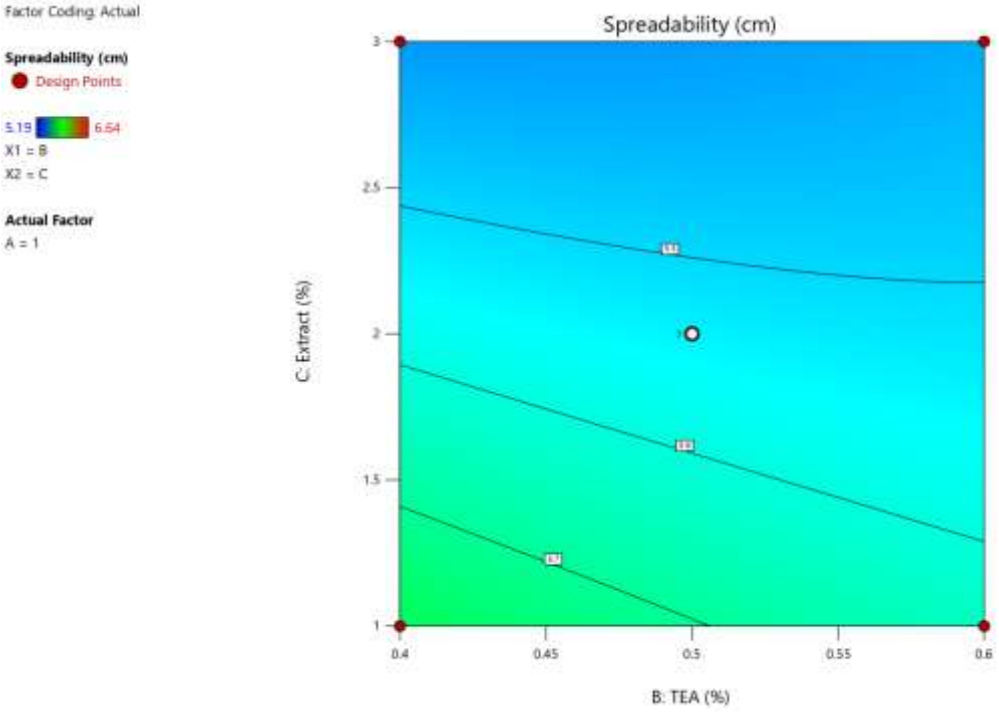


Figure 7.13 Contour plot (2D) showing the combined effect of TEA and Extract on Spreadability

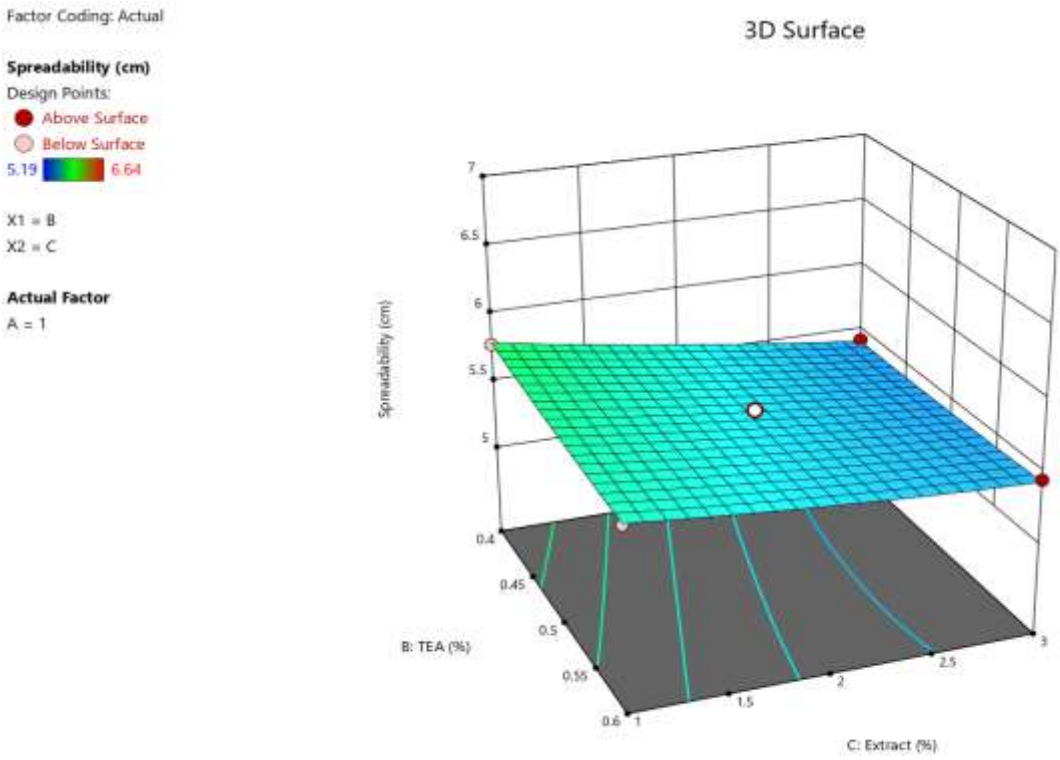


Figure 7.14 Response surface (3D) showing the combined effect of Extract and TEA on Spreadability

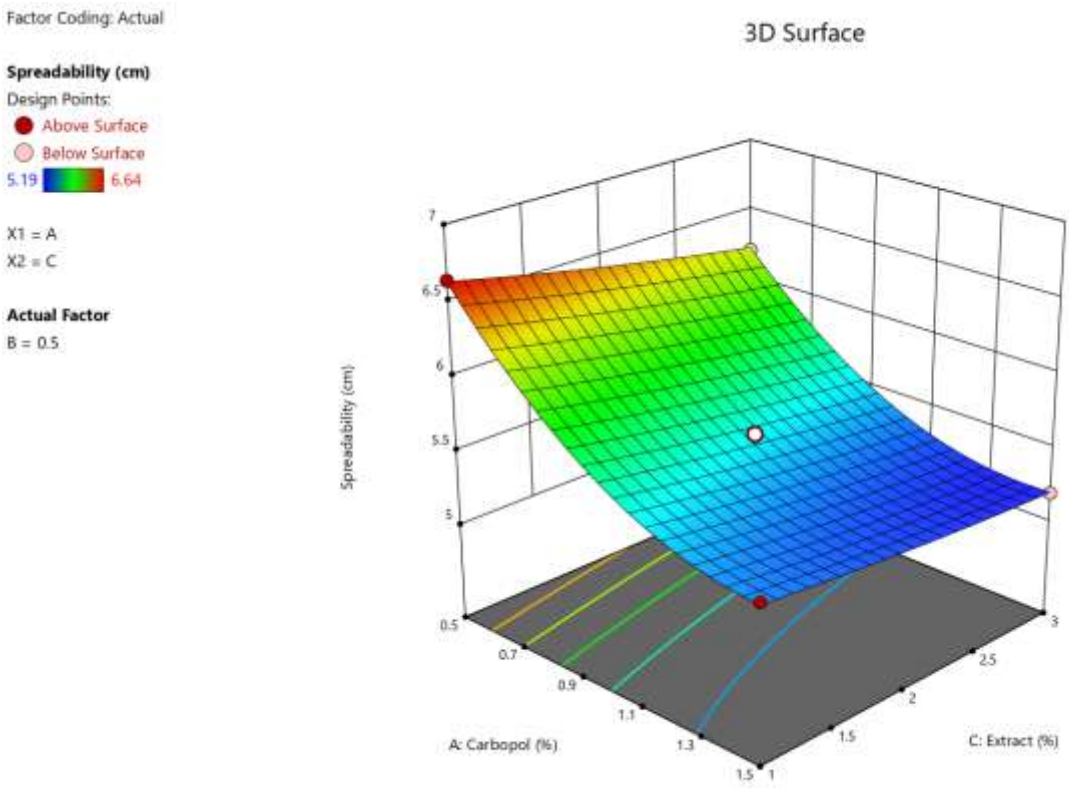


Figure 7.15 Response surface (3D) showing the combined effect of Extract and Carbopol on Spreadability

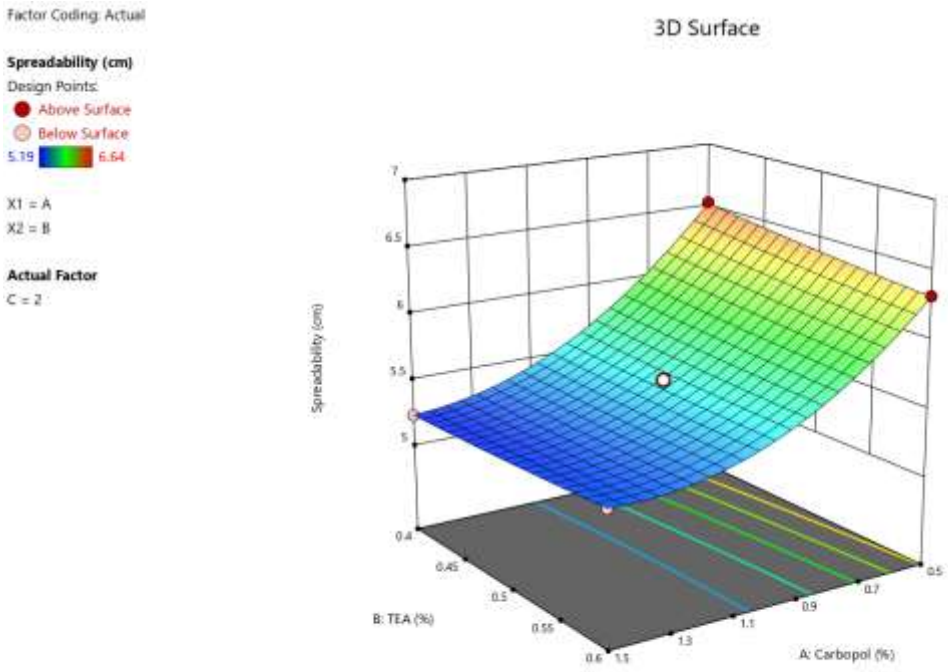


Figure 7.16 Response surface (3D) showing the combined effect of TEA and Carbopol on Spreadability

7.3.2.3 Influence of investigated parameters on pH

A) Statistical Analysis for pH

Table 7.17 The statistical analysis of the design for pH

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.0339	0.8396	0.7403	
2FI	0.5998	0.0283	0.8232	0.5046	
Quadratic	**0.0003**	**0.4538**	**0.9919**	**0.9669**	**Suggested**
Cubic	0.4538		0.9933		**Aliased**

As shown in Table 7.16, the best model to fit the experimental results of pH in Gel is the quadratic model and was chosen for further evaluation.

