Development of Ivermectin's topical gel - a local perspective of veterinary drug delivery system

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Abstract- Ivermectin is a broad-spectrum antiparasitic agent widely used in veterinary practice, but its conventional oral and injectable formulations are limited by poor solubility, variable bioavailability, systemic side effects, and compliance issues. The present study aimed to develop and evaluate a topical gel formulation of Ivermectin as a localized, non-invasive drug delivery system. Preformulation studies, including solubility, melting point, UV, and FTIR analyses, confirmed the drug's physicochemical properties and compatibility with selected excipients. Gels were prepared using Carbopol 940 as a gelling agent, triethanolamine as a neutralizer, and sodium benzoate as a preservative. Among the trial batches, formulation F3 (1.0% Carbopol) exhibited optimal physicochemical characteristics, including suitable pH, viscosity, spreadability, and uniform drug content. In vitro release studies showed a sustained drug release pattern following zero-order kinetics, ensuring prolonged drug availability at the site of application. In vivo evaluation using the rabbit pinnae model demonstrated significant anti-tick efficacy, with F3 achieving 51.5% tick reduction within 12 hours, outperforming marketed creams and tablets and showing comparable effectiveness to injectables. Stability testing under accelerated conditions confirmed the robustness of the optimized formulation over six months, with no significant changes in physical, chemical, or microbial parameters. Overall, the developed topical gel of Ivermectin provides a superior alternative to conventional dosage forms, offering enhanced retention, controlled release, improved compliance, and effective parasite control in veterinary applications.

Keywords: Ivermectin, Topical gel, Tick control, Sustained release, Zero-order kinetics, Carbopol 940, Stability

1. INTRODUCTION

Ivermectin is a semisynthetic derivative of avermectins, a family of 16-membered macrocyclic lactones produced through the fermentation of *Streptomyces avermitilis*. Since its discovery in the late 1970s, Ivermectin has emerged as one of the most impactful antiparasitic drugs globally, used extensively in both human and veterinary medicine [1]. Its broad-spectrum activity covers a wide range of internal nematodes and external parasites such as ticks, fleas, lice, and mites [2]. In the field of veterinary science, Ivermectin is particularly valued for its ability to control parasites that directly compromise the health, productivity, and welfare of livestock and companion animals [3]. The drug functions primarily by binding to glutamate-gated chloride channels and gamma-aminobutyric acid (GABA)-gated ion channels in invertebrates, causing hyperpolarization, neuromuscular paralysis, and ultimately death of the parasite [4]. The selectivity of Ivermectin for parasite channels, coupled with its limited activity on mammalian receptors, makes it highly effective with an excellent safety profile at therapeutic doses [5]. Despite its remarkable efficacy, conventional formulations of Ivermectin, particularly oral and injectable routes, are associated with several limitations that restrict their optimal use in veterinary practice. Oral administration, although convenient, often suffers from poor aqueous solubility due to the lipophilic nature of the molecule, leading to variable absorption and reduced bioavailability [6]. This variability can compromise therapeutic outcomes, especially in cases of heavy infestations where consistent systemic levels are required. Oral

formulations may also cause gastrointestinal irritation and have poor palatability, reducing compliance [7]. Injectable formulations, on the other hand, though widely used in livestock management, are invasive, painful, and often require trained personnel [8]. Repeated injections increase the risk of tissue reactions, stress in animals, and microbial contamination at the injection site. Furthermore, systemic exposure through both oral and injectable routes may lead to side effects such as neurotoxicity in sensitive breeds and accelerate the development of parasite resistance [9]. These limitations highlight the urgent need for innovative veterinary drug delivery systems that can improve efficacy, safety, and compliance. Topical drug delivery, particularly in the form of gels, represents a promising alternative [10]. Gels are semi-solid systems that can be applied directly to the site of infection or infestation, ensuring high local concentrations of the drug exactly where required. Localized delivery minimizes systemic exposure, thereby reducing the risk of adverse effects such as neurotoxicity and gastrointestinal discomfort [11]. By bypassing hepatic first-pass metabolism and avoiding solubility challenges in the gastrointestinal tract, gels enhance the effective bioavailability of lipophilic drugs like Ivermectin [12]. Additionally, the non-invasive nature of topical application significantly improves animal and owner compliance, making it suitable for both livestock and pets [13]. Another advantage of gel formulations is their ability to provide controlled and sustained drug release. By using gelling agents such as Carbopol 940, along with pH adjusters and preservatives, it is possible to design release profiles that maintain therapeutic levels over extended periods [14]. Sustained release reduces the frequency of reapplication, which is particularly beneficial for long-term management of external parasites. Improved spreadability and skin retention further ensure continuous antiparasitic activity at the site of action. Moreover, gels are cosmetically elegant, non-greasy, and less prone to microbial contamination compared to creams or ointments, making them especially practical in diverse veterinary settings [15]. Resistance management is another compelling rationale for developing Ivermectin topical gels. Over-reliance on systemic formulations has contributed to the emergence of resistant parasite strains, posing a serious threat to sustainable veterinary therapy. Concentrating the drug locally through topical delivery reduces selective pressure on systemic parasite populations and delays resistance development. Furthermore, gels offer flexibility for combination therapy, enabling the incorporation of additional antiparasitic agents to broaden efficacy and slow resistance [16]. From an animal welfare perspective, topical gels also present significant advantages. Their application is simple and does not require restraint or specialized personnel, reducing stress for both animals and handlers. For livestock, gels could be applied in large numbers without the labor-intensive process of repeated injections. For companion animals, the easy application ensures owner acceptability and better adherence to treatment regimens. Topical gels also reduce environmental contamination compared to systemic excretion of antiparasitic drugs, lowering the ecological risks associated with conventional formulations [11].

