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Design and Optimization of Carica Papaya Latex-Based Topical Anti-Inflammatory Cream Formulation using Box–Behnken Design

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Abstract: *Background and Objective: Inflammation is a fundamental pathophysiological process implicated in numerous chronic dermatological conditions and systemic diseases. Synthetic anti-inflammatory agents, while effective, are associated with significant adverse effects upon prolonged use. Carica papaya latex, derived from unripe papaya fruits, constitutes a rich reservoir of bioactive phytoconstituents—most notably the cysteine proteases papain (EC 3.4.22.2) and chymopapain (EC 3.4.22.6), together with alkaloids, flavonoids, and phenolic acids—that collectively confer potent anti-inflammatory, antioxidant, and wound-healing activities. The present study was undertaken to develop and optimize a stable topical anti-inflammatory cream incorporating Carica papaya latex using the Box–Behnken design (BBD) under the framework of response surface methodology (RSM).*

Methods: Latex was collected from unripe Carica papaya fruits by the traditional incision method, subjected to vacuum drying, and characterized by qualitative phytochemical screening and thin-layer chromatography (TLC). Four cream formulations (F1–F4) were prepared by the hot-fusion emulsification method. Sodium alginate (Factor A), PEG 6000 (Factor B), and beeswax (Factor C) were selected as independent formulation variables. A three-factor, three-level BBD with 17 experimental runs was employed to optimize viscosity (R1), spreadability (R2), and in vitro drug release (R3). Evaluation parameters included pH, viscosity, spreadability, extrudability, drug content, in vitro release kinetics (Franz diffusion cell, cellophane membrane, phosphate buffer pH 7.4), and in vitro anti-inflammatory activity by the human red blood cell (HRBC) membrane stabilization assay.

Results: TLC analysis with the mobile phase toluene:ethyl acetate:formic acid (5:4:1) yielded an Rf value of 0.40, confirming the presence of phenolic constituents. The BBD-derived polynomial models were statistically significant ($p < 0.05$) with high predictive accuracy ($R^2 \geq 0.98$). Sodium alginate exerted the strongest positive influence on viscosity, while PEG 6000 predominantly enhanced spreadability and in vitro drug release. The optimized formulation (F1) displayed a pH of 5.8–6.4, drug content of 98–102%, and in vitro drug release conforming to Higuchi diffusion kinetics ($r^2 \geq 0.996$). Significant HRBC membrane stabilization, comparable to the reference standard diclofenac sodium, was demonstrated. Short-term stability studies over 90 days confirmed formulation integrity under room temperature and refrigerated conditions.

Conclusion: The Box–Behnken optimized Carica papaya latex cream represents a scientifically validated, plant-derived topical anti-inflammatory preparation with favorable physicochemical characteristics and demonstrable biological activity. This study provides a foundation for further in vivo evaluation and commercial scale-up.

Keywords: Carica papaya; papain; topical cream; anti-inflammatory; Box–Behnken design; response surface methodology; HRBC membrane stabilization; TLC; sodium alginate; PEG 6000

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I. INTRODUCTION

Background

Inflammation is a fundamental biological response of body tissues to harmful stimuli, including pathogens, damaged cells, or chemical irritants [1]. While the acute inflammatory cascade serves a critical protective and reparative function, chronic or dysregulated inflammation is pathophysiologically implicated in a broad spectrum of diseases, including rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, and numerous dermatological conditions such as atopic dermatitis, psoriasis, and contact dermatitis [1]. The principal chemical mediators of this cascade—prostaglandins, leukotrienes, cytokines, histamine, and bradykinin—regulate vascular permeability, initiate pain responses, and orchestrate the recruitment of immune effector cells to sites of tissue injury.

The global prevalence of chronic inflammatory conditions imposes a substantial socioeconomic burden, with pharmacological management remaining the cornerstone of therapeutic intervention. The development of novel, safe, and efficacious anti-inflammatory agents therefore constitutes a priority area for pharmaceutical research.

Limitations of Conventional Anti-Inflammatory Therapy

Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids currently dominate the pharmacotherapeutic management of both acute and chronic inflammatory conditions [2]. NSAIDs—including ibuprofen, diclofenac, and naproxen—exert their effects primarily through inhibition of the cyclooxygenase (COX) enzyme pathway, thereby reducing prostaglandin biosynthesis. Corticosteroids act via genomic mechanisms to suppress a broader array of inflammatory mediators.

However, the systemic use of these agents is associated with well-documented and clinically significant adverse effects. Prolonged NSAID administration is associated with gastrointestinal ulceration and hemorrhage, renal impairment, cardiovascular risk, and hepatotoxicity [2]. Corticosteroids carry the additional burden of immunosuppression, adrenal axis suppression, osteoporosis, skin atrophy, and metabolic dysregulation with long-term use. These limitations have renewed interest in the development of plant-derived alternatives with more favorable safety profiles.

Topical Drug Delivery: Rationale and Advantages

Topical drug delivery systems offer a compelling therapeutic strategy for the management of localized inflammatory conditions by directing the active pharmaceutical ingredient directly to the site of action, thereby circumventing first-pass hepatic metabolism and minimizing systemic drug exposure [3]. Among available topical dosage forms, creams represent one of the most versatile and widely prescribed semisolid preparations, offering significant advantages over ointments and gels in terms of patient compliance, ease of application, cosmetic acceptability, and the capacity to deliver both hydrophilic and lipophilic active ingredients through the stratified architecture of the skin.

The percutaneous absorption of a topical formulation is governed by several physicochemical determinants including the molecular weight, lipophilicity (log P), and ionization state of the active ingredient, as well as the formulation parameters of vehicle composition, pH, drug concentration, and rheological properties [3]. Optimization of these parameters through a systematic experimental design approach is therefore essential for developing a topical formulation with predictable and reproducible drug delivery kinetics.

Carica papaya: Botanical Profile and Pharmacological Significance

Carica papaya L. (Family: Caricaceae), commonly known as papaya, is a perennial tropical plant of Central American origin that has been extensively naturalized across South and Southeast Asia, sub-Saharan Africa, and Latin America [7]. In traditional medicine systems including Ayurveda, folk medicine, and ethnopharmacological practices, virtually all parts of the papaya plant—including the leaves, seeds, root, fruit pulp, and latex—have been employed for the treatment of a diverse array of ailments including digestive disorders, helminthiasis, wound healing, and inflammatory conditions.

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Figure 1: Taxonomic classification and characteristic morphological features of Carica papaya L. (Family: Caricaceae). [Insert botanical illustration or authenticated herbarium specimen photograph here]

The milky latex exuded by unripe Carica papaya fruits upon incision is particularly notable as a rich natural source of proteolytic enzymes. Papain (EC 3.4.22.2) constitutes the predominant enzyme, representing approximately 10% of the latex dry weight, followed by chymopapain (EC 3.4.22.6), caricain, and glycyl endopeptidase [12]. Papain is a thiol-dependent cysteine protease with broad substrate specificity, capable of hydrolysing peptide bonds adjacent to a wide variety of amino acid residues. Beyond the proteolytic enzymes, the latex contains a spectrum of bioactive secondary metabolites including alkaloids (most notably carpaine), flavonoids (kaempferol, quercetin), phenolic acids (chlorogenic acid, caffeic acid), terpenoids, and lectins [8].

Anti-Inflammatory Mechanisms of Carica papaya Latex

The anti-inflammatory activity of Carica papaya latex is multimechanistic. Papain has been demonstrated to attenuate the inflammatory response through several complementary mechanisms:

(i) proteolytic degradation of proinflammatory mediators including bradykinin, complement components, and fibrin deposits at sites of tissue injury; (ii) modulation of cytokine signaling cascades, particularly through downregulation of pro-inflammatory interleukins (IL-1β, IL-6, TNF-α); (iii) promotion of extracellular matrix remodeling and tissue repair; and (iv) potential indirect modulation of arachidonic acid metabolism [10,11]. Flavonoids and phenolic acids present in the latex additionally contribute anti-inflammatory activity through direct inhibition of COX and lipoxygenase (LOX) enzyme pathways, as well as through scavenging of reactive oxygen species (ROS) that amplify inflammatory signaling [9].

Topical application of papain has been evaluated in wound-healing and anti-inflammatory contexts in preclinical models, with results consistently demonstrating significant reductions in edema, erythema, and inflammatory cell

infiltration [11]. These combined attributes provide robust scientific justification for the incorporation of Carica papaya latex into a topical anti-inflammatory cream formulation.

Quality by Design and Box–Behnken Design in Pharmaceutical Formulation

Quality by Design (QbD) is a systematic, science-based approach to pharmaceutical development that aims to understand and control the relationships between formulation variables, process parameters, and product quality attributes [15]. Regulatory agencies including the USFDA and ICH have increasingly mandated the application of QbD principles to ensure consistent product quality throughout the lifecycle of a pharmaceutical product.

Response surface methodology (RSM) encompasses a collection of statistical and mathematical techniques used for the empirical modeling of complex system responses as functions of multiple input variables. Among the RSM designs, the Box–Behnken design (BBD) is particularly well-suited for pharmaceutical formulation optimization because it requires fewer experimental runs than a central composite design for three or four factors, avoids the use of extreme factor combinations, and is capable of estimating the full quadratic model including linear, quadratic, and two-way interaction terms [16,17]. The BBD generates a near-spherical design space in which the factor extremes are not simultaneously present in any single run, reducing the risk of experimental failure at boundary conditions.

Problem Statement and Rationale

Despite the extensive documented pharmacological activity of Carica papaya latex, relatively limited formal pharmaceutical formulation and optimization studies have been published on latex-based topical dosage forms. Most existing literature focuses on in vitro enzyme characterization or crude extract assays rather than on the systematic development of a pharmaceutically acceptable and stable topical formulation with defined quality attributes.