B) ANOVA Analysis for pH

Table 7.18 The ANOVA for pH.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.87	9	0.2074	191.15	< 0.0001	significant
A-Carbopol	0.2415	1	0.2415	222.59	< 0.0001	
B-TEA	1.39	1	1.39	1285.21	< 0.0001	
C-Extract	0.0001	1	0.0001	0.1037	0.7605	
AB	0.0380	1	0.0380	35.05	0.0020	
AC	0.0081	1	0.0081	7.47	0.0412	
BC	0.0006	1	0.0006	0.5760	0.4821	
A^2	0.0156	1	0.0156	14.38	0.0127	
B^2	0.1590	1	0.1590	146.52	< 0.0001	
C^2	0.0000	1	0.0000	0.0000	1.0000	
Residual	0.0054	5	0.0011			
Lack of Fit	0.0036	3	0.0012	1.34	0.4538	not significant
Pure Error	0.0018	2	0.0009			
Cor Total	1.87	14				

Factor coding is **Coded**. Sum of squares is **Type III – Partial.** The **Model F-value** of 191.15 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, AC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 1.34 implies the Lack of Fit is not significant relative to the pure error. There is a 45.38% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

The pH was increased with an increasing concentration of TEA used in accordance with a p-value of <0.0001. TEA is difficult to evaporate at room temperature, has an ammonia odor, and can form a solid or liquid depending on its temperature and the value of its purity.

Table 7.19 ANOVA study results for pH

Parameters	**Results of Response**	**Parameters**	**Results of Response**
Std. Dev.	0.0329	**R^2**	0.9971
Mean	5.84	**Adjusted R^2**	0.9919
C.V. %	0.5644	**Predicted R^2**	0.9669
		Adeq Precision	43.9675

The **Predicted R^2** of 0.9669 is in reasonable agreement with the **Adjusted R^2** of 0.9919; i.e. the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 43.968 indicates an adequate signal. This model can be used to navigate the design space.

C) Mathematical Model for pH

To evaluate the effect of various factors on pH, contour plots and 3D plots were referred to along with the value of ANOVA. From Table 7.18, we can observe that with change in the combination of various levels of factors, the final response, i.e., pH confirming the effect of various factors. Looking closely at different factor involved provide us a better understanding

of the extent of the impact. The equation talks about the type of effect that is positive or negative.

Table 7.20 Coded equation for pH

pH	=
+5.76	
+0.1737	A
+0.4175	B
+0.0038	C
+0.0975	AB
-0.0450	AC
+0.0125	BC
-0.0650	A^2
+0.2075	B^2
+0.0000	C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The acidity degree (pH) is a very important parameter in gel preparations since gel is a topical skin (4.5–6.5) to avoid irritation or erythema on the skin. This test shows that the pH of the gel preparation ranges from 5 to 6.5, which is still in the human skin's pH range (Table 7.8). The quadratic design model has a significant effect on the acidity degree of gel preparations, with a p-value of 0.0001 (less than 0.0500), as shown in Table 7.16. Significant factors on acidity are A, B, and B2 because they have p-values, respectively, of 0.0016; <0.0001, and 0.0005. The insignificance of the"lack of fit" with an F-value of 6.41 and a p-value of 5.23%, indicates that the quadratic design model is appropriate for analyzing the acidity test data, but this model has a low probability because its p-value is less than 10%.

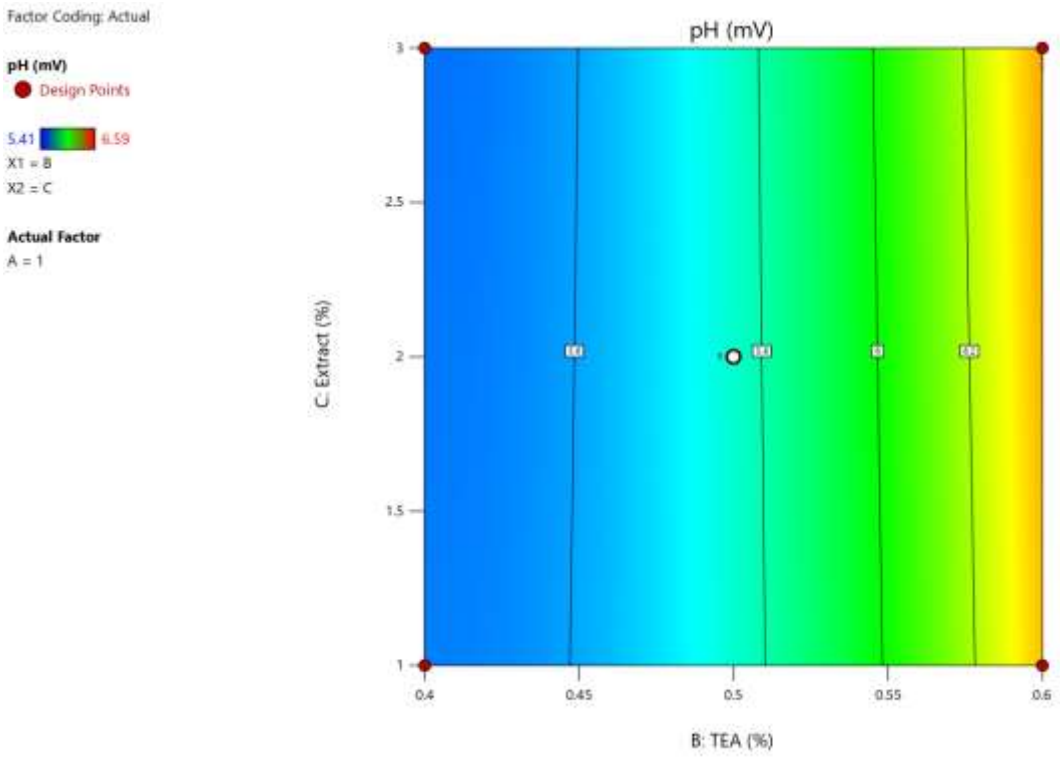


Figure 7.17 Contour plot (2D) showing the combined effect of TEA and Extract on pH

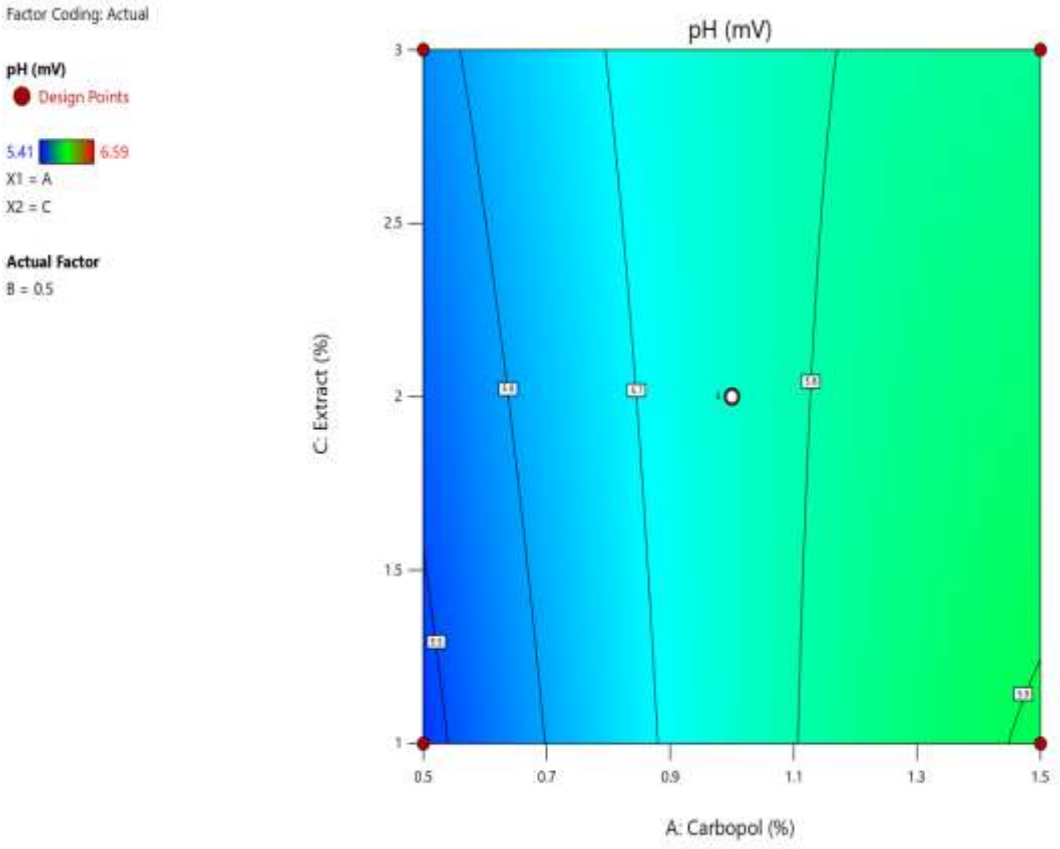


Figure 7.18 Contour plot (2D) showing the combined effect of Carbopol and Extract on pH

