FORMULATION AND EVALUATION OF TOPICAL MELOXICAM EMULGELS

ALEX NJOROGE MWANGI

U53/6896/2017

A DISSERTATION RESEARCH SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF PHARMACY IN INDUSTRIAL PHARMACY OF THE UNIVERSITY OF NAIROBI

DECLARATION OF ORIGINALITY

Name of student	Alex Njoroge Mwangi
Registration No:	U53/6896/2017
College	Health Sciences
School	Pharmacy
Department	Pharmaceutics and Pharmacy Practice
Course Title	Master of Pharmacy in Industrial Pharmacy
Title of project	Formulation and Evaluation of Topical Meloxicam Emulgels

DECLARATION

1. I understand what plagiarism is and I am aware of the University's policy in this regard.

2. I declare that this dissertation is my original work and has not been submitted anywhere for examination, award of a degree or publication. Where other people's work has been used, it has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

3. I have not used the services of any professional agencies to produce this work.

4. I have not allowed and shall not allow anyone to copy my work with the intention of passing it off as his/her work.

5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with the plagiarism policy of the University of Nairobi.

Signature.......................................Date......................................

Alex N. Mwangi

SUPERVISORS' APPROVAL

This dissertation research has been submitted with our approval as the University supervisors.

Signature…………………………….Date……………………….

Dr. Shital Maru Mahindra, PhD

Chairperson, Department of Pharmaceutics and Pharmacy Practice,

School of Pharmacy,

University of Nairobi

Signature…………………………….Date……………………….

Dr. Peter M. Njogu, PhD

Lecturer, Department of Pharmaceutical Chemistry,

School of Pharmacy,

University of Nairobi

DEDICATION

Dedicated to my family and friends

TABLE OF CONTENTS

DEFINITION OF TERMS

Formulation – the design and development of a product

Evaluation – thorough assessment of a product to determine its characteristics. In the case of a medicinal product, this will include determination of pharmaceutical and pharmacological profile

Topical – refer to skin as the route of administration and not necessarily the targeted system for drug delivery

Meloxicam – is a non-steroidal anti-inflammatory drug which falls under the oxicams class

Emulgel – is a combined dosage form of both an emulsion and a gel

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

API	–	Active pharmaceutical ingredient
AS	–	Ankylosing spondylitis
BCS	–	Biopharmaceutical classification system
BP	–	British Pharmacopoeia
COX	–	Cyclooxygenase
DMARDs	–	Disease modifying anti-rheumatic drugs
FD	–	Franz diffusion
FTIR	–	Fourier transform infrared
GIT	–	Gastro intestinal tract
h	–	Hour(s)
HPLC	–	High performance liquid chromatography
ICH	–	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IR	–	Infrared
IUPAC	–	International Union of Pure and Applied Chemistry
JIA	–	Juvenile idiopathic arthritis
JRA	–	Juvenile rheumatoid arthritis
KBr	–	Potassium bromide
KH_2PO_4	–	Potassium dihydrogen orthophosphate
NaOH	–	Sodium hydroxide
NSAID	–	Non-steroidal anti–inflammatory drug
NMT	–	Not more than
OA	–	Osteo arthritis
PsA	–	Psoriatic arthritis
qs	–	*Quantum satis*; amount which is enough
RA	–	Rheumatoid arthritis
RSD	–	Relative standard deviation
SC	–	Stratum corneum
TNF	–	Tumor necrosis factor
TRPV1	–	Transient Receptor Potential cation channel subfamily V member 1
USP	–	United States Pharmacopeia

ABSTRACT

Background: Meloxicam is one of the non-steroidal anti-inflammatory drugs (NSAIDs) and is commonly used for the symptomatic treatment of pain and inflammation associated with rheumatic diseases such as osteoarthritis. Meloxicam and other NSAIDs are predominantly available as oral dosage forms. In the recent past, topical dosage forms of NSAIDs are on the rise especially for management of chronic conditions, as they have reduced gastrointestinal, cardiovascular and other systemic side effects associated with oral medications. Consequently, they have resulted in improved patient compliance. From a survey of literature to date, there is no topical meloxicam product in the global market, whether as a gel or as an emulgel. This study therefore sought to formulate and evaluate emulgels of meloxicam for topical application, as an alternative product for management of rheumatic diseases.