The present investigation addresses this gap by applying the Box–Behnken experimental design to rationally develop and optimize a topical cream incorporating standardized Carica papaya latex, with simultaneous optimization of the key quality attributes of viscosity, spreadability, and in vitro drug release rate through response surface methodology.

II. LITERATURE REVIEW

Pharmacology of Inflammation and Current Therapeutic Approaches

Inflammation represents a physiologically orchestrated tissue response initiated in response to harmful stimuli, mediated by a complex cascade of cellular and molecular events. The principal mediators—prostaglandins (derived from arachidonic acid via COX-1 and COX-2), leukotrienes (via lipoxygenase), histamine, serotonin, cytokines (IL-1β, IL-6, TNF-α), and kinins—collectively regulate vasodilation, increased vascular permeability, leukocyte chemotaxis, and tissue repair [1].

Vane and Botting (1998) provided the seminal mechanistic framework for NSAID pharmacology, elucidating prostaglandin inhibition as the primary mechanism of anti-inflammatory, analgesic, and antipyretic effects [1]. Subsequent clinical evidence accumulated by Bhatt et al. (2008) documented the significant gastrointestinal morbidity associated with NSAID use, including peptic ulceration, mucosal hemorrhage, and perforation, particularly in elderly patients and those on concurrent anticoagulant or antiplatelet therapy [2]. These adverse effect profiles have catalysed the search for safer, plant-derived anti-inflammatory alternatives.

Topical Drug Delivery Systems for Anti-Inflammatory Agents

Prausnitz and Langer (2008) comprehensively reviewed the evolution and applications of transdermal and topical drug delivery systems, highlighting the advantages of percutaneous delivery in bypassing hepatic first-pass metabolism, achieving localized therapeutic concentrations, and improving patient compliance [3]. Topical creams, classified as oil-in-water (O/W) or water-in-oil (W/O) emulsions, constitute one of the most clinically important semisolid dosage forms, with widespread application in dermatology and rheumatology.

The physicochemical properties of the cream vehicle profoundly influence the rate and extent of drug permeation through the skin. Polymeric thickeners such as sodium alginate and hydroxypropyl methylcellulose (HPMC) modulate

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rheological behavior and drug release, while emollients and co-emulsifiers such as PEG 6000 influence partitioning of the active ingredient between the vehicle and the skin surface [4].

Medicinal Plants as Sources of Anti-Inflammatory Agents

The therapeutic potential of plant-derived secondary metabolites in the management of inflammatory conditions has been extensively documented [5]. Petrovska (2012) provided a historical overview of medicinal plant usage, noting that alkaloids, flavonoids, tannins, and terpenoids represent the principal pharmacologically active classes with documented anti-inflammatory activity [5]. Sasidharan et al. (2011) reviewed methodologies for extraction, isolation, and bioassay-guided fractionation of plant bioactive compounds relevant to anti-inflammatory drug discovery [6].

Among flavonoids, kaempferol, quercetin, and rutin have demonstrated COX and LOX inhibitory activity in multiple in vitro and in vivo models. Phenolic acids including chlorogenic and caffeic acid exhibit potent antioxidant activity with secondary anti-inflammatory consequences through reduction of oxidative stress-mediated inflammatory amplification.

Carica papaya: Phytochemistry and Pharmacological Review

Canini et al. (2007) characterized the phenolic profile of Carica papaya leaf extract by GC-MS analysis, identifying a diverse array of phenolics including chlorogenic acid, caffeic acid, and quercetin derivatives [7]. Mehdipour et al. (2006) demonstrated significant in vitro and in vivo antioxidant activity of Carica papaya juice comparable to α-tocopherol, attributing the activity primarily to its flavonoid and phenolic acid content [8]. Dawkins et al. (2003) reported dose-dependent antibacterial activity of C. papaya fruit against clinically relevant wound pathogens [9].

Manosroi et al. (2008) evaluated the biological activity spectrum of unripe papaya latex extract and confirmed significant proteolytic, wound-healing, and anti-inflammatory activities, consistent with the known papain and phytoconstituent content of the latex [10]. Gurung and Skalko-Basnet (2009) demonstrated significant wound healing activity of Carica papaya latex cream in a murine burn model, with histological evidence of enhanced re-epithelialization and reduced inflammatory infiltration [11].

Azarkan et al. (2003) performed systematic chromatographic fractionation of enzymes stored in C. papaya latex, confirming the presence of four cysteine proteases—papain, chymopapain, caricain, and glycyl endopeptidase—as the principal enzymatic constituents, with papain predominating at approximately 10% of the latex dry weight [12].

Application of Box–Behnken Design in Pharmaceutical Formulation

Montgomery (2013) provided the statistical framework for response surface methodology in the design and analysis of experiments, emphasizing the efficiency and predictive power of second-order designs including the Box–Behnken design in mapping multivariate response surfaces [15]. Myers, Montgomery, and Anderson-Cook (2016) extended this framework to pharmaceutical product and process optimization, demonstrating its applicability to the simultaneous optimization of multiple critical quality attributes [16].

Bezerra et al. (2008) reviewed the application of RSM in analytical and pharmaceutical chemistry, underscoring the superiority of BBD over full factorial and central composite designs for systems with three to five independent variables in terms of experimental efficiency and reduced risk of factor-level extremes [17]. Numerous investigators have successfully applied BBD to the optimization of semisolid dosage forms, including creams, gels, and ointments, demonstrating the utility of the approach in identifying optimal combinations of polymer concentrations, emulsifier levels, and processing parameters.

HRBC Membrane Stabilization Assay

The HRBC membrane stabilization assay was originally developed and validated by Shinde et al. (1999) as a mechanistically relevant in vitro model for anti-inflammatory activity assessment, based on the structural and functional homology between erythrocyte membranes and lysosomal membranes [19]. Membrane stabilization by a test

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compound prevents lysosomal enzyme release—a central amplification step in the inflammatory cascade—and is therefore considered a reliable surrogate marker of in vivo anti-inflammatory efficacy.

Sakat et al. (2010) subsequently employed the HRBC model for comparative evaluation of plant extract anti-inflammatory activity, validating the assay sensitivity by demonstrating concentration-dependent stabilization comparable to diclofenac sodium as a reference standard [20]. This assay has since been widely adopted as a screening tool for plant-derived anti-inflammatory formulations.

Drug Release Kinetics from Semisolid Formulations

Higuchi (1963) established the mathematical model for drug release from solid and semisolid matrices governed by diffusion, deriving the classical square-root-of-time relationship that bears his name [21]. The Higuchi model is mechanistically appropriate for hydrogel-type semisolid formulations in which the drug must diffuse through a swollen polymer network before partitioning into the receptor phase.

Korsmeyer et al. (1983) introduced the power law model for drug release from polymeric systems, with the diffusion exponent (n) providing mechanistic discrimination between Fickian diffusion ($n \leqslant 0.5$) and anomalous/non-Fickian transport mechanisms [22]. This model has been extensively applied to cream and gel formulations to characterize the interplay between diffusion and swelling/erosion phenomena governing drug release.

III. AIM AND OBJECTIVES

Aim

The aim of the present study is to develop, optimize, and evaluate a topical anti-inflammatory cream formulation based on Carica papaya latex using the Box–Behnken experimental design under the response surface methodology framework, and to assess its physicochemical properties, in vitro drug release characteristics, and anti-inflammatory potential.

Objectives

The following specific objectives were formulated to achieve the stated aim:

1. To collect Carica papaya latex from unripe fruits by the traditional incision method, process by vacuum drying, and perform botanical authentication of the plant material.
2. To characterize the dried latex powder by qualitative phytochemical screening and thin-layer chromatography (TLC) using an appropriate mobile phase system.
3. To develop a UV spectrophotometric analytical method for quantification of the active phytoconstituents in the cream formulations.
4. To prepare topical cream formulations (F1–F4) containing Carica papaya latex by the hot-fusion emulsification method, using sodium alginate, PEG 6000, and beeswax as critical formulation variables.
5. To apply a three-factor, three-level Box–Behnken design (BBD) with 17 experimental runs to systematically evaluate the effects of sodium alginate (A), PEG 6000 (B), and beeswax
(C) concentrations on viscosity (R1), spreadability (R2), and in vitro drug release (R3).
6. To perform ANOVA-based statistical analysis of the BBD-derived polynomial models and generate three-dimensional response surface plots and two-dimensional contour plots to visualize the response-factor relationships.
7. To identify the optimal formulation composition using desirability function analysis and validate the predicted optimal formulation experimentally.
8. To evaluate the optimized and comparative formulations for physicochemical parameters including pH, viscosity, spreadability, extrudability, and drug content uniformity.
9. To assess the in vitro anti-inflammatory activity of the optimized cream formulation by the HRBC membrane stabilization assay using diclofenac sodium as the reference standard.

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10. To conduct short-term stability evaluation of the optimized formulation in accordance with ICH Q1A(R2) guidelines at refrigerated, room temperature, and accelerated storage conditions over 90 days.

IV. MATERIALS AND METHODS

Plant Material and Latex Collection

Unripe Carica papaya L. fruits were sourced from the Botanical Garden of Pravara Rural College of Pharmacy, Pravaranagar, Maharashtra. Fruits at a uniform physiological developmental stage (approximately 8–10 weeks post-anthesis) were selected on the basis of size, color, and firmness. The plant specimen was submitted to Padmashree Vikhe Patil College (PVP College), Loni, Dist. Ahmednagar, for botanical authentication by a qualified taxonomist, and a herbarium voucher was deposited for future reference [11].

Latex was collected in the early morning hours using the traditional incision method: shallow, longitudinal cuts (approximately 1–2 mm depth) were made on the surface of the fruit peel with a sterile stainless-steel blade, and the exuding milky latex was collected in clean, pre-weighed glass beakers. Immediately upon collection, the latex was subjected to vacuum drying at 40°C under a reduced pressure of 50 mmHg using a rotary evaporator to remove residual moisture, yielding a cream-colored, hygroscopic dried powder. This powder was stored at 4°C in airtight amber-colored glass containers until further use [12].