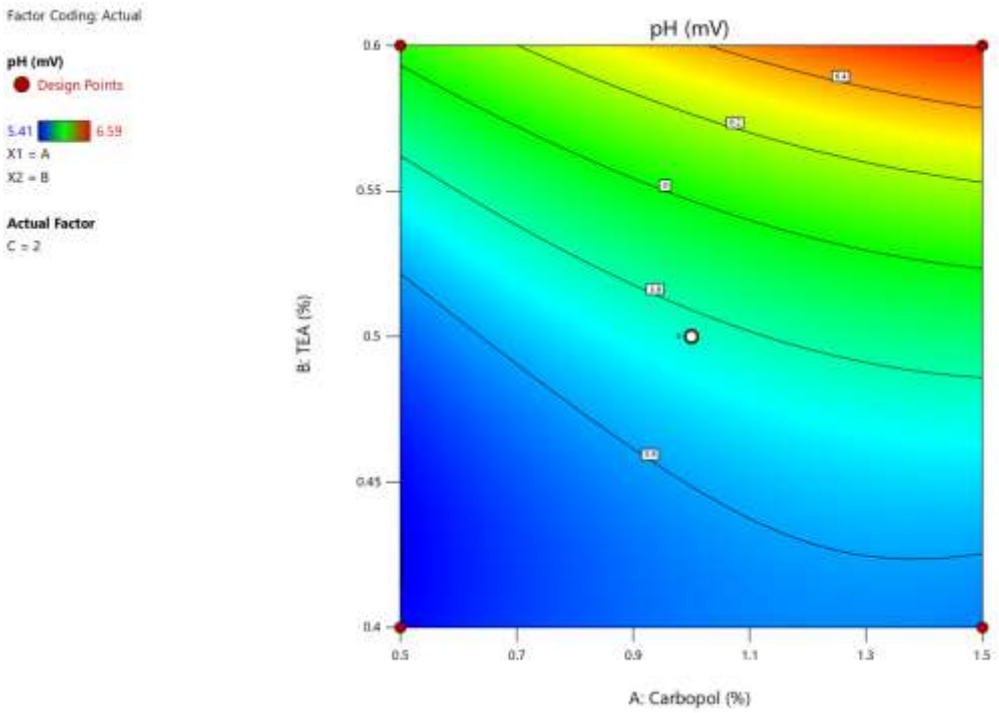


Figure 7.19 Contour plot (2D) showing the combined effect of Carbopol and TEA on pH

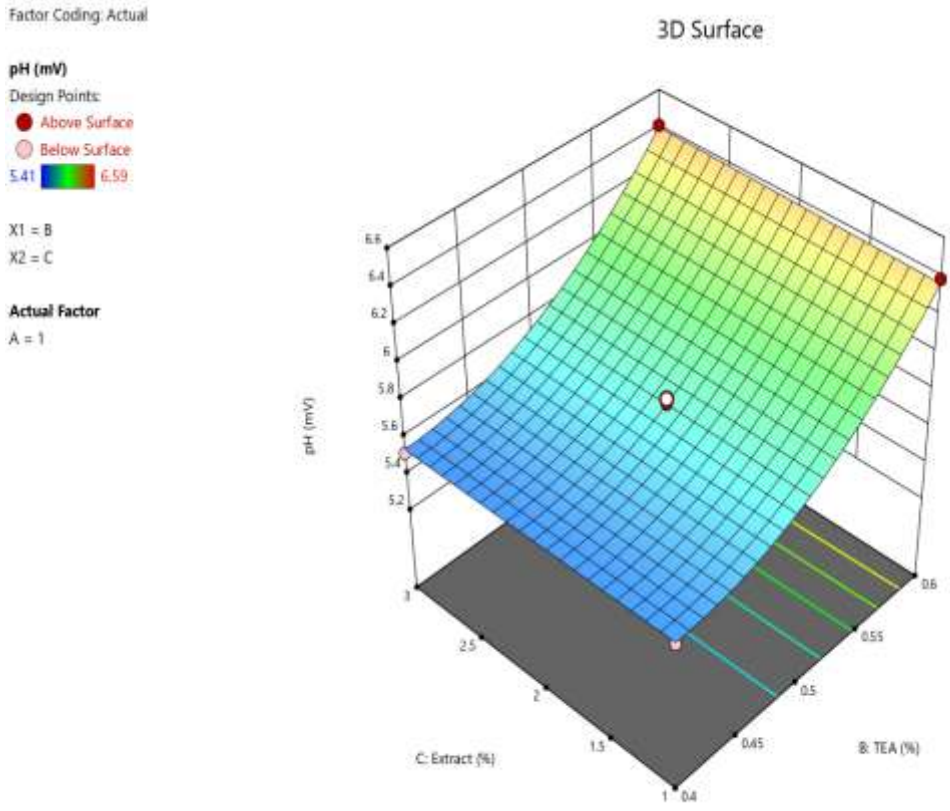


Figure 7.20 Response surface (3D) showing the combined effect of Extract and TEA on pH

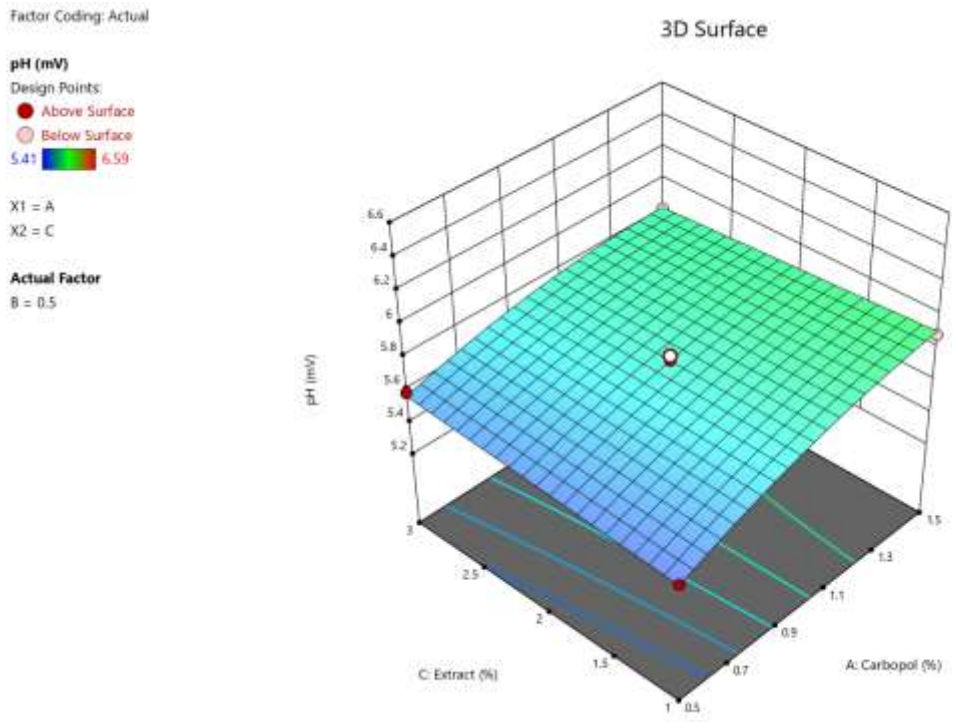


Figure 7.21 Response surface (3D) showing the combined effect of Extract and Carbopol on pH

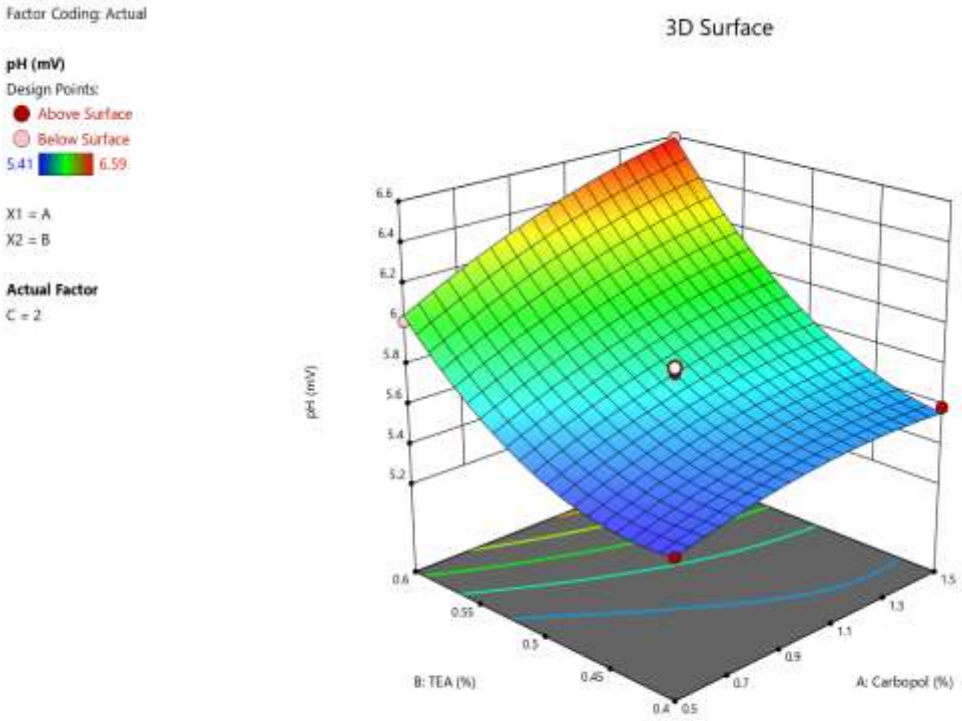


Figure 7.22 Response surface (3D) showing the combined effect of TEA and Carbopol on pH

❖ **Optimization using Desirability plot**

A desirability plot gives the optimum value of variables to get desired responses. A desirability plot was generated (Fig. 23) using Design Expert 13.0.

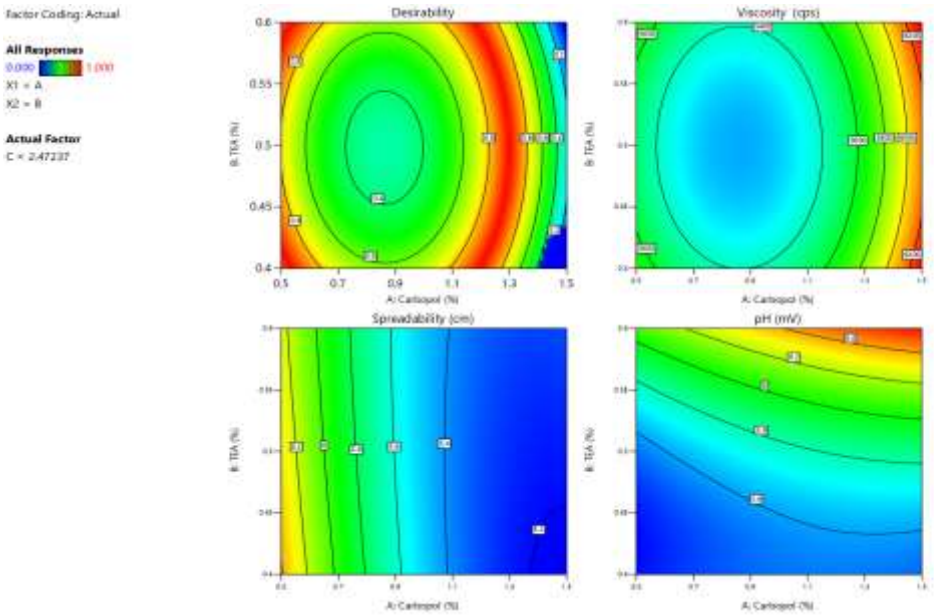


Figure 7.23 Desirability plot

7.3.3 Establishment of Design Space

ICH Q8 (2008) defines "Design Space" as a "multidimensional combination and interaction input variables and process parameters that have been established to provide assurance of quality." The composite desirability function based on the set constraints was used to determine the conditions that would result in an optima formulation design.