Methods: This research study employed a 3^2 factorial design and utilized both quantitative and qualitative approaches. Carbopol 934 and menthol were the two factors under study as gelling agent and penetration enhancer, respectively. The Design Expert® software was used to analyze their relationship with four responses namely viscosity, spreadability, cumulative drug permeation at 1 h and cumulative drug permeation at 8 h. The software was also used to decrypt an optimized formulation. Drug excipient compatibility studies were conducted using fourier transform infrared spectroscopy.

Results and Discussion: Meloxicam active pharmaceutical ingredient and excipients used in the emulgels were found to be compatible. All formulations were translucent, homogenous and with no observable grittiness or phase separation upon visual examination. Their pH was between 5.7 (F7) and 6.5 (F2), viscosity between 20426 mPa.s (F1) and 42336 mPa.s (F8), spreadability between 7.0 cm (F8) and 9.9 cm (F10) and the percentage drug content was between 90.7 (F7) and 109.9 (F9). The order of percentage cumulative drug permeation after 1 h and after 8 h from the highest to the lowest was: F11>F10>F3>F2>F1>F9>F6>F5>F8>F4>F7 and F11>F10>F3>F2>F1>F6>F5>F9>F8>F4>F7, respectively.

Conclusion: This study has shown that it is possible to formulate meloxicam emulgels that have high pharmaceutical quality and are pharmacologically active. Further optimization could potentially provide a safe and efficacious alternative for symptomatic treatment of pain and inflammation associated with rheumatic diseases as well as other inflammatory conditions.

CHAPTER ONE: INTRODUCTION

1.0 INTRODUCTION

The title of this study is 'formulation and evaluation of topical meloxicam emulgels'. This chapter introduces the orientation of the research. The following is discussed: background information, problem statement, research objectives, study justification as well as study delimitation.

1.1 BACKGROUND

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID). The NSAIDs are globally given for the symptomatic management of pain as well as inflammation associated with rheumatic diseases such as rheumatoid arthritis (RA), osteo-arthritis (OA), ankylosing spondylitis (AS) and juvenile rheumatoid arthritis (JRA) among others (Au et al., 2014; Syngle, 2006). The NSAIDs are predominantly available as oral dosage forms and thus taken orally. Since they pass through the gastrointestinal tract (GIT) into systemic circulation, they undergo first pass hepatic metabolism that reduces systemic exposure and also cause undesirable GIT side effects which include nausea, vomiting, diarrhoea, heartburn and ulcers, among others. In addition, cardiovascular and other systemic side effects are more pronounced (Wongrakpanich et al., 2018).

Topical dosage forms of NSAIDs are on the rise especially for management of chronic conditions, as they have reduced GIT, cardiovascular and other systemic side effects, and they have proven to be safe and efficacious. Consequently, they have resulted in improved patient compliance (da Silva and Woolf, 2010). Topical formulations available in the market are mostly gels and include diclofenac, ibuprofen, ketoprofen, naproxen and piroxicam among others. Emulgels of NSAIDs are very rare and it is only one brand of diclofenac emulgel that is currently registered in Kenya. From a survey of literature to date, there is no topical meloxicam product in the global market, whether as a gel or as an emulgel.

An emulgel is a combined dosage form of both an emulsion and a gel. It is a better formulation with numerous advantages of both a gel and an emulsion. They include ability to incorporate both hydrophilic and hydrophobic drugs, enable controlled release of drugs, improved formulation stability, reduced cost of production and they are more aesthetically appealing since

they are emollient, thixotropic, spread and removed with ease, bio-friendly, not greasy, do not stain and their appearance is more pleasing (Verma et al., 2018; Nikumbh et al., 2015).

To improve their efficacy, NSAIDs may be co-formulated with rubefacients. Rubefacients are substances that cause irritation and reddening of the skin upon topical application, since they cause capillary dilation and increased blood flow to the skin. They are commonly used to temporarily relieve minor pain that is related to arthritis, back ache, muscle strains, sprains, bruises and stiffness. Examples of rubefacients include capsaicin, menthol, methyl salicylate, camphor and isopropanol among others (Jorge et al., 2010; Moss et al., 2014).

