Synthesis of a Novel Formulation for Enhancing the Drug Absorption of a Commercially Available Topical Analgesic Cream.

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H-12 ISLAMABAD, PAKISTAN

December, 2021

Synthesis of a novel formulation for enhancing the drug absorption of a commercially available topical analgesic cream.

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A thesis submitted in partial fulfillment of the requirements for the degree of MS Biomedical Sciences and Engineering

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Acknowledgements

First and foremost, all praise to Almighty Allah the creator of the universe. Who gave me the strength and courage when it was needed the most. all regards to the Prophet Muhammad (P.B.U.H) who paved us to the right path.

The names that are part of my acknowledgments duly deserve to be acknowledged on account of the support and help they provided.

My utmost gratitude is for my supervisor Dr. Murtaza Najabat Ali, who mentored my project. I am truly in his debt for his invaluable advice and insights that he shared with me and the opportunities that he gave me to learn. Thank you, Sir: for catapulting me to higher grounds and for the little words of encouragement. I have hugely benefitted from your skills as my mentor I am obliged to Dr. Syed Omer Gilani (SMME), Dr. Emad Uddin (SMME), Dr. Muhammad Bilal Khan Niazi (SCME) for their valuable advices and relentless assistance. Thank you for trusting me and supporting me in my project, making everything easy for me.

A bundle of thanks to Zunaira Qureshi for lending me a helping hand every time I asked for. Thank you for tolerating me. Thank you for everything you've done for me and especially for the humor. Last but not least, I am grateful to my mother and father for her invaluable support and prayers.

“Dedicated to my parents”

Abstract

Background: Topical analgesic drugs are used for numerous painful situations which may include acute conditions such as strain, muscle sprains and chronic conditions which are typically known as osteoarthritis of hand or knee, or neuropathic pain. The commercially available treatment options offer slow Drug release profile leading to delayed effective dose build up and delayed onset of pain relief**.** Therefore, the aim of the current study was to develop and evaluate a novel formulation of a commercially available analgesic cream for enhanced drug absorption and rapid onset of pain relief.

Methodology: The study was designed to develop a topical cream formulation containing carbopol-934 gelling agent, drug release enhancers and API (Methyl salicylate and menthol).

Result: The results of the optimized formulation exhibited rapid drug release profile and good physiochemical properties like PH, homogeneity and viscosity. There was no significant difference in the fresh formulation and that after 12 days of freeze thaw cycle in terms of pH, spreadability and drug release profile. There was also no statistically significant difference in the drug release profile and physiochemical properties of the analgesic cream after it was subjected to accelerated shelf-life test. Furthermore, the in vivo study confirmed that the application of novel formulation resulted in rapid and effective analgesic effect than that of the commercially available analgesic agent and no erythema and edema appeared after the novel formulation was applied.

Conclusion: Therefore, it was concluded that the formulation could be promising alternative for the topical analgesic treatment. However, further preclinical, clinical and long-term stability studies are required.

Key words: Topical drug delivery, Carbopol gel, Novel formulation, *In vitro* permeability study, Mice Analgesic activity, Dermal irritant, Stratum corneum, Methyl salicylate

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List of Abbreviations

PBSPhosphate buffer solution

SCStratum corneum

API Active pharmaceutical ingredients

WHO World health of organization

ICH International council of harmonization

CAGR Compound annual growth rate

(**TTX-S)**Tetrodotoxin-Sensitive

NSAIDs Non-steroid anti-inflammatory response

PAF Platelet activating factor

GIT Gastrointestinal tract

FTIR Fourier transforms infrared

RSD Relative standard deviation

CDR Cumulative drug release

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Introduction

The skin is divided into three layers: (a) dermis (SC), (b) epidermis and (c) stratum corneum (SC), which is covered by a complex network of capillaries.In addition, hair follicles, sweat glands and appendages are spread across the skin in varied numbers and sizes depending on the body sites. The SC is divided into two-compartment first one is keratinized cells which are implant in a multilamellar,lipidmatrix primarily consisting of ceramides and neutral lipids. It's roughly 10 mm thick (5–7) and made up of about 20 cell layers.(Herkenne et al., 2008) topical medication is applied externally and is absorbed through the skin. The skin contains several layers includingepidermis,stratum corneum, and dermis it contains appendages that includes sweat glands, sebbaceous glands and hair follicles.

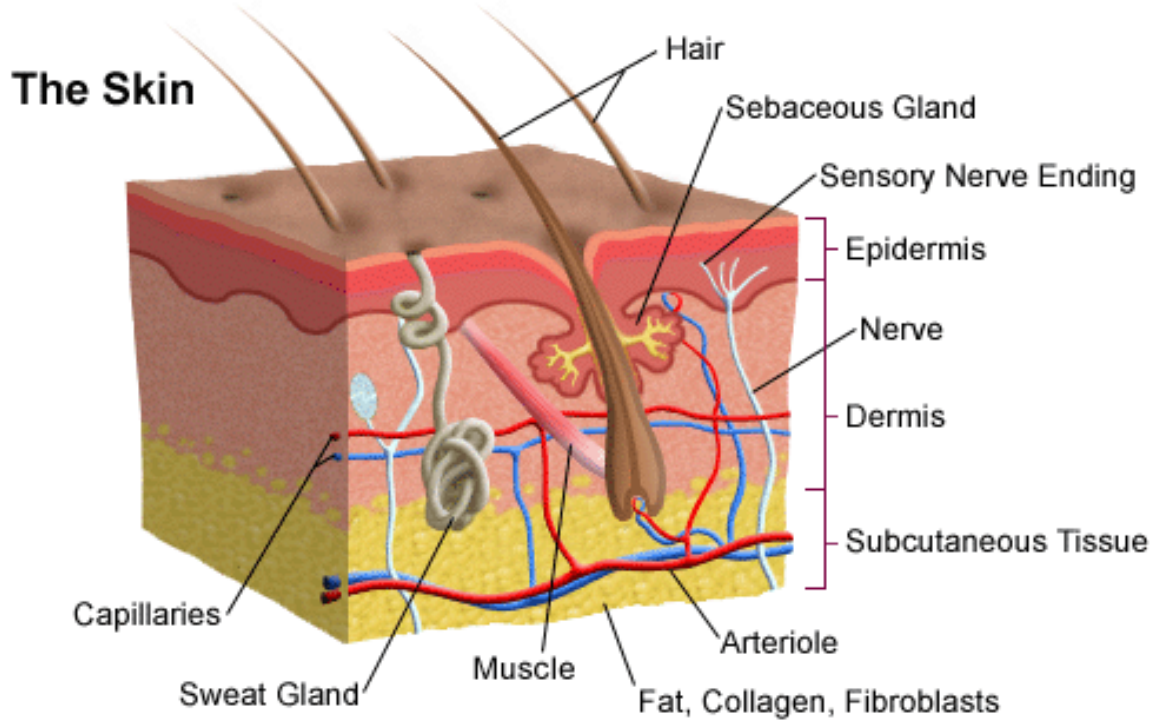


Figure 1: Route of absorption

Moving on there are different types of pain relievers which are NSAIDs, opoids and local anesthetics. Our main focus is on NSAIDS. NSAIDs block the manufacturing of prostaglandins that cause inflammation .Basically; NSAIDS block the effects of enzymes cox-2 and cox-1 which inhibit the making of prostaglandins. Currently, there are two creams that are mostly being use first one is voltral and second one is wintogeno and the active pharmaceutical ingredient that are being used in the formulation are NSAIDs and other are the excipient high is used as a penetration enhancers and permeation of drug. However their limitations are less NNT (number needed to be treated) 1.5 (voltral), slow drug release profile leading to delayed effective dose built up,delayed onset of pain relief. For medical purposes oil-in-water and water-in-oil emulsion are widely used and as carriers for delivering medications to the skin. Emulsions have a certain beauty to them and may be readily rinsed off as needed. They're also quite good at penetrating the skin.(Magdy I Mohamed, 2004)Furthermore, the formulation has maintained the greasiness, viscosity and appearanceof emulsions. Water-in-oil emulsions are more extensively used for the treatment of dry skin and emollient applications, whereas oil-in-water emulsions are more beneficial as water washable medication bases and for general cosmetic uses. (Bhanu et al., 2011)Thixotropic, greaseless, readily spreadable, easily removable, emollient, nonstaining, compatible with a variety of excipients, and water-soluble or miscible are only a few of the benefits of dermatological gels.. (Bhanu et al., 2011)Emulgels are emulsions that are gelled by combining with a gelling agent. They can be oil-in-water or water-in-oil emulsions. (Kostenbauder & Martin, 1954)(Sintov & Botner, 2006)(Patel & Patel, 2009)Therefore,They have a high level of patient acceptance since they combine the benefits of both emulsions and gels.(Arora & Mukherjee, 2002; Bhaskar, Anbu, Ravichandiran, Venkateswarlu, & Rao, 2009; Naito & Tominaga, 1985; Parsaee, Sarbolouki, & Parnianpour, 2002; Wang & Fang, 2008). An

emulgel is a combination of emusion and a gel. It is a formulation that combines the advantages of both gel and emulsion technologies. It is possible to incorporate both hydrophilic and hydrophobic drugs, which allows for controlled drug release. It is also possible to improve stability, lower the cost of production, and increase aesthetic appeal because they are thixotropic, emollient, spreadable with ease, non-staining, bio-friendly and non-greasy among other characteristics. (Amit Verma, Jain, Tiwari, & Jain, 2018)(Nikumbh, Sevankar, & Patil, 2015).NSAIDs may be used with rubefacients to boost their efficacy. Rubefacients are topical medicines that promote reddening of the skin via dilatation of the capillaries and increased blood flow. Rubefacients are often used to pain caused by arthritis, stiffness, and sprain back discomfort and muscle strains, bruising. Common rubefacients include capsaicin, menthol, camphor, meloxicam, and isopropanol.(Jorge, Feres, & Teles, 2011), (Moss et al., 2014).

Most nonsteroidal anti-inflammatory drugs (NSAIDs) used in topical formulations show lesser effective and have a lesser time pain effect when compared to methyl salicylate, and they have a greater number of side effects. Furthermore, because most of these medicines include single active pharmaceutical ingredient (API), which are available from other sources. As a result, there is a pressing need to develop alternative goods that may address these inadequacies. The increased therapeutic usefulness of pharmaceuticals, in turn, will aid to enhance patients' compliance and adherence to medications, resulting in an overall improvement in the patient's overall quality of life. Because of their reported analgesic properties, menthol and thymol are frequently used in topical analgesic formulations (Beer, Lukanov, & Sagorchev, 2007)(Haeseler et al., 2002). Researchers from all over the world are attempting to improve topically active medicine absorption past the skin's major barrier. In light of these facts, we've decided to conduct

scientific research on some unique laboratory-prepared topical analgesic drugs that can be administered for lengthy periods of time without creating significant systemic accumulation. According to a review of the literature, there is no global market for a combined topical product of methyl salicylate and Arnica in the form of a gel or an emulgel. A FDC containing methyl salicylate and Arnica is also unknown; therefore it represents a unique opportunity to develop a new topical therapy option for rheumatism. “The goal of this study was to formulate methyl salicylate and Arnica emulgels separately and as an FDC with capsaicin, and to evaluate them pharmaceutically for quality and compliance with compendial requirements, as well as pharmacologically for efficacy, as well as to formulate the emulgel and optimise the formulation through in-vitro and ex-vivo diffusion studies. The study's secondary goal is to assess the anti-inflammatory effects of the herbo-synthetic semisolids dosage form when applied topically. Methyl salicylate and camphor were chosen as model drugs from a synthetic source, and arnica Montana was chosen as a model medication from a herbal source for this project. The study's findings may bring various benefits in current pain treatment, such as improved patient acceptance with fewer synthetic pharmaceutical compounds and improved safety features of herb-synthetic dose.”

Topical medication delivery is a popular strategy for local and systemic therapies, and it's frequently utilised to treat inflammatory illnesses like dermatological diseases and musculoskeletal injuries. (Kaur, 2013)(Mitkari, Korde, Mahadik, & Kokare, 2010) Topical administration has a number of advantages over traditional dosing forms, particularly in terms of avoiding some major systemic side effects. (Whitehouse, 2011) When a medicine is applied topically, it is able to enter deeper into the skin, resulting in improved absorption. (Glavas-Dodov et al., 2003)(Rupal, Kaushal, Mallikarjuna, & Dipti, 2010) Topical formulation avoids

drug processing in the gastrointestinal issues, liver and inconveniences of intravenous therapy, as well as various risk are associated with the absorption conditions such as enzyme presence, gastric emptying time and pH. Furthermore, the drug's bioavailability is improved, and its action happens immediately at the site of action. (Bhasha, Khalid, Duraivel, Bhowmik, & Kumar, 2013)(Sharma, Pawar, & Jain, 2012) In the delivery method for topical medications, a wide range of pharmacological dose forms can be used. Gels, creams, and ointments are the most popular, followed by sprays and liquid solutions. (Gisby & Bryant, 2000)(ASHNI Verma, Singh, Kaur, & Jain, 2013) By expanding the resident time at the injection site, drug's resistance time on the skin can improve by topical delivery with gels as well as the distribution and release of the material. (Karadzovska, Brooks, Monteiro-Riviere, & Riviere, 2013) . Furthermore, for a range of clinical circumstances, transdermal delivery of various medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs), utilising gel has been shown to be helpful. (Cevc & Blume, 2001) but we need proper instructions and time. So, topical medication is more helpful, it is easy to use and give instant relieve of pain.

Chapter 2

Literature review

1.1 Pain

Pain is associated with unpleasant emotional and sensoryexperience.

1.1.1 Statistics

On the subject of today's note. Chronic pain (CP) affects one in every five individuals worldwide, or around 1.5 billion people, and the prevalence of CP rises with age. Pain is recognised as a worldwide health issue, and pain management is included in the Universal Declaration of Human Rights' promise of the best achievable level of health. According to a report from CDCP on the health status of Americans, about 20% of adults — 50.2 million people — suffer from chronic pain on a daily or weekly basis. The global topical pain relief market was worth USD 7,481 million in 2017, and is expected to grow at a CAGR of 7.4% from 2018 to 2025, reaching USD 13,276 million.

- Increase in prevalence of arthritis is the major factor.
- Lesser side effects caused due to topical analgesic.
- High demand for topical pain relief by sports player.

1.1.2 Types of Pain

Pain is typically divided into three types: inflammatory pain, neuropathic, andnociceptive, which is distinguished by three characteristics: symptoms, causes, and syndromes.

The response to potentially damaging stimuli or actual by our bodies' sensory nerve systemsis known as nociception. Nociceptors are present at sensory nerve endings that are triggered by

such stimuli and are activated at the earliest stage of pain sensations. Primary afferent nociceptors (C- and A-fibers) these are the noiceptors that are activated to unpleasant stimuli occur in our body.Ma, #141, 2007.

Nociceptive pain is of two type phantom pain and visceral pain which is classified as superficial pain and deep somatic pain. Generally speaking, C fibers are present in the deep somatic tissues for example muscles and joints. A-fibers are stimulated by heat or mechanical stimulation and producing a sensation, prickling and short-lived discomfort. In response to heat or mechanical stimulation, A-fibers are activated, producing a prickling, short-lived discomfort sensation. In contrast, chemical, thermal and mechanical stimuli activate C-fiber, resulting in poor localization of pain and a minor pain perception. Primary afferent neurons receptors have three major functions they can stimulate the inhibitory response, excitatory and sensitizing response when the receptors are triggered and the pain threshold reached to the brain so, the impulses reach to the PNS and medulla (cranial) through the afferent. These nociceptors which are the type of quiet nociceptors those are present in the brain. Silent nociceptors are present in the viscera, and they are responsible for pain perception and these afferent nerve fibers lack any anatomical features that would allow them to respond to noxious stimuli. Instead, these receptors are only stimulated during the inflammation processes when the chemical mediators are produced.