7.3.3.1Overlay Plot for predicted design space

The experimental design was used for numerous responses: Viscosity, Spreadability and pH. Overlay plot (Fig. 7.24) can be obtained by superimposing contour plots of all three responses, which displays possible response values in the factor space. The region highlighted in yellow is where a slight variation in the critical variables won't affect the final response and the response will be in the desired range. Areas that do not fit the optimization criteria are shaded gray, while design space is accepted colored yellow. Fig. 7.24 shows an overlay plot based on the desirability criteria.

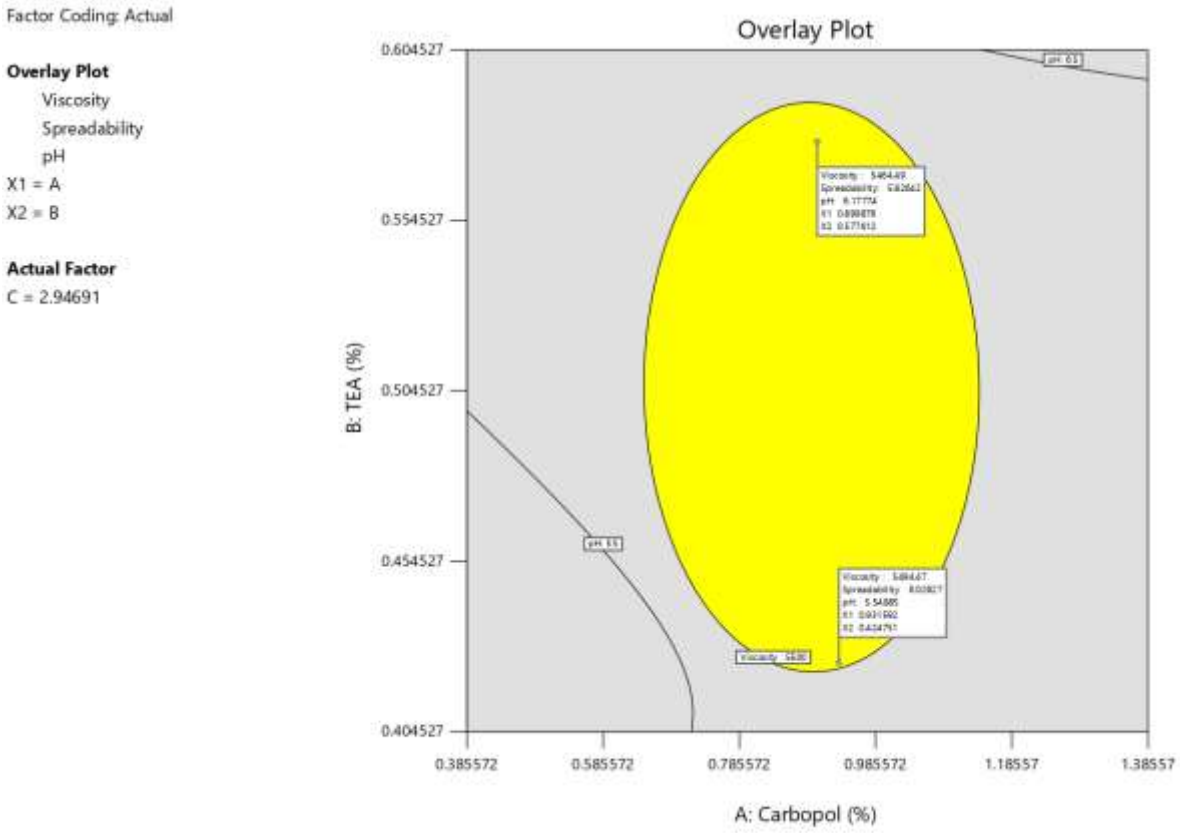


Figure 7.24 Overlay plot

Table 7.21 Composition of optimized batch of Polyherbal gel by Overlay Plot

Batch	**Carbopol 934 % (X1)**	**Triethanolamine (TEA)% (X2)**	**Parameters**	**Predicted value**	**Observed value**	**% Error**
A	0.93	0.42	Viscosity (cp)	5494	5490	0.07
			Spreadability (g.cm/sec)	6.02	5.89	2.2
			pH	5.54	5.34	3.7
B	0.89	0.57	Viscosity (cp)	5464	5422	0.77
			Spreadability (g.cm/sec)	5.82	5.71	1.9
			pH	6.1	5.9	3.3

Gel formulation is more preferred, among the other topical semisolid preparations, since it has long residence time on the skin, high viscosity, moisturizing effect on flaky skin due to their occlusive properties, more bio adhesiveness, less irritation, independent of water solubility of active ingredient, ease of application and better release characters.

7.3.4 Evaluation of optimised batch of Polyherbal gel

1)Appearance and Homogeneity- The appearance, texture, and transparency of the prepared gels were examined visually. The physical appearance of Polyherbal gel was shown in figure.

Figure 7.24 Appearance of developed Polyherbal Gel

2) pH- Prepared gels were found to be homogeneous and in good appearance and consistency. The pH values of all the formulations were in the close range of neutral pH 5-6.5 and hence it caused no skin irritation, which is also supported by skin irritation study.

3) Viscosity- Polymers were included in the designed topical formulations in order to provide a prompt release of drug and to achieve as well as to maintain the drug concentration within the therapeutically effective range. The value between 4900-6300 cp was reported for topical gel formulation developed using Carbopol 934 polymers.

4) Spreadability- The spreadability of Polyherbal gel was found to be 6. 21g.cm/s, demonstrates the good spreadability of the formulated gel.

5) Extrudability

The extrusion of the gel from the tube plays a crucial role in its application and patient acceptance. Gels that are too thick may not easily extrude from the tube, while gels that are too

thin may flow too quickly. Therefore, an appropriate consistency is necessary to ensure smooth extrusion. All gel formulations demonstrated good extrudability.

Table 7.22 Evaluation of optimised batch of Polyherbal gel

Evaluation parameter	**Value of parameter**
Appearance and Homogeneity	Brownish Transparent
pH	5.7
Viscosity	5600 cp
Spreadability	5.89 g.cm/s
Extrudability	Good

7.3.5 Evaluation of Polyherbal Spray

Table 7.23 Evaluation parameters of developed Polyherbal Spray

Parameters (n=3)	**Results**
pH	6.7
Clarity of solution	Clear
Delivery rate of Polyherbal spray	0.83 g/sec
Spray pattern	2.56 cm
Area covered by each spray	5.5 cm^2
Leakage from container	0.17 %

Leakage from container

The leakage test for nine Sprays were significantly different, but the results still met the requirement of USP. The product passed the average leakage test if the rate per year for the nine containers is not more than 3.5% of the net fill weight.

Stability studies

Table 7.24 Stability studies of Both Formulations

Formulation	Physiological Parameters	0 month	1 month	2 months	3 months	6 months
Polyherbal Gel	**Appearance**	Brownish transparent	Brownish transparent	Brownish transparent	Brownish transparent	Brownish transparent
	pH	5.2	5.5	6.0	5.7	5.8
	Viscosity (cps)	5200	5500	5679	5577	6006
	Spreadability (g.cm/s)	5.5	5.9	5.7	5.9	6.0
Polyherbal Spray	**pH**	6.3	6.2	6.4	6.1	6.1
	Clarity of solution	Clear	Clear	Clear	Clear	Clear
	Delivery rate (g/sec)	0.77	0.74	0.78	0.81	0.77
	Spray pattern (cm)	2.5	2.5	2.6	2.4	2.9
	Area covered by each spray (cm^2)	5.5	5.5	5.7	5.4	6
	Leakage of container (%)	0.11	0.15	0.16	0.16	0.13

7.3.6 Assay of developed Polyherbal Gel and Polyherbal Spray

1) Quantification of Vasicine in developed Polyherbal Gel and Spray

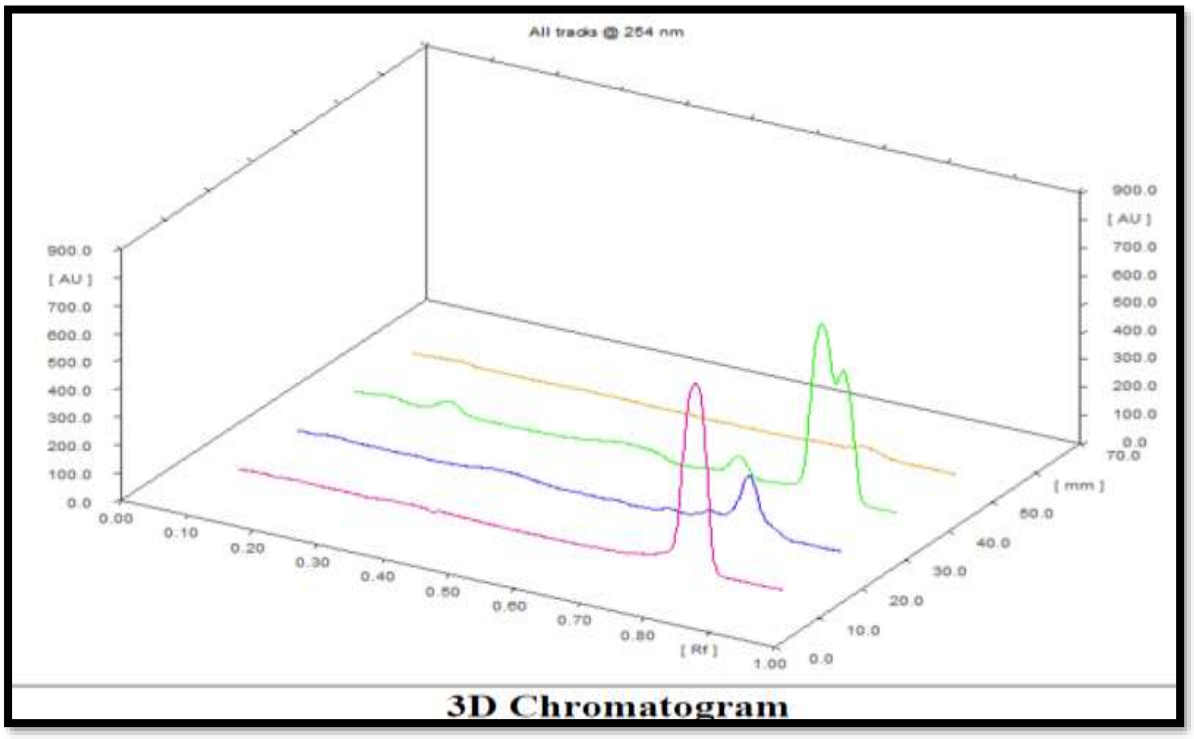


Figure 7.25 3D Chromatogram of Vasicine

R_f value

Spot No.	T1	T2	T3	T4
1	--	--	0.13	--
2	--	--	0.23	--
3	--	0.38	--	--
4	--	0.40	--	--
5	--	--	0.50	--
6	--	--	0.51	--
7	--	0.65	0.65	--
8	--	0.72	--	--
9 (Vasicine)	**0.78**	**0.78**	**0.78**	**0.78**
10	--	--	0.83	--