1.2 PROBLEM STATEMENT

For symptomatic management of inflammation and acute or chronic pain associated with several types of rheumatic diseases and muscle pains, topical NSAIDs are preferred over oral NSAIDs. This is because they cause no or reduced GIT side effects as they mostly have localized activity and undergo minimal systemic circulation. Rubefacients are also commonly used to alleviate such pain.

Compared to meloxicam, most of the NSAIDs used in the available topical formulations are less potent and have a shorter duration of action with more side effects. Most formulations available are gels and currently it is only one topical NSAID emulgel, diclofenac emulgel that is registered in Kenya. From a survey of literature to date, there is no topical meloxicam product in the global market, whether as a gel or as an emulgel. A combination of meloxicam and rubefacients is also non-existing in the market.

Consequently, less potent products with shorter duration of action and more side effects are being used. Most of these products contain a single active pharmaceutical ingredient (API) and therefore lack the improved efficacy as a result of drug regimen combination. There is therefore a need to develop alternative products to alleviate these shortcomings. This will in turn help improve patients' compliance and adherence to medications prescribed, therapeutic usefulness of medicaments and thereby improve patients' quality of life.

This study therefore sought to formulate and evaluate emulgels of meloxicam in combination with rubefacients, for topical application. It was hoped that the emulgels would be successfully developed and characterized, and that they would be safer, more efficacious and of higher quality than the existing alternatives.

2

1.3 STUDY JUSTIFICATION

It was expected that this study would provide a procedure for formulating and evaluating meloxicam emulgels as well as an emulgel containing both meloxicam and capsaicin for enhanced activity. The study findings could potentially provide a safe and efficacious alternative for symptomatic management of pain along with inflammation linked to arthritis as well as other musculoskeletal pains. A better alternative can in turn lead to improved patients' compliance to medication and thereby improve their quality of life.

The study findings were disseminated through a power-point presentation to the Department of Pharmaceutics and Pharmacy Practice, School of Pharmacy, University of Nairobi. In addition, hardcopies of the dissertation were deposited in the same Department and also in the university library for future reference. Further, key findings of this study will be published in a peer-reviewed journal.

Furthermore, this study related to one of the priorities of the country, of providing quality and affordable healthcare to all citizens. Thus, potential beneficiaries of this study would also include the local and global pharmaceutical manufacturers.

1.4 RESEARCH OBJECTIVES

1.4.1 Broad objective
The broad objective of this study was to formulate meloxicam emulgels for topical application and evaluate them pharmaceutically for quality and compliance with compendial requirements as well as pharmacologically for efficacy.

1.4.2 Specific objectives
This research project had three specific objectives and these were;

1. Preformulation studies on meloxicam to determine its suitability for formulation as an emulgel.
2. Formulation of meloxicam emulgels and a combination emulgel containing both meloxicam and capsaicin.
3. Evaluation of the formulated emulgels using *in vitro* and *in vivo* methods.

1.5 STUDY DELIMITATION

This research work focused on formulation of 0.5%w/w meloxicam emulgels. Capsaicin was incorporated as a rubefacient in formulation of the combination emulgel at a concentration of 0.025% w/w. Other concentrations were not covered. *In vivo* evaluation of potential skin irritation by the formulated emulgels was not done.

CHAPTER TWO: LITERATURE REVIEW

2.0 INTRODUCTION

This section deliberates on common rheumatic diseases along with their management, as well as NSAIDs dosage forms available for treatment of rheumatic diseases. In addition, key formulation and evaluation aspects of emulgels are discussed.

2.1 RHEUMATIC DISEASES

Rheumatology is the study of the diagnosis and treatment of rheumatic diseases. Rheumatic diseases, sometimes referred to as musculoskeletal diseases, are a group of diseases that affect the joints, bones, muscles, tendons, ligaments and other connective tissues (Syngle, 2006). These diseases have common signs and symptoms which include pain especially in the joints, degeneration and inflammation that is characterized by reddening, swelling and warmth in the affected areas (Syngle, 2006).