Neuropathic Pain

Allodynia is typically associated with neuropathic pain, which is characterized as a nerve injury or dysfunction. Repetitive non-painful receptor stimulation causes allodynia, a kind of central pain sensitization.Due to the sensitization process that occurs as a result of such repetitive stimulation, a pain response is elicited by a stimulus that would normally be considered non-painful. Because in our bodies' defence mechanisms neuropathic pain does not play a role, it is

referred to as "pathologic" pain. It might manifest as a persistent sensation or as episodic occurrences.The most prevalent causes of this sort of pain include inflammation or metabolic conditions including tumors, diabetes,herpes zoster infection, toxin, trauma and neurological diseases. Central nervous system plays an important part in this process which provides sensitizations. Neuropathic pain is produced by somatosensory nervous system nerve injury, although it can also be caused by CNS and PNS problems.

The neurochemistry of injured axons can be altered as a result of complicated reactions triggered by peripheral nerve compression, stretching, or transaction, followed by spontaneous hyperexcitability at the location. In the modulation of neuronal excitabilityNav channels play a major role, and it is the initial step in action potential. Nav channels are activated by Nociceptors during neuropathic pain. High activation of threshold show Slow tetrodotoxin-sensitive (TTX-S), fast tetrodotoxin-resistant (TTX-R) and persistent TTX-R with lower activation thresholds are the three forms of dorsal root ganglion in Na+ channel.(DRG). #142, Narahashi, 1964 TTX is a neurotoxin that operates as a Nav channel blocker, inhibiting the firing of action potentials generated in neurons by binding to the Nav channels. #142, Narahashi, 1964(Narahashi, Moore, & Scott, 1964)

Inflammatory Pain

Inflammation is produced by our body in response to damaging stimuli by removing necrotic cells tissue repair process begins. The first responders to an inflammatory reaction are neutrophils, which travel via the bloodstream to the site of injury. Allodynia, Hyperalgesia, and sympathetic are the three responses of inflammation. Mast cell degranulation can also be induced by inflammation and the platelet activating factor (PAF)is released and the stimulation of 5-HT release from platelets that circulates. The activation and sensitization of primary afferent neurons

causes' redness permanent loss of function are the cardinal indications of inflammation. Free arachidonic acid (AA)that is release from the phospholipids is then induced by the localised inflammatory response, which is then transformed into prostaglandins (PG) via the cyclooxygenase (COX) pathways.

Inflammationpain is divided into acute and chronicpain. Acute inflammatory pain is lasting less than three and it starts as a reaction to damaging stimuli that are ordinarily mediated by the A-fibers.Plasma concentration and leukocytes at the site of the injury contributes to the inflammatory response. The condition known as chronic inflammatory pain, arises when inflammation persists for a longer period of time than expected and is mediated by C-fibers.. (Basbaum, Bautista, Scherrer, & Julius, 2009).A gradual shift of mononuclear cells can also be seen at the site of inflammation. Inflammatory pain induces a rise in afferent input to the DH of the spinal cord, resulting in central sensitization. Histamine, 5-HT, leukotrienes, glutmate, nerve growth factors (NGF) kinins, adenosine triphosphate (ATP), PG, nitric oxide (NO), NE, and protons are some of the mediators released at the site of injury. These chemical inflammatory mediators are created by necrotic tissues during the inflammatory process and interact with nociceptors in the inflamed area to activate them.

Because tissue injury induces the release of chemical mediators that cause pain (prostaglandins, bradykinins), it is associated to acute pain.(Ezeja, Omeh, Ezeigbo, & Ekechukwu, 2011).An analgesic, or painkiller, is a drug that blocks pain receptors without inducing drowsiness. (Ezeja et al., 2011) Pain reliever can be applied topically and taken orally and present in the form of gels, patches, tablets, sprays, ointments and other forms. Oral pain medications are routinely used to treat acute and chronic pain, while they can have systemic side effects. Because topical analgesics have fewer side effects and provide the same analgesic relief as oral analgesics, they

have more promise.(Argoff, 2013)it has the ability to produce an effective pain reduction less systemic absorption and give the targeted drug delivery , ease of dose termination, avoidance of first pass medication, lesser side effects painless which increases the patient adherence and acceptance, are all advantages of topical or local application of analgesics or anaesthetics to a target.(Tadicherla & Berman, 2006), (Jafri et al., 2019)

1.1.3 Pain Mechanism

Pain is pretty important for us and that is a sort of defense mechanism. Pain mechanism is activated by noxious stimuli. When the noxious stimuli occur the nociceptors are activated. These nociceptors bring this information to the spinal cord which undergoes three steps transduction, modulation and transmission. Transduction occurs in the following order along the nociceptive pathway: the stimuli are converted into chemical signal and then the chemical signal is converted into electrical events in neurons and it transferred into synapses in the chemical forms, the next mechanism would be transmission. This information is transferred into the spinal cord through the dorsal horn which is the back of the spinal cord. The nociceptors then release chemicals here, and then neurotransmitters transfer the information from post-synaptic terminal to pre-synaptic terminal.The basic illustration on pain transmission is illustrated in Figure

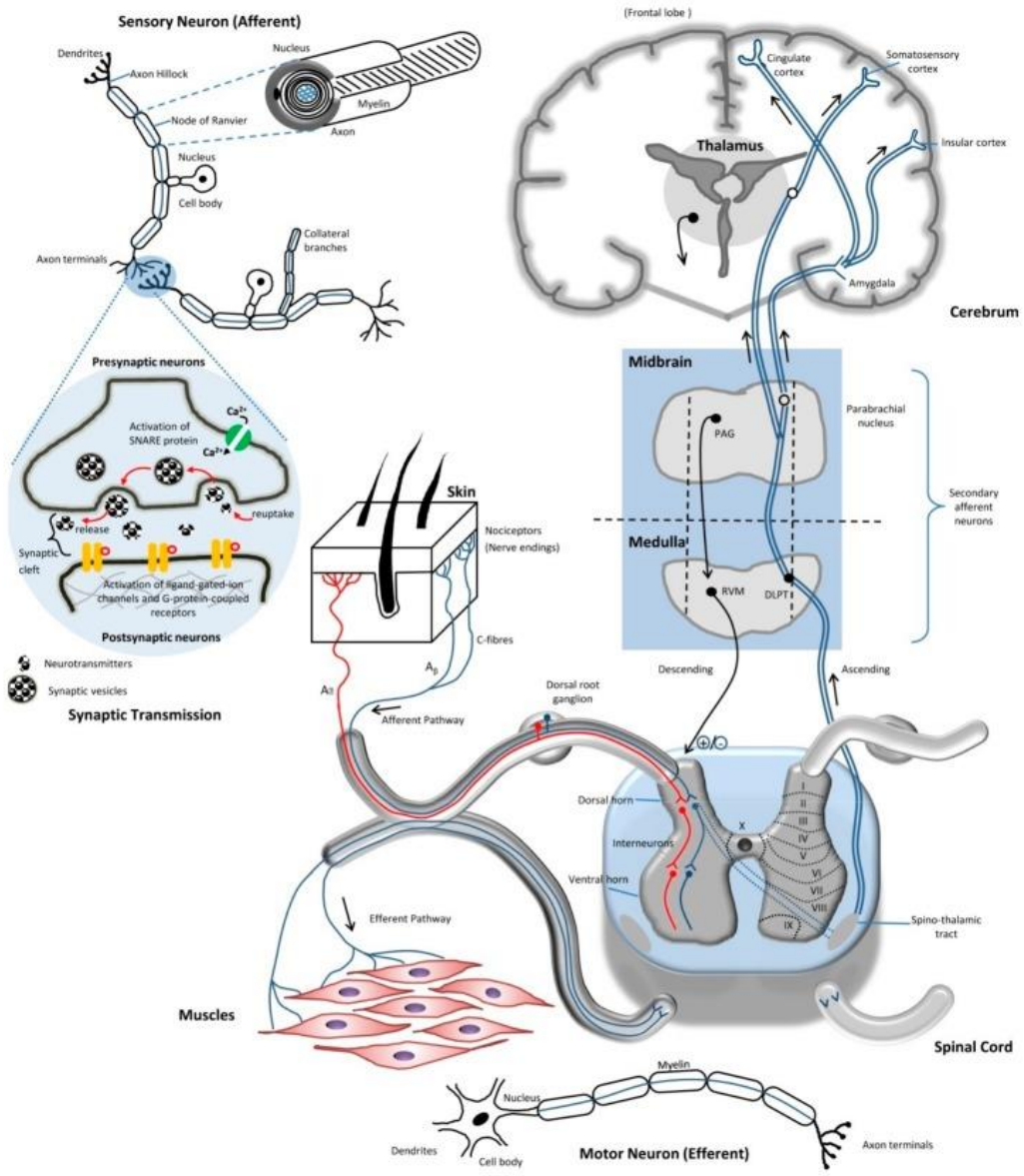


Figure 2 Pathway of Pain Mechanism

1.1.4 Degree of Pain

Pain are differentiated on the basis of the duration of damage it has caused to the skin (Yam et al., 2018) Pain categorization is summarized in Table below.

Table 1: Types of Pain

Characteristics	Acute Pain	Chronic Pain
Timing	Short duration period	Long duration period
Sign	Sign of tissue damage	No, sign of tissue damage
CNS involvement	CNS intact	CNS dysfunction
Severity	Severity involve with damage	Severity not involved with damage

1.2 Treatment of Pain

A range of painful disorders are treated with topical analgesics. Acute injuries include strains and muscle pains and sprains. Chronic pain is knee pain, lower back pain, osteoarthritisor neuropathic pain. Topical analgesic medications are those that are applied externally to the body skin or mucous membranes to relieve pain; they are either rubbed on the skin or fashioned into patches or plasters that are put on the skin. Painful cutaneous ulcers, wounds of various kinds, including surface wounds or wounds inside the body due to surgery or pain due to infiltration by needles; and painful eye conditions, especially perioperatively, such as after cataract surgery, are all examples of painful conditions that could be treated by direct application of drugs. Each of these, as well as other scenarios, might be defined as a medication application on the skin.

1.2.1 Background

Pain is something that almost everyone goes through at some point in their lives. Acute discomfort lasts for a brief time, usually less than three months, and progressively fades as the wounded tissues heal. Pain that lasts three to six months or longer is considered chronic. Acute pain conditions such as tension headaches, migraines, and acute low back pain are among the top

10 most common conditions worldwide. Painful conditions, which include neck pain, osteoarthritis, low back pain and other musculoskeletal diseases.(Lozano et al., 2012)

Pain causes significant employment and loss of quality of life, as well as increased health-care expenses. People in pain want it to go away, and knowing this has led to the conclusion that what we consider to be meaningful outcomes in pain trials, namely a big reduction in pain or being in a low pain state, should be driven by this. (Moore, Derry, Eccleston, & Kalso, 2013).

Acute pain (tendonitis, sprains, strains) and chronic pain(neuropathic pain, low back pain osteoarthritis) are treated with topical analgesics. Guidelines prescribe topical analgesics for the pain of osteoarthritis and neuropathic pain.(Khaliq, Alam, & Puri, 2007; Nüesch et al., 2010)

How the intervention might work

Externally applied topical drugs are absorbed through the skin. They have actions that are localized to the application site, with systemic uptake or dissemination. This contrasts with transdermal application, in which the medication is applied externally and absorbed through the skin, but its impact is dependent on systemic distribution.

A topical product must first enter through the skin to be effective. Individual medications have varying levels of penetration, and some formulations include ingredients that aid skin penetration and result in increased drug concentrations in tissues. To maximise penetration, a balance of lipid and water solubility is required, and the use of prodrug esters has been recommended as a method of increasing permeability. Skin penetration is also dependent on the formulation. Creams are often less successful than gels or sprays, according to experiments using artificial membranes or human epidermis, however emerging formulations such as microemulsions may have higher promise.(Derry et al., 2017)

1.2.2 Pain Relievers

Topical NSAIDs

NSAIDs reversibly block the enzyme cyclooxygenase COX, which mediates the formation of prostaglandins and thromboxane A2 and is now known to have two isoforms, COX1 and COX2. (FitzGerald & Patrono, 2001)The COX2 format is inhibited, which lowers inflammation and generates analgesic effects. Prostaglandins play a key role in physiological processes, including regulating renal blood flow and endothelial tone, maintaining the gastric mucosal barrier. Inflammatory and nociceptive (pain) processes are also influenced by them. NSAIDs have the ability to inhibit COX enzymes locally and peripherally with minimal systemic absorption is the basis for topical administration.As a result, their application is restricted to conditions in which the pain is superficial and localised, such as joint and skeletal muscle discomfort.

Pain relief can be achieved when the medicine can be present at high concentration at the site of action inhibit COX enzymes. Topical NSAIDs are thought to work by reducing symptoms caused by periarticular and intracapsular structures on a local level. NSAIDs administered topically to the skin to inhibit COX2. On the other hand, are a fraction of those seen in plasma after oral administration (typically less than 5%). Topical treatment has the potential to reduce systemic adverse effects by lowering medication concentrations in the body. We know that persistent use of topical NSAIDs reduces upper gastrointestinal bleeding, but we don't know much about the impact on heart and renal failure that are linked to oral NSAID use. For hand or knee osteoarthritis, current UK guidelines recommend topical NSAIDs above oral NSAIDs, COX2 inhibitors, or opioids.

Topical Rubefacients

Rubefacients (mostly including salicylates) produce skin irritation and reduce tendons, joints and pain in muscles, as well as musculoskeletal symptoms , by counter irritating the skin.(Anand & Bley, 2011). These chemicals stimulate the skin to redden by forcing the blood vessels in the skin to widen, giving the sensation of warmth. The concept of a counterirritant is that stimulation of sensory nerve endings affects.

The classification of which substances should be categorised as rubefacients has been a source of debate. Salicylates are pharmacologically linked toNSAIDs and aspirin, but their main effect in topical applications (typically as amine derivatives) is to irritate the skin. Topical NSAIDs, on the other hand, permeate the skin and underlying tissues, inhibiting COX enzymes as stated above. As rubefacients, we'll use salicylates and nicotinate esters.

Topical Capsaicin

Capsaicin is the main component of chilli peppers that causes them to be spicy when consumed. “It binds to nociceptors (sensory receptors in the skin that send signals that induce pain perception), specifically the TRPV1 receptor, which regulates calcium and sodium ion transport across the cell membrane. Binding opens the ion channel (allowing sodium and calcium ions to enter), causing depolarization and the formation of action potentials, which are felt as itching, pricking, or burning sensations. Repeated applications or high concentrations cause a long-lasting effect known as 'defunctionalisation,' which is caused by a combination of events that overwhelm the cell's normal processes and can lead to reversible nerve terminal degeneration.”Pain from a variety of chronic illnesses, including postherpetic neuralgia (PHN), peripheral diabetic neuropathy (PDN), osteoarthritis, and rheumatoid arthritis, as well as pruritus and psoriasis, is treated with topical creams containing low concentrations of capsaicin.

Capsaicin creams normally contain 0.025 percent or 0.075 percent capsaicin, however 0.25 percent creams are available in some countries.(Anand & Bley, 2011)

Methyl Salicylate

Methyl salicylate is a type of nonsteroidal anti-inflammatory medicine (NSAID) used to treat pain.(Bhanu, Shanmugam, & Lakshmi, 2011). It comes in topical dose forms such as creams, lotions, spray and ointments, as well as oral and parentral dosage forms. (Bhanu et al., 2011)Methyl salicylate (wintergreen oil) is an organic ester produced naturally.