Track T1: Standard Vasicine
Track T2: Vasaka Extract
Track T3: Polyherbal Gel
Track T4: Herbal Spray

HPTLC Plate

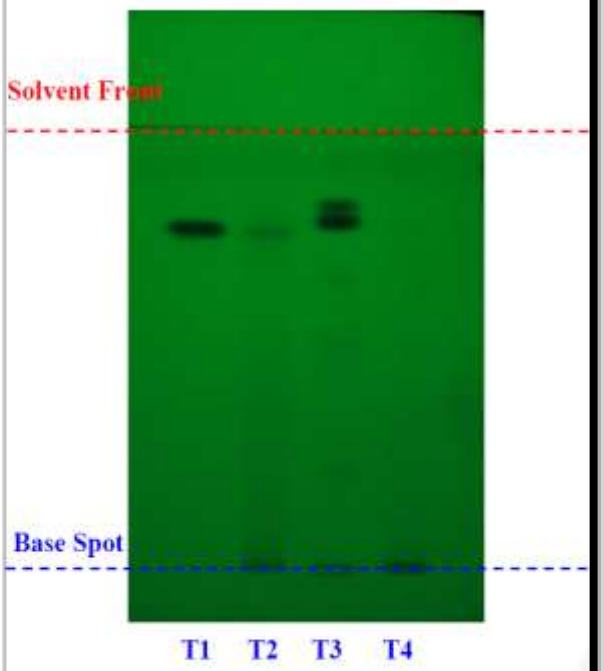


Figure 7.26 R_f values and HPTLC Plate of Vasicine

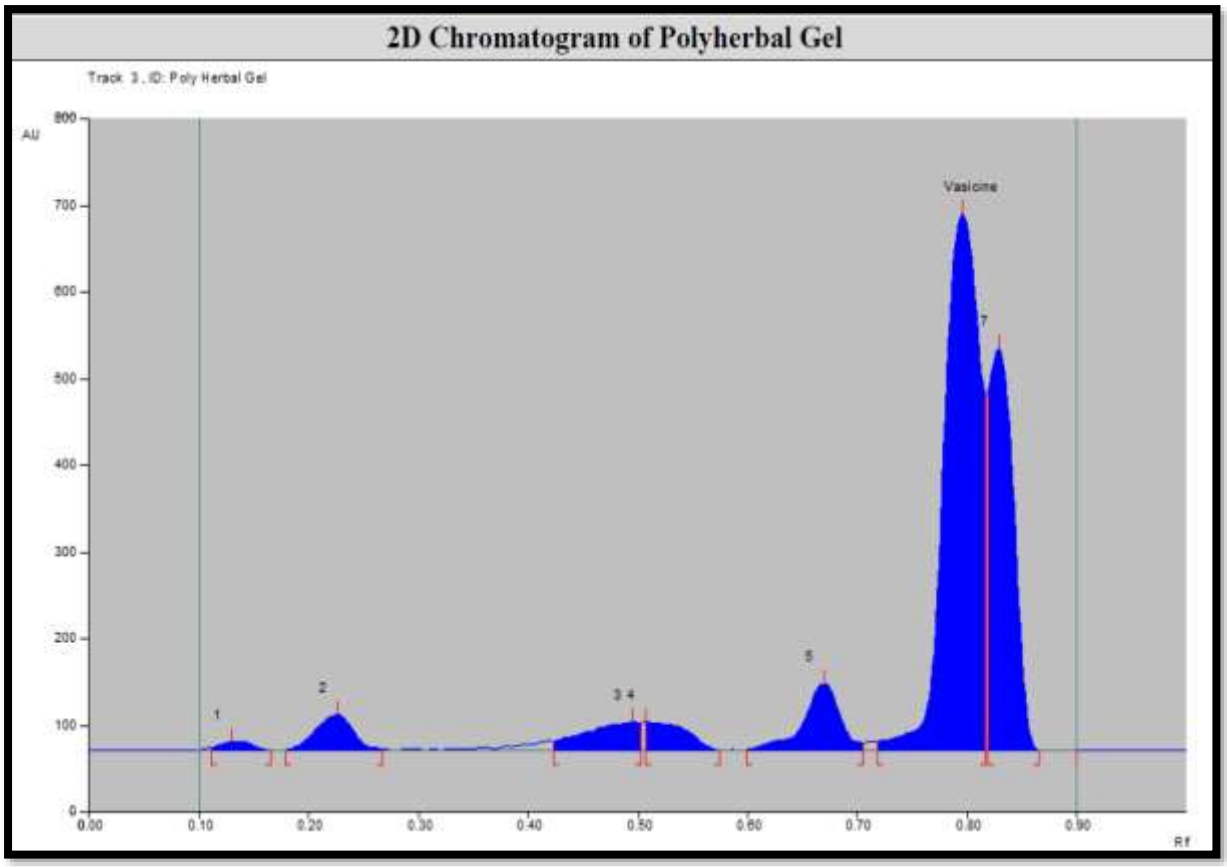


Figure 7.27 2D Chromatogram of Vasicine in Polyherbal Gel

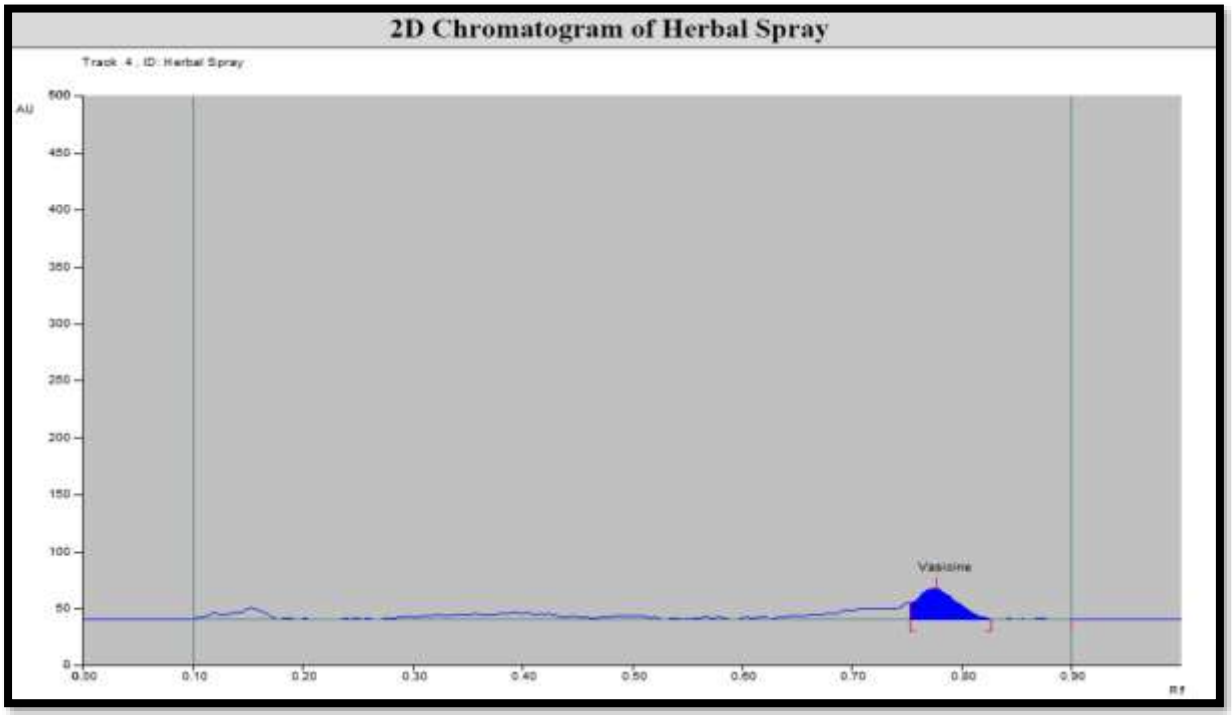


Figure 7.28 2D Chromatogram of Vasicine in Polyherbal Spray

2) Quantification of Rutin in developed Polyherbal Gel and Spray

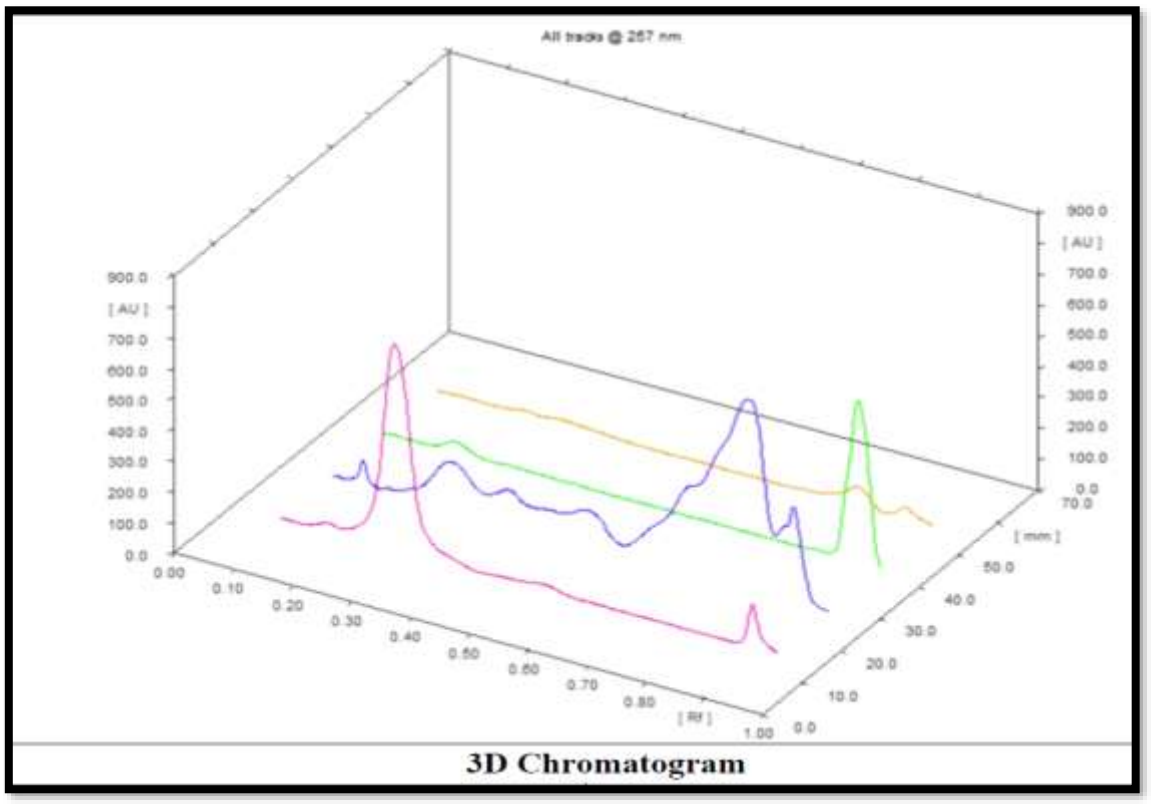


Figure 7.29 3D Chromatogram of Rutin

R_f value

Spot No.	T1	T2	T3	T4
1	--	0.13	--	--
2 (Rutin)	**0.25**	**0.25**	**0.25**	**0.25**
3	--	0.38	--	--
4	--	0.51	--	--
5	--	0.79	--	0.79
6	--	0.86	0.86	0.86

Track T1: Standard Rutin
Track T2: Rose Extract
Track T3: Polyherbal Gel
Track T4: Herbal Spray