There are more than 200 rheumatic diseases and the common ones are the different types of arthritis and spondyloarthropathies, gout, scleroderma, lupus, inflammatory bowel disease and polymyalgia rheumatica. Arthritis is a portmanteau that is derived from two Greek words, '*arthron*' and '*itis*' and they mean "joint" and "inflammation" respectively. It is commonly used to refer to various conditions characterized by pain and inflammation of the joints (Syngle, 2006) such as OA, RA and JRA among others. Common spondyloarthropathies include ankylosing spondylitis (AS) and psoriatic arthritis (PsA) (Maruotti et al., 2014).

Several factors are acknowledged as causes of rheumatic diseases. A good number of the diseases are attributed to idiopathic causes. For those with known causative factors, most are caused by autoimmune disorders. Others are as a result of genetic disorders and exposure to environmental influences such as cigarette smoke and pollution. The risk of getting rheumatic disease is usually higher in women than in men.

Rheumatoid arthritis is a chronic autoimmune inflammatory ailment which mostly attacks the synovial tissue. Its symptoms include chronic pain, irreversible joint damage, stiffness and functional impairment. It is more prevalent in women than men (Syngle, 2006). Osteoarthritis is the most common type of chronic arthritis which results from deterioration of the articular cartilages. Unlike most rheumatic diseases, it is not an autoimmune disorder. The risk factor of

OA is age-related and is higher in the elderly above 55 years (Zatarain, 2007). Juvenile rheumatoid arthritis refers to conditions that have chronic arthritis in common. It is the most common type of arthritis in children and its cause is unknown. As such, it is also referred to as juvenile idiopathic arthritis (JIA) (Lovell, 2008).

Ankylosing spondylitis is 'a form of spondyloarthropathy associated with chronic inflammation of sacroiliac joints and the spine as well as some extraspinal lesions involving the eye, bowel and the heart' (Van der Heijde, 2008). Ankylosis refers to the fusion of the spinal joints, either partially or wholly to form a single unit. Psoriatic arthritis, also a spondyloarthropathy, is related to psoriasis (Au et al., 2014; Klippel et al., 2008). Psoriasis is a noncontagious disease that mainly manifests on the skin as flaky patches that are reddish and get inflamed on scratching. Other than psoriasis, PsA also has various musculoskeletal and dermatological manifestations (Coates and Helliwell, 2017).

2.2 MANAGEMENT OF RHEUMATIC DISEASES

2.2.1 Treatment modalities

Management of rheumatic diseases can be broadly divided into two categories, which are pharmacologic and nonpharmacologic therapies. Each disease follows a customized management strategy which most of the times involves combination of both pharmacologic and nonpharmacologic therapies. Early diagnosis and treatment of a disease is likely to result in a better long-term prognosis.

2.2.2 Pharmacological modalities

Pharmacologic therapy comprises several drugs which include disease modifying anti-rheumatic drugs (DMARDS), biologics, NSAIDS, analgesics, rubefacients, glucocorticoids, colchicine and allopurinol. The DMARDs are a group of diverse unrelated pharmacological compounds and exert anti-inflammatory, immunomodulatory or immunosuppressive effects. Such agents include methotrexate, azathioprine, hydroxychloroquine, sulfasalazine and leflunomide (Coates and Helliwell, 2017; Van der Heijde, 2008). Biologics are sometimes also classified as biologic DMARDs. They include tumor necrosis factor (TNF) inhibitors (e.g. etanercept, adalimumab and golimumab), interleukin inhibitors (e.g. anakinra and tocilizumab) and B-cell-directed therapy agents (e.g. rituximab) (Coates and Helliwell, 2017; Van der Heijde, 2008; Syngle, 2006).

The NSAIDs are the most commonly used agents as adjuvants to the rheumatic disease-modifying agents. They produce a mild to moderate anti-inflammatory and analgesic effect by inhibiting cyclooxygenase (COX) enzymes which catalyse formation of pro-inflammatory prostaglandins. Selective NSAIDs such as celecoxib inhibit COX-2 enzymes whereas nonselective NSAIDs inhibit both COX-1 and COX-2 enzymes and are hence associated with more side effects. They include indomethacin, diclofenac, ibuprofen, naproxen, piroxicam and meloