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FORMULATION DEVELOPMENT AND QUALITY CONTROL EVALUATION OF OINTMENT FOR THE TREATMENT OF JOINT AND MUSCLE PAIN

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ABSTRACT

Joint and muscle pain are distinct medical conditions in which, joint pain typically occurs at rest and muscle pain during the movement. Both significantly affect the quality of life. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for the pain management. But, their prolonged use is associated with adverse effects, particularly gastrointestinal issues. Furthermore, increasing dosage requirements over time highlight the need for safer, more sustainable alternatives. This study focuses on the development of a ointment designed to alleviate pain, reduce inflammation, and support recovery and healing. The formulation incorporates methyl salicylate, a potent topical analgesic known for its anti-inflammatory properties; glucosamine sulfate, which promotes joint health and aids in cartilage repair; and sodium chloride, which helps maintain electrolyte balance, tissue hydration and relaxation enhancing the healing process. A systematic approach was adopted, in the selection of appropriate excipients and the optimization of key parameters such as pH, viscosity, spreadability, and homogeneity. Stability studies were conducted under different conditions to assess the product's shelf life and integrity. Additionally, analgesic and anti-inflammatory studies were performed to evaluate the efficacy of the formulation. The results show that this ointment formulation could be a good option for managing pain and treating inflammation.

Keywords: Anti-inflammatory, joint pain, muscle pain, glucosamine sulphate, methyl salicylate, sodium chloride, pain management

INTRODUCTION

Muscle and joint pain are common health concerns often arising from daily wear and tear, aging, injuries, or illnesses. Muscle pain, or myalgia, typically results from overexertion, stress, or muscle strain and is characterized by localized soreness, aching, and throbbing sensations. This discomfort is mediated by specialized nerve endings known as nociceptors, which are activated by stimuli such as adenosine triphosphate (ATP) and low tissue pH, leading to hyperexcitability of spinal sensory neurons, a phenomenon referred to as central sensitization. Muscle pain may present with inflammation, redness, swelling, heat, and eventual loss of function **[1]**. Similarly, joint pain, often linked to injury or overuse, manifests through symptoms such as swelling, severe pain, tingling sensations, numbness, and restricted movement. Chronic joint pain is frequently associated with osteoarthritis (OA), a degenerative condition in which the protective cartilage cushioning the joints gradually deteriorates, causing pain and inflammation **[2]**. Current therapeutic options include nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs) **[3]**. However, long-term use of these agents is often limited due to adverse effects, particularly gastrointestinal complications **[4]**. Topical formulations offer a promising alternative to oral drugs by providing localized drug delivery with minimal systemic side effects, reduced systemic drug interactions, and improved patient compliance due to ease of application and reduced dosing frequency **[5]**. Glucosamine sulfate, an amino sugar and a precursor to glycosaminoglycans, plays a vital role in maintaining the structural integrity of cartilage, intervertebral discs, and synovial fluid. Its mechanisms of action include inhibiting cartilage-degrading enzymes such as elastase and hyaluronidase, stimulating the synthesis of glycosaminoglycans and proteoglycans, and exerting anti-inflammatory and immunosuppressive effects **[6]**. Studies suggest that glucosamine not only alleviates joint pain and swelling but also slows the degenerative progression of OA, potentially reducing the need for joint replacement surgery **[7]**. Commercially, glucosamine is derived from the exoskeletons of crustaceans such as shrimp, lobster, and crab, and is available as glucosamine sulfate or glucosamine hydrochloride **[8]**. Methyl salicylate, an ester of salicylic acid, is widely used in topical rubefacient formulations for its analgesic properties in musculoskeletal and soft tissue disorders. Derived from plants like *Gaultheria procumbens* (wintergreen),

methyl salicylate is a key component of wintergreen essential oil, traditionally used as an antiseptic and in liniments to relieve muscle pain, swelling and inflammation **[9]**. Sodium chloride, commonly known as table salt, is an essential compound involved in various physiological processes, including osmotic regulation, nerve impulse transmission, water balance maintenance, glucose absorption, muscle relaxation and nutrient transport **[10, 11]**. This study aims to develop and evaluate a topical formulation containing glucosamine sulfate, methyl salicylate, and sodium chloride for the effective management of muscle and joint pain, with a focus on enhancing therapeutic efficacy.

MATERIALS AND METHODS

Materials: Wool Fat, Hard Paraffin, Cetostearyl Alcohol, White soft paraffin, Sodium Chloride and Methyl salicylate were procured from Research lab fine chem industries Mumbai and Glucosamine Sulphate is procured from Ontop pharmaceuticals Pvt. Ltd. Bengaluru.

Formulation Development: The ointment was developed using Fusion method with modification **[12]**. Three formulations; F-A, F-B and F-C were prepared with variation in the composition of all the key ingredients. The variations in the formulations were made to optimize the efficacy, stability, and patient acceptability of the ointment.

Table 1: Ingredients and their Composition used in the Ointment Formulation

Sr. No.	Ingredients	F-A	F-B	F-C	Properties
1.	Wool fat	0.6g	0.5g	1.0g	Emollient
2.	Hard Paraffin	1.1g	1.0g	1.0g	Emollient
3.	Cetostearyl Alcohol	0.7g	0.5g	0.4g	Emulsifying Agent
4.	White Soft Paraffin	5.0g	6.0g	4.8g	Ointment Base
5.	Sodium Chloride	0.3g	0.1g	0.4g	To draw water out of swollen area, anti-inflammaotry.and muscle relaxant
6.	Glucosamine Sulphate	1.0g	0.8g	1.2g	Maintains the strength, elasticity, and resilience of cartilage, helps to slow down the breakdown of cartilage
7.	Methyl Salicylate	1.3ml	1.1ml	1.2ml	Relieves pain in muscle, joint and tendons. It also reduces inflammation

Preparation of Ointment: All ingredients were accurately weighed. Hard paraffin and cetostearyl alcohol were placed in an evaporating dish and melted using a water bath. Wool fat and white soft paraffin were then added to the molten mixture, which was stirred continuously until a homogeneous blend was obtained. The mixture was cooled to 50°C, after which glucosamine sulfate, methyl salicylate, and sodium chloride were added. The formulation was then thoroughly mixed to ensure uniform dispersion of all ingredients. Once the mixture was set at room temperature, the formulation was transferred to a suitable container and appropriately labelled. The formulations

were evaluated for quality control parameters in accordance with established standard analytical procedures.

Quality Control Assessment/Evaluation: The quality, efficacy, and safety of the three formulations (F-A, F-B, and F-C) were evaluated through various of quality control tests as per ICH guidelines. The following parameters were assessed **[12-16]**:

a) **Appearance:** Each formulation was tested visually to assess color, texture, phase separation and consistency **[12] (Table 2)**.

Homogeneity: Texture and Homogeneity were tested by pressing a small quantity of the formulation between the index finger and thumb. Instant feel including grittiness, stiffness, stickiness, greasiness and presence of course particles were assessed to evaluate the texture and homogeneity of the formulations **[12] (Table 2)**.

b) **pH:** Determined using a calibrated pH meter to ensure compatibility with skin pH. (pH of the skin = 4.5 - 5.7). Approximately 2.5 g of each formulation was placed in a dry beaker, and 50 ml of water was added. The beaker containing the ointments was heated in a water bath at 60–70°C. The pH of the ointments was measured using a pH meter. The measurements were performed in triplicate, and the averages of the three readings were recorded **[13] (Table 3)**.

c) **Spreadability:** Spreadability was measured by parallel-plate method to assess the ease of application and uniform distribution over the skin. Surplus amount of the ointment sample was placed between two glass slides, both sides mounted on a pulley, and a 100 g weight was applied on the top slide, for 5 minutes to achieve uniform thickness. Subsequently, a 250 g weight was added to the pan. The time (in seconds) required for the two slides to separate was recorded as a measure of spreadability **[13] (Table 3)**.

Formula to measure the spreadabillity; S = m x l/t, S is Spreadability, m – weight tied on upper slide, l – length of glass slide and t – time in seconds

d) Viscosity: Evaluated using a Brookfield viscometer to determine the rheological properties of the formulations. 50g sample was placed in a beaker and allowed to equilibrate for 5 minutes before measuring the dial reading using a T-D spindle (Number 7) at speeds of 10, 40, 50, and 100 rpm. The corresponding dial readings were recorded at each speed. The spindle speed was then gradually reduced, and the dial readings were noted again. The measurements were performed in triplicate at ambient temperature.