HPTLC Plate

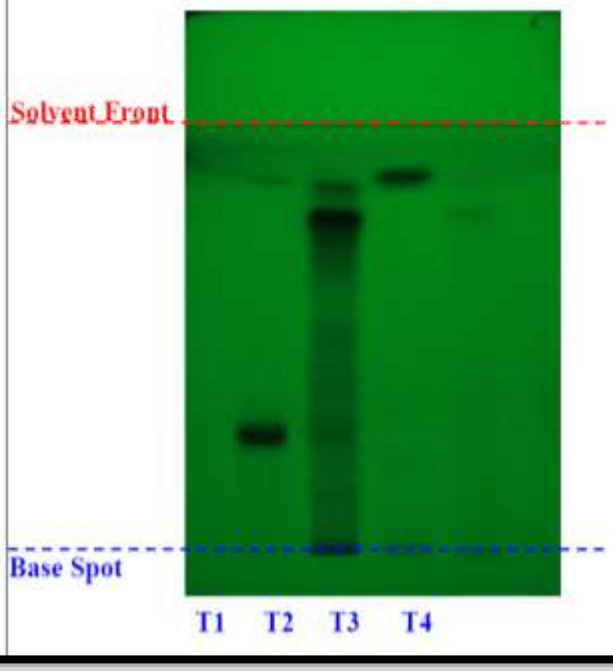


Figure 7.30 R_f values and HPTLC Plate of Rutin

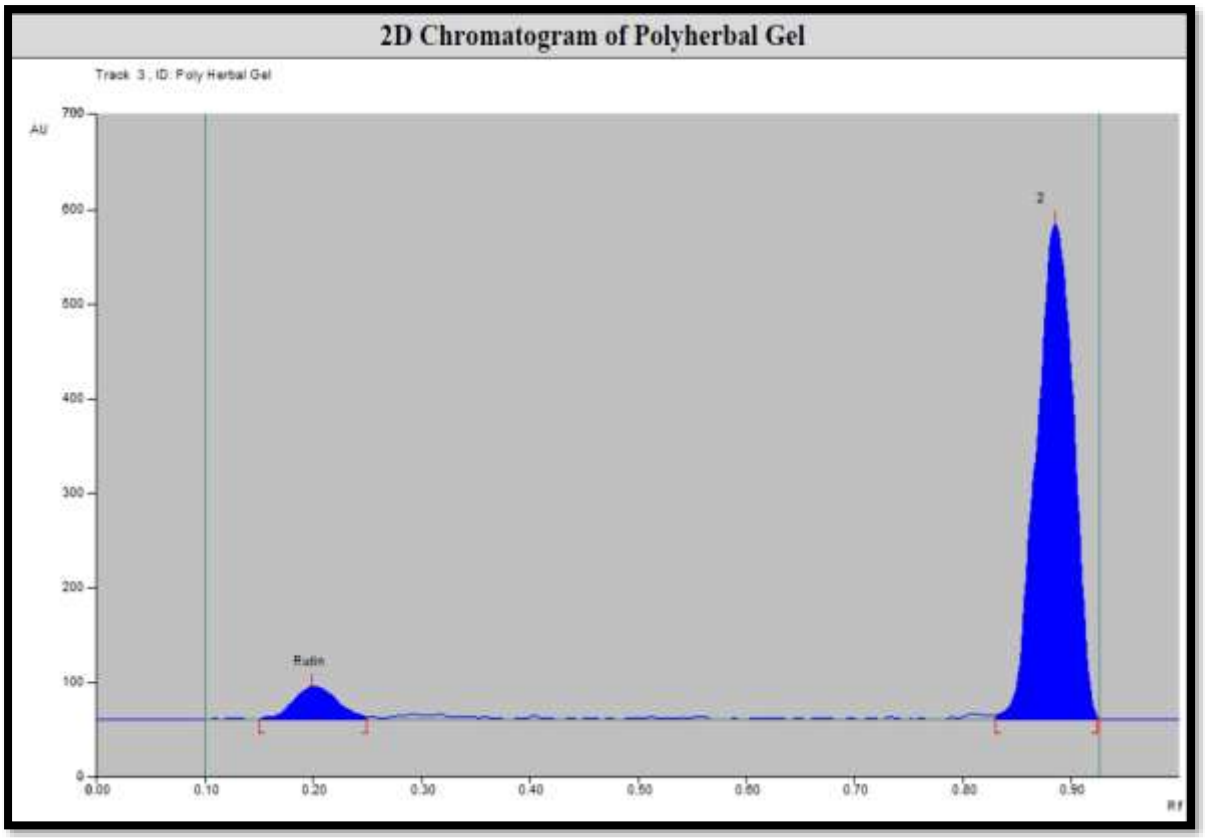


Figure 7.31 2D Chromatogram of Rutin in Polyherbal Gel

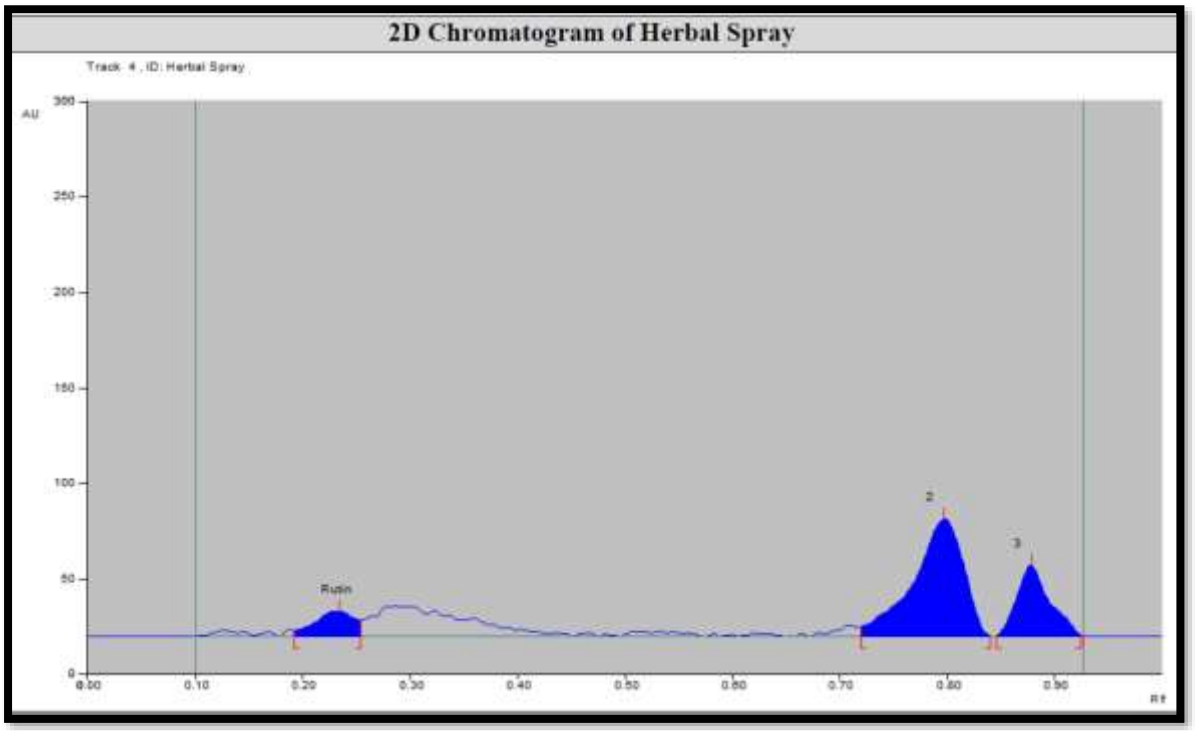


Figure 7.32 2D Chromatogram of Rutin in Polyherbal Spray

3) Quantification of Gallic acid in developed Polyherbal Gel and Spray

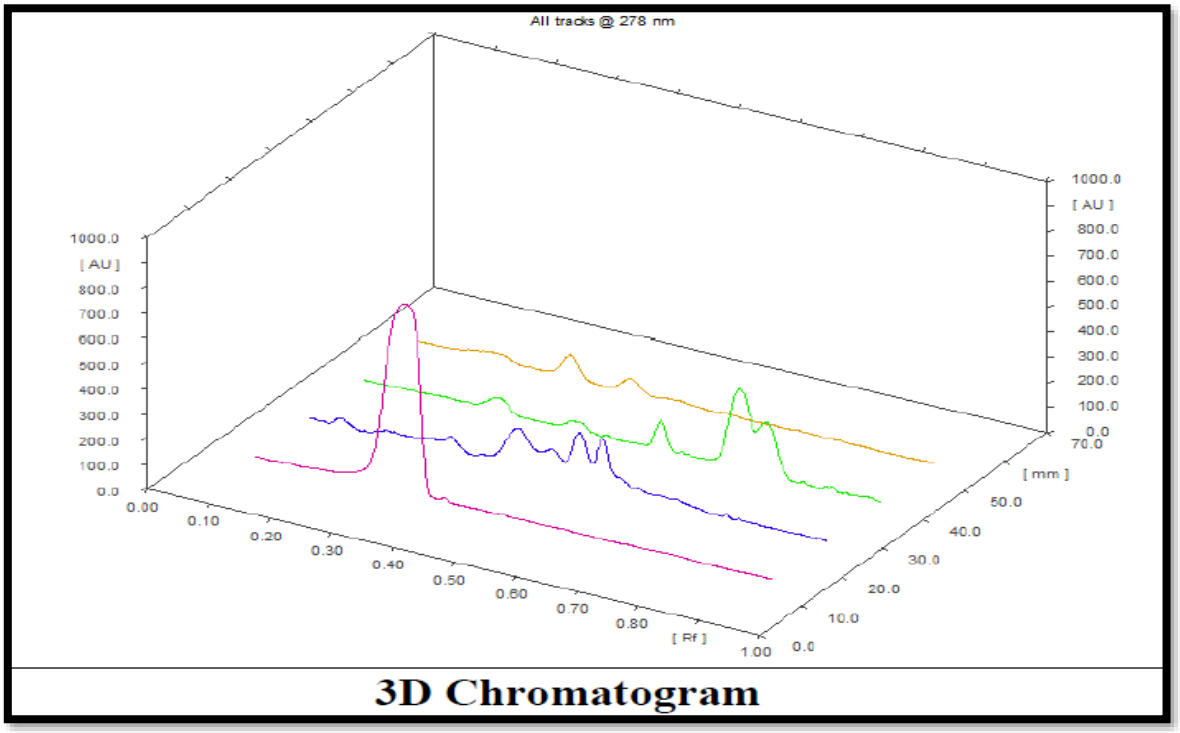