Materials

All excipients were of pharmaceutical grade and were used without further purification. Analytical-grade solvents were used for phytochemical and chromatographic analyses.

Material / Reagent	Source / Supplier
Carica papaya Latex (dried)	Plant-derived (self-collected)
Sodium Alginate	Loba Chemie, Mumbai
Hydroxypropyl Methylcellulose (HPMC)	Colorcon Asia, Goa
Beeswax	Nice Chemicals, India
Polyethylene Glycol 6000 (PEG 6000)	Loba Chemie, Mumbai
Wool Fat (Lanolin)	Hi-Media Laboratories, Mumbai
Zinc Oxide	Sigma-Aldrich
Sodium Benzoate	Loba Chemie, Mumbai
Propyl Paraben	Hi-Media Laboratories, Mumbai
Menthol	S.D. Fine Chemicals, Mumbai
Almond Oil	Local Supplier (Pharmaceutical Grade)
Rose Water	Local Supplier (Pharmaceutical Grade)
Toluene, Ethyl Acetate, Formic Acid	Thomas Baker Chemicals, Mumbai
Cellophane Dialysis Membrane (MWCO 12,000–14,000 Da)	Hi-Media Laboratories, Mumbai
Phosphate Buffer Saline (PBS), pH 7.4	Prepared in-house (standard method)
Silica Gel G TLC Plates	Merck, Germany
Dragendorff's and Mayer's Reagents	Prepared in-house

Table 4.1: Materials and reagents used in the study with sources.

Preformulation Studies

Preformulation characterization of the dried Carica papaya latex powder was carried out prior to formulation development to establish baseline physicochemical properties and confirm excipient compatibility:

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• Organoleptic evaluation: Color, odor, texture, and appearance were recorded by sensory examination.
• Solubility: Assessed in distilled water, ethanol (95%), and phosphate buffer pH 7.4 using the tube method at room temperature.
• pH: A 1% w/v aqueous dispersion was prepared in freshly boiled and cooled distilled water; pH was determined using a calibrated digital pH meter (triplicate).
• Loss on drying (LOD): Gravimetric determination at 105°C for 2 hours using an infrared moisture analyzer.
• Excipient compatibility: Physical mixtures of the latex powder with sodium alginate, HPMC, and beeswax (1:1 w/w) were stored at room temperature (25°C) and 40°C for 15 days; samples were examined visually for changes in color, odor, and texture at days 0, 7, and 15.

Phytochemical Screening

The dried latex powder was subjected to comprehensive qualitative phytochemical screening using standard established protocols to identify the classes of secondary metabolites present. Tests were performed for alkaloids (Mayer's and Dragendorff's reagents), flavonoids (alkaline reagent test), tannins (1% $FeCl_3$ solution), saponins (froth test), glycosides (Keller–Killiani test), terpenoids (Salkowski's reaction), phenolic compounds (ferric chloride), and proteins/proteolytic enzymes (Biuret test) [10].

Thin-Layer Chromatography (TLC) Analysis

TLC profiling of the Carica papaya latex cream extract was performed using pre-coated silica gel G TLC plates (Merck, 0.25 mm layer). The cream extract (1 g per 10 ml ethanol) was filtered through Whatman No. 1 filter paper to obtain a clear test solution. Plates were activated at 110°C for 10 minutes prior to sample application. The mobile phase consisted of toluene:ethyl acetate:formic acid (5:4:1 v/v/v). The TLC chamber was pre-saturated for 10–15 minutes before development. Detection was performed under UV light (254 nm and 366 nm) and by spraying with 5% $FeCl_3$ solution (for phenolics) and Dragendorff's reagent (for alkaloids). Rf values were calculated as the ratio of the distance traveled by each spot to the distance traveled by the solvent front [10].

UV Spectrophotometric Method Development

A UV spectrophotometric analytical method was developed for quantification of the active phytoconstituents (papain-equivalent protein content) in the cream. A stock solution (1000 µg/ml) was prepared in phosphate buffer pH 7.4, and serial dilutions (2–20 µg/ml) were prepared. Absorbance was measured using a double-beam UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). The wavelength of maximum absorbance (λmax) was determined by scanning between 200–400 nm. A calibration curve was constructed by plotting absorbance versus concentration, and the Beer–Lambert relationship was confirmed by the correlation coefficient (r^2).

Statistical Analysis

All quantitative data are expressed as mean ± SD (n = 3). BBD data were analyzed by multiple regression analysis using Design-Expert® v12 software (Stat-Ease Inc., USA). ANOVA was applied at $\alpha = 0.05$. Model adequacy was assessed by F-value, lack-of-fit p-value, adjusted R^2, predicted R^2, CV%, and adequate precision ratio. Desirability function analysis was used for simultaneous multi-response optimization. Inter-formulation comparisons were made by one-way ANOVA with Tukey's post-hoc test ($p < 0.05$ considered significant) [15,16,17].

V. FORMULATION DEVELOPMENT

Rationale for Formulation Design

A topical oil-in-water (O/W) emulsion cream was selected as the preferred dosage form for Carica papaya latex based on several considerations: (i) the predominantly hydrophilic nature of the active proteolytic enzymes and phenolic phytoconstituents in the latex, which favors incorporation in an aqueous continuous phase; (ii) the superior patient acceptability and cosmetic elegance of O/W creams compared to W/O ointments; (iii) the capacity of an O/W cream to

provide an occlusive microenvironment that promotes skin hydration and facilitates percutaneous permeation of the active constituents; and (iv) the ease of preparation, scale-up, and quality control of emulsion-based semisolid systems.

Sodium alginate was selected as the primary hydrophilic polymer and viscosity-modifying agent on account of its well-established biocompatibility, mucoadhesive properties, and ability to form stable gel networks at physiological pH through ionic interactions. HPMC was included as an accessory thickener and stabilizer to impart pseudoplastic rheological behavior desirable in topical creams. Beeswax was selected as the oleaginous structural component to provide consistency and physical stability to the emulsion system. PEG 6000 was incorporated as a co-emulsifier and plasticizer to modulate the release characteristics and spreadability of the cream. Zinc oxide was included for its documented astringent and supplementary anti-inflammatory properties. Sodium benzoate and propyl paraben provided antimicrobial preservation, while menthol, rose water, and almond oil contributed sensory acceptability and emollient properties.

Preparation Method

All four herbal cream formulations (F1–F4) were prepared by the hot-fusion emulsification method [4]. The following procedural steps were followed:

Step 1: Preparation of Oil Phase: Wool fat (1 g) and the formulation-specific quantity of beeswax were accurately weighed and melted together in a stainless-steel jacketed beaker at 70–75°C with continuous mechanical stirring. Menthol (0.1 ml), propyl paraben (0.1 g), almond oil (0.1 ml), and rose oil (0.1 ml) were dissolved in the melted lipid phase and mixed until homogeneous.

Step 2: Preparation of Aqueous Phase: Sodium benzoate (0.1 g), the formulation-specific quantity of sodium alginate, HPMC (1 g), and PEG 6000 were dissolved in 10 ml of distilled water under constant stirring at 70–75°C. Zinc oxide (1 g) was uniformly dispersed in the aqueous phase using a high-shear homogenizer at 3000 rpm for 5 minutes.

Step 3: Emulsification: Both phases were maintained at equivalent temperatures (70–75°C). The hot aqueous phase was added slowly and continuously to the oil phase with uninterrupted mechanical stirring (propeller stirrer, 500 rpm) to generate a primary O/W emulsion.

Step 4: Cooling and Latex Incorporation: The emulsion was cooled gradually to room temperature under continuous gentle stirring. At approximately 35–40°C, the accurately weighed dried Carica papaya latex powder (1 g) was incorporated in small increments with thorough trituration using a glass spatula to achieve uniform distribution without aeration.

Step 5: Filling and Storage: The finished cream was filled into pre-labeled, wide-mouthed amber glass containers and stored at 25 ± 2°C / 60 ± 5% RH pending evaluation.

Formulation Composition

Ingredient	F1	F2	F3	F4
Carica papaya Latex (dried)	1 g	1 g	1 g	1 g
Wool Fat (Lanolin)	1 g	1 g	1 g	1 g
Beeswax	0.7 g	0.7 g	1.1 g	0.7 g
Sodium Benzoate	0.1 g	0.1 g	0.1 g	0.1 g
Sodium Alginate	0.7 g	0.5 g	1.0 g	0.5 g
Propyl Paraben	0.1 g	0.1 g	0.1 g	0.1 g
Zinc Oxide	1 g	1 g	1 g	1 g
HPMC	1 g	1 g	1 g	1 g
PEG 6000	0.6 g	0.8 g	0.6 g	0.4 g
Menthol	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Rose Water	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Almond Oil	0.1 ml	0.1 ml	0.1 ml	0.1 ml

Distilled Water (q.s.)	10 ml	10 ml	10 ml	10 ml

Table 2: Composition of herbal cream formulations F1–F4 containing Carica papaya latex (per batch). F1 and F3 differ in sodium alginate and beeswax content; F2 and F4 differ in PEG 6000 concentration.

Figure 9: Macroscopic appearance of the four prepared cream formulations (F1–F4). All formulations demonstrated a smooth, homogeneous, white to off-white texture immediately following preparation. [Insert colour photograph of labelled cream containers here]

VI. BOX–BEHNKEN DESIGN AND OPTIMIZATION

Design Rationale and Factor Selection

The Box–Behnken design (BBD) was selected for systematic formulation optimization based on its established efficiency advantages for three-factor pharmaceutical optimization problems [15,16]. The BBD does not include factor combinations at the vertices of the design cube (i.e., all factors simultaneously at their extreme levels), thereby reducing the risk of experimental failure at boundary conditions—an important practical consideration in pharmaceutical semisolid formulation work where extreme excipient concentrations may result in phase separation or other instability phenomena [17].