Counter-irritation is used in the treatment of pain because it can alter the sensory nerve endings. (Derry, Matthews, Wiffen, & Moore, 2014)The underlying musculoskeletal pain and discomfort is thought to be masked by this irritation. When topically applied, methyl salicylate entered the skin by passing through skin layers and act on the targeted site by inhibiting the COX enzyme and reducing the production of prostaglandins both locally and peripherally.

NSAIDs are the first-line treatment for lupus, ankylosing spondylitis, rheumatoid arthritis and osteoarthritis, among other rheumatic disorders.
.(Syngle, 2006), (Au, Kim, Goldminz, Alkofide, & Gottlieb, 2014). They can reduce the first-pass hepatic metabolism, minimizes systemic exposure and reduce the effect of gastro intestinal side effects such as diarrhea, peptic ulcer, vomiting, nausea and heartburnbecause they reach systemic circulation via the gastrointestinal tract (GIT). Furthermore, systemic side effects such as cardiovascular adverse effects and nephrotoxicity may be more noticeable. (Duangjit, Opanasopit, Rojanarata, & Ngawhirunpat, 2013) , (Wongrakpanich, Wongrakpanich, Melhado, & Rangaswami, 2018).

(da Silva & Woolf, 2010)The most common topical NSAID formulations on the market include creams and gels containing naproxen, ibuprofen, piroxicam, Diclofenac and ketoprofen. Only a few NSAID emulgels have been developed. Several botanicals, including turmeric, aloe vera, clove, cinnamon, ginger, and others, have been claimed to have anti-inflammatory properties in addition to these synthetic substances.

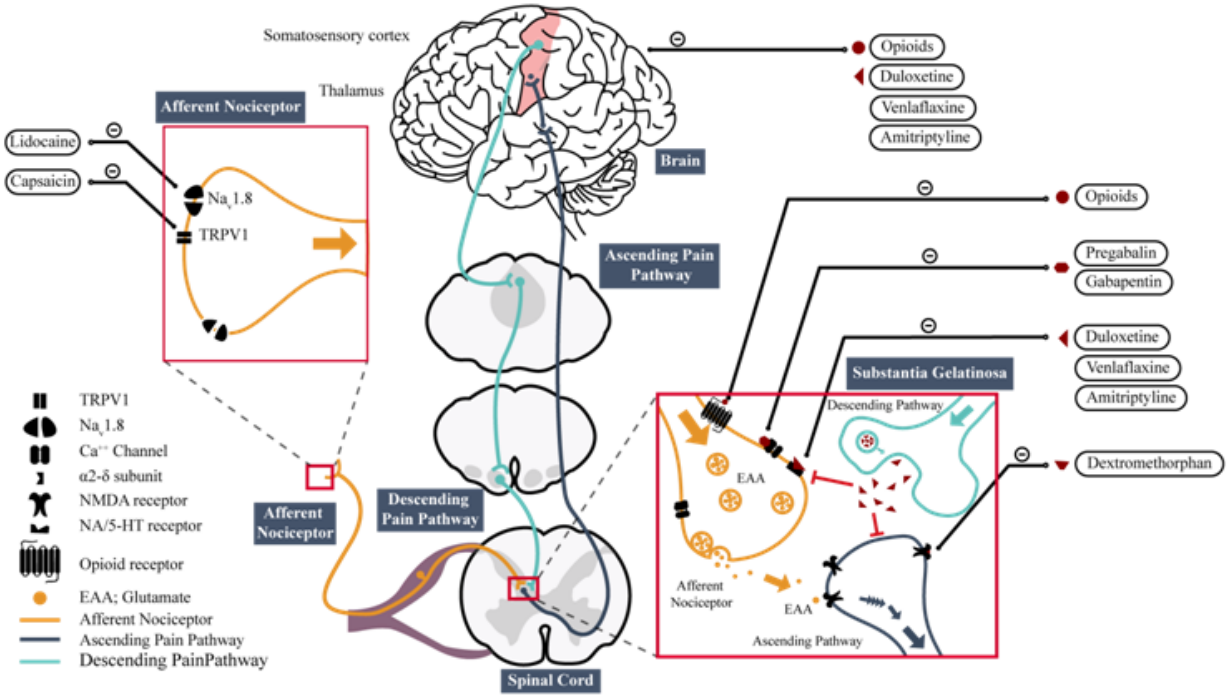


Figure 3 Pain reliever

1.3 Various Marketed EmulgelFormulation

Emulgel is a commercially available product that comes in a variety of forms, some of which are included in the table below. VoltarenEmulgel is a topical analgesic gel that lowers swelling and relieves shoulder and back pain. VoltarenEmulgel is a non-greasy, white, pleasant-smelling gel that comes in a 100g tube and contains diclofenac sodium 1 percent w/w as the active ingredient (as diclofenac diethylamine). DiclomaxEmulgel, developed by Torrent Pharma, is another

emulgel used to treat inflammation of the joints, muscles, tendons and ligaments. Miconazole nitrate and hydrocortisone are the active ingredients in Miconaz H emulgel, which has bactericidal, fungicidal, anti-inflammatory, and antipruriginous qualities. It is made by Medical Union Pharmaceuticals.(Kumar, Singh, Antil, & Kumar, 2016)

Table 2: Marketed Emulgel

Brand name	**Active pharmaceutical drug (API)**	**Excipient**	**Manufacturer**	**Use**
Voltaren	Diclofenac Diethylamine	Permeation enhancer Propylene glycol Emulsifier Macrogol cetostearyl	GlaxoSmithKline GSK.	Anti-inflammatory
Wintogenocream	Methyl Salicylate,Menthol	Permeation enhancer Glycerin, Emulsifier cetomacrogol, cetostearyl alcohol.	Actavis UK Limited	Anti-inflammatory

Materials and Method

All of the materialshas a pharmaceutical quality. Methyl salicylate API, while capsaicin and other excipients.Carbopol-934 (gelling agent),glycerine (Moisturizer),Capsicum Oleoresin Topical (Treat minor aches and pains of the muscles), Eucalyptol Topical (Anti-inflammatory),Thymol Topical (Relaxing smooth muscles), Cetomacrogl (Enhancing the moisturizer effect), Isopropyl myristate (Skin penetration enhancer), Arnica Montana (Antiseptic, anti-inflammatory, and pain relieving properties), liquid paraffin (oil phase vehicle), Cetostearyl alcoholE216(propylparaben), E218(methyl paraben) (emulsifying agents and surfactants, preservative), Camphor oil (Topical analgesic and anesthetic used to relieve pain). Menthol (drug release enhancer and rubefacient),cetomacrogol1000 (moisturizer, used to treat dry skin conditions such as eczema)and purified water.

Table 3: Phases of Novel Formulation

Phase of cream formulation	**Ingredients**
API	Methyl salicylate, Menthol, Camphor, Arnica Montana
	Eucalyptol, Thymol, Capsicum oboresin, Glycerine
Excipients	Isopropyl myristatae, parrafin oil, cetomacrogol 100, cetaryl alcohol.
	Methyl paraben, propyl paraben
	Carbomer 934

2.1 Selection Criteria

The reason for selecting these drugs was, if we use other synthetic drugs with methylsalicylate may induce an increase in the risk or severity of bleeding and gastrointestinal bleeding, as well as a decrease in the therapeutic efficacy of Methyl salicylate. Reason for selecting these excipients was physical and chemical stability, No interference with drug bioavailability, Physiological inertness.

2.2 Methodology Followed

2.2.1 Formulation of Emulgels

Composition

Each emulgel was made in a single 100-gram batch. The preparation was accomplished in three steps, as detailed in Steps 1–3.(Kapoor et al., 2014)(Kapadiya et al., 2016) these are the following composition that I made in the first composition.I haven't addedglycerine but I used carbomer 1.5%. In the second composition I used 2% glycerin and 0.75% carbomer. In the final composition I used 10gglycerine and 1.5% carbomer. The viscosity depends on the polymer concentration 1.5% had the highest viscocity. The gel viscosity plays an important role in controlling drug permeation. Glycerineconcentratin was optimized during drug solution preparation, the drug was not dissolved properly and it was slightly heated to dissolve the drug. Cetomacrgol 1000 concentration was prepared with 4% reduced better emulsion compare to other two formulations.

Table: 4 Composition of Novel Formulation (% w/w).

Drugs	Formulation 1	Formulation 2	Formulation 3 Final
Methyl salicylate	12.20%	12.20%	12.20%%
Eucalyptus	0.11%	0.11%	0.11%
Arnica	1.5%	1.5%	1.5%
Capsicum oleoresin	0.30%	0.30%	0.30%
Glycerin	-	2%	10%
Thymol	0.11%	0.11%	0.11%
Camphor	0.5%	0.5%	0.5%
Menthol	2.60%	2.60%	2.60%
Isopropyl myristate	10%	10%	10%
Parrafin oil	1%	2%	2%
Cetomacrogol 100	1.25%	4%	4%
Cetaryl alcohol	3%	3%	3%
Methyl paraben	0.1%	0.2%	0.2%
Propyl paraben	0.5%	0.1%	0.1%
Carbomer	1.5%	0.75%	1.5%

Step 1: Aqueous phase/Preparation of the gel base

In a planetary mixer, propyl paraben and methyl paraben were dissolved in hot water. To this finely sieved carbomer 934 was dispersed by continuous stirring at constant speed. Once the uniform dispersion attained without lumps and bubbles, it is heated up to 75°C.The gel base was

adjusted to a pH of 5–7. Because the skin's pH is about 5.5, and a acceptable pH range is 5–7 which is safe to use and it can avoid skin irritation.

Figure 4: Aqueous Phase

Step 2: Oil phase

The phase inversion approach was used to make the oil-in-water (o/w) emulsion. Dissolving the cetostearyl alcohol, cetomacrogol 1000, liquid paraffin oil and isopropyl myristate were heated to 75°C and then mix slowly to above aqueous phase with continuous stirring until a fine emulsion forms while, Carpobol was dissolved in hot water to create the water phase. The aqueous phase was gradually added to the above phase. Both phases were heated on a hot plate to 70°–80°C.The emulsion was then allowed to cool at room temperature.

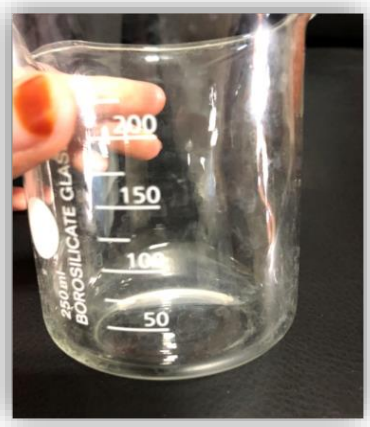


Figure 5a: Oil Phase

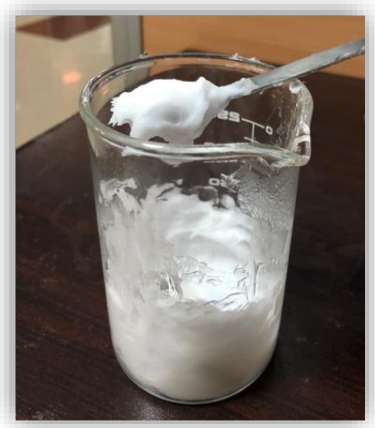


Figure 5b: Mixture of Oil & Aqueous Phase

Step 3: Drug solution preparation:

Exact quantity of methyl salicylate, Arnica, camphor, thymol, menthol, glycerine, eucalyptus, capsicum oleoresinwas accurately weighed and added to the preformed emulsion to get gelled emulsion (emulgel). The gel base was added to the emulsion 1:1 ratio with continuous and gradually blending with the help of a stirring rod at room temperature to generate the desired emulgel. The final recipe was placed in a jar with a label, and the % yield was calculated.

Figure 6a: Drug Solution Preparation

Figure 6b: Cream

2.2.2 Evaluation of the Novel Formulation

For evaluation and analysis, three formulation samples were prepared. The findings of quantitative analyses are presented as average values.

2.3 Physical Examination

Homogeneity,colour,grittiness, clarity actual phase separation were all visually checked in each formulation.

2.3.1 PH Measurement

PH is used by pharmaceutical companies to monitor chemical reactions in the manufacturing process. The speed of reactions is determined by the pH of a solution, and the pH can be used to estimate the reaction's end point. For example, when making antibiotics, the pH of the fermentation process must be maintained to ensure a high yield and antibacterial characteristics. Because medicines and cosmetics are ingested or applied to the skin of humans, the greatest quality control is essential. The consequences of a large pH differential between skin and cosmetic items could be disastrous. If a drug has the wrong pH, it can become poisonous.

Method

Before measuring the pH, one-gram Novel formulation was diluted to 100 mL with distilled water and to withstand for 2 hours. (Panday, Shukla, Sisodiya, Jain, & Mahajan, 2015)

2.3.2 Viscosity measurement

The viscosity of a substance is a measurement of its resistance to motion when subjected to a force.

The formula for calculating viscosity is straightforward:

Shear stress / shear rate = viscosity

The result is usually represented in centipoise (cP), which is equal to 1 millimetre of mercury per second (millipascal second).The force per unit area necessary to shift one layer of fluid in proportion to another is known as **shear stress.**The **shear rate** is a measurement of how fast intermediate layers move in relation to one another.

2.3.3 Non-Newtonian Fluids vs. Newtonian Fluids

The formula's creator, Isaac Newton, believed that the viscosity of a fluid would remain constant independent of changes in shear rate at a certain temperature and shear stress.

He was partially correct. Water and honey are two examples of fluids that act in this way. Non-Newtonian fluids are classified as thixotropic, rheopectic, pseudoplastic, dilatant, and plastic. When measuring each of these fluid kinds, different considerations are required.

When producers collect data on a material's viscosity, they can forecast the real world examples of the substance are , if the viscosity of toothpaste is incorrect, it can be difficult to pump out of the tube or pump out too much.

Knowing a material's viscosity has an impact on how production and transportation operations are built.

Method

At room temperature, the viscosity was measured. The torque values were taken on a scale ranging from 15% to 95% of the base scale. The L4 spindle was employed, with a speed of 25 rotations per minute.

2.3.4 SpreadabilityStudies

Spreadabilityof Novel Formulation was measured by 0.5 g of emulgel by putting on a glass slab at 1 cm diameter circle that had already been pre-marked. A glass slab was pre-weight put on top, and a weight of approximately 1 kg on the upper glass slab for 5 minutes. The emulgel spread and diameter of the circle was increased, which was assessed with electroniVernier callipers.(Bachhav & Patravale, 2010; Shinde, Parmar, & Easwaran, 2019; Singh & Bedi, 2016)

2.3.5 Determination of content uniformity

To determine the consistency of API content in the formulations, the API content in each formulation was analysed. By rotating for nearly half an hour, a 0.5g novel formulation containing 136.6 mg of medication was dispersed in 50 mL freshly prepared phosphate buffer (pH 7.4). After that, 5 mL of the solution was added to 20 mL of phosphate buffer solution. To determine the drug content, UV–Vis spectrophotometer values at 302 nm were taken.

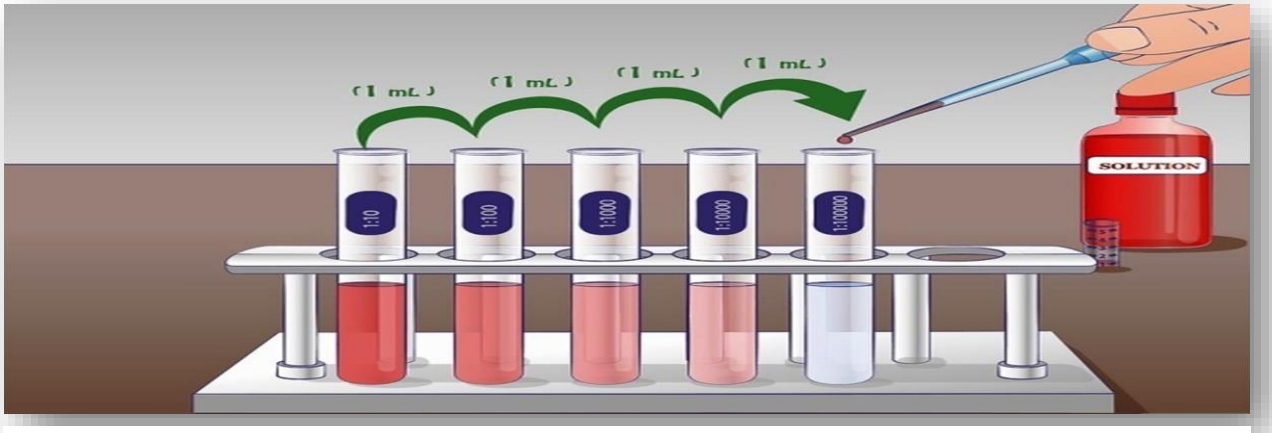


Figure 7: Dissolution Process

UV-VIS Spectrophotometry

Ultraviolet-Visible absorption spectroscopy is the most commonly used technique that helps to identify presence of components in sample. When a beam of light passes through the sample, it provides the absorption spectra of sample as an output by reflecting the beam.

$$A = -\log(T)$$

UV-Vis spectroscopy obeys the Beer-Lambert law where absorbance of the sample is directly proportional to the molar concentration of the sample present in the cuvette. The molar absorptivity also called absorption value is used to identify entities present in the sample. The equation of Beer-Lambert law is:

A = log (I0/I) = Ecl

UV- Vis spectroscopy is the foremost confirmatory test that is used to verify the synthesis of transfersome nanoparticles. Hence, for this very purpose, Jasco V-650 spectrophotometer model was used in the range of 200-400 nm and samples were measured. PBS was used as a relative reference.

CHALLENGE OF INSOLUBILITY OF DRUG IN PBS

Drug solution is hydrophobic it does not dissolve in PBS. If we checked the absorption peak in the UV-spectra it had a lot of distortions, clear peak didn't appear.

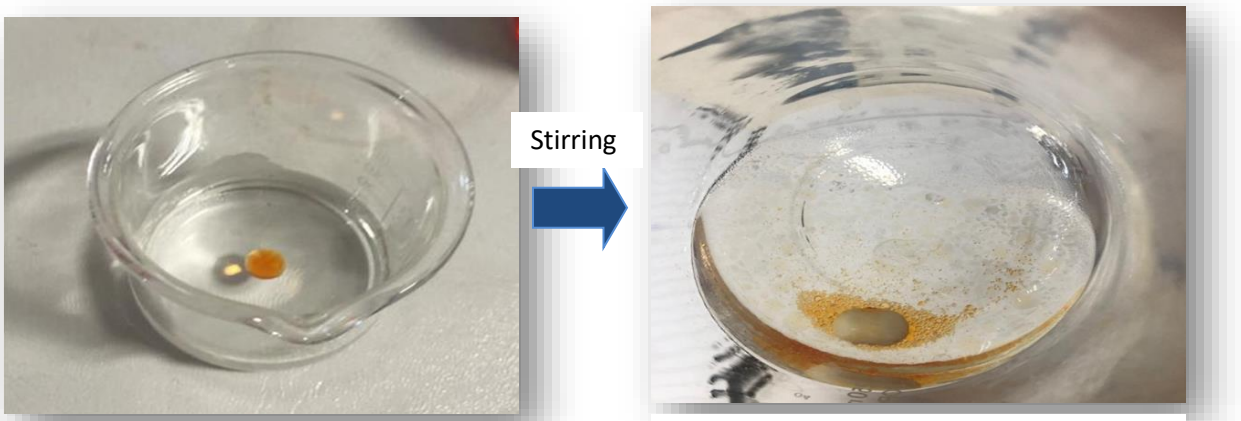


Figure 8a: Drug in PBS

Figure 8b: Drug in PBS

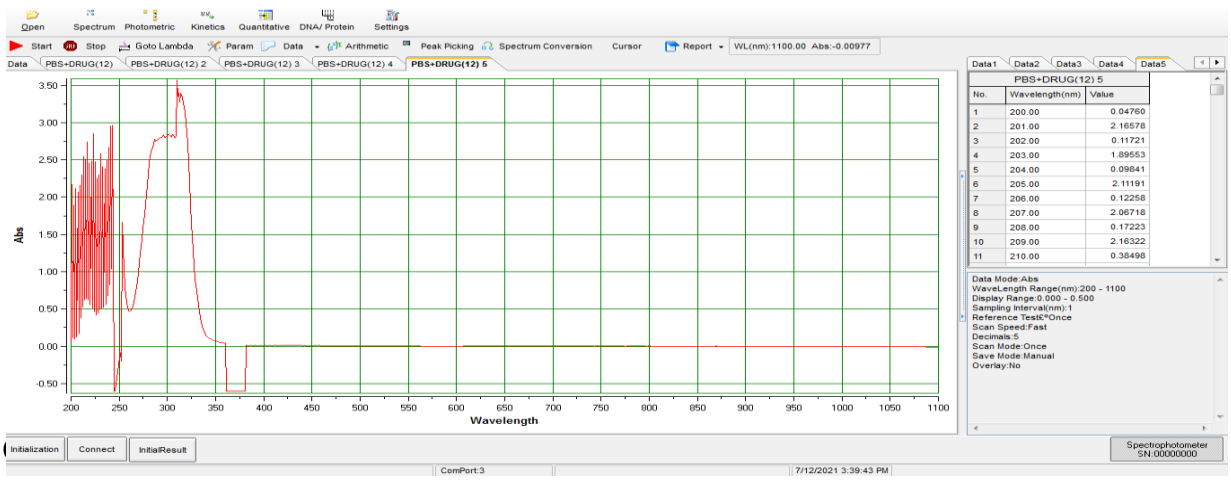


Figure 9: Challenge of Insolubility of Drug in PBS

Standard curve was needed to ensure precision and accuracy of your measurement. It s necessary when you are trying to quntify the concentration of an unknown.According to European Pharmacopoeia 5.17.1.there are two method has been given one is Maintain sink condition or Dissolution media may contain, surfactants (tween 80). I used sink condition which resolved the problem and clear peak had been shown in the UV-spectra and standard curve was formed.

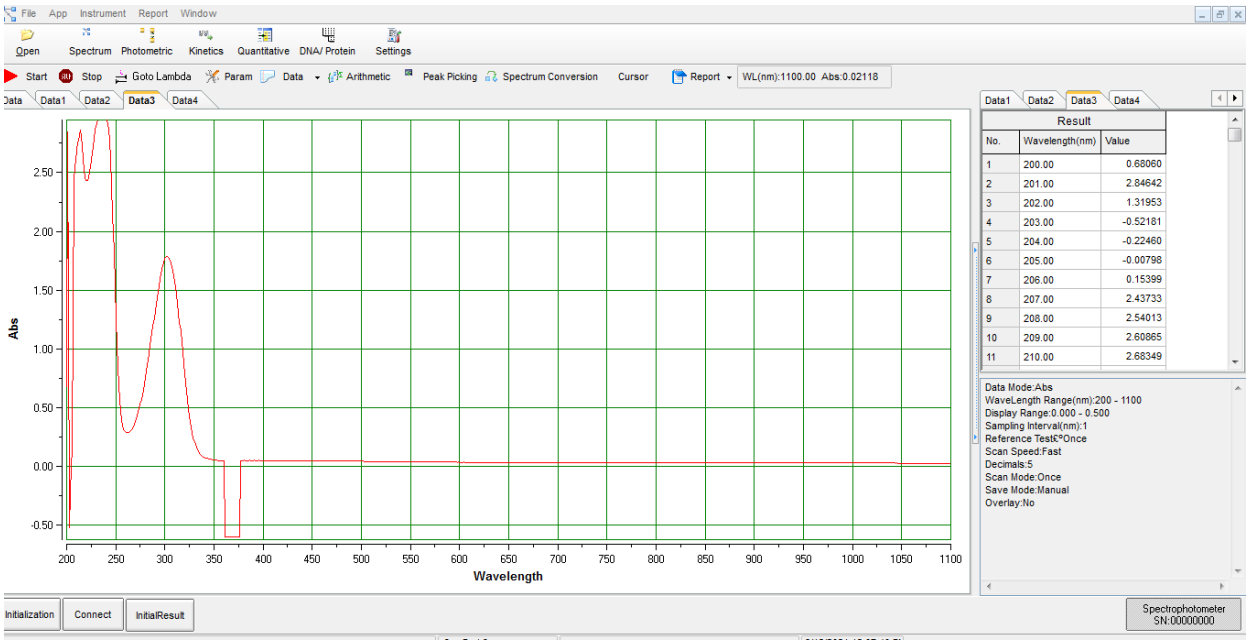


Figure 10: Challenge of Insolubility of Drug in PBS is Solved

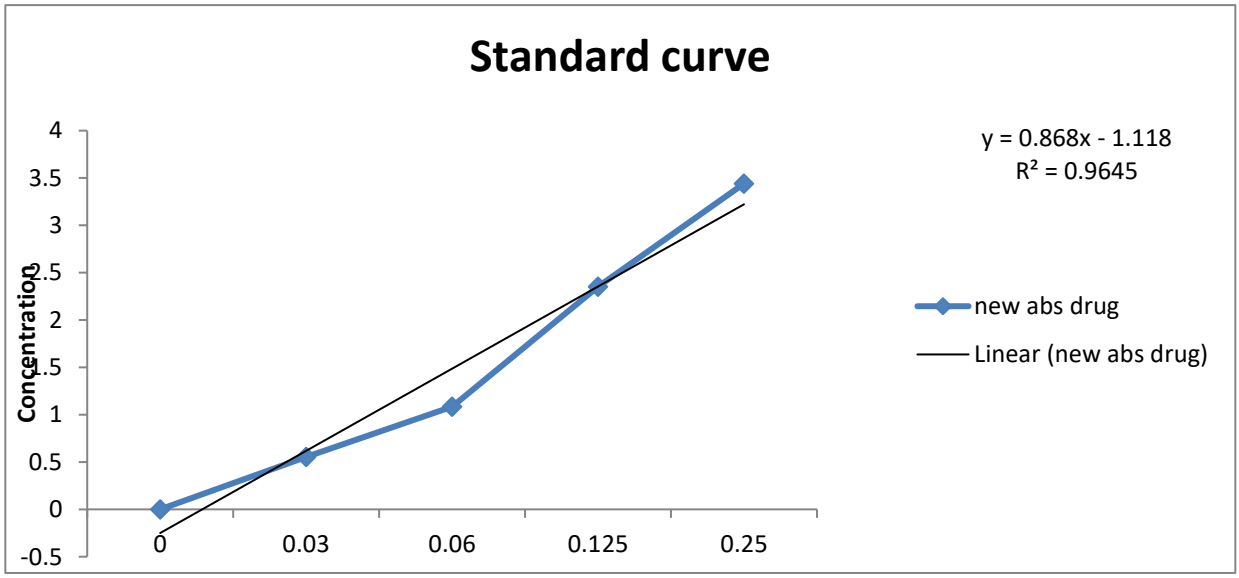


Figure 11: Standard Curve

2.3.6 Centrifugation Test

In a tapered test tube, 30 g of formulation was added to perform the centrifugation test. The sample gel was centrifuged at 3000 rpm for 30 minutes at room temperature during the centrifugation process.

2.3.7 Fourier Transform InfraRed Analysis (FTIR)

Compatibility study using FT-IR

FTIR is a technique that is used to identify the functional groups or bonds present in pure compounds, mixtures or used for their comparison. Every bond between different elements absorbs light energy at different frequencies. This light absorbance is measured by FTIR and produced as a graph identifying the bonds present in sample. The interaction between prepared API and excipients was studied by using Perkin-Elmer Spectrum-100 spectroscopy at wavelength ranging from 400 cm-1 to 4000 cm-1 along with blankPBS. KBr disc method was used to analyze the samples. KBr was heated at 110^0C to evaporate any traces of water. Hydraulic press was used to make pellet of KBr. Samples were loaded in chamber individually and submitted to IR radiation to detect the functional groups. The spectrum of infrared light to check the spectra for the medicine, infrared spectroscopy was used, and the spectrum was obtained in the 4000-400/cm range. For each shift in drug peaks in the spectrum of a physical mixture of drug, the interaction between drug and excipients was noted.

Stimulated drug release studies

In general, drug solubility and stability, test sensitivity, and the method used to determine which release media are used for production. Despite the fact that maintaining sink conditions is preferred, non-sink circumstances have been used. Agitation, which is typically used to prevent

dosage forms from aggregating during an in vitro release study, is dependent on the apparatus used. Similarly, the type of in vitro procedure used determines sampling and buffer replenishment (complete or partial).

Percentage of Novel Formulation drug penetration through each synthetic membrane was calculated and plotted against time. The steady-state slope of the curve was used to compute API flux. At each time point, a CV was calculated for the total API content in the receptor chamber using equation M1.

%cv = (standard deviation/mean)*100

2.4 In vitro drug release

The tests were carried out on a Franz cell array provided by GlaxoSmithKline (Harlow, UK). This system is built up of 27 bespoke receptors, donors, and four heated magnetic stirrer chambers, each with eight blocks where Franz cells are placed. The Franz cells had a receptor volume of 11.7 0.1 mL and an effective diffusion area of 59.6 3.1 mm2.

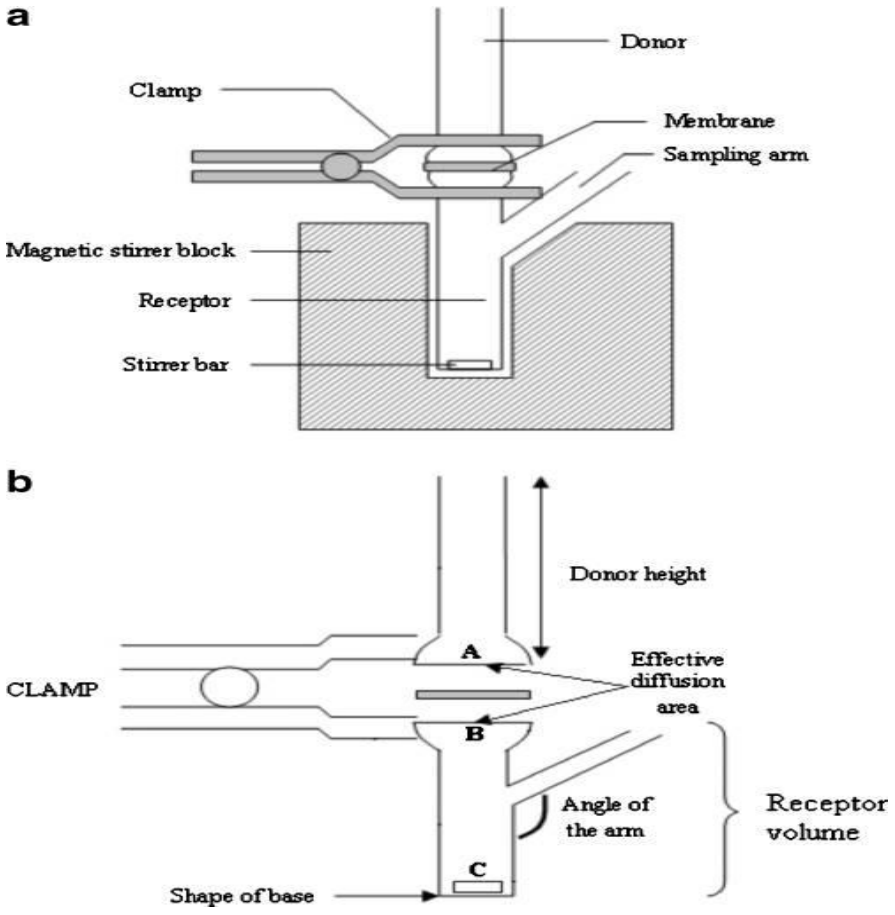


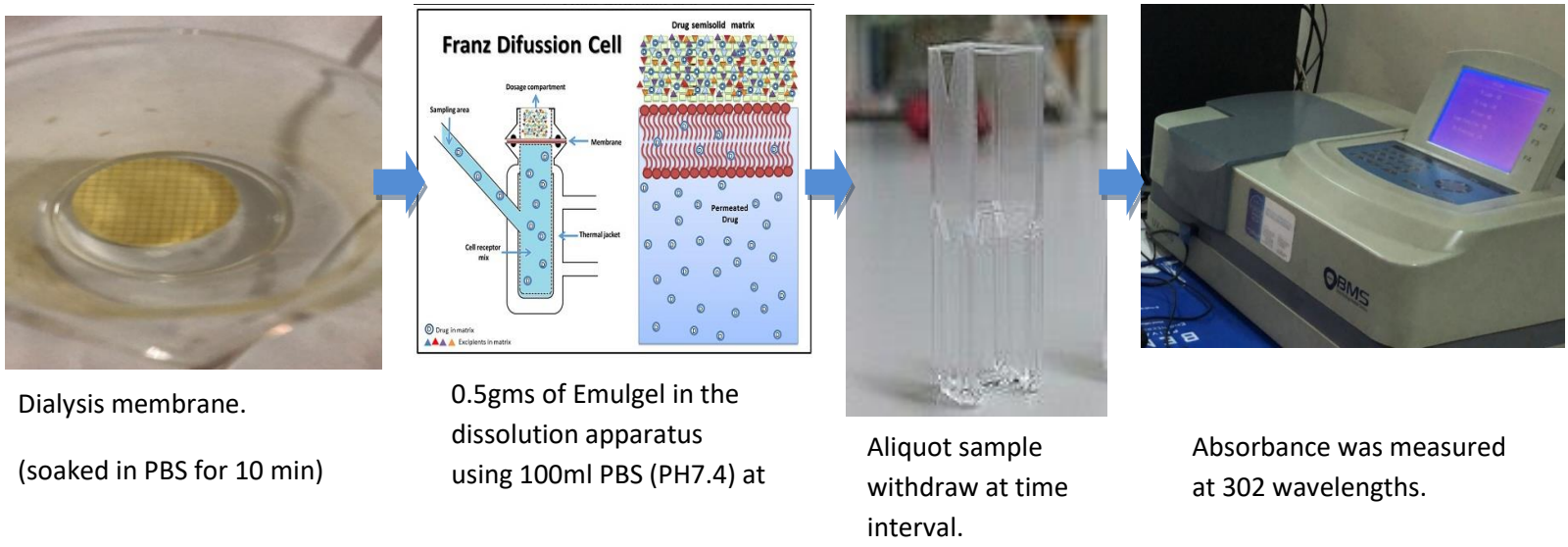
Figure 12: Franz Diffusion Cell

The Franz diffusion cell has been evolved into a research approach for assessing skin permeability, providing information into the connections between skin and medication. The testing was important for new formulation and also control toxicity and quality of the formulation.(Ueda et al., 2009). Franz diffusion cells are typically employed using human or animal skin that has been removed. When living organism skin is not available, synthetic membranes are either used in place of it. It is necessary to utilise synthetic membranes in Franz cell drug diffusion experiments for two reasons: to simulate skin and to ensure quality control. PDMS (polymethylsiloxane) is an example of a synthetic membrane that has been frequently utilised to mimic the skin due to its hydrophobicity and rate-limiting qualities, which are similar to those of the skin.(Twist & Zatz, 1986)(Pellett, Castellano, Hadgraft, & Davis, 1997). Synthetic

membranes for quality control, on the other hand, should have minimal drug diffusion resistance and solely serveas a means of separating the formulation from the receptor medium. Pores are common in synthetic membranes, which are referred to as 'porous membranes. Much research has been done in the last two decades to measure topical medication diffusion utilising porous synthetic membranes. A large number of Synthetic and semi synthetic membranes are available on the market. Typically, Most of the porous synthetic membranes used in Franz diffusion cells (such as polysulfone and cellulose acetate) are taken from separation and filtering applications and repurposed for use in Franz diffusion cells. The literature indicates that researchers have used synthetic membranes made of a variety of pore sizes, thickness and materials, in their experiments. Silicone, cellulose, and polysulfone are the most often encountered membranes.(Ng, Rouse, Sanderson, & Eccleston, 2010)

“A modified Franz diffusion (FD) cell with a diffusion area of 6.2cm2 was used. Before usage, the cellulose nitrate membrane was soaked for 30 minutes in freshly made phosphate buffer pH 7.4. Each donor and receptor compartments of the modified FD cell were divided into two compartments, with one gram of the test gel dispersed on the surface of the cellulose nitrate membrane that separated them. After that, the cell was placed inside the dissolving vessel of the dissolution tester equipment to be tested. The receptor compartment consisted of a 100 mL vessel that was filled with phosphate buffer pH 7.4 that served as the dissolving solution for the experiment. This was more than enough medium to keep sink conditions from becoming unstable.The circulating water jacket kept the water bath at 37°C, and the assembly was spun at 50 rotations/min utilising USP dissolving equipment 2.(Magdy Ibrahim Mohamed, Abdelbary, Kandil, & Mahmoud, 2019)(Farghaly, Aboelwafa, Hamza, & Mohamed, 2017)(Fauzee, Khamanga, & Walker, 2014). To maintain a constant volume, a 10 mL sample was drawn at

appropriate time intervals and replaced with an equivalent amount of new dissolving media. UV–Vis spectrophotometry at 302 nm was used to analyse the aliquots, and the cumulative released drug was estimated as a function of time for 2 hours."(Pednekar, Dandagi, Gadad, & Mastiholimath, 2015)(Haneefa, Easo, Hafsa, Mohanta, & Nayar, 2013)



Dialysis membrane.

(soaked in PBS for 10 min)

0.5gms of Emulgel in the dissolution apparatus using 100ml PBS (PH7.4) at

Aliquot sample withdraw at time interval.

Absorbance was measured at 302 wavelengths.

Figure 13: Method of Drug Release Profile

2.5 Shelf-Life Studies

Extreme temperature extremes, such as freezing or overheating, are not unusual during the transportation of products. As a result, cream must be able to endure some temperature variations during transportation.

Stability testing includes freeze-thaw cycle testing, which helps you to see Whether or not your formula will remain stable under a variety of settings. This test subjected your sample to a series of drastic changes in temperature, rapid temperature variations similar to those encountered during typical shipping and handling procedures. The use of freeze-thaw stability testing,

especially for liquid-based cosmetics, is strongly recommended. Phase separation may occur in certain items, which might have a deleterious impact on their intended function.

Freeze-thaw testing is freezing the product for 24 hours (about -4°C) and then letting it thaw at room temperature for another 24 hours. The sample is then subjected to a higher temperature (about 40°C) for 24 hours before being returned to ambient temperature for another 24 hours. The sample is analysed for significant alterations. This marks the conclusion of one cycle. If no significant changes are observed after three freeze-thaw cycles, you can be confident that the stability of your product is adequate for transit.

2.5.1 Freeze-Thaw Cycle

Novel formulation was submitted to a freeze-thaw cycle; the test took 12 days and six cycles. The material was kept at a specific temperature for 24 hours in each cycle. The refrigerator temperature was 4°C, while the oven temperature was 40°C.

2.5.2 Stability Studies

Pharmaceutical product was evaluated by the set of tests designed stability in order to determine its shelf-life and use period under specific packaging and storage conditions.

Exaggerated storage conditions are used in studies to speed up the pace of chemical breakdown and physical change of a medicine as part of the formal stability testing method. In addition to those derived from real-time data, the information received this way. Stability studies can be used to examine long-term chemical effects in non-accelerated situations, as well as the impact of short-term deviations from regular operating procedures. Storage conditions should be

labelled, as they may develop during transit. The outcomes of the accelerated. Physical changes are not typically predicted by testing investigations.

Table 5: Main Objectives of Stability Testing

Objective	Study type	Use
To pick appropriate formulas and containerclosure systems (in terms of stability).	Accelerated	Evaluation of the Novel Formulation
To ascertain the shelf-life and storage conditions	Real-time and Accelerated	Product evaluation and registration dossier preparation
To back up the claimed shelf-life	Real-time	Registration dossier
To ensure that no changes have been made to the formulation or manufacturing process that could jeopardize the product's stability.	Real-time and Accelerated	Quality assurance of Novel product, including quality control

The manufacturer must continue to undertake real-time stability testing to confirm the expiration date and storage conditions predicted previously. The data required to certify a provisional shelf-life must be received by the registration agency. Other outcomes During GMP inspections, the status of ongoing stability studies are confirmed. To ensure the quality and safety of products on a national scale, with an emphasis on degradation. Health authorities should monitor the stability and quality of market preparations. To bring the product to market, a follow-up inspection and testing programme will be implemented. Additional stability tests are required after the product has been registered. Whenever big changes to the composition or manufacturing process are made, packing or preparation procedure the results of these investigations must be made public. The proper drug regulatory agencies were notified.

The International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (Technical Requirements for Registration of Pharmaceuticals for Human Use) criteria was used to conduct the stability tests. For three months, the improved During storage in a stability chamber, compositions were exposed to accelerated stability conditions of 40°C and 75 percent relative humidity. After then, they were checked at one-month intervals for a total of three months for organoleptic characteristics, pH, spreadability, and drug content. (Blessy, Patel, Prajapati, & Agrawal, 2014)

2.6 In V*ivo* Studies

2.6.1 Animals

Male Balb/c mice (2-3 weeks) were obtained from the Atta-ur Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST) Laboratory Animal House for this work. Animals (n = 12) were housed in conventional polypropylene cages and kept in standard laboratory settings, which included a constant temperature of 25°C and a relative humidity of 60% with a 12 hour light/dark cycle and free access to feed and drink**.**

2.6.2 Determination of Analgesic Activity

The experiment was conducted using male mice weighing, taken from Animal house of ASAB, National University of sciences, Pakistan. The animal were kept in plastic cages with soft bedding 3 per formulation ingredients formulation code Menthol(g), Glycerin(%w/w), Methyl salicylate(g), Camphor (g), Arnica (%w/w), Eucalyptus (%w/w), capsaicin (%w/w). Three cages are kept in typical circumstances, including a light and dark cycle, as well as food and tap water. Before the experiments, they were given a week to acclimate. The animal skin investigations received ethical approval from NUST's research ethics committee. The mice were separated into

three groups, each with three mice, and the test was conducted using the hot plate method. The animals had already been accustomed to the hot-plate twice. The carbopol gel base was used as a control group.(Adzu, Amos, Kapu, & Gamaniel, 2003). Second group were served as standard group treated with the marketed formulation and the third group treated with the formulation. A 0.5gm cream was applied to the area of the right hind paw one hour prior to treatment. The mice were placed on a heated plate that was kept at 55°C for the duration of the experiment. The hot-plate delay was measured as the time it took for a hind paw licking or a jump off the surface to occur. The animals were evaluated at 0 minutes (baseline), 15 minutes, 30 minutes, 45 minutes, and 60 minutes. The cut-off time was 30s to prevent tissue damage(Rasool, Abu-Gharbieh, Fahmy, Saad, & Khan, 2010)(Jafri et al., 2019)

1. Allow the animals to acclimatize and calm without movement of tail.

2.Weigh and mark the animals

3.Cream were applied to the tail

4.Mice dropped on hot plate at 55 degree

Figure 14: Method of Analgesic Activity

2.6.3 Dermal Irritant Test

In metal cages with perforated floors, three young adult mice were housed. Water and ordinary mouse food were supplied to the animals. The room temperature was kept between 22 and 23 degrees Celsius, with a relative humidity of 30 to 70 percent. The lighting was set to provide 12

hours of artificial light each day (8 a.m. to 8 p.m.). Hair patch was removed on the back of the mouse and clean 24 hours before the test (dose administration), exposing approximately 4 cm2 of skin. The Novel formulation mixture was applied equally to a 4 cm2 patch of each mouse's carefully cut skin. The skin reaction was measured at the applied site and subjectively evaluated and scored once daily for the first 1, 24, 48, and 72 hours after the treatment (post test observation period)in the following order. (Aiyalu, Govindarjan, & Ramasamy, 2016)

2.7 Statistical Analysis

Each analysis was duplicated, and the new formulation was tested in triplicate. The significance of the effects of formulation factors following the freeze-thaw cycle was determined using the It was done using the Student's t-test, which was implemented in the most recent version of the Graph Pad Prism software (Graph Pad Software Inc., San Diego, CA, USA), with p values less than 0.05 considered significant.

Results and Discussion

Initially the formulation was prepared to optimize the concentration of polymer carbomer 934, the spreadabilty was high but its consistency is very thin and the viscosity was low. In formulation 02 1.5% carbopol showed good spreadability, consistency and viscosity.

Glycerine concentration was optimized with the formulation during the drug solution preparation; the drug was not dissolved properly. Increasing the concentration of the glycerine and slightly heated, to dissolve the drug. (Higashi, Kiuchi, & Furuta, 2010),(Padmawar, Herbals, Bhadoriya, & Mechanics, 2018),10% glycerine concentration was good.

To optimize the cetomacrogol 1000 concentration 4% give better emulsion than 1.25%. In this formulation there were no drug particles when viewed under microscope, it gives evidence that drug was dissolved properly.

3.1 Compatibility Study of Using FTIR

FTIR is a technique that is used to identify the functional groups or bonds present in pure compounds, mixtures or used for their comparison. The interaction between prepared API and excipients was studied by using Perkin-Elmer Spectrum-100 spectrocopy at wavelength ranging from 400 cm-1 to 4000 cm-1 along with blank PBS. KBr disc method was used to analyze the samples. The infrared spectra output was recorded in a graph and interpreted to determine the bond stretching in functional groups by using essential FTIR software.

The identification of the formulation by FTIR spectroscopy resulted in the production of a spectrum. In pure drug, bands at 3339.958/cm were due to N-H stretching of secondary amine, bands at 2961.3066/cm were due to O-H stretching of carboxylic acid, bands at 2930.438/cm

were due to C-H aromatic ring stretching, bands at 1757.439/cm were due to C=O of carboxyl ion, and bands at 1685.413/cm were due to C=C ring stretching. In addition, cream and drug solution had all of the features peaks of the pure drug; as a result, it was showed that the drug and excipients has no interactions between them.

It is concluded that PH of formulation is accelerated at room temperature condition. A good topical preparation should have a suitable PH with skin and the accepted PH is 4.2-6.5. (Kartini, Winarjo, Fitriani, & Islamie, 2017) The formulations show 6.02 PH which is acceptable. If the PH is too acidic than it will make skin irritable.

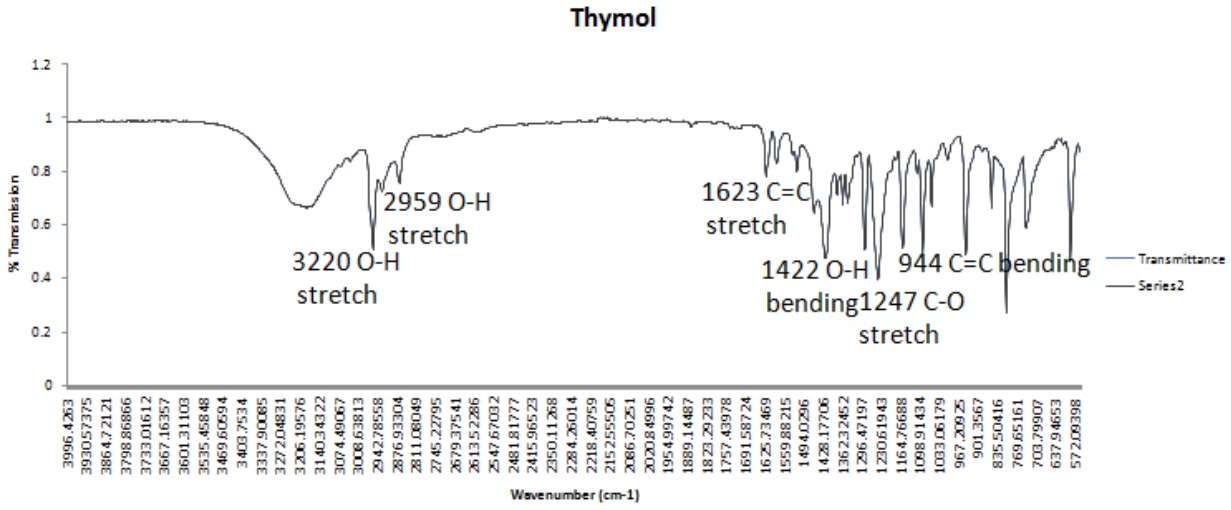


Figure15.1: FTIR of Thymol

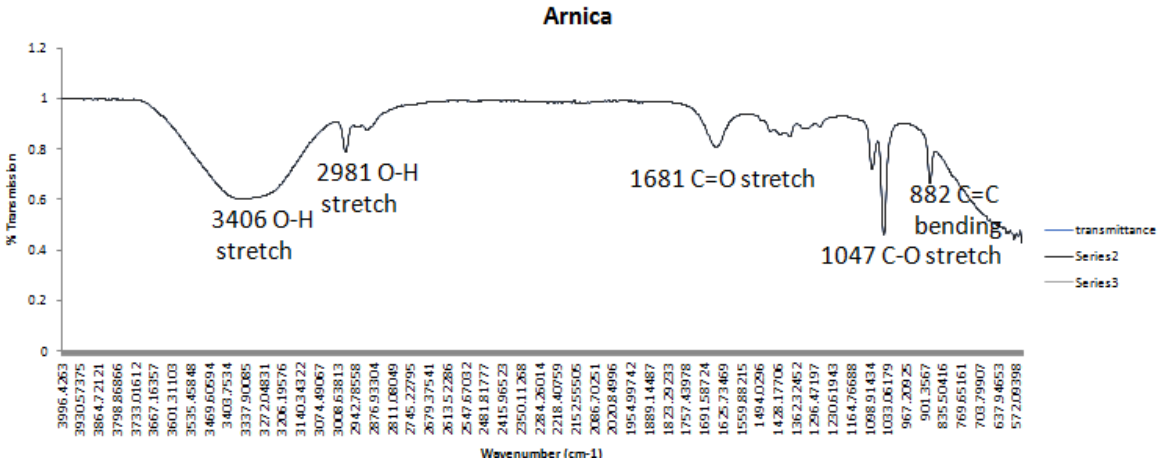


Figure 15.2: FTIR of Arnica

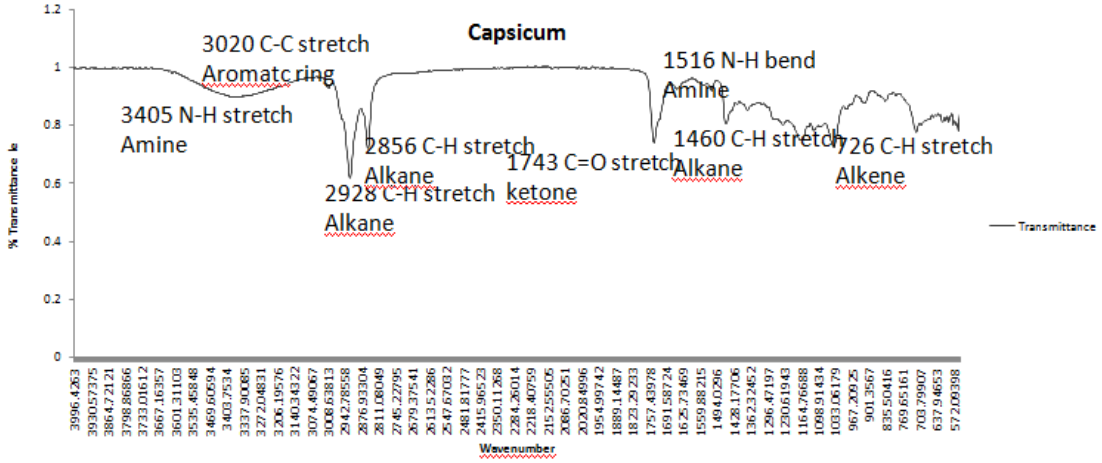


Figure15.3: FTIR of Capsicum

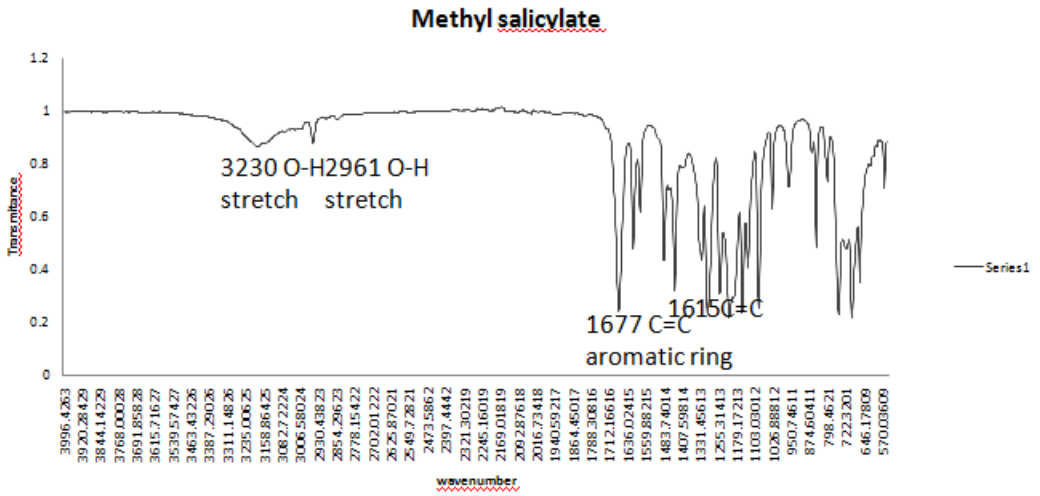


Figure 15.4: FTIR of Methyl salicylate

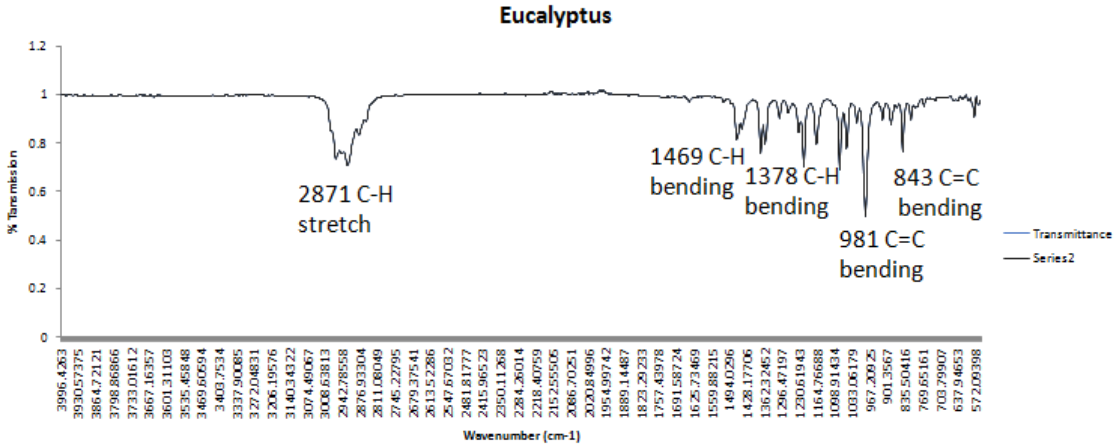


Figure 15.5: FTIR of Eucalyptus

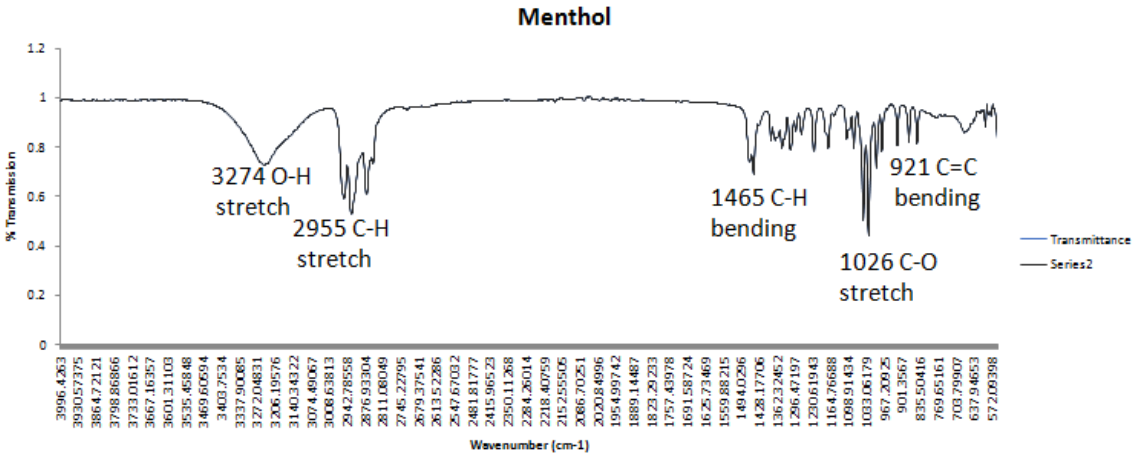


Figure 15.6: FTIR of Menthol

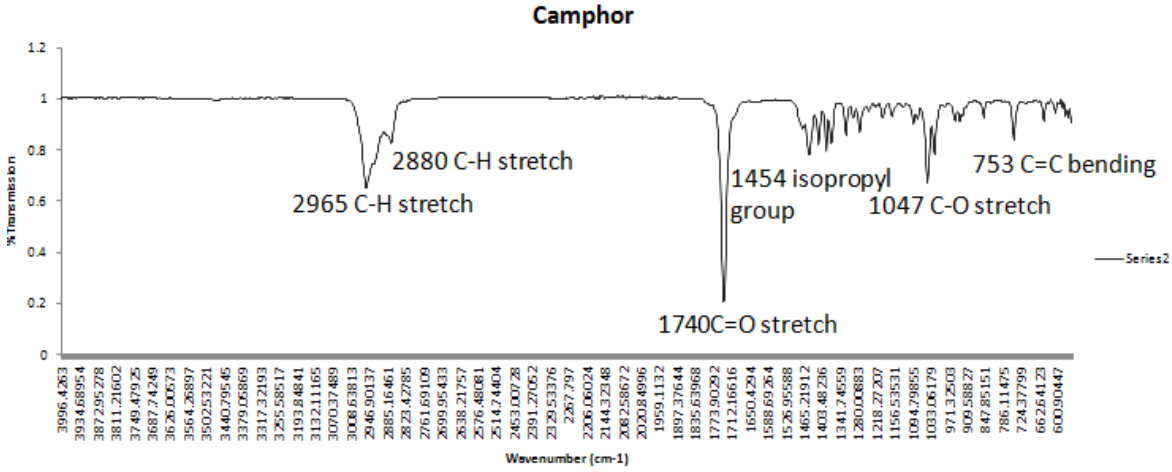


Figure15.7: FTIR of Camphor

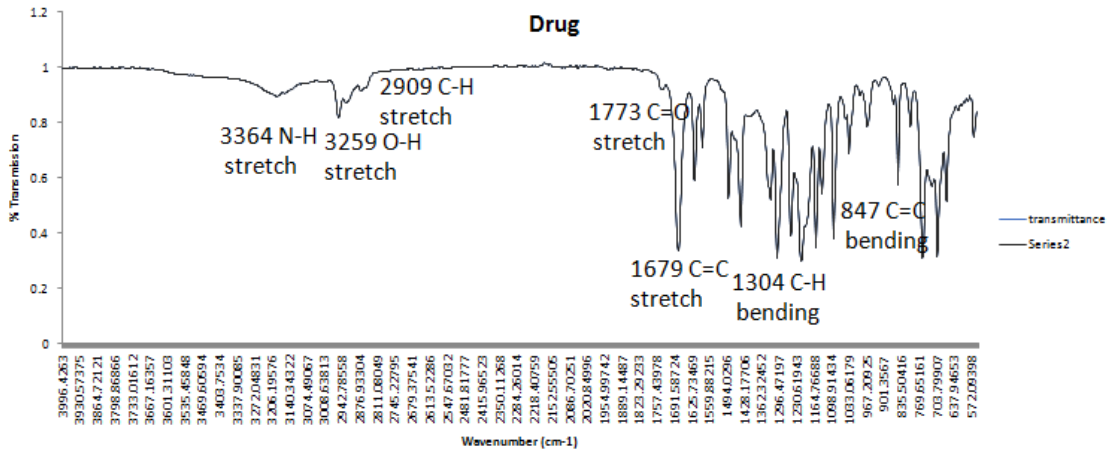


Figure: 15.8 FTIR of Drug solution

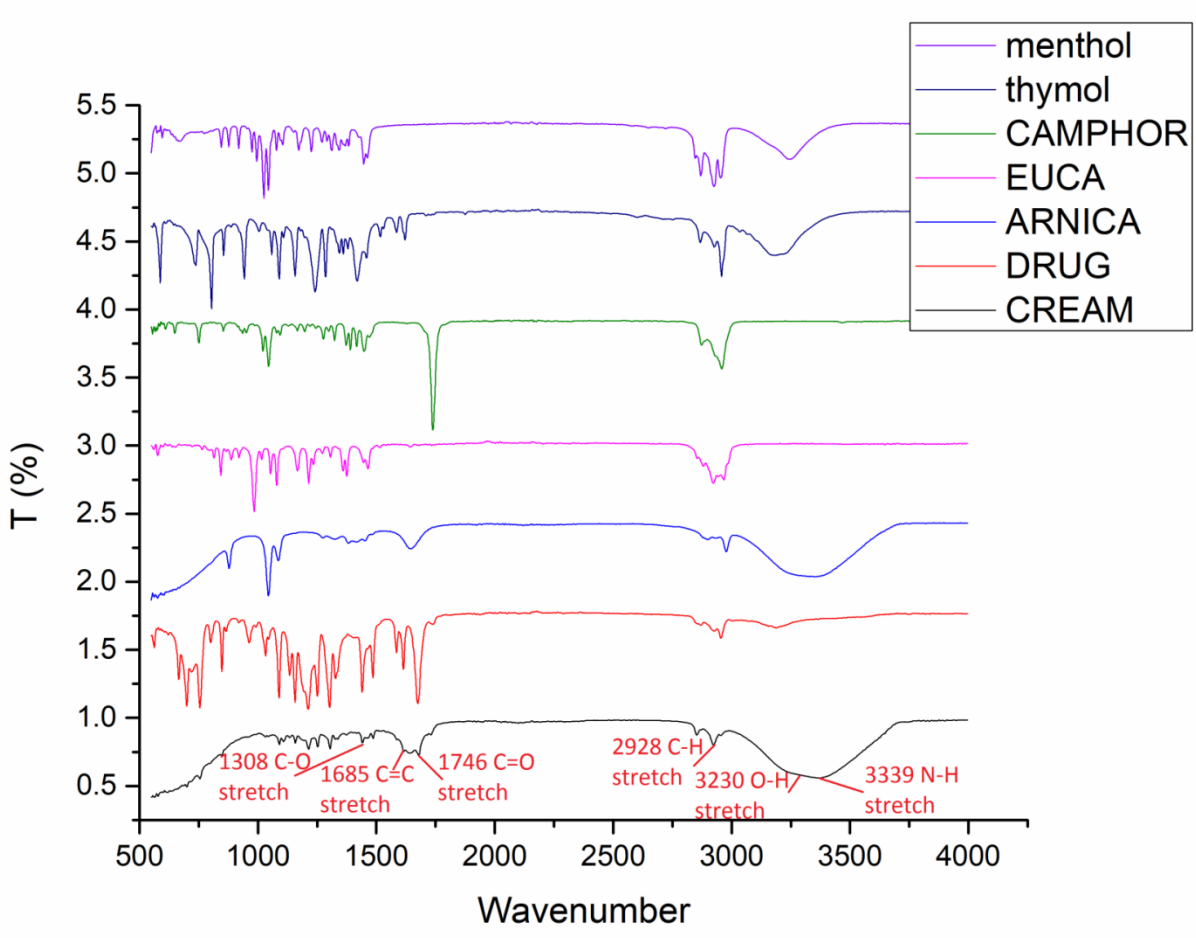


Figure 15: FTIR of Cream

Compatibility study between API and the excipients

According to the results, there was no evident alteration or chemical group interaction between the API and each excipient acquired FTIR spectra of the binary mixes. All of the prominent peaks in the API spectrum were present in the binary mixes' spectra. Overlying of the API and related excipient peaks was blamed for a few modest alterations in the spectra. This indicates that there will be no contact between the medicine and the excipient, and that their physicochemical qualities are compatible.

3.2 Physical Examination

3.2.1 pH Measurement

At the time of the visual assessment, all formulations (novel formulation, and marketed formulations)showed translucent, homogeneous emulgels that seemed to be cream and did not have any obvious grittiness. Novel formulation colour was a light orange. The formulations had an average percentage yield of 97.6%, with an RSD of 1.8 percent. All of the formulations had a pH of 5–7, which was ideal and equal to marketed formulation.

Table 6: Acceptable Range

Acceptable Range(ICH)	**4.5-6.5**
Wintogeno	6
Voltral	7
Novel Formulation	6.02

Figure 16: Homogenous Appearance of Novel Formulation

3.2.2Viscosity measurement

The formulations' viscosity ranged from 36,200 to 122,400 cps, with new Formulations having the lowest and maximum viscosity. Emulgels with 0.5 percent carbopol-934 by weight had the lowest viscosity, while those with 1.5 percent had the greatest. This finding is consistent with the notion in the literature that increasing. When all other factors remain constant, the concentration of polymer in a formulation increases the viscosity of the formulation.(Mwangi et al., 2021)

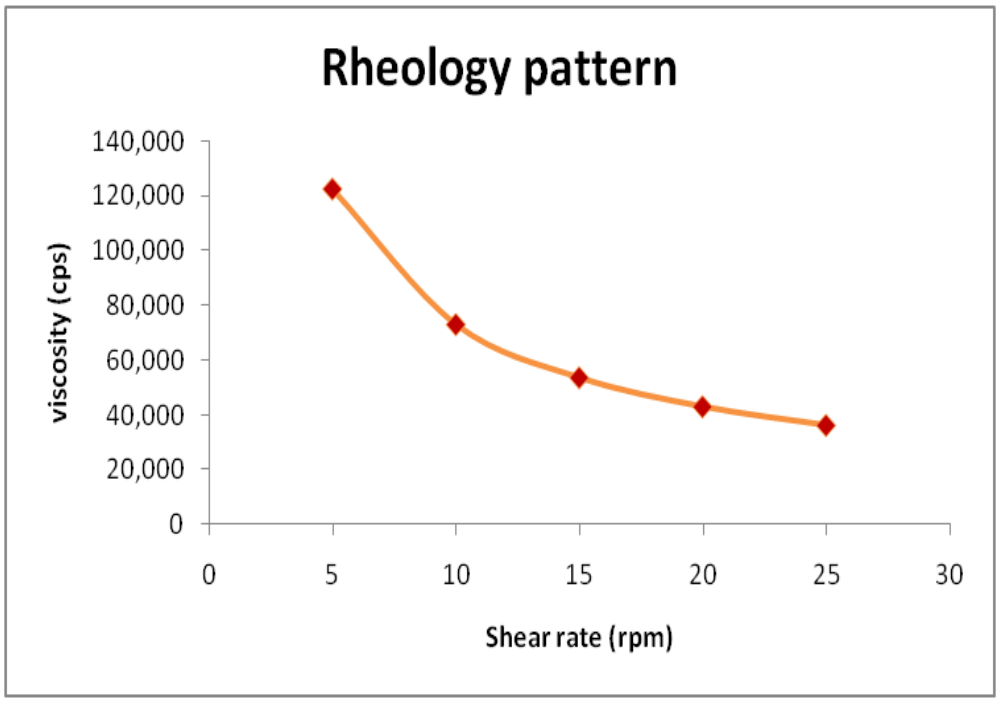


Figure 17: Rheology Pattern

3.2.3Spreadability studies

The increase in diameter of the novel formulation was used to determine their spreadability. The spreadability of a polymer was discovered to be influenced by its concentration and viscosity. Novel formulations viscocity increased as the amount of polymer in them grew, and the spreadability of the formulations decreased as a result. (Panday et al., 2015), The spreadability of novel Formulations 9.0 cm was measured, and this can be attributed to the carbopol-934 concentration of 1.5 percent weighted average. Because of their high spreadability, emulgels are simple to apply, which enhances the surface area accessible for drug penetration in turn. Generally speaking, spreadability values more than 7.5 cm indicate good spread ability, as demonstrated by new Formulations. (Bachhav & Patravale, 2010) Our Novel formulation and marketed formulation showed equal results.