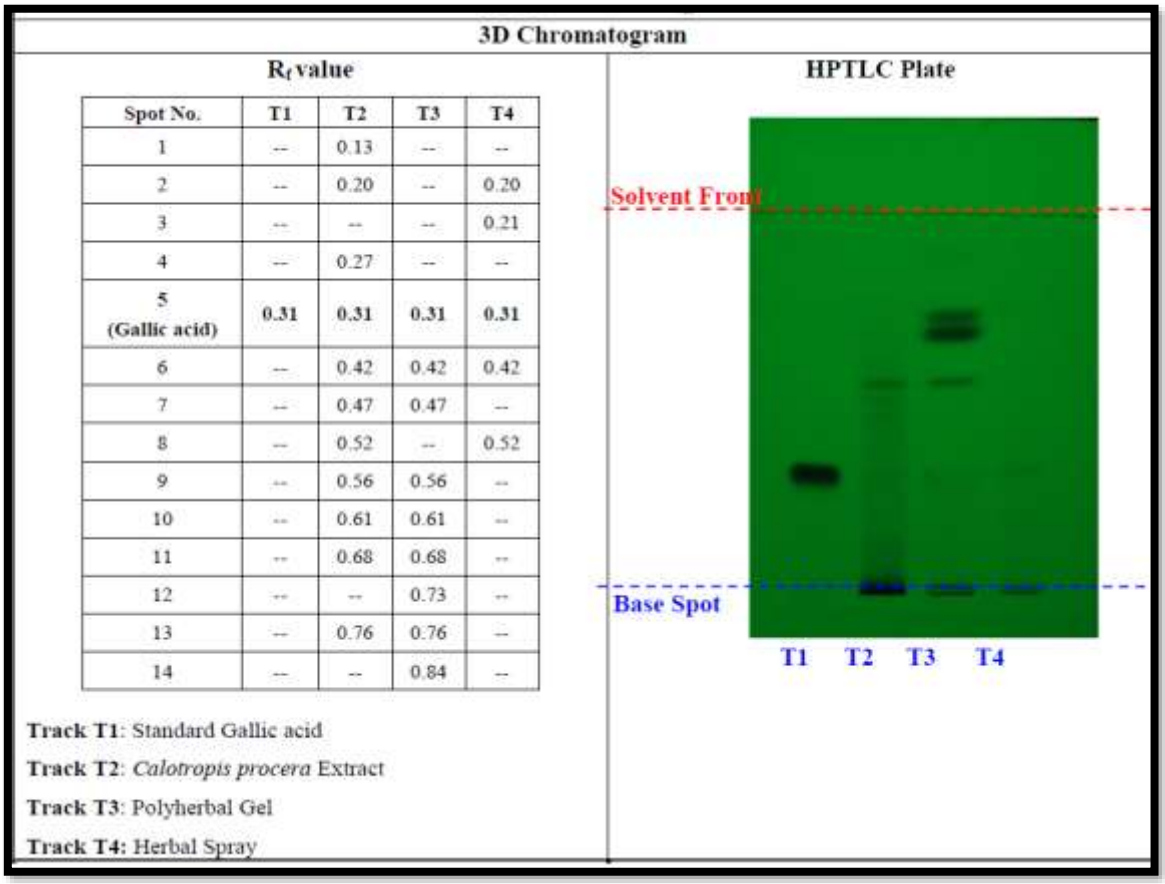


Figure 7.33 3D Chromatogram of Gallic acid



Spot No.	T1	T2	T3	T4
1	--	0.13	--	--
2	--	0.20	--	0.20
3	--	--	--	0.21
4	--	0.27	--	--
5 (Gallic acid)	**0.31**	**0.31**	**0.31**	**0.31**
6	--	0.42	0.42	0.42
7	--	0.47	0.47	--
8	--	0.52	--	0.52
9	--	0.56	0.56	--
10	--	0.61	0.61	--
11	--	0.68	0.68	--
12	--	--	0.73	--
13	--	0.76	0.76	--
14	--	--	0.84	--