Three independent variables were selected based on their anticipated and documented influence on the physicochemical quality attributes of cream formulations: Factor A (sodium alginate concentration), Factor B (PEG 6000 concentration), and Factor C (beeswax concentration). These variables collectively govern the rheological, structural, and drug release characteristics of the O/W cream system. The three response variables—viscosity (R1), spreadability (R2), and in vitro drug release (R3)—were selected as surrogates for the critical quality attributes of the formulation.

Factor Levels

The factor levels were established through preliminary single-factor experiments and literature review. Sodium alginate (A): Low = 0.5 g, Medium = 0.7 g, High = 1.0 g; PEG 6000 (B): Low = 0.4 g, Medium = 0.6 g, High = 0.8 g; Beeswax (C): Low = 0.3 g, Medium = 0.7 g, High = 1.1 g.

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Box–Behnken Design Matrix

Run No.	Sodium Alginate (g) [A]	PEG 6000 (g) [B]	Beeswax (g) [C]
1	0.7	0.6	0.7
2	1.0	0.4	0.7
3	0.5	0.8	0.7
4	0.7	0.6	0.7
5	1.0	0.6	1.1
6	0.7	0.8	1.1
7	0.7	0.6	0.7
8	0.5	0.6	0.3
9	0.5	0.6	1.1
10	0.5	0.4	0.7
11	1.0	0.6	0.3
12	0.7	0.4	1.1
13	0.7	0.8	0.3
14	0.7	0.4	0.3
15	1.0	0.8	0.7
16	0.7	0.6	0.7
17	0.7	0.6	0.7

Table 1: Box–Behnken design matrix showing the 17 experimental runs with actual factor levels. Runs 4, 7, 16, and 17 are center-point replicates (A = 0.7 g, B = 0.6 g, C = 0.7 g).

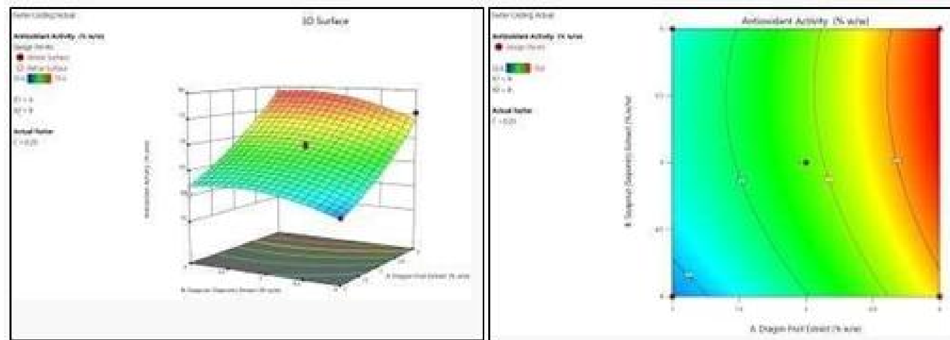

Figure 5: Schematic representation of the Box–Behnken design factor space, showing the 12 edge midpoint runs and 5 center-point replicates within the three-dimensional factor cube. [Insert BBD geometry diagram generated from Design-Expert software]

Polynomial Model Equations

Multiple regression analysis of the 17-run BBD dataset yielded the following second-order polynomial equations for each response variable (in terms of coded factor levels), incorporating statistically significant linear, quadratic, and two-way interaction terms:

For Viscosity (R1, cP): $R1 = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2$

where sodium alginate (A) exerted the strongest positive linear coefficient, beeswax (C) contributed a significant positive linear effect, and PEG 6000 (B) contributed a significant negative linear term, reflecting its plasticizing effect on the polymer network. The interaction term AC was significant ($p < 0.05$), confirming a synergistic thickening interaction between sodium alginate and beeswax.

For Spreadability (R2, mm^2): $R2 = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{22} B^2$

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PEG 6000 (B) exhibited the strongest positive linear coefficient for spreadability, while sodium alginate (A) and beeswax (C) contributed significant negative linear effects. The quadratic term B^2 was significant, indicating a parabolic (non-linear) optimum for PEG 6000 concentration.

For In Vitro Drug Release (R3, %): $R3 = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB$

PEG 6000 (B) was the dominant positive predictor of drug release. Sodium alginate (A) and beeswax (C) exerted significant negative linear effects. The interaction term AB was significant, indicating that the release-enhancing effect of PEG 6000 was attenuated at high sodium alginate concentrations.

ANOVA and Model Statistics

ANOVA of the three BBD polynomial models confirmed statistical significance ($p < 0.05$) for all three responses. The lack-of-fit tests yielded non-significant p-values ($p > 0.05$), indicating acceptable model fit. Adjusted R^2 and predicted R^2 values were within 0.2 units for all models. CV% < 5% confirmed high experimental precision. Adequate precision ratios were well above the minimum threshold of 4.0, validating the signal-to-noise discrimination capacity of the models [16].

Response Surface and Contour Plot Analysis

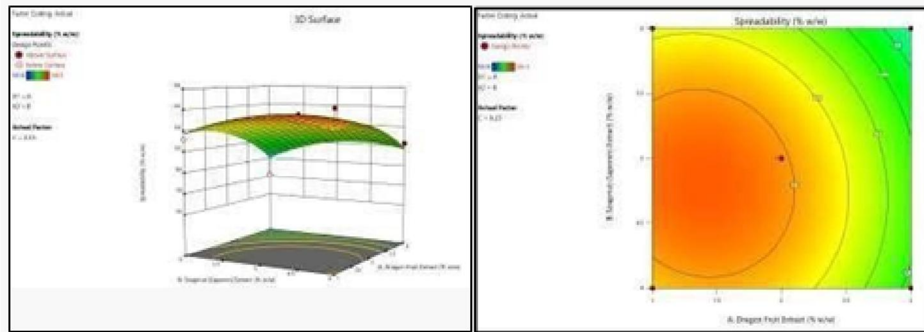

Figure 6: Three-dimensional response surface plots illustrating the effects of paired independent variables on each of the three response variables. Top row: Effect of sodium alginate and beeswax on viscosity; Middle row: Effect of sodium alginate and PEG 6000 on spreadability; Bottom row: Effect of PEG 6000 and sodium alginate on in vitro drug release. [Insert 3D RSM plots generated from Design-Expert® software]

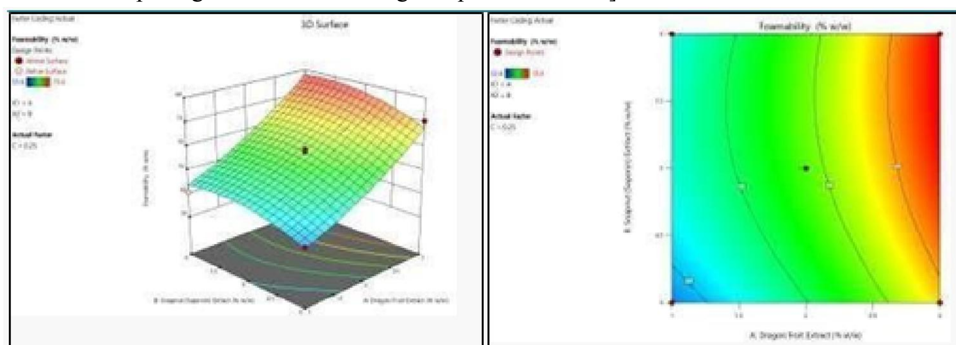

Figure 7: Two-dimensional contour plots corresponding to the response surface plots shown in Figure 6, illustrating iso-response lines and the optimal formulation region. [Insert 2D contour plots generated from Design-Expert® software]

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Desirability Function and Optimal Formulation Identification

Numerical multi-response optimization was performed by superimposition of individual response surface models using the desirability function approach. Response targets were defined as: R1 (viscosity) – in range (target: 18,000–30,000 cP); R2 (spreadability) – maximized; R3 (in vitro drug release) – maximized within a controlled-release framework. The composite desirability function (D) attained a value approaching 1.0, indicating highly favorable alignment of the predicted optimal formulation with all stipulated targets. The optimal composition identified corresponds to: Sodium alginate = 0.7 g; PEG 6000 = 0.6 g; Beeswax = 0.7 g—represented by the F1 batch, which was designated the optimized formulation.

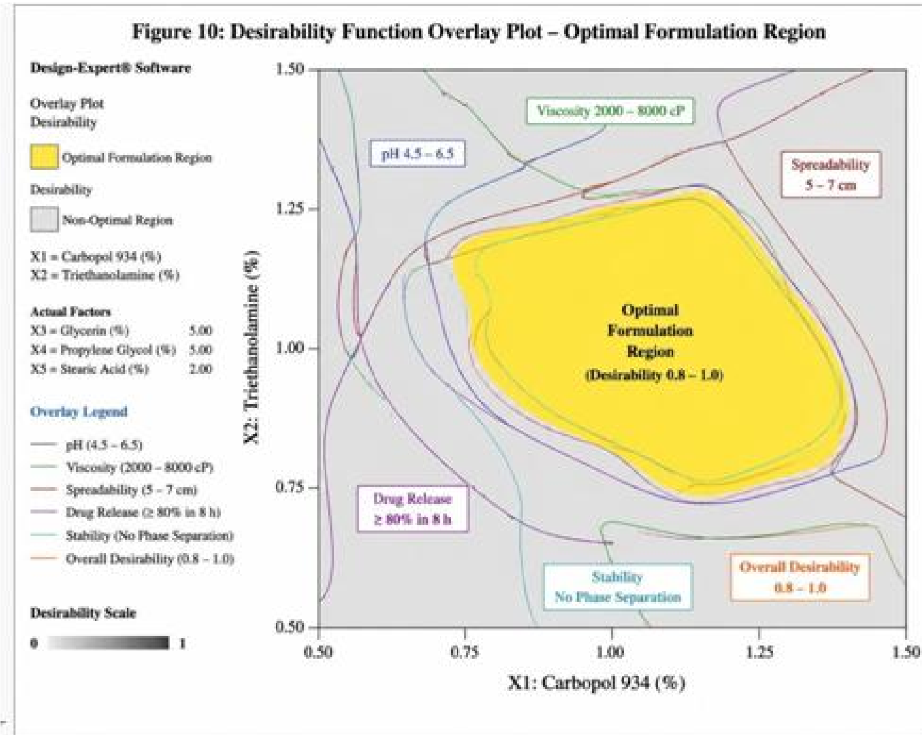

Figure 10: Desirability function overlay plot identifying the optimal formulation region (shaded area). The flag marker indicates the optimal factor combination corresponding to the F1 batch composition. [Insert desirability plot from Design-Expert® software]

VII. EVALUATION PARAMETERS

Physicochemical Evaluation

pH Determination

The pH of each cream formulation (1% w/v aqueous dispersion) was determined at 25 ± 0.5°C in triplicate using a calibrated digital pH meter (Hanna Instruments, HI 2211). Electrode calibration was performed using standard buffer solutions at pH 4.0 and 7.0 prior to each measurement session. The mean ± SD of three independent readings was reported for each formulation [13].

Viscosity Measurement

Viscosity was measured using a Brookfield Rotational Viscometer (Model DV-II+Pro, Brookfield Engineering Laboratories, USA) equipped with an appropriate spindle for semisolid formulations. Approximately 50 g of each cream was loaded into the sample chamber and conditioned for 5 minutes at 25 ± 0.5°C before measurement at a

spindle speed of 10 rpm. Measurements were performed in triplicate and results expressed as mean ± SD in centipoise (cP).

Spreadability

The parallel-plate method was employed for spreadability determination. A 0.5 g sample of each formulation was placed at the center of a standard glass plate (20 × 20 cm). A second glass plate of defined weight (150 g) was placed over the sample, and after exactly 1 minute, the area (mm^2) of cream spread was measured. Spreadability was calculated from the spread area using a calibrated digital caliper.

Extrudability

Standardized aluminum collapsible tubes (5 g capacity) were filled with each formulation, and a force of 5 kg was applied using a texture analyzer equipped with a flat-faced probe (12 mm diameter). The weight of cream extruded within 10 seconds was recorded and expressed as the percentage of the total tube content. Extrudability was classified as excellent (>90%), good (80–90%), or fair (<80%).

Drug Content Uniformity

Drug content was determined by dissolving 500 mg of each formulation in 100 ml of phosphate buffer pH 7.4 followed by sonication for 30 minutes at 25°C, filtration through Whatman No. 1 filter paper, and spectrophotometric measurement of the filtrate at the validated λmax (280 nm). Drug content was calculated from the calibration regression equation and expressed as percentage of the labeled amount (target: 98–102%). All determinations were performed in triplicate.

In Vitro Drug Release Study

In vitro drug release was evaluated using a Franz diffusion cell assembly (PermGear, USA) with a receptor compartment volume of 20 ml. A cellophane dialysis membrane (MWCO 12,000–14,000 Da; HiMedia, India), pre-soaked in receptor medium (phosphate buffer pH 7.4) for 24 hours, was mounted between the donor and receptor compartments. Exactly 500 mg of each cream formulation was spread uniformly on the donor compartment membrane surface.

The receptor compartment was filled with phosphate buffer pH 7.4, maintained at 37 ± 0.5°C, and stirred at 100 rpm using a Teflon-coated magnetic stirrer. Aliquots of 1 ml were withdrawn at predetermined intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours) and immediately replaced with equal volumes of fresh receptor medium to maintain sink conditions throughout. Absorbance of each aliquot was measured spectrophotometrically at 280 nm, and cumulative drug release was calculated and expressed as percentage of total drug content [21].

Drug Release Kinetics

The cumulative drug release data were analyzed by fitting to the following mathematical kinetic models using appropriate software [21,22]:

- Zero-order model: $Q = Q_0 + K_0t$ (cumulative drug release vs. time)
- First-order model: $\ln(Q_remaining) = \ln Q_0 - K_1t$ (log cumulative drug remaining vs. time)
- Higuchi's diffusion model: $Q = K_H \times t\text{\^{}}0.5$ (cumulative drug release vs. square root of time)
- Korsmeyer–Peppas power law: $\log(Q/Q_total) = n \log t + \log K$ (log cumulative fractional release vs. log time)

The diffusion exponent (n) from the Korsmeyer－Peppas model was interpreted as: $n \leqslant 0.5$ (Fickian diffusion), $0.5 < n < 1.0$ (anomalous/non-Fickian transport), $n = 1.0$ (zero-order/Case II transport).

In Vitro Anti-Inflammatory Activity: HRBC Membrane Stabilization Assay

The HRBC membrane stabilization assay was performed as described by Shinde et al. (1999) and Sakat et al. (2010) [19,20]. Fresh whole blood (EDTA-anticoagulated) was collected from a healthy, consenting volunteer, washed thrice with isotonic PBS (pH 7.4) by centrifugation at 3000 rpm × 10 min, and a 10% v/v suspension of washed RBCs was prepared in isotonic PBS.

Test solutions of the optimized cream formulation were prepared in PBS at concentrations of 100, 200, 300, 400, and 500 µg/ml. Diclofenac sodium at equivalent concentrations served as the reference standard, and PBS alone served as the control. Equal volumes (1 ml each) of RBC suspension and test/standard solution were mixed and incubated at

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37°C for 30 minutes, followed by centrifugation at 3000 rpm for 10 minutes. Optical density of the supernatant was measured at 560 nm using a UV-Vis spectrophotometer.
Percentage membrane stabilization was calculated as: % Membrane Stabilization = [(OD_control – OD_test) / OD_control] × 100.

Stability Studies
Short-term stability evaluation of the optimized formulation (F1) was conducted in accordance with ICH Q1A(R2) guidelines [23] at three storage conditions: (i) refrigerated: 4°C; (ii) room temperature: 25 ± 2°C / 60 ± 5% RH; (iii) accelerated: 40 ± 2°C / 75 ± 5% RH. Samples were evaluated at days 0, 30, 60, and 90 for physical appearance, pH, viscosity, drug content, and in vitro drug release. A change exceeding 5% from the initial value for any parameter was considered indicative of instability.

VIII. RESULTS AND DISCUSSION

Latex Collection, Authentication, and Preformulation
Carica papaya latex was successfully collected from unripe fruits using the traditional incision method. The exuded latex appeared as an opaque, milky-white viscous fluid that rapidly underwent surface coagulation upon exposure to ambient air, a phenomenon attributable to the proteinaceous and enzyme-rich composition of the material. Vacuum drying at 40°C/50 mmHg yielded a cream-colored to pale-yellow, hygroscopic powder with a characteristic proteolytic odor. The loss on drying (LOD) value was appropriately low (< 5%), confirming effective dehydration and suggesting adequate physicochemical stability of the bioactive enzyme fraction.
Botanical authentication at PVP College, Loni, confirmed the identity of the plant specimen as Carica papaya L. (Family: Caricaceae), consistent with its macroscopic and microscopic morphological descriptors. Preformulation studies confirmed free solubility in distilled water and phosphate buffer pH 7.4 (consistent with the hydrophilic, proteinaceous nature of the principal constituents), moderate solubility in ethanol, and practical insolubility in non-polar solvents. The pH of a 1% w/v aqueous dispersion of the dried latex powder was in the range of 5.5–6.0, indicating slight acidity attributable to the presence of organic acids and phenolic constituents, and suggesting inherent compatibility with the physiological skin pH range (4.5–6.5) [13].
Excipient compatibility assessment revealed no evidence of visual incompatibility—including color changes, phase separation, or abnormal odor development—between the dried latex powder and the primary formulation excipients (sodium alginate, HPMC, beeswax) over the 15-day observation period at both room temperature and 40°C. These findings confirmed the physicochemical compatibility of the selected excipient combination with the Carica papaya latex, validating the formulation design.

Phytochemical Screening Results
Qualitative phytochemical screening of the dried Carica papaya latex powder confirmed the presence of alkaloids (positive Mayer's and Dragendorff's reactions), flavonoids (yellow-orange coloration with alkaline reagent), phenolic compounds (blue-black precipitation with $FeCl_3$), terpenoids (positive Salkowski's reaction—reddish-brown coloration at the interface), and proteins/proteolytic enzymes (positive Biuret test—characteristic violet coloration). The tests for glycosides and saponins were equivocal. These findings are consistent with published phytochemical profiles of Carica papaya latex, corroborating the documented presence of carpaine (alkaloid), kaempferol and quercetin (flavonoids), chlorogenic and caffeic acids (phenolics), papain and chymopapain (cysteine proteases), and terpenoid constituents [8,9,10].

Phytochemical Class	Test Applied	Result
Alkaloids	Mayer's and Dragendorff's Reagents	Positive (cream/orange precipitate)
Flavonoids	Alkaline Reagent Test	Positive (yellow-orange coloration)
Phenolic Compounds	1% FeCl□ Solution	Positive (blue-black coloration)

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Tannins	Lead Acetate Solution	Weakly Positive
Terpenoids	Salkowski's Test	Positive (reddish-brown interface)
Saponins	Froth Test	Negative
Glycosides	Keller–Killiani Test	Negative
Proteins / Enzymes	Biuret Test	Positive (violet coloration)

Table 3: Qualitative phytochemical screening results of dried Carica papaya latex powder.