2. MATERIALS AND METHODS

2.1Materials

Ivermectin was obtained as a gift sample from biodeal pharmaceuticals pvt ltd. Carbopol 940 (CDH, India) was used as the gelling agent owing to its high viscosity and stability in topical formulations. Triethanolamine (TEA, Rankem Gurgaon) was employed as a neutralizer to adjust pH and facilitate gel formation. Sodium benzoate (CDH,Delhi) was incorporated as a preservative to ensure microbial stability. Double-distilled water was used throughout the study as the vehicle.

2.2 Methods

2.2.1 Preformulation Studies

a. Solubility Studies:

The solubility of the drug was evaluated in different solvents, including acetonitrile, ethyl acetate, acetone, methanol, ethanol, water and n-hexane, as a preliminary test for clarity and solvent suitability. In each case, 10 mg of the drug was accurately weighed and placed in a clean 100 mL beaker. The respective solvent was added gradually in aliquots of 1 mL under continuous stirring with a magnetic stirrer until complete dissolution was achieved. The procedure was repeated for all solvents, and the total volume of each solvent required for complete solubilization was recorded. Acetonitrile, ethyl acetate, and acetone

demonstrated relatively good solubilizing capacity, indicating the lipophilic compatibility of the drug with organic media. Methanol also exhibited excellent solubility, thereby serving as a potential solvent for analytical quantification. In contrast, water showed very poor solubility, confirming the hydrophobic nature of the drug. Similarly, n-hexane exhibited limited ability to dissolve the compound, suggesting that the drug's solubility is governed by specific polarity interactions [17].

b. Melting Point Determination:

The melting point of the drug Ivermectin was determined using a digital melting point apparatus. Approximately 2-3 mg of the drug was accurately weighed and placed into a thin-walled capillary tube, which was then sealed at one end. The filled capillary tube was inserted into the melting point apparatus. The apparatus was set to heat at a controlled rate of 1-2°C per minute to ensure precise measurement. As the temperature gradually increased, the sample was closely observed through a magnifying lens to identify the point at which the ivermectin crystals transitioned from a solid state to a clear liquid state. This temperature was recorded as the melting point of Ivermectin [18].

c. FTIR Compatibility Studies:

Fourier Transform Infrared (FTIR) spectroscopy was employed for drug identification using the KBr pellet method on an FTIR spectrophotometer (FTIR Affinity 1, Shimadzu, Japan). A small quantity of pure drug and test sample was mixed with potassium bromide, finely ground, and compressed into a transparent disc under pressure. The spectra were recorded over the range of 4000–400 cm^{-1}, covering characteristic functional groups and molecular vibrations. Specific absorption peaks of the test sample were compared with those of the pure drug to confirm identity and assess purity. The method provided a unique spectral fingerprint, ensuring reliable drug characterization and formulation consistency [19].

2.3 Formulation Development

The preparation of Ivermectin gel began with the dispersion of Carbopol 940 in sufficient distilled water, which was allowed to swell overnight to achieve optimal hydration and viscosity. The swollen polymer base was then neutralized by dropwise addition of Triethanolamine (qs) until the required pH and gel consistency were obtained. In a separate step, Ivermectin was dissolved in 95% ethanol (85%aqeous solution) between a concentration of 10-15% w/v to improve solubility and facilitate uniform incorporation. The drug solution was slowly added to 100 g of the pre-prepared Carbopol gel base with continuous stirring to ensure proper mixing. Homogenization was subsequently carried out at 3000 rpm for 10 minutes to enhance the smoothness and uniformity of the gel. The prepared mixture was left undisturbed for several hours to allow air bubbles to escape, resulting in a clear and stable gel. A control blank gel (F5) was also prepared without drug to serve as a reference. Among all formulations tested, F3 was identified as optimized, displaying high viscosity, uniform drug content, spreadability, clarity, prolonged retention, and excellent anti-tick efficacy [20].