Figure 18: Spread Ability Measurement Using Vernier Caliper

3.2.4Centrifugation

Centrifugation test is performed to check the oil and water separation. There is no phase separation was appeared in the marketed formulation and Novel formulation.

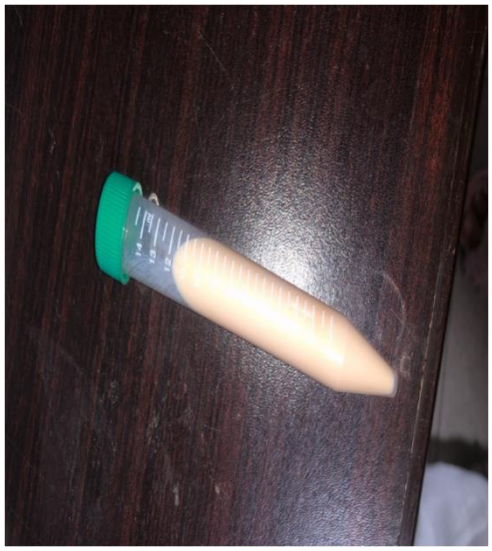


Figure19: Formulation after Centrifugation

3.2.5 Uniformity of Novel Formulation

The Novel formulation percentage was 96.5% with a RSD of 0.5%. This percentage shows uniformity of drug content.

This was the comparative analysis through physiochemical evaluation in which it was clearly seen, that there is no change in the marketed formulation and our Novel formulation.

Table 7: Physiochemical Evaluations

Formulation	Color	Homogeneity	Centrifugation	PH	Spread ability
Wintogeno	Creamy white	Homogeneous.	No, phase separation	6.00	9cm
Voltral gel	White	Homogeneous.	No, phase separation	7.02	9.5cm
Novel formulation		Homogeneous	No, phase separation	6.02	9cm

3.3 Shelf-Life

3.3.1 Freeze Thaw Cycle

In this study we checked the shelf-life of our product and the marketed formulation at different environmental conditions. There was no difference in aspect of Novel formulation before and after freeze thaw cycle. The below table shows the physical and chemical values of Novel formulation before and after twelve days of the freeze thaw cycle.

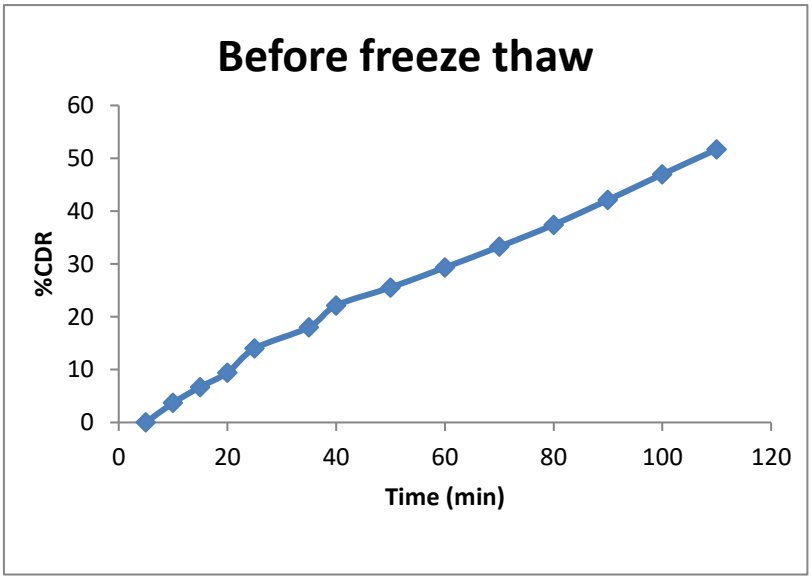


Figure 20a: Before Freeze thaw cycle

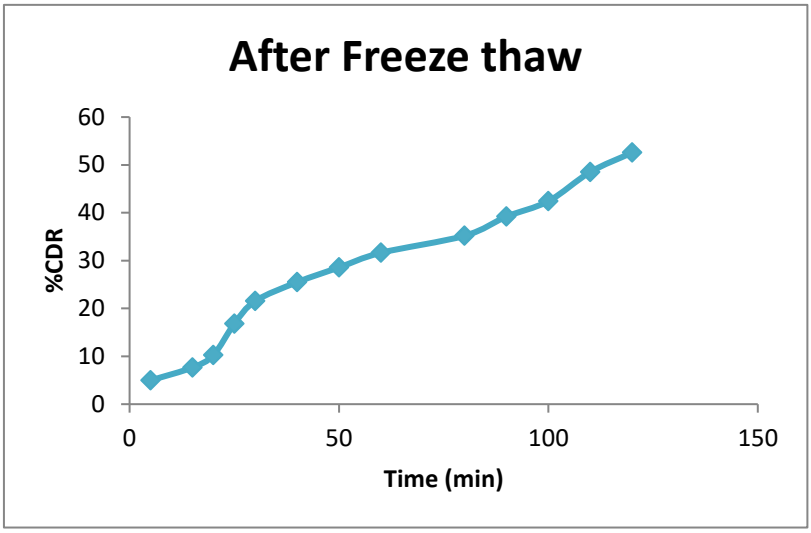


Figure 20b: After Freeze thaw cycle

Table 8: Results of evaluation of the preliminary stability of novel formulation before and after the freeze-thaw

Parameter	**Before freeze thaw results**	**After freeze thaw results**
PH	6.02	6.02
Viscosity (cPs)	122,400	122,400
Spread ability (cm)	9cm	9cm
Homogeneity	Homogeneous	Homogeneous
Drug release 2hr (%)	52.02±0.22	56.40±0.15
Centrifugation	No, phase separation	No, phase separation
P value	0.68	0.68
Significance	No	No

3.3.2 Stability studies

Table summarizes the optimized formulations' three-month stability statistics. Their look was similar at first and after three months in accelerated stability circumstances. These findings suggest that the prepared emu gels are physiochemical stable.

Table 9: Accelerated Stability Study of the Optimized Formulations

Formulation	Month	Color	PH	Viscosity	Drug Content (%)	%CDR	P Value	Significance
Cream	0	Light Orange	6	122,400	96.5±3.7	53±0.22	0.771	No
	1	Light Orange	6	-	65.8±3.7	52±0.22		No

Result: Physical appearance, viscosity, pH medication, and in-vitro drug release profile did not alter much. This demonstration showed that there was no significant drug degradation ($p>0.05$, student t test) over the study period, and the formulations were stable.