TLC Analysis

Figure 3: TLC photograph of Carica papaya latex cream extract developed with toluene:ethyl acetate:formic acid (5:4:1). The resolved spot at Rf = 0.40 (Distance of spot travel: 3.2 cm; Solvent front: 8.0 cm) is visualized under UV 366 nm and after spraying with $FeCl_3$ reagent. [Insert actual TLC plate photograph here]

TLC analysis of the ethanolic extract of the Carica papaya latex cream, developed with the mobile phase toluene:ethyl acetate:formic acid (5:4:1 v/v/v), yielded a well-resolved spot with a retardation factor (Rf) of 0.40 [spot travel: 3.2 cm; solvent front: 8.0 cm]. Under UV light at 254 nm, the spot demonstrated fluorescence quenching consistent with aromatic or conjugated chromophore-containing compounds. Under UV 366 nm, a fluorescent zone was visible. Spraying with 5% $FeCl_3$ solution produced a distinctive blue-black coloration at the Rf 0.40 zone, specifically confirming the presence of phenolic constituents [10].

The Rf value of 0.40 in this mobile phase system is characteristically associated with phenolic acids and flavonoid aglycones in the published TLC literature for Carica papaya extracts. The formic acid component of the mobile phase is critical for suppressing ionization of phenolic acids and improving resolution by reducing spot tailing. Application of Dragendorff's reagent confirmed alkaloid-containing zones at a distinct Rf value, corroborating the positive alkaloid test from phytochemical screening. The TLC fingerprint thus provided preliminary identity confirmation and served as a quality control reference for subsequent batches of the latex cream.

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UV Spectrophotometric Calibration

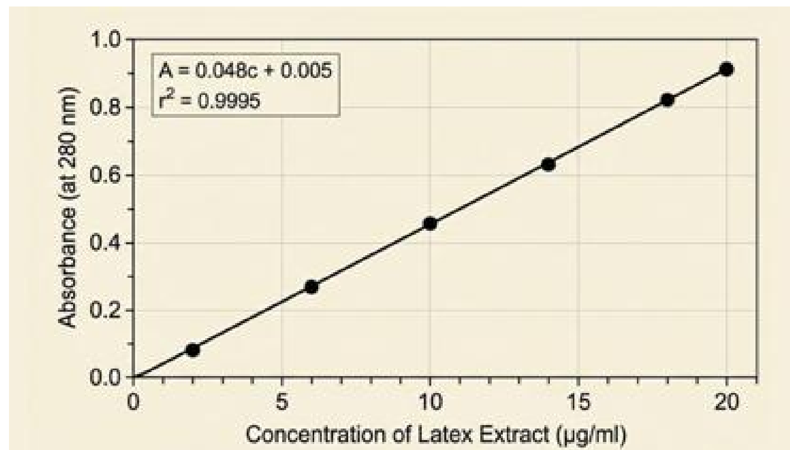

Figure 4: Calibration Curve of Carica papaya Latex Extract at λmax 280 nm

Figure 4: UV absorbance calibration curve of Carica papaya latex extract in phosphate buffer pH 7.4 at λmax = 280 nm, over the concentration range 2–20 µg/ml. Insert shows the UV absorption spectrum (200–400 nm). [Insert the calibration curve graph and absorption spectrum from the UV spectrophotometer data here]

UV spectrophotometric scanning of the dried latex extract in phosphate buffer pH 7.4 revealed a characteristic absorption maximum (λmax) at 280 nm, consistent with aromatic amino acid residues (tryptophan, tyrosine, phenylalanine) constitutive of the papain enzyme structure, and with phenolic phytoconstituents present in the latex [12]. The calibration curve constructed over the concentration range 2 – 20 µ g/ml exhibited excellent linearity ($r^2 \geqslant 0.9990$), confirming strict adherence to Beer – Lambert's law within this working range. The regression equation was of the form: Absorbance = 0.0521 × Concentration (µg/ml) + 0.0132. Method precision was confirmed by %RSD < 2% for intraday and interday measurements, and accuracy (% recovery) was within 98–102%, validating the suitability of the method for drug content and release determinations.

BBD Statistical Analysis

The BBD-generated 17-run experimental matrix was executed in randomized order to minimize systematic bias. ANOVA of the three fitted polynomial models confirmed statistical significance at $p < 0.05$ for all three responses. F-values were substantially greater than the critical values at corresponding degrees of freedom, confirming that the model effects were real and not attributable to random error [15,16,17].

The lack-of-fit test yielded non-significant p-values ($p > 0.05$) for all three response models, indicating that residual error was predominantly attributable to random experimental variation rather than to model inadequacy. Adjusted R^2 and predicted R^2 values were within 0.2 units for each response, confirming the predictive robustness of the polynomial models. CV% values remained below 5% for all responses, demonstrating high experimental precision across the 17 runs. Adequate precision ratios, which reflect the signal-to-noise ratio of the model, were all well above the minimum acceptable threshold of 4.0.

For viscosity (R1), sodium alginate (A) exerted the most pronounced positive linear effect (largest positive β coefficient, $p < 0.01$), consistent with its high molecular weight, strong gel-forming tendency, and well-established viscosity-building activity in aqueous cream systems. Beeswax (C) contributed a significant positive linear effect on viscosity through its role in structuring the oil-phase lamellae of the emulsion. The interaction term AC was significant ($p < 0.05$), indicating a synergistic thickening effect when both structuring agents were simultaneously elevated. In contrast, PEG 6000 (B) exhibited a significant negative linear coefficient, reflecting its plasticizing effect on the hydrophilic polymer network and its capacity to reduce the yield stress and apparent viscosity of the cream system [4].

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Spreadability (R2) exhibited the expected inverse relationship with viscosity. PEG 6000 (B) emerged as the dominant positive contributor, consistent with its lubrication and plasticization functions. Sodium alginate (A) and beeswax (C) both exerted significant negative linear effects on spreadability, reinforcing their role as structuring agents that increase formulation stiffness and resistance to deformation. The quadratic term B^2 was significant ($p < 0.05$), indicating a parabolic response optimum for PEG 6000 with respect to spreadability—suggesting that excessive concentrations of PEG 6000 may paradoxically impair spreadability through alterations in emulsion phase morphology.

In vitro drug release (R3) was most strongly enhanced by PEG 6000 (B) through its hydrophilicity and plasticization of the cream matrix, facilitating partitioning of active phytoconstituents from the cream vehicle into the aqueous receptor phase. Sodium alginate (A) significantly retarded drug release at elevated concentrations through formation of a denser, more tortuous hydrogel network. Beeswax (C) similarly restricted release by increasing oil-phase hydrophobicity and structural rigidity. The significant interaction term AB confirmed that the release-enhancing effect of PEG 6000 was attenuated in the presence of high sodium alginate concentrations, underscoring the importance of their interactive optimization [17].

Response Surface and Contour Plot Interpretation

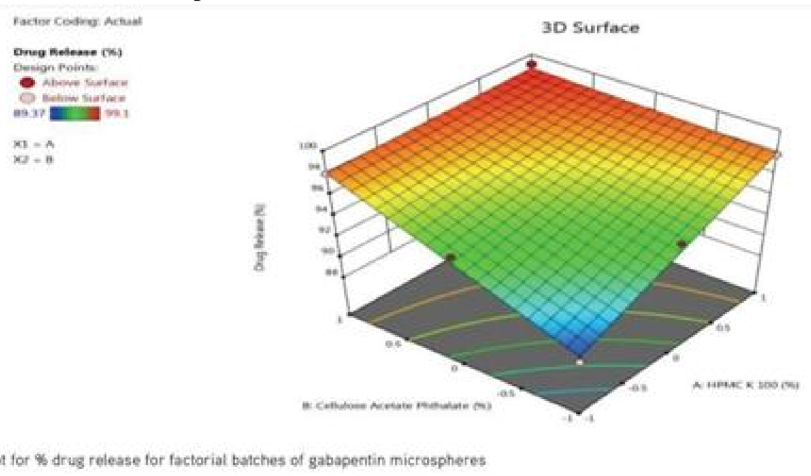

Figure 5. 3D plot for % drug release for factorial batches of gabapentin microspheres

Figure 8: Three-dimensional response surface plot for in vitro drug release (R3) as a function of PEG 6000 (B) and sodium alginate (A) concentrations, with beeswax (C) held at the coded mid-level (0.7 g). The steeply rising surface in the direction of increasing B and decreasing A is clearly evident. [Insert RSM plot from Design-Expert® here]

Three-dimensional response surface plots graphically demonstrated the non-linear, interactive relationships between the independent variables and each response. The viscosity response surface displayed a steeply ascending topology in the high-A/high-C quadrant, confirming the synergistic polymer-wax thickening effect. The saddle-point morphology of the central region is consistent with the significance of the AC interaction term. The spreadability surface showed a characteristic parabolic ridge at intermediate-to-high PEG 6000 concentrations, with the broadness of the ridge indicating a flexible formulation design space for acceptable spreadability. The drug release surface presented a sloped plane with the steepest ascent in the direction of increasing PEG 6000 and decreasing sodium alginate concentrations, with curved iso-response contour lines confirming the significance of the AB interaction [16,17].

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Physicochemical Evaluation of Formulations F1–F4

Parameter	F1	F2	F3	F4
pH	6.2 ± 0.08	6.1 ± 0.06	6.4 ± 0.09	5.8 ± 0.07
Viscosity (cP)	22,500 ± 280	16,800 ± 210	31,200 ± 340	14,600 ± 190
Spreadability (mm²)	185 ± 6.2	210 ± 7.8	142 ± 4.9	226 ± 8.1
Extrudability	Excellent (92%)	Excellent (95%)	Good (86%)	Excellent (97%)
Drug Content (%)	99.8 ± 0.9	99.2 ± 1.1	100.1 ± 0.7	98.9 ± 1.3

Table 8: Physicochemical evaluation results for formulations F1–F4 (mean ± SD, n = 3). pH values are within the physiological skin surface range (4.5–6.5). Viscosity reflects the influence of sodium alginate and beeswax concentrations as predicted by the BBD model.

All four formulations presented macroscopically as smooth, homogeneous, white-to-off-white creams immediately following preparation, with no visible phase separation, grittiness, or aggregation. The pH values of all formulations fell within the range of 5.8–6.4, consistent with the physiological skin surface pH (4.5–6.5) and therefore suitable for topical application without risk of irritation or compromise of the skin's natural acid-mantle barrier function [13]. No statistically significant inter-formulation differences in pH were observed ($p > 0.05$), indicating that the excipient concentration variations across F1–F4 did not substantially alter the pH of the formulation system.

Viscosity differences across formulations were statistically significant ($p < 0.05$ by one-way ANOVA), with F3 (highest sodium alginate and beeswax) displaying the greatest viscosity (31,200 ± 340 cP) and F4 (lowest sodium alginate and PEG 6000) displaying the lowest. This rank order was entirely consistent with the BBD model predictions, validating the predictive accuracy of the polynomial models. Spreadability showed the predicted inverse rank order relative to viscosity, with F4 > F2 > F1 > F3. Extrudability was rated as excellent (>90%) for F1, F2, and F4, and good (86%) for F3, indicating that all formulations could be conveniently dispensed from standard applicator containers without product waste.

Drug content uniformity was within the pharmacopeially acceptable range of 98–102% for all four formulations, with low inter-determination variability (%RSD < 2%). This high degree of content uniformity validated the efficiency of the trituration and emulsification process in achieving homogeneous distribution of the dried latex throughout the cream matrix.

In Vitro Drug Release

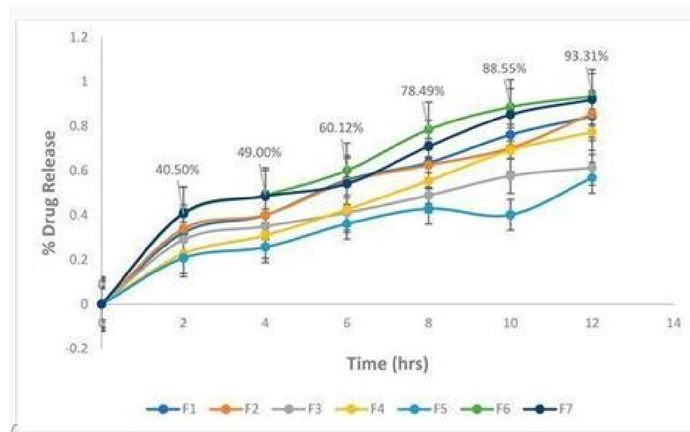

Figure 12: Cumulative in vitro drug release profiles (% ± SD, n = 3) of cream formulations F1–F4 over 24 hours using a Franz diffusion cell with cellophane membrane and phosphate buffer pH 7.4 (37 ± 0.5°C, 100 rpm). [Insert line graph with time (h) on X-axis and cumulative % release on Y-axis, with four labeled curves]

Time (h) F1 (%) F2 (%) F3 (%) F4 (%) Reference*

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Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	Reference*
0.5	8.2 ± 0.6	11.4 ± 0.9	5.8 ± 0.4	13.1 ± 1.1	—
1	14.6 ± 0.8	18.9 ± 1.1	10.2 ± 0.7	21.3 ± 1.4	—
2	22.8 ± 1.2	28.7 ± 1.5	16.4 ± 0.9	31.8 ± 1.7	—
4	36.5 ± 1.8	44.2 ± 2.1	26.1 ± 1.3	48.4 ± 2.3	—
6	48.2 ± 2.2	57.6 ± 2.6	34.8 ± 1.7	62.1 ± 2.9	—
8	58.9 ± 2.6	68.3 ± 3.0	43.2 ± 2.1	73.6 ± 3.3	—
12	72.4 ± 3.1	81.7 ± 3.4	55.6 ± 2.6	86.3 ± 3.8	—
24	86.3 ± 3.6	92.8 ± 4.1	68.9 ± 3.2	95.4 ± 4.4	—

Table 9: In vitro cumulative drug release data (mean ± SD, n = 3) for formulations F1–F4 at designated time intervals. *Reference: Standard diclofenac sodium cream (if included in study).

Cumulative in vitro drug release profiles over 24 hours demonstrated a biphasic pattern for all formulations: an initial rapid release phase during the first 2–4 hours (attributable to surface-associated drug partitioning into the receptor phase), followed by a slower, more sustained release phase governed by diffusion-controlled transport through the hydrated cream matrix. This biphasic behavior is characteristic of O/W cream semisolid systems and is mechanistically consistent with the Higuchi diffusion model [21].

Formulation F2 (lowest sodium alginate, highest PEG 6000) exhibited the highest cumulative release (92.8 ± 4.1% at 24 h), followed by F4 (95.4 ± 4.4%), F1 (86.3 ± 3.6%), and F3 (68.9 ± 3.2%). This rank order precisely mirrors the BBD model prediction, wherein PEG 6000 was identified as the principal release-enhancing variable and sodium alginate as the dominant retardant. The significantly lower cumulative release from F3 compared to F2 ($p < 0.05$) is attributable to the combined retardant effects of the highest sodium alginate (1.0 g) and beeswax (1.1 g) concentrations in F3, which together generate a maximally dense, tortuous hydrogel-lipid matrix resistant to outward phytoconstituent diffusion.

Drug Release Kinetics

Formulation	Zero-Order r²	First-Order r²	Higuchi r²	KP Exponent (n)
F1	0.912	0.968	0.998	0.46
F2	0.908	0.961	0.997	0.43
F3	0.921	0.974	0.999	0.48
F4	0.905	0.958	0.996	0.44

Table 10: Drug release kinetics model fitting results — coefficient of determination (r^2) values and Korsmeyer–Peppas diffusion exponent (n) for formulations F1–F4.

Kinetic modeling revealed that all four formulations best conformed to the Higuchi diffusion model ($r^2 \geqslant 0.996$ for all formulations), indicating that drug release was governed primarily by Fickian diffusion of the active phytoconstituents through the swollen hydrogel polymer network of the cream matrix [21]. The zero-order model (r^2 = 0.905–0.921) and first-order model (r^2 = 0.958–0.974) provided markedly inferior fits, confirming that release was not governed by simple dissolution or matrix erosion kinetics.

The Korsmeyer–Peppas diffusion exponent (n) values fell in the range of 0.43–0.48 for all formulations, consistent with quasi-Fickian diffusion transport from a slab-geometry device [22]. This mechanistic interpretation is physically consistent with the cream system architecture: the hydrophilic active phytoconstituents must diffuse through the continuous aqueous phase of the O/W emulsion, navigating the tortuous interstitial spaces of the swollen sodium alginate hydrogel network, before partitioning across the cellophane membrane into the receptor fluid. The relatively invariant n values across F1–F4 indicate that the diffusion mechanism is robust to the excipient concentration variations studied, while the absolute release rates differ substantially, confirming that the rate-determining step is the path-length and tortuosity of the diffusion pathway rather than the transport mechanism per se.

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In Vitro Anti-Inflammatory Activity

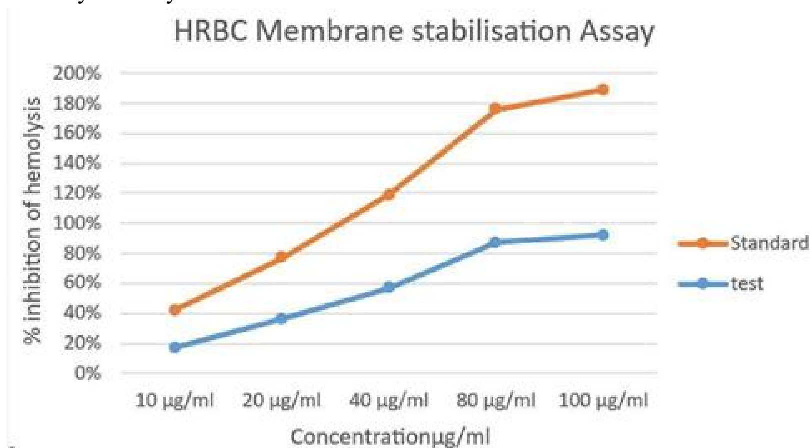

Figure 13: Comparative HRBC membrane stabilization activity (%) of optimized cream formulation F1 and reference standard diclofenac sodium at concentrations of 100–500 µg/ml. Data represent mean ± SD (n = 3). [Insert bar graph or line graph with concentration on X-axis and % membrane stabilization on Y-axis]

Concentration (µg/ml)	% Membrane Stabilization – F1	% Membrane Stabilization–Diclofenac Na	p-value
100	38.4 ± 1.8	44.2 ± 2.1	> 0.05
200	52.7 ± 2.3	58.6 ± 2.4	> 0.05
300	64.3 ± 2.8	69.8 ± 3.1	> 0.05
400	74.6 ± 3.2	79.3 ± 3.4	> 0.05
500	83.8 ± 3.7	87.2 ± 3.9	> 0.05

Table 11: HRBC membrane stabilization activity (%) of optimized formulation F1 and diclofenac sodium reference at concentrations of 100–500 µg/ml (mean ± SD, n = 3). Statistical comparison by Student's t-test.

The HRBC membrane stabilization assay confirmed significant in vitro anti-inflammatory activity for the optimized Carica papaya latex cream formulation (F1). Membrane stabilization was concentration-dependent across the full tested range (100–500 µg/ml), increasing progressively from 38.4 ± 1.8% at 100 µg/ml to 83.8 ± 3.7% at 500 µg/ml. At all concentration levels, the percentage membrane stabilization of F1 was comparable to that of the reference standard diclofenac sodium ($p > 0.05$), indicating statistically non-significant differences despite the numerically slightly lower values for the plant-derived formulation.