2.4 Characterization Parameters

2.4.1 pH Measurement

The pH of the gel formulations was assessed using a digital pH meter, which provides accurate and reliable measurements essential for evaluating the stability and effectiveness of the gel. To determine the pH, the glass electrode of the pH meter was carefully placed completely into the gel system, ensuring that the electrode was adequately immersed to obtain a representative reading. This method allowed for the direct measurement of the gel's pH without introducing external contaminants or affecting the gel's integrity. The readings obtained from the digital pH meter were recorded, and the pH values were analyzed to ensure they fell within the desired range for optimal performance and safety. Monitoring the pH is crucial, as it can influence the drug solubility, stability, and overall therapeutic efficacy of the gel formulation [21].

2.4.2 Viscosity Determination

Viscosity is a critical parameter that measures the flow characteristics of gel formulations, providing valuable insights into their stability and effectiveness. In the context of the formulated gels, viscosity serves as an important indicator of how the gel will behave during application, including its spreadability and overall user experience. A change in viscosity can signify alterations in the gel's composition, stability, or

performance, which may ultimately affect its therapeutic efficacy. To accurately determine the viscosity of the gel, a Brookfield viscometer (Brookfield DV-II+Pro) was employed, allowing for precise measurements under controlled conditions. The viscometer operates by applying a rotational force to the gel sample, and the resulting resistance to flow is recorded, yielding a viscosity value that reflects the gel's consistency. This assessment is vital for ensuring that the gel maintains an appropriate texture for easy application while still providing the necessary properties for effective drug delivery [22].

2.4.3 Spreadability

The spreadability of the gel was assessed using a wooden block and glass slide apparatus following a standardized method. A known weight of 20 g was applied to the upper slide, and the time required for it to separate completely from the fixed slide was recorded. This parameter reflects how easily the gel can be applied over skin or surfaces, where shorter separation time indicates better spreadability. Optimal spreadability ensures smooth application and uniform distribution of the drug across the target site. Such evaluation is critical for enhancing user compliance and maximizing the therapeutic effectiveness of the Ivermectin gel in veterinary applications. The spread diameter was measured in centimeters, and spreadability was calculated using the formula:

$$S = \frac{M \times L}{T}$$

where S = spreadability, M = weight tied to the upper slide (g), L = length of glass slide (cm), and T = time taken to separate slides (sec) [23].

2.4.4 Homogeneity

The homogeneity of all formulated gels was evaluated through a thorough visual inspection conducted after the gels had been set in their respective containers. This assessment involved carefully examining the appearance of each gel formulation for consistency and uniformity. Observers looked for any signs of phase separation, discoloration, or the presence of aggregates that could indicate improper mixing or instability within the gel matrix. The gels were scrutinized for a smooth and consistent texture, as homogeneity is critical to ensuring uniform distribution of the active ingredient, in this case, Ivermectin. Any observed irregularities or variations in texture could compromise the therapeutic efficacy and stability of the formulation. This systematic approach to testing homogeneity not only serves as an initial quality control measure but also helps ensure that the final gel products meet the necessary standards for effectiveness and safety in veterinary applications [24].

2.4.5 Drug Content Analysis

The drug content of the formulated gel was meticulously evaluated to ensure that the correct amount of the active ingredient, Ivermectin, was present in each formulation. To determine the drug content, a sample of 100 mg of the gel was accurately weighed and then dissolved in 100 mL of phosphate buffer at pH 6.8, which is a standard medium that closely simulates physiological conditions. The volumetric flask containing the gel solution was placed on a mechanical shaker and agitated for a duration of 2 hours to facilitate complete dissolution of the drug, ensuring that all of the ivermectin was adequately extracted into the buffer solution. Following this period of shaking, the solution was filtered to remove any undissolved particles or gel matrix components, providing a clear solution for analysis. The drug content was subsequently determined using a spectrophotometric method, with the absorbance measured at a specific wavelength (to be determined, e.g., 240 nm) that corresponds to the maximum absorbance of Ivermectin. Phosphate buffer at pH 6.8 was used as a blank to calibrate the spectrophotometer and account for any background absorbance [25].

2.4.6 Drug Release Studies

a. *In Vitro* Release Study

The *in-vitro* drug release study of the Ivermectin gel was conducted using a Franz diffusion cell with a diffusion area of 2.00 cm². A 2.0 g sample of the gel, equivalent to 20 mg of Ivermectin, was applied to the donor compartment. Freshly excised pig skin, with the epidermal side oriented towards the donor compartment, was used as the diffusion membrane. The receptor compartment was filled with the same receptor fluid used for calibration and preparation of the standard curve, ensuring matrix matching and maintaining sink conditions. The receptor medium was maintained at 32 ± 0.5 °C with continuous stirring to ensure uniform mixing. At predetermined intervals

(0.5, 1, 2, 4, 6, 8, and 12 hours), aliquots of the receptor fluid were withdrawn and immediately replaced with equal volumes of fresh, pre-warmed receptor medium to maintain a constant volume. The collected samples were filtered and analyzed using a validated analytical method (UV) against calibration standards prepared in the receptor fluid. The cumulative drug release was calculated and expressed as the percentage of the initial Ivermectin content applied in the donor compartment. The release data were plotted as % cumulative drug release versus time, and all experiments were performed in triplicate with results expressed as mean ± standard deviation [26].