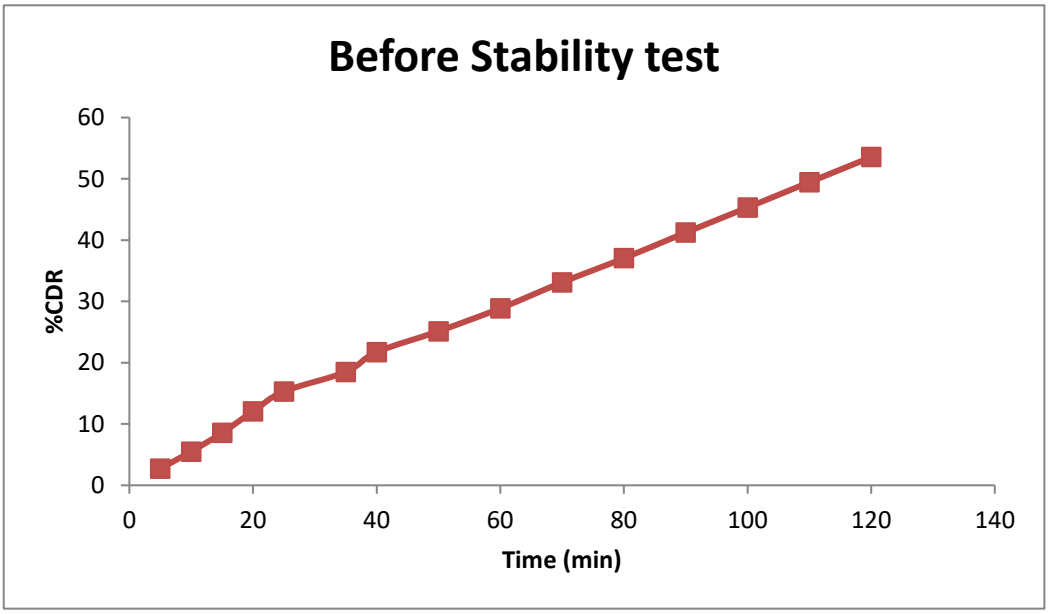


Figure 21a: Before Stability Test

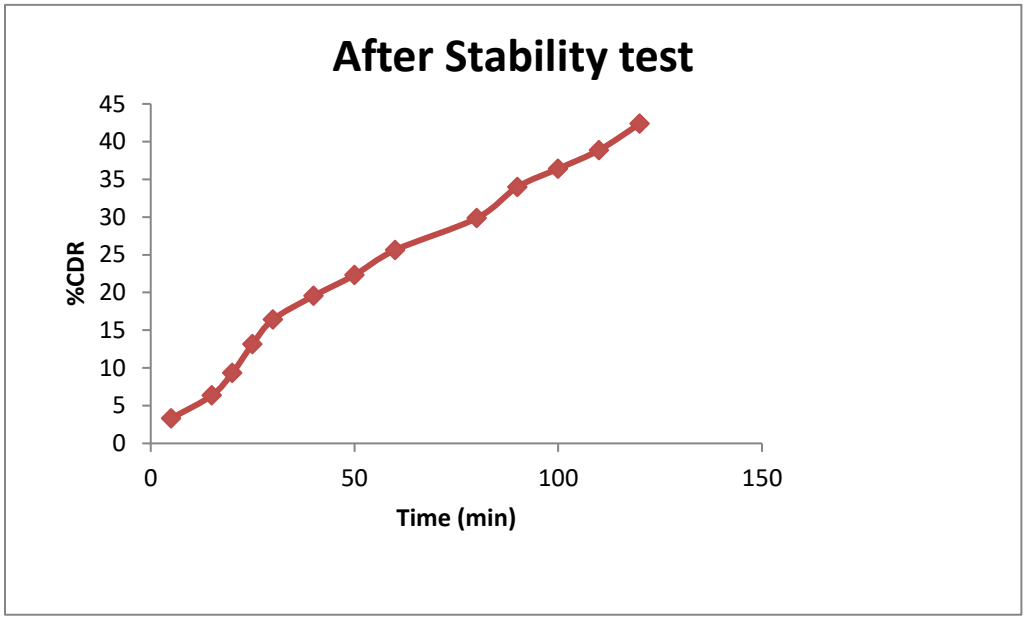


Figure 21b: After Stability Test

3.4 *IN VITRO* drug release study

Novel formulation penetration as a percentage of overall permeation is displayed. The durations of the releases were 1 hour and 2 hours, respectively. The Novel Formulation, which contained 0.5 percent w/w carbopol-934 and 11 percent w/w menthol, had a maximum drug release of 53 percent after two hours. Its release was aided by the presence of carbopol-934 and menthol in the formulation. Low polymer content causes a low viscosity and minimal flow resistance. (Hascicek, Bediz-Ölçer, & Gönül, 2009).On the other hand, high menthol concentrations resulted in dramatically increased drug release. Its strategy for improving drug release may be to create a Novel Formulation, which would increase solubility, or to work synergistically with glycerine in the formulation to increase drug-partition coefficient and hence overall release.(Murthy, 2020)(Sinha & Kaur, 2000)

In vitro result shows that Novel formulation release was more. When compare to other two formulations which shows that diffusion study result of Novel formulation was significant with that of marketed formulation.

Table 10: Data of Statistical Analysis of Emulgel

Emulgels	30 min	60 min	90 min	120 min
Wintogeno	8.31±0.82	7.34±0.81	26.69±0.79	15.33±1.45
Voltral	3.16±0.57	18.21±0.19	10.42±0.38	33.85±2.046
Novel formulation	18.64±2.01	29.02±3.27	41.39±4.28	53.53±4.97

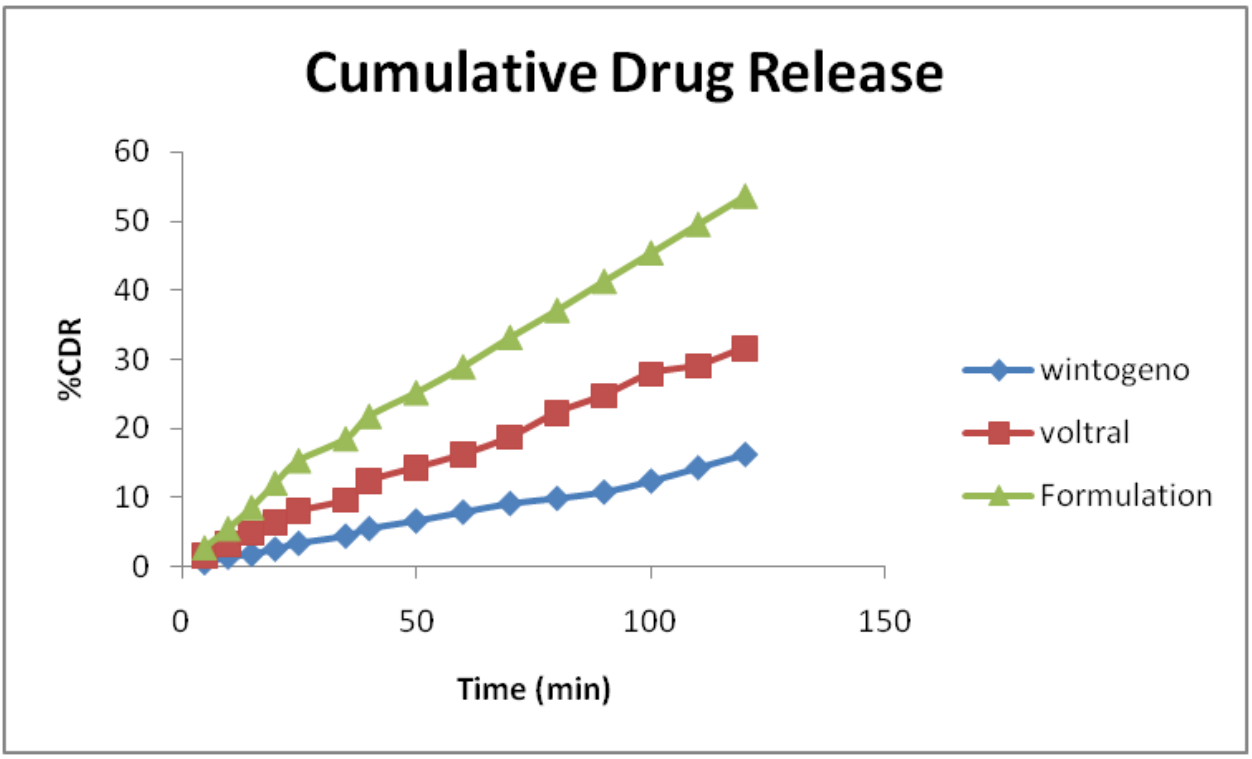


Figure 22: Drug release profile of the Formulation

3.5 *In Vivo* Studies

3.5.1 Analgesic test

Analgesic effectiveness of the test formulation was checked on the topical application by the tail flick method, as well as a standard medicine and a control sample. All test samples demonstrated substantial analgesic action, according to the data obtained after 15 minutes. With time, Novel Formulation demonstrated a progressive rise in analgesic activity.

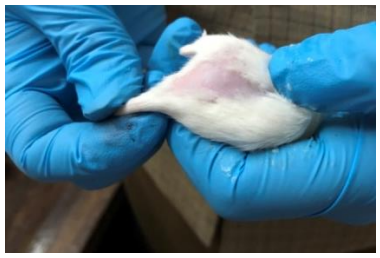


Figure 23a: Control (0-Hr)

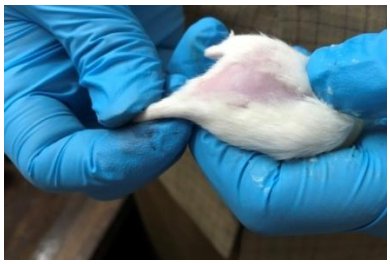


Figure 22b: Control (24hr)

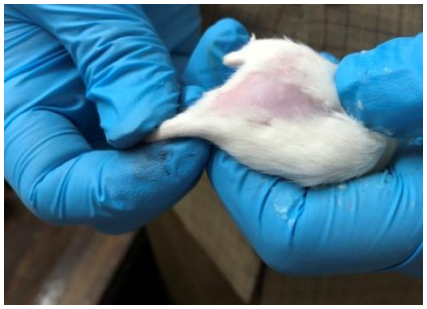


Figure 23c: Standard (0 Hr)

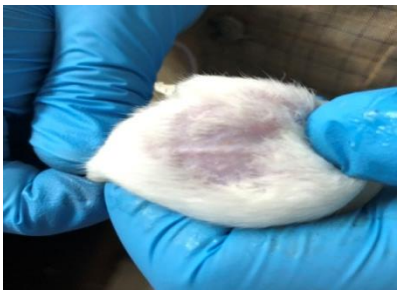


Figure 23d: Standard (24 Hr)

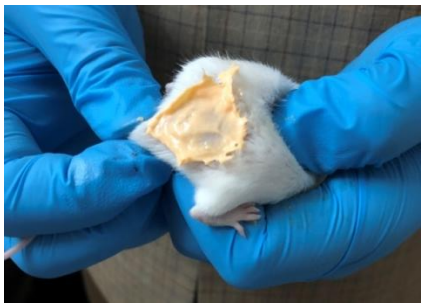


Figure 23e: Test (0 Hr)

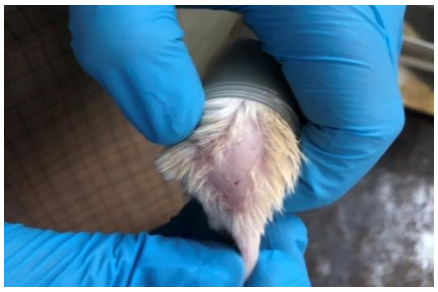


Figure 23f: Test (24 Hr)

Figure 23: Analgesic Test

Table 11: Study Design

1.Standard group	**2.Control group**	**3.Test group**
3 Mice	3 Mice	3 Mice
Wintogeno	Gel base Carbopol	Formulation

Table 12: Analgesic Activity of Test Form

Sr. #	**Sample**	**Pre drug reaction time (0 min)**			
		0min	30min	45min	60min
1	Blank	5	51%	46%	34%
2	Standard	4.7	62%	59.17%	64%
3	A	6	47.8%	66%	78%

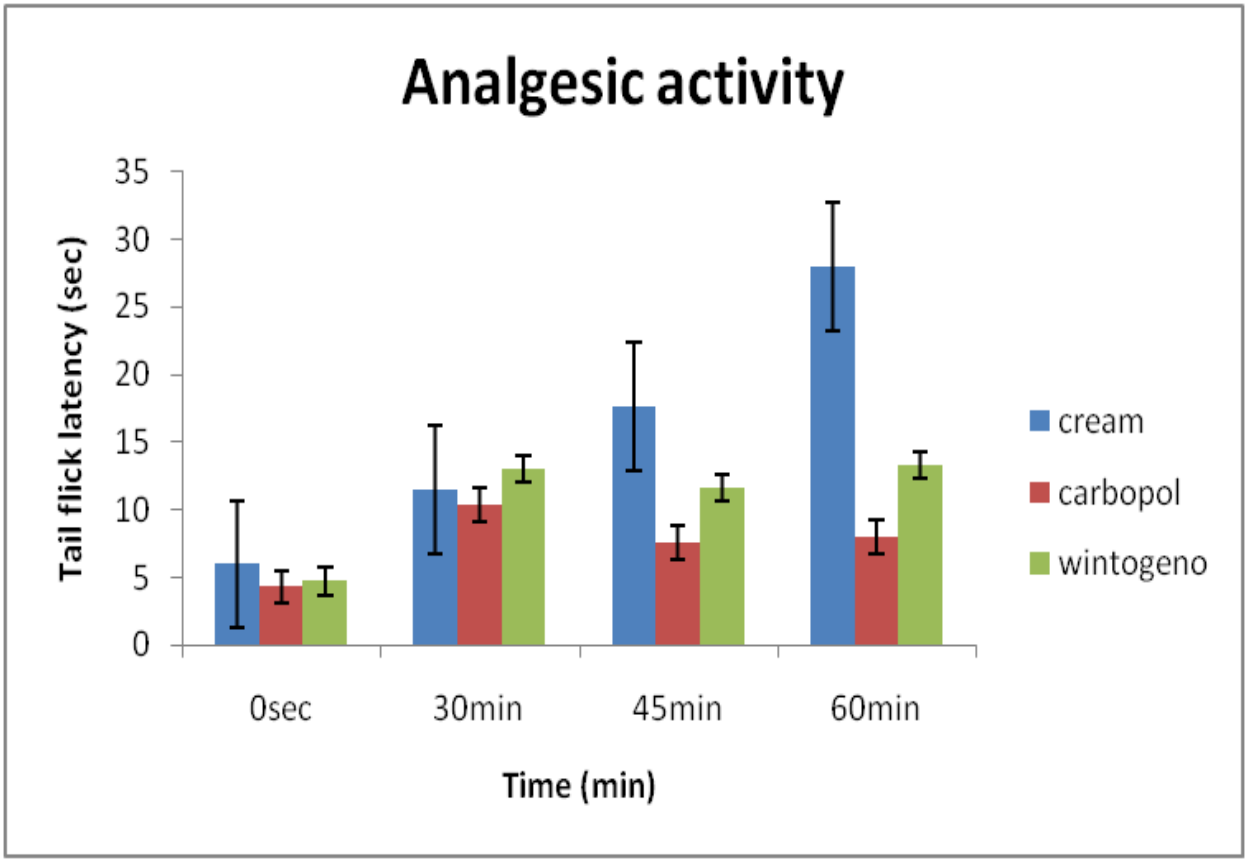


Figure 24: Analgesic activity test by tail flick method

3.5.2 Dermal irritant test

Skin irritation testing using a small layer of test and control samples demonstrated no harmful effects such as inflammation, itching, redness, irritation, skin infection or any other symptom on the test subject's skin harmful consequences. During the claimed monitoring period, all of the animals were healthy, active, and attentive, with typical behavior, and no mortality was detected. This demonstrates that none of the samples were harmful.

Table 13: Draize Scoring System

Oedema and Erythema formation (Draize scoring system)	
No oedema	0
Very slight oedema (barely perceptible)	1

Slight oedema (edge of area well defined by definite raising)	2
Moderate oedema (raised approximately 1mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible: 4	

Table 14: Oedema Dermal Irritant Test

Grading and time intervals (Results)			
Animals	Control group (24hr)	Standard group (24hr)	Test group (24hr)
1	0	0	0
2	0	0	0
3	0	0	0

Table 15: Erythema Dermal Irritant Test

Grading and time intervals (Results)			
Animals	Control group (24hr)	Standard group(24hr)	TestGroup(24hr)
1	0	0	0

2	0	0	0
3	0	0	0

3.6 Statistical Analysis

Short-term stability investigations were carried out for three months, and the results revealed that there was no statistically significant difference ($p>0.05$, student t test) in physical appearance, pH, viscosity, drug content, or in vitro release profile. The results of accelerated stability studies of the optimized formulation are presented in the following table.

Group 1	Voltral
Group 2	wintogeno
Group 3	Cream

Table 16: Data of Statistical Analysis

Comparison formulations	**At 30 min**		**At 60 min**		**At 90 min**		**At 120 min**	
-	P value	Significance	P value	Significance	P value	Significance	P value	Significance
Group 1 vs. group 2	0.009	Yes	0.004	Yes	0	Yes	0.003	Yes
Group 2 vs. group 3	0.012	Yes	0.017	Yes	0.018	Yes	0.01	Yes
Group 3 vs. group 1	0.023	Yes	0.08	No	0.071	No	0.042	Yes

Conclusion

According to the results obtained from this study, it was concluded that the Novel Formulation was successfully incorporated into the carbopol formulation to obtain a gel. The Novel formulation possessed good physiochemical properties with optimum amount of drug release with good stability. In-vitro drug release study confirmed that the topical delivery of novel formulation is better than the marketed product. No interaction was observed among the drugs/excipients in FTIR spectrum. In-vivo studies showed analgesic activity. Therefore, the novel formulation could be a promising alternative for the topical treatment. However, it can be commercialized after successful pre-clinical and clinical Trials

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