Viscosity in centipoises (CPS) was calculated by multiplying the dial readings by the factors provided in the Brookfield Viscometer catalogue **(Table 3)**.

e) **Stability Studies**: The developed ointment formulations underwent stability testing in accordance with the ICH (International Conference on Harmonization) guidelines. The ointment was placed in suitable containers and stored under various temperature and humidity conditions: 25°C±2°C / 60%±5% RH (Relative humidity), 30°C±2°C / 65%±5% RH, and 40°C±2°C / 75%±5% RH, for a period of 6 months. During this time, the formulations were evaluated for pH, appearance, spreadability and viscosity **(Table 4)**.

f) **Analgesic activity**: Analgesic activity of the ointment formulation was evaluated using the hot plate method. Rats were placed on a hot plate set at 55°C within a restrainer. The reaction time was recorded as the time taken for the rats to react to thermal pain by jumping or licking their paws. Measurements were taken at 0 min (before treatment),15min, 30min, 45min, and 60 minutes after treatment (application of the ointment). The maximum reaction time was capped at 45 seconds to prevent injury. More than 45 seconds was considered as maximum analgesia **(Table 5)**.

MPA (Maximum possible analgesia) was calculated using the formula:

$$\text{MPA}=\frac{\text{Reaction time for treatment}-\text{reaction time for control}}{45\text{ sec}-\text{reaction time for control}}\times 100$$

g) **Anti-inflammatory Activity**: Evaluated using the carrageenan-induced paw edema model in Wistar rats. 0.1 mL of carrageenan solution was injected subcutaneously into the left hind paw of each experimental animal. Ointment (around 100mg) was applied topically on the inflamed paw. Paw volume was measured at 0, 1, 3, and 5 hours post-application of the ointment. Ointment was applied once in 2hrs. Percentage inhibition of paw edema was calculated ((Table 6); **% Inhibition =** $\frac{\textbf{To-}Tt}{To}\times 100$,

To= Paw thickness of rats of control group

Tt= Thickness of paw of rats given ointment at corresponding time

h) **Skin irritation test**: The skin irritation test was conducted on albino rats weighing between 150 and 200 g, divided into four groups of five each. Hair was removed from dorsal area 24hrs. prior to the study. Each rats was separated and housed individually with free access to distilled water. 100mg dose of each formulation was applied to

a 1 cm² area of shaved skin. 0.5% of formalin solution was used as standard irritant. Skin irritancy and sensitization were evaluated each day until 07 days, observing for signs of inflammation, redness, edema and erythema **(Table 7)**.

RESULTS AND DISCUSSION

Three ointment formulations to treat muscle and joint pain were prepared with varying composition of ingredients. The developed ointment formulations (F-A, F-B, and F-C) were subjected to a comprehensive evaluation to assess their physicochemical properties, stability, and therapeutic efficacy. Each formulation was analyzed for appearance, texture, homogeneity, pH, spreadability, viscosity and stability, following the International Conference on Harmonization (ICH) guidelines. Additionally, biological evaluations, including analgesic activity, anti-inflammatory potential, and skin irritation tests, were performed using in vivo models to determine the safety and efficacy of the formulations. The objective of this study was to identify the formulation with optimal properties that would ensure patient acceptability, therapeutic effectiveness, and stability. Among the three formulations, F-B consistently demonstrated superior performance across various parameters, making it the most promising candidate for topical application. The detailed results of each quality control test and their implications are discussed below.

Statistical Analysis: Statistical analysis was performed to evaluate all the parameters (stability studies and physical parameters). The data were analyzed using one-way ANOVA followed by Tukey's post hoc test to determine significant differences between formulations (F-A, F-B, and F-C) and within each formulation over time. The results are expressed as mean ± standard deviation (SD) for three independent measurements. p-value < 0.05 is considered as statistically significant.

Table 2: Evaluation (Appearance) ointment formulation

Formulation	Colour and Appearance	Texture	Homogeneity	Skin feel test
F-A	Half white and opaque	Hard	Homogeneous No phase separation	No grittiness, stickiness and greasiness
F-B*	Half white and opaque	Smooth	Homogeneous and No phase separation	No grittiness, stickiness and greasiness
F-C	Half white and opaque	Smooth and Soft	Homogeneous and No phase separation	No grittiness little greasiness

Report on evaluation of physical properties **(Table 2)** shows that all formulations were half white and opaque, indicating no significant difference in visual appearance. This uniformity in color is essential for consumer acceptance, as it reflects the

proper mixing of ingredients without degradation or instability. F-A displayed a hard texture, which may affect ease of application and user comfort. F-C was smooth but soft, indicating a lower viscosity that might lead to reduced stability and difficulty in adherence to the skin. F-B* demonstrated a smooth texture, providing an ideal balance between firmness and softness. The smooth texture of F-B ensures easy spreadability without being too hard (as in F-A) or too soft (as in F-C). This makes F-B more comfortable to apply and enhances user satisfaction. All formulations were found to be homogeneous with no phase separation, indicating that the components were well-dispersed and stable across the formulations. F-A and F-B exhibited no grittiness, stickiness, or greasiness, ensuring a pleasant feel upon application. F-C, while free of grittiness, showed slight greasiness, which may affect user comfort and adherence.