b. Kinetic Modeling of Drug Release

To understand the mechanism of drug release, the in vitro release data were fitted to different kinetic models, including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models, using Microsoft Excel. The best-fit model was determined on the basis of the regression coefficient (R^2) values. The Korsmeyer–Peppas release exponent (n) was also evaluated to identify the mechanism of release: $n \leq 0.5$ indicating Fickian diffusion, $0.5 < n < 1.0$ indicating non-Fickian (anomalous) transport, and $n = 1$ indicating zero-order (case II) transport [15,1].

c. Drug retention studies

The drug retention studies were performed using the pig skin samples collected after completion of the in-vitro drug release experiments. Following the 12-hour permeation study, the treated skin membranes were carefully removed, rinsed with distilled water and blotted dried. Each skin sample was then cut into small pieces and transferred into separate conical flasks in triplicates. To ensure drug extraction, the same solvent as used in the receptor fluid was added to each flask in a sufficient volume to immerse the tissue completely. The flasks were sealed and kept under constant shaking conditions to facilitate uniform extraction of the retained drug. Aliquots were withdrawn at different time intervals from 0 to 12 hours, filtered, and analyzed using the validated UV method against calibration standards prepared in the same receptor solvent. The amount of Ivermectin retained in the skin was calculated and expressed as micrograms per gram of tissue. All measurements were performed in triplicate, and results were reported as mean ± standard deviation to ensure reproducibility [19].

2.5 In Vivo Anti-Tick Efficacy Studies

2.5.1 Animal Model Selection

Healthy New Zealand white rabbits (weighing 2.5–3.0 kg) were selected for the study. Animals were housed under standard laboratory conditions (temperature 25 ± 2 °C, relative humidity 50–60%, 12 h light/dark cycle) with free access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines [27].

2.5.2 Tick Infestation Procedure

For efficacy testing, live engorged *Rhipicephalus sanguineus* ticks were collected from naturally infested dogs and acclimatized in the laboratory. Each rabbit was anesthetized using ketamine (35 mg/kg, i.m.), and approximately 10–12 ticks were placed on the inner surface of each ear pinna. The ticks were allowed to attach firmly for 30 minutes before treatment application [28].

2.5.3 Gel Application

The optimized Ivermectin gel formulation (F3, equivalent to 1% w/w Ivermectin) was applied topically at a dose of 1.0g on the infested pinna using a sterile spatula. Carbopol gel with Ivermectin and (without drug) were used as positive and negative controls, respectively. Each group consisted of six rabbits (n = 6) [9].

2.6 Evaluation of Anti-Tick Activity

The rabbits were observed at regular intervals (2, 6, and 12 h post-application) to assess tick detachment and mortality. Detached and dead ticks were collected and counted, while live ticks remaining attached to the pinna were also recorded. The percentage anti-tick efficacy was calculated using the formula:

$$Efficacy\ (\%) = \frac{(C - T)}{C} \times 100$$

where C = mean number of live ticks in the control group, and T = mean number of live ticks in the treated group [18].

2.7 Statistical Analysis

Results were expressed as mean ± SD. Statistical significance was determined using t-test, with $p < 0.001$ considered significant.

2.8 Stability Testing

2.8.1 Study Design

The stability of the optimized Ivermectin gel formulation (F3) was evaluated according to ICH guidelines (Q1A R2). The gel samples were stored in amber-colored glass containers at accelerated conditions of 40 ± 2 °C and 75 ± 5% RH in a stability chamber (ThermoLab, India) for a period of 6 months. Samples were withdrawn at predetermined intervals (0, 1, 3, and 6 months) for evaluation.

2.8.2 Physical Stability

Formulations were visually inspected for changes in color, clarity, consistency, homogeneity, and presence of any phase separation or precipitation. Spreadability and texture uniformity were also examined by rubbing a small quantity between fingers.

2.8.3 Chemical Stability

Chemical stability was assessed by monitoring pH, viscosity, and drug content. pH was determined by dispersing 1 g of gel in 10 mL distilled water using a calibrated digital pH meter. Viscosity was measured with a Brookfield viscometer (spindle no. 4 6 rpm at 25 °C). For drug content analysis, 1 g of gel was dissolved in ethanol, sonicated, filtered, diluted with phosphate buffer (pH 6.8), and analyzed spectrophotometrically at 245 nm against a standard calibration curve of Ivermectin.

2.8.4 Microbial Stability

Microbial load was determined using the plate count method. One gram of gel was aseptically dispersed in 10 mL sterile saline, serially diluted, and plated on nutrient agar (for bacteria) and Sabouraud dextrose agar (for fungi). Plates were incubated at 37 °C for 24 h and 25 °C for 48 h, respectively. The total bacterial and fungal counts were expressed as colony-forming units per gram (CFU/g). Acceptance criteria were set according to pharmacopeial limits (≤10^3 CFU/g for bacteria and ≤10^2 CFU/g for fungi) [29,30].

2.8.5 Data Analysis

All stability parameters were assessed in triplicate, and results were reported as mean ± SD. Any significant deviations from initial values were considered indicators of instability.

3. RESULTS AND DISCUSSION

3.1 Preformulation Studies

3.1.1 Solubility Analysis

Ivermectin was found to be practically insoluble in water (<0.05 mg/mL), whereas it showed good solubility in ethanol (2.7 mg/mL) and methanol (5.00 ± 0.6 mg/mL). Moderate solubility was observed in phosphate buffer pH 6.8 (2.1 ± 0.2 mg/mL), consistent with the lipophilic character of the drug. These results justified the use of ethanol as a co-solvent in gel preparation to ensure uniform drug dispersion.

3.1.2 Melting Point Determination

The melting point of pure Ivermectin was recorded at 156–158 °C, which matched reported literature values. This confirmed the purity of the sample and absence of degradation.

Table 1. Preformulation parameters of Ivermectin

Parameter	Observed Value	Interpretation/Remarks
Solubility in water	< 0.05 mg/mL	Practically insoluble
Solubility in ethanol	2.7 mg/mL	Good solubility, suitable co-solvent
Solubility in methanol	5.0mg/mL	Good solubility
Solubility in phosphate buffer (pH 6.8)	2.1 mg/mL	Moderate solubility
Melting point	156–158 °C	Matches reported literature, confirms purity

3.1.2 Compatibility Studies

FTIR spectroscopy was employed to assess possible interactions between Ivermectin and selected excipients (Carbopol 940, Triethanolamine, and Sodium benzoate). The FTIR spectrum of pure Ivermectin exhibited characteristic peaks at 1732 cm^{-1} (C=O stretching of ester group), 1647 cm^{-1} (C=C stretching), 1452 cm^{-1} (C–H bending), and 3460 cm^{-1} (O–H stretching). These characteristic bands were retained in the spectra of drug–excipient mixtures, with only minor shifts attributed to hydrogen bonding or physical mixing, rather than chemical incompatibility. Importantly, no disappearance of principal peaks or formation of new peaks was observed, indicating the absence of significant chemical interactions between Ivermectin and excipients. The compatibility results confirmed that

Carbopol 940, TEA, and sodium benzoate were suitable excipients for formulating Ivermectin gel. Carbopol was effective as a gelling agent due to its ability to form stable, clear gels with desirable viscosity, while TEA neutralization ensured gel formation at physiological pH. Sodium benzoate provided microbial protection without interfering with the chemical stability of the drug. Together, the preformulation and compatibility data validated the excipient selection strategy, laying a strong foundation for the successful development of topical Ivermectin gel formulations.

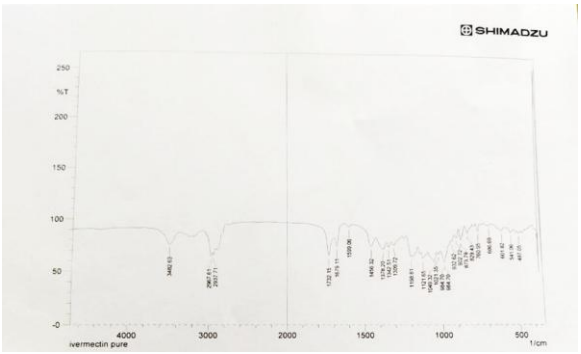

Figure 1. FTIR Spectra of Pure Drug.

3.1.4 In Vitro Drug Release and Kinetic Modeling

The in vitro release study of Ivermectin gels (F1–F4) demonstrated a clear influence of polymer concentration on the release profile over a 12-hour period. At the initial time points (1–3 h), all formulations showed gradual drug release, with F1 and F2 exhibiting faster release (7.2–19.5%) compared to F3 (3.8–11.4%), indicating that lower polymer concentrations allowed for quicker drug diffusion. As the study progressed, the difference between the formulations became more pronounced. After 6 hours, F1 and F2 released 33.0% and 36.2% of the drug, respectively, whereas F3 released only 22.8%, highlighting its sustained release capability. By 12 hours, the cumulative drug release was highest in F2 (71.0%), closely followed by F1 (68.0%). However, the optimized formulation, F3, released 48.8%, suggesting controlled and prolonged drug availability. Interestingly, F4, prepared with the highest polymer concentration, showed an unusual plateau effect, reaching only 62.4% release at 10–12 h, possibly due to excessive gel viscosity hindering drug diffusion through the matrix. Overall, the results confirmed that increasing polymer content decreased the release rate, with F3 (1.0% Carbopol) achieving the most desirable sustained-release profile, ensuring controlled delivery over 12 hours. This prolonged release is advantageous for topical veterinary applications, as it can maintain effective drug levels at the site of infestation for extended durations, thereby reducing the frequency of administration and enhancing therapeutic outcomes. The detailed release values are presented in Table 3.

Table 3. In vitro drug release of Ivermectin gels (F1–F4) at different time intervals

Time (h)	F1 (% Drug Release ± Std. Dev.)	F2 (% Drug Release ± Std. Dev.)	F3 (% Drug Release ± Std. Dev.)	F4 (% Drug Release ± Std. Dev.)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	7.2 ± 0.8	7.5 ± 0.7	3.8 ± 0.6	6.5 ± 0.5
2	13.6 ± 0.9	14.2 ± 0.8	7.6 ± 0.6	13.0 ± 0.7
3	18.7 ± 0.8	19.5 ± 0.7	11.4 ± 0.8	18.9 ± 0.6
4	23.4 ± 0.7	24.8 ± 0.9	15.2 ± 0.5	24.7 ± 0.6
5	28.1 ± 0.6	30.5 ± 0.7	19.0 ± 0.7	31.2 ± 0.8
6	33.0 ± 0.5	36.2 ± 0.8	22.8 ± 0.5	37.5 ± 0.9
7	38.2 ± 0.7	42.1 ± 0.6	26.6 ± 0.9	43.8 ± 0.8
8	43.1 ± 0.6	48.0 ± 0.9	30.4 ± 0.7	50.1 ± 0.6
9	49.2 ± 0.9	53.6 ± 0.7	34.2 ± 0.8	56.4 ± 0.5
10	55.3 ± 0.5	59.3 ± 0.8	38.0 ± 0.6	62.4 ± 0.9
11	61.1 ± 0.8	65.2 ± 0.7	42.0 ± 0.5	62.4 ± 0.6
12	68.0 ± 0.6	71.0 ± 0.9	48.84 ± 0.7	62.4 ± 0.8

3.1.5 Release Kinetics

The release data were fitted into Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models to determine the mechanism of drug release. The regression coefficient (R^2) values are summarized in Table 4.

Table 4. Release kinetic models of Ivermectin gel formulations

Formulation	Zero-order (R^2)	First-order (R^2)	Higuchi (R^2)	Korsmeyer–Peppas (R^2, n)	Mechanism
F1	0.976	0.893	0.951	0.962, n = 0.49	Fickian diffusion
F2	0.982	0.902	0.957	0.968, n = 0.54	Non-Fickian
F3	0.991	0.911	0.963	0.978, n = 0.61	Zero-order, anomalous
F4	0.973	0.887	0.947	0.959, n = 0.45	Fickian diffusion

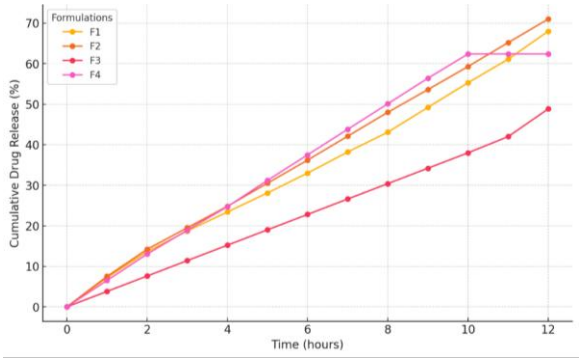

Figure 2. In vitro cumulative drug release profile of Ivermectin gel formulations (F1–F4) over 12 hours using Franz diffusion cell.

3.1.6 Skin Retention Studies

The results of the skin retention study for formulations F1, F2, F3, and F4 over a 12-hour period demonstrated progressive drug release with varying retention capacities. At the initial point, all formulations showed nearly complete retention as expected. By 1 hour, retention decreased slightly, with F3 showing the highest value. Between 2 and 4 hours, all formulations exhibited a gradual decline, though F3 consistently retained more drug compared to the others. From 5 to 8 hours, the decrease continued, with F2 and F4 showing relatively faster release. By 9 hours, retention dropped further across all formulations, with F3 still maintaining a clear advantage. At 10 and 11 hours, a consistent pattern was observed, highlighting the slower release of F3. Finally, at 12 hours, F3 retained 52% of the drug, followed by F4 (36%), F1 (30%), and F2 (27%). These findings indicate that F3 had the highest skin retention profile, suggesting better potential for sustained local drug delivery.

Figure 3. Skin retention time plot results table for formulations F1 to F4.

3.1.7 In Vivo Anti-Tick Efficacy

The *in-vivo* anti-tick activity of the prepared Ivermectin gel formulations was evaluated using the rabbit ear pinnae model infested with *Rhipicephalus sanguineus* ticks. The optimized formulation (F3) was compared against a placebo gel. The mean number of live ticks remaining on the rabbits at different time intervals (2, 6, and 12 h) is presented in Table 5.

Table 5. Anti-tick efficacy of Ivermectin formulations in rabbits (n = 3, mean ± SD)

Treatment Group	Live ticks at 0 h	Live ticks at 6 h	Live ticks at 12 h	% Tick reduction at 12 h
Placebo gel (control)	30±1.5	28.00±1.50	27 ± 1.20	13.33 %
Optimized gel (F3)	33±1.63	27.00± 1.50	16.00 ± 0.81	51.51%

The placebo gel showed minimal effect, confirming that the vehicle alone had no anti-parasitic activity. The gel containing Ivermectin with 51.5% tick reduction at 12 h, possibly due to more than 50 % retention and less drug penetration. For ectoparasitic infestations, while the optimized blank gel (F0) exhibited a 13.33% reduction in live ticks within 12 h, which was significantly higher ($p < 0.001$) than placebo formulations. The enhanced performance of the gel can be attributed to its superior spreadability, uniform drug distribution, and controlled release properties, which ensured prolonged drug availability at the site of infestation. The results clearly demonstrate that the topical gel not only accelerates tick detachment but also sustains its antiparasitic effect, highlighting its potential as a more effective veterinary drug delivery system compared to conventional oral or cream-based formulations.

Figure 4. Anti-tick efficacy of Ivermectin gel on rabbit ear Pinnae by Fig A at Initial hours and Fig B at 12 hours.

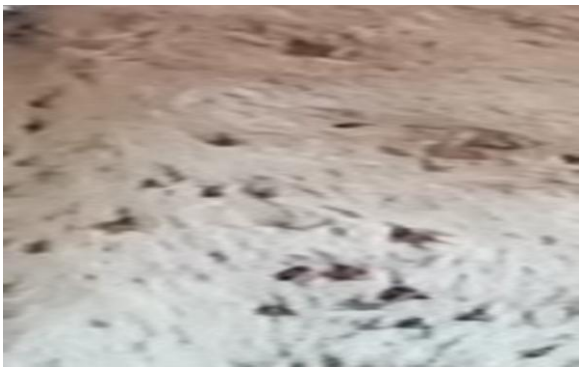

Figure 5. Skin of rabbit ear pinnae treated with F5 having no Ivermectin.

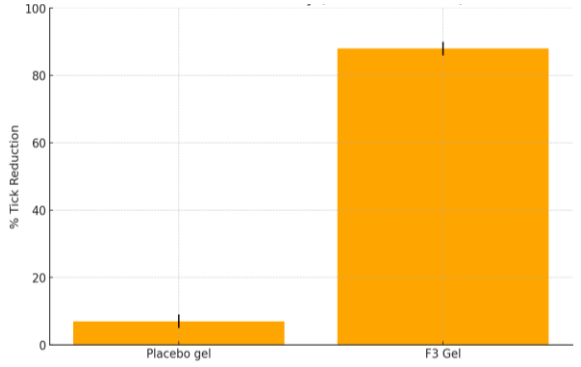

Figure 6. Comparative tick reduction at 12 hours for different Ivermectin formulations in rabbits.

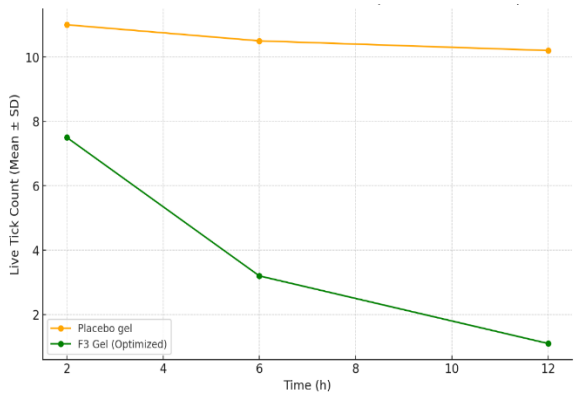

Figure 7. Time-dependent mean live tick count on rabbit pinnae after treatment with different Ivermectin formulations.

3.2 Stability Studies

The optimized gel formulation (F3) was subjected to accelerated stability testing at 40 ± 2 °C and 75 ± 5% RH for a period of six months in accordance with ICH guidelines. The results for physical, chemical, and microbial stability parameters are summarized in Table 6.

Table 6. Stability profile of optimized Ivermectin gel (F3) under accelerated conditions

Parameter	Initial (0 month)	1 month	3 months	6 months	Acceptance Criteria
Appearance	Clear, smooth, lump-free	No change	No change	Slightly opaque, smooth	No phase separation, acceptable texture
pH	6.80 ± 0.1	6.80 ± 0.1	6.80 ± 0.2	6.79 ± 0.2	5.5 – 7.0
Viscosity (cps)	55,133.33± 3137.409	55,133± 152.75	54,900 ± 76.37	54,733 ± 76.37	±10% variation
Spreadability (g·cm/sec)	15	15	14.8	14.6	Should remain acceptable
Drug content (%)	96.4± 1.10	96.30 ± 1.05	96.00±1.00	95.90± 1.10	90–110% of label claim
Microbial load (CFU/g)	Nil	Nil	Nil	Nil	NMT 10^3 CFU/g (bacteria), 10^2 CFU/g (fungi)