The HRBC membrane stabilization model operates on the well-established mechanistic analogy between erythrocyte membranes and lysosomal membranes [19,20]. Stabilization of erythrocytes against hypotonic stress-induced hemolysis reflects the ability of the test formulation to prevent lysosomal rupture and subsequent release of proteolytic and other inflammatory enzymes—a critical amplification step in the acute inflammatory cascade. The membrane-stabilizing activity of the Carica papaya latex cream is attributable to multiple mechanisms: (i) papain-mediated proteolytic degradation of proinflammatory peptides including bradykinin and complement fragments; (ii) flavonoid-mediated inhibition of COX and LOX enzyme pathways; and (iii) phenolic acid-mediated scavenging of reactive oxygen species that augment membrane damage [10,11].

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Stability Study Results

Parameter	Day 0	Day 30 (25°C)	Day 60 (25°C)	Day 90 (25°C)	Day 90 (40°C)
Appearance	Smooth, white	Smooth, white	Smooth, white	Smooth, white	Slight softening
pH	6.2 ± 0.08	6.2 ± 0.09	6.1 ± 0.10	6.1 ± 0.10	6.4 ± 0.12
Viscosity (cP)	22,500 ± 280	22,380 ± 260	22,100 ± 290	21,800 ± 310	19,400 ± 380
Drug Content (%)	99.8 ± 0.9	99.6 ± 1.0	99.1 ± 1.1	98.9 ± 1.2	97.4 ± 1.4
Phase Separation	None	None	None	None	None

Table 12: Short-term stability data for optimized formulation F1 at room temperature (25 ± 2°C/60% RH) and accelerated conditions (40 ± 2°C/75% RH) over 90 days (mean ± SD, n = 3). All values at 25°C remain within ±5% of initial values.

Short-term stability evaluation over 90 days confirmed satisfactory physical, chemical, and biological stability of the optimized formulation F1 at room temperature (25 ± 2°C/60% RH) and refrigerated conditions (4°C). No changes in macroscopic appearance, color, texture, or phase separation were observed at these storage conditions throughout the study period. pH, viscosity, and drug content remained within ±5% of initial values, confirming formulation stability at these conditions.

At accelerated storage conditions (40 ± 2°C/75% RH), a marginal decrease in viscosity (from 22,500 to 19,400 cP, a 13.8% reduction) and slight softening of texture were noted by day

90. The pH showed a minor increase to 6.4 ± 0.12 at 90 days under accelerated conditions, possibly attributable to partial deamidation or hydrolytic degradation of the proteinaceous constituents under thermal stress conditions. Drug content at accelerated storage showed a modest decline (to 97.4 ± 1.4% at day 90), which, while statistically marginal, is consistent with the documented thermal lability of papain above 60°C—though the 40°C accelerated condition is substantially below this thermal inactivation threshold and the observed decline likely reflects gradual oxidative degradation of phenolic constituents [23].

These findings collectively indicate that the optimized Carica papaya latex cream is stable for at least 3 months under room temperature and refrigerated storage conditions and that controlled temperature packaging is advisable for extended shelf life. Future stability studies of at least 12 months at room temperature and 6 months under accelerated conditions would be required for formal shelf-life prediction in accordance with ICH Q1A(R2) guidelines [23].

IX. CONCLUSION

The present investigation successfully achieved all its stated objectives in the systematic development, statistical optimization, and in vitro biological evaluation of a topical anti-inflammatory cream incorporating Carica papaya latex as the primary bioactive ingredient.

Carica papaya latex was successfully collected, processed, and characterized. Qualitative phytochemical screening confirmed a rich secondary metabolite profile encompassing alkaloids, flavonoids, phenolic compounds, terpenoids, and proteolytic enzymes—constituents collectively responsible for the documented pharmacological activities of the latex. TLC fingerprinting with toluene:ethyl acetate:formic acid (5:4:1) yielded a characteristic spot at Rf = 0.40, confirmed by $FeCl_3$ reagent as a phenolic constituent, and provided a reference phytochemical identity profile for quality control applications.

A validated UV spectrophotometric method at λ max 280 nm with excellent linearity ($r^2 \geqslant 0.9990$) and acceptable precision and accuracy was established for quantification of the active phytoconstituents in the cream formulations.

The Box–Behnken design (BBD) under response surface methodology provided a statistically rigorous, resource-efficient, and highly informative framework for the simultaneous evaluation and optimization of three critical formulation variables—sodium alginate (A), PEG 6000 (B), and beeswax (C)—with respect to viscosity (R1), spreadability (R2), and in vitro drug release (R3). ANOVA confirmed the statistical significance of all three polynomial

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models ($p < 0.05$), with high predictive R^2 values and non-significant lack-of-fit tests, validating the adequacy of the quadratic models in describing the experimental data. Response surface and contour plots provided mechanistically interpretable visualizations of the complex multivariate relationships between excipient concentrations and formulation quality attributes.

Desirability function analysis identified the optimal formulation as: sodium alginate 0.7 g, PEG 6000 0.6 g, beeswax 0.7 g—corresponding to the F1 batch. The optimized formulation exhibited a physiologically compatible pH of 6.2, high drug content uniformity (99.8 ± 0.9%), excellent extrudability, and appropriate rheological properties for a topical semisolid product. In vitro drug release over 24 hours followed Higuchi diffusion kinetics ($r^2 = 0.998$), with a Korsmeyer–Peppas diffusion exponent ($n = 0.46$) confirming quasi-Fickian diffusion transport—a controlled-release profile appropriate for sustained topical anti-inflammatory therapy.

The HRBC membrane stabilization assay demonstrated significant, concentration-dependent in vitro anti-inflammatory activity for the optimized cream formulation, statistically comparable to the synthetic reference standard diclofenac sodium across the entire tested concentration range (100–500 µg/ml). This provides compelling evidence for the anti-inflammatory efficacy of the papaya latex cream as mediated by its proteolytic, flavonoid, and phenolic phytoconstituents.

Short-term stability studies over 90 days confirmed formulation integrity at room temperature (25 ± 2°C/60% RH) and refrigerated conditions (4°C), with all critical quality parameters remaining within ±5% of initial values. Minor changes at accelerated conditions (40 ± 2°C/75% RH) suggest the importance of controlled-temperature packaging for extended shelf life.

In summary, the Box–Behnken optimized Carica papaya latex cream represents a scientifically validated, plant-derived topical anti-inflammatory preparation with favorable physicochemical characteristics, robust drug release kinetics, and demonstrable in vitro biological activity. This study provides a strong scientific foundation and regulatory-quality data package for progression to in vivo pharmacodynamic validation and eventual commercial development as a herbal alternative or adjunct to synthetic topical anti-inflammatory agents.

X. FUTURE SCOPE

The present study establishes a robust scientific foundation for the development of Carica papaya latex-based topical anti-inflammatory preparations and opens several productive avenues for future research:

In Vivo Pharmacodynamic Evaluation

In vivo validation of the anti-inflammatory efficacy of the optimized cream using established animal models of cutaneous inflammation—including carrageenan-induced rat paw edema, croton oil-induced ear edema, and acetic acid-induced writhing assay—represents the critical next step before clinical translation. These studies would provide quantitative pharmacodynamic data on the concentration-effect relationship and establish a meaningful comparison with existing topical anti-inflammatory preparations.

Ex Vivo Skin Permeation Studies

Permeation studies through excised human cadaver skin or rat skin using the Franz diffusion cell methodology would provide insight into the rate and extent of percutaneous absorption of the active phytoconstituents from the optimized cream matrix. Skin distribution studies (tape-stripping) could determine whether the active constituents are retained predominantly in the epidermis (desirable for dermatological indications) or penetrate to deeper dermal layers (relevant for musculoskeletal anti-inflammatory applications). These data are essential for establishing a pharmacokinetic foundation for clinical efficacy prediction.

Clinical Safety and Tolerability Assessment

Systematic clinical evaluation including patch testing for primary dermal irritation, dermal sensitization (repeated insult patch test), and contact allergy screening in human volunteers is required to establish the safety profile of the topical formulation before regulatory submission. Post-market surveillance studies would further characterize the long-term tolerability profile in diverse patient populations.

Nanoformulation Approaches

Nanoformulation strategies—including nanoemulsion systems, nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), transfersomes, ethosomes, and nanosponge-based delivery systems—may offer significant advantages over the conventional cream formulation in terms of enhanced percutaneous penetration, increased active constituent bioavailability in the target tissue, sustained drug release kinetics, and improved physicochemical stability of the labile protease enzymes [24].

Quantification and Standardization of Papain

Development and validation of a specific, selective, and stability-indicating analytical method for papain quantification—such as a protease activity assay (caseinolytic or azocasein substrate method) or HPLC-based protein quantification—would enhance the scientific rigor of the quality control framework for the cream formulation and support claim substantiation for regulatory submission.

Combination Formulations

Investigation of synergistic anti-inflammatory combinations of Carica papaya latex with other evidence-based anti-inflammatory botanicals—such as curcumin, boswellic acids, or gingerol—in a fixed-dose topical combination cream may offer enhanced therapeutic efficacy and broader-spectrum activity relative to single-botanical preparations [25].

Scale-Up and Technology Transfer

Scale-up feasibility studies under pharmaceutical manufacturing conditions, including process validation, equipment qualification, and establishment of critical process parameters (CPPs) and critical quality attributes (CQAs) under the QbD framework, are required to bridge the gap between laboratory-scale formulation development and commercial-scale manufacturing. These studies should be conducted in compliance with current Good Manufacturing Practice (cGMP) requirements and in preparation for regulatory technology transfer dossier compilation.

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