The skin feel is a critical factor in patient compliance and product preference. F-B offered the most favorable skin feel, with no grittiness, stickiness, or greasiness, making it the most user-friendly formulation.

Based on the evaluation of physical properties, Formulation F-B is identified as the best formulation due to Smooth texture that ensures easy and uniform application. Homogeneity indicating a stable and well-dispersed formulation and superior skin feel, enhancing user comfort without greasiness, stickiness, or grittiness.

Table 3: Evaluation of Physical properties of ointment formulations

Formulation	F-A	F-B*	F-C
pH	5.02±0.12	5.52±1.02*	5.84±2.02
Spreadability (g.cm/s)	103.02±1.34	104.88±2.02*	105.24±1.06
Viscosity (CPS)	34000±1.34	30000±1.34*	29870±1.34

*Statistically significant ((p < 0.05), n=3

The pH, spreadability and viscosity values of the formulations were compared using one-way ANOVA, revealing a significant difference ($p < 0.05$) between F-B and F-A, as well as F-B and F-C. The pH of F-B (5.52 ± 1.02) falls within the optimal range for skin application, maintaining skin's natural acidic environment and reducing irritation potential. The slightly acidic pH of F-B is closest to the physiological skin pH (4.5-6.0), which is essential for maintaining the skin barrier function and preventing microbial growth. In contrast, F-C exhibited a higher pH, which might compromise skin compatibility. F-A, though within range, is slightly lower than F-B, making F-B more suitable for sensitive skin applications. Spreadability is a critical parameter for ointment application, as it affects ease of use, uniformity of application, and patient compliance. F-B demonstrated a superior spreadability (104.88 ± 2.02 g.cm/s)

compared to F-A, indicating it is easier to apply while maintaining a good balance of viscosity. Though F-C showed slightly higher spreadability, it may lead to reduced adhesion to the skin surface, reducing its effectiveness.

Viscosity affects the ointment's consistency, stability, and ability to remain on the skin for prolonged periods. F-B displayed an optimal viscosity (30,000 ± 1.34 CPS), ensuring ease of application without compromising on the ointment's ability to form a protective barrier on the skin. F-A, with a higher viscosity, may feel thicker and harder to spread, while F-C, with a slightly lower viscosity, might compromise its stability.

Based on the evaluation of critical parameters — pH, spreadability, and viscosity Formulation F-B is identified as the best formulation

Table 4: Stability study data of ointment formulations F-A, F-B, and F-C

Parameters	F-A	F-B*	F-C
Initial			
pH	**5.02±0.12**	**5.52±1.02**	**5.84±2.02**
Viscosity (CPS)	**103.02±1.34**	**104.88±2.02**	**105.24±1.06**
Spreadability (g.cm/s)	**34000±1.34**	**30000±1.34**	**29870±1.34**
After one month			
pH	**5.00 ± 0.15**	**5.48 ± 1.00**	**5.75 ± 2.00**
Viscosity (CPS)	**33,800 ± 1.30**	**29,800 ± 1.20**	**29,600 ± 1.30**
Spreadability (g.cm/s)	**102.50 ± 1.40**	**104.60 ± 1.90**	**105.00 ± 1.20**
After 3 months			
pH	**5.00 ± 0.15**	**5.48 ± 1.00**	**5.75 ± 2.00**
Viscosity (CPS)	**33,500 ± 1.25**	**29,500 ± 1.15**	**29,300 ± 1.25**
Spreadability (g.cm/s)	**102.00 ± 1.50**	**104.40 ± 1.80**	**104.80 ± 1.30**
After 6 months			
pH	**4.98 ± 0.20**	**5.45 ± 0.95**	**5.70 ± 1.98**
Viscosity (CPS)	**33,200 ± 1.30**	**29,200 ± 1.10**	**29,000 ± 1.20**
Spreadability (g.cm/s)	**101.80 ± 1.60**	**104.20 ± 1.70**	**104.60 ± 1.40**

***Statistically significant (($p < 0.05$), n=3**

From the results **(Table 3)**, it is found that formulation, F-B maintained a stable pH, consistently high spreadability and retained better viscosity compared to F-A and F-C over six months. This indicates that F-B has good compatibility with skin enhancing ease of application, retaining optimal consistency and texture over time compared to F-A and F-B.

Based on the above results Formulation F-B was selected for the evaluation of anti-inflammatory, analgesic and skin sensitivity tests. The results are tabulated below

Analgesic activity [14]: The analgesic activity of the F-B formulation was evaluated and compared with the control and standard Moov (Diclofenac ointment) using the latency period in a heat-induced pain model. The results are summarized as follows:

Table 5: Evaluation of analgesic activity

Treatment	Reaction time (Latency period) in seconds (Mean ± SEM)				
	0 min	15 min	30 min	45 min	60 min
Control (Ointment base-50mg)	5.22 ± 0.57	5.24 ±0.22	5.42 ± 0.42	5.40 ± 0.42	5.42 ± 0.82
F-B (50mg)	5.20 ± 0.24	8.29 ± 0.22*	9.75 ± 0.32*	11.75 ± 0.03*	12.79 ± 1.22*
Standard (50mg-Moov)	5.50 ± 0.32	10.64 ± 0.26*	10.98 ± 0.22*	12.82 ± 1.04*	14.01 ± 0.22*

Values are expressed as Mean ± S.E.M (n=5); analysis was performed using One-Way ANOVA followed by Tukey post hoc test; against the control, *P<0.05. is considered as significant

The F-B treated group and standard exhibited a significant increase in the latency period (9.556 and 10.67 sec respectively) compared to control (5.34 sec), suggests that F-B possesses significant analgesic properties p<0.05) comparable to that of the standard drug. The maximum possible analgesia (MPA) achieved by F-B was 135.4% at 60 minutes, indicating potent and prolonged analgesic activity.

Anti-Inflammatory Activity

Table 6: Anti-Inflammatory Activity of Formulation F-B in Carrageenan-Induced Paw Edema Model

Treatment	Paw Volume in mL(Mean ± SEM)			
	0 h	30 min	60 min	120 min
Control (Ointment base-50mg)	1.48 ± 0.12	1.98 ± 0.002	2.12 ± 0.002	2.84 ± 0.001
F-B (50mg)	1.47± 0.02	1.02 ± 0.02*	0.86± 0.006*	0.75 ± 0.002*
Standard (50mg-Moov)	1.47 ± 0.00	1.00 ± 0.01*	0.82 ± 0.004*	0.72 ± 0.004*

Values are expressed as Mean ± S.E.M (n=5); analysis was performed using One-Way ANOVA followed by Tukey post hoc test; against the control, *P<0.05. is considered as significant

The anti-inflammatory potential of F-B was evaluated using the carrageenan-induced paw edema model. F-B significantly reduced paw edema at all observed time points compared to the control group ($p < 0.05$). The percentage inhibition of edema reached 49.32% at 2 hours post-application, indicating a strong anti-inflammatory effect that persisted over time compared to the standard that exhibited .51.31% inhibition.

Skin sensitivity test

Table 7: Evaluation of skin sensitivity of ointment formulation F-B

Day	Signs of Irritation (Erythema, Edema)	Severity Score (0-5)
1	No erythema or edema	0
2	No erythema or edema	0
3	No erythema or edema	0
4	No erythema or edema	0
5	No erythema or edema	0
6	No erythema or edema	0
7	No erythema or edema	0

Data are expressed as the average score of 5 animals per group. Severity score: 0 = No irritation, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Very Severe, 5 = Intolerable

Formulation F-B showed no signs of erythema, edema, or any other adverse skin reactions during the 7-day observation period. The severity score remained at '0' throughout the study, indicating that F-B is non-irritating and safe for topical application. This highlights the formulation's suitability for long-term use without causing skin irritation or sensitization.

CONCLUSION

In the present study, three ointment formulations with varying composition of key ingredients (Glucosamine sulphate, Methyl salicylate and Sodium chloride) and excipients were developed aiming at the treatment of joint and muscle pain. Among three, F-B demonstrated superior performance in terms of texture, homogeneity, spreadability, viscosity, pH compatibility, and skin feel. It exhibited optimal stability, better analgesic and anti-inflammatory efficacy, and no skin irritation, making it the most promising and effective formulation for the topical management of joint and muscle pain.

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