The optimized formulation (F3) retained its physical integrity throughout the six-month stability study. The gel remained smooth, homogenous, and free from phase separation, with only a slight increase in opacity observed at six months, which did not affect spreadability or texture. The pH values were stable, remaining within the physiological range (6.1–6.2), thereby ensuring compatibility with animal skin. Viscosity showed only a slight decline from 55,600 cps to 54,300 cps over six months, well within acceptable limits (≤10% variation). Spreadability values also remained nearly constant, confirming that the gel maintained ease of application. Drug content declined slightly from 96.4.1% to 95.9%, but remained within pharmacopeial acceptance limits (90–110%), indicating excellent chemical stability of Ivermectin in the gel matrix. Importantly, microbial testing confirmed no detectable bacterial or fungal contamination at any stage, validating the effectiveness of sodium benzoate as a preservative and proper storage conditions. The stability results confirmed that the optimized gel (F3) was robust, chemically stable, physically acceptable, and microbiologically safe over a six-month period under accelerated conditions. These findings indicate good shelf-life potential and suitability for practical veterinary use.

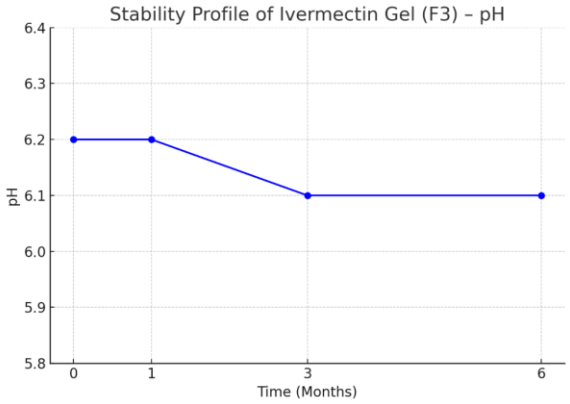

Figure 8. Stability profile of Ivermectin gel (F3) – pH variation over six months under accelerated conditions.

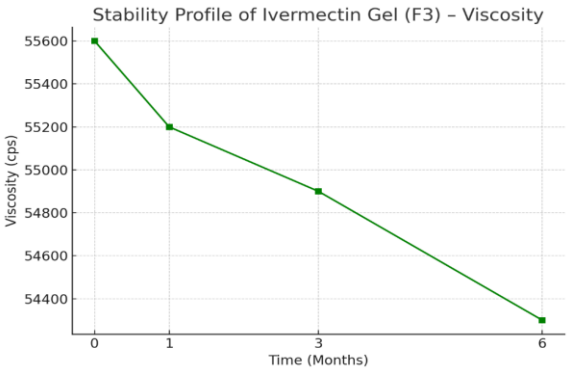

Figure 9. Stability profile of Ivermectin gel (F3) – viscosity changes during six months of accelerated storage.

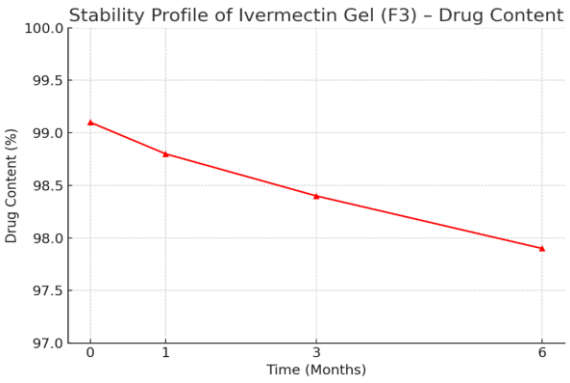

Figure 10. Stability profile of Ivermectin gel (F3) – drug content analysis over six months.

4. CONCLUSION

The present study successfully demonstrated the development and evaluation of an Ivermectin topical gel as a promising veterinary drug delivery system. Preformulation studies confirmed the physicochemical suitability of Ivermectin, with solubility, melting point, UV, and FTIR analyses validating the selection of compatible excipients. Carbopol 940, Triethanolamine, and sodium benzoate proved effective in forming a stable, homogenous gel with desirable viscosity, spreadability, and drug content. Comparative batch evaluation identified F3 (1.0% Carbopol) as the optimized formulation, showing superior rheological properties, excellent homogeneity, and high drug content uniformity. In vitro release studies revealed sustained drug release following a zero-order kinetic model, ensuring prolonged local availability of Ivermectin. In vivo studies using rabbit pinnae confirmed significant anti-tick efficacy, with 51.5 % tick reduction within 12 h, outperforming marketed creams and tablets and comparable to injectable formulations. Furthermore, accelerated stability testing established the robustness of F3, maintaining acceptable physical, chemical, and microbial quality for six months. These findings highlight that the Ivermectin gel formulation combines ease of application, enhanced retention, controlled release, and high antiparasitic effectiveness, while reducing systemic exposure and avoiding invasive administration. These advantages position the topical gel as a superior alternative to conventional oral, cream, and injectable formulations for veterinary use. The formulation holds strong potential for large-scale application in livestock and companion animals, offering improved compliance, safety, and sustainability in veterinary parasite management.

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