Figure 7.34 R_f values and HPTLC Plate of Gallic acid

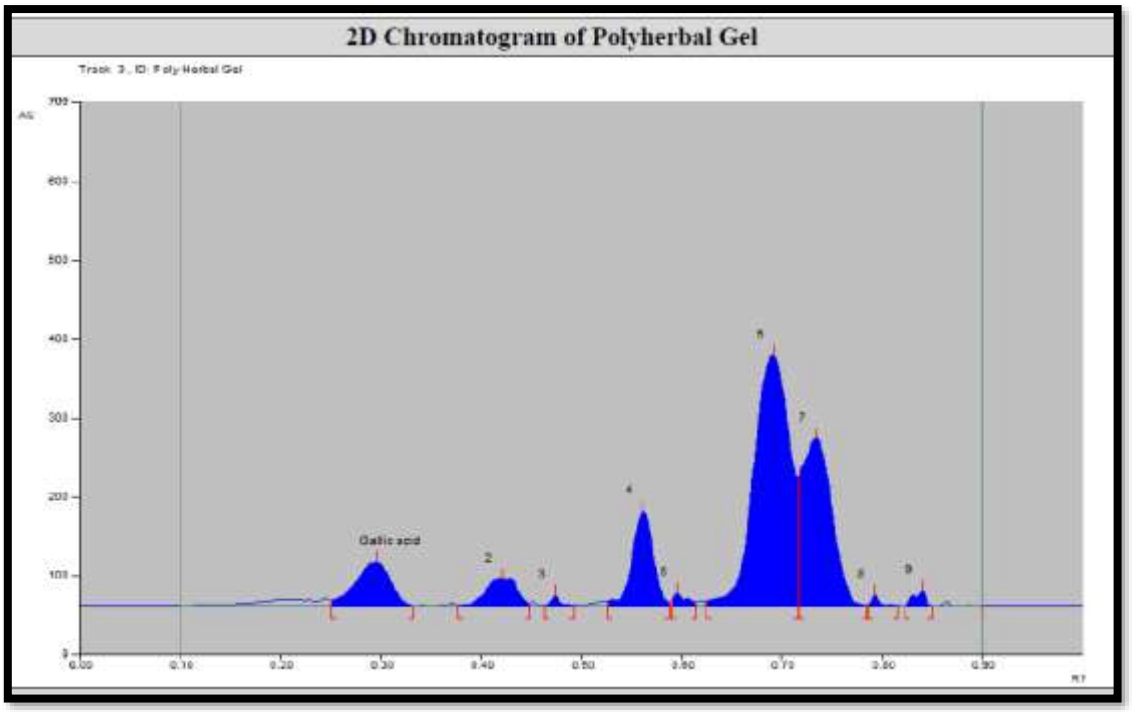


Figure 7.35 2D Chromatogram of Gallic acid in Polyherbal Gel

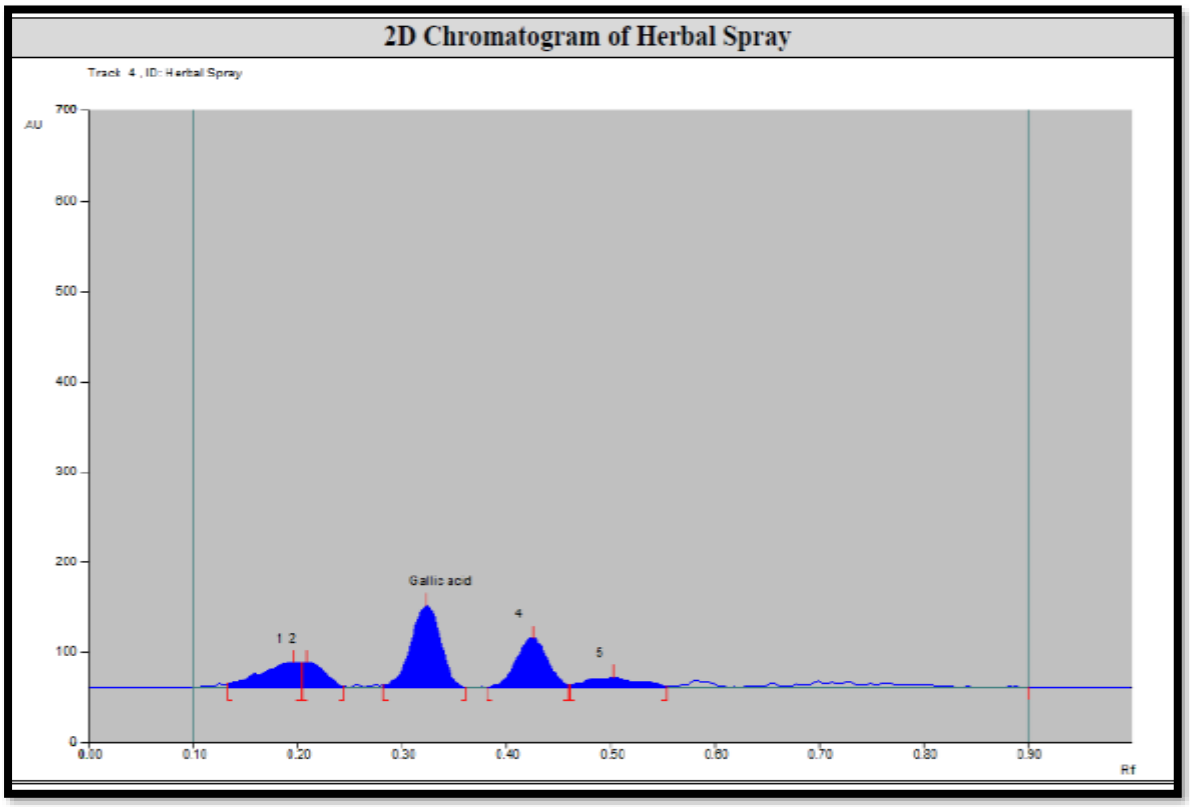


Figure 7.36 2D Chromatogram of Gallic acid in Polyherbal Spray

Table 7.25 Assay data of both the formulations

Formulation	Vasicine	Rutin	Gallic acid
Polyherbal Gel	0.151 %	0.031 %	0.058 %
Polyherbal Spray	0.038 %	0.012 %	0.026 %

7.4 Conclusion

Herbal topical gels represent a promising avenue for delivering natural therapeutic agents directly to the skin, harnessing the benefits of traditional herbal medicine in a modern and user-friendly format. The development of such formulations requires a thorough understanding of herbal pharmacology, formulation science, and regulatory requirements. By combining the wisdom of traditional medicine with contemporary scientific approaches, herbal topical gels can offer safe, effective, and sustainable solutions for a wide range of dermatological and inflammatory conditions.

The quadratic model in the Box-Behnken Design was used to produce the optimum formulation of *Adhatoda vasica, Rosa indica* and *Calotropis procera* extracts in polyherbal gel, with a composition of: Carbopol 934 (1%), TEA (0.4%), and Extracts (3%) [The concentration of the extract (3%) was selected based on the result of Design of experiment and to achieve better therapeutic activity within safe range.], which produce pH of (5-6.5), a spreadability of (5-7 g.cm/s), Good Extrudability and viscosity of (4900-6300 cps). Results have shown that gel formulations are good in appearance, homogeneity, extrudability, and spread ability. Synergistic effect between extracts of *Adhatoda vasica. Rosa indica*, and *Calotropis procera* in the herbal formulation Gel and Spray.

Herbal sprays represent a modern, convenient, and effective way to deliver the benefits of traditional herbal medicine. The development of such formulations requires a comprehensive understanding of herbal pharmacology, formulation science, and regulatory requirements. By integrating the wisdom of traditional herbal medicine with contemporary scientific methodologies, herbal sprays can offer safe, effective, and user-friendly solutions for a wide range of health conditions. This study aims to contribute to the growing field of herbal medicine by developing a novel herbal spray formulation that meets the therapeutic needs of modern healthcare.

Polyherbal Spray with the composition of Water, Ethanol and Extracts (3%), which produce clear, pH of (6.5-7), a delivery rate (0.7-0.91 g/s), Spray pattern (2-3 cm) Area covered by each spray (5-7 cm^2) and Leakage from container (0.1-0.2 %).

Both the optimized polyherbal gel and spray formulations have shown significant potential as effective and safe treatments for inflammatory conditions. The systematic development process ensured that both formulations met the desired quality and efficacy standards. The use of natural herbal extracts not only enhances the therapeutic potential but also reduces the likelihood of adverse effects commonly associated with synthetic drugs. These formulations offer a viable, natural, and cost-effective alternative for patients, promoting better health outcomes and greater acceptance in the realm of herbal medicine.

Future studies may focus on further clinical evaluations and exploring the broader applications of these formulations in various inflammatory and dermatological conditions. The success of these formulations underscores the value of integrating traditional herbal knowledge with modern scientific techniques to develop innovative healthcare solutions.

7.5 References

1. General guidelines for drug development of Ayurvedic formulation, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Government of India, New Delhi.
2. Surver, C. and Davis, F.A., Bioaviability and Bioequivalence, In Walter, K.A. (Ed.) Dermatological and Transdermal Formulation, Marcal Dekker, INC. NewYork, 119, 2002, pg no. 403, 323,326,32,403.
3. Banker G.B.S., Rodes C.T., "Modern Pharmacist", 2nd edition, Vol. 40, Marcel Dekker,New York, 1979, 263- 273, 283,286-287,299-311.
4. Lemberger A.P., "A Hand Book of Non-Prescription Drug", American Pharmaceutical Association, Washington, 1973, 161.
5. Mithal B.M., and Saha R.N., "A Hand Book of Cosmetics", Ist edition, Vallabh Prakashan Delhi, 2003, 11-17,21-22,37-38,61-89,90-93,177,214-215
6. Aniket Raval, Research J. Topical and Cosmetic Sci. 6(2): July-Dec. 2015 page 91-126 DOI: 10.5958/2321-5844.2015.00013.8.
7. Nayank S.H., Nkhat P.D., and Yeole P.G., "The Indian Pharmacist", Vol. III, No. 27, Sept. 2004, 7-14. 27. Jain N.K., "Pharma Times", May 2000, 21. 28.
8. Misra A.N., "Controlled and Novel Drug Delivery", CBS Publishers and Distributors, New Delhi,1997, 107- 109. 29. Nandu S, "Indian Journal of Pharmaceutical Science" Vol. 60. No.4., 1998, 185-188.
9. Mishr B, "Indian Journal of Experimenal Biology", 1990, 28,1001. 31. Lee V.H.L., and Robinson J.R., "Journal of Pharmaceutical Science" 1979. 68,673.
10. Banker G.B.S., Rodes C.T., "Modern Pharmacist", 2nd edition, Vol. 40, Marcel Dekker,New York, 1979, 263- 273, 283,286-287,299-311
11. ICH Q8(R2), Pharmaceutical Development, Guideline, Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_ Guideline.pdf [last accessed 4 Nov 2016.
12. Box GEP, Hunter WG, Hunter JS, Statistics for Experimenters- An introduction to Design, Data Analysis and Model Building, John Wiley and Sons, New York, 1978.

13. Brasil JL, Martins LC, Ev RR, Dupont J, Dias SLP, Sales JAA, Airoldi C, Lima EC. Factorial design for optimization of flow injection preconcentration Procedure Copper (II) determination in natural waters, using 2-aminomephylpyridine grapfted silica gel as adsorbant and spectrophotometric detection. Int. J. Environ. Anal. Chem. 2005: 15: 475-491.
14. https://www.theopeneducator.com/doe/Response-Surface-Methodology/Box-Behnken-Response-Surface-Methodology
15. Jadhav KR, Shetye SL, Kadam VJ. Design and evaluation of microemulsion based drug delivery system. Int J Adv Pharm Sci 2010; 1:156-66
16. Wood JH, Catacalos G, Lieberman SV. Adaptation of commercial viscometers for special applications in pharmaceutical rheology. II. Severs extrusion rheometer. J Pharm Sci 1963; 52:375-8.
17. ICH Guidelines. Stability Testing of New Drug Substances and Products. ICH Guidelines; 1993. 37.
18. Singh M, Mittal V. Formulation and evaluation of herbal gel containing ethanolic extract of Ipomoea fistulosa. Indo-Iran J Sci Res 2014; 3:14.
19. Agrawal SS, Paridhavi M, Herbal Drug Technology, University Press (India) Pvt. Ltd. 2007: 663-672.
20. The Ayurvedic Formulary of India, Anonymous, 1978, Government of India, Delhi.
21. Indian Pharmacopoeia, Vol I and II. New Delhi: Controller of publication, Ministry of Health and Family Welfare, Govt of India.
22. Ramesh C. Nagarwal, A. Srinatha,and Jayanta K. Pandit, In Situ Forming Formulation: Development, Evaluation, and Optimization Using 3^3 Factorial Design, AAPS PharmSciTech, Vol. 10, No. 3, September 2009. DOI: 10.1208/s12249-009-9285-3
23. Sita V G and Pradeep Vavia, Bromocriptine Nanoemulsion-Loaded Transdermal Gel: Optimization Using Factorial Design, In Vitro and In Vivo Evaluation, AAPS PharmSciTech (2020) 21: 80. DOI: 10.1208/s12249-020-1620-8.
24. Patel, N. A., Patel, N. J., & Patel, R. P. (2009). *Design and Evaluation of Transdermal Drug Delivery System for Curcumin as an Anti-Inflammatory Drug. Drug Development and Industrial Pharmacy, 35(2), 234–242.* doi:10.1080/03639040802266782
25. Yatoo, M. I., Gopalakrishnan, A., Saxena, A., Parray, O. R., Tufani, N. A., Chakraborty, S., … Iqbal, H. M. N. (2018). *Anti-Inflammatory Drugs and Herbs with Special Emphasis on Herbal Medicines for Countering Inflammatory Diseases and Disorders*

– *A Review. Recent Patents on Inflammation & Allergy Drug Discovery, 12.* doi:10.2174/1872213x12666180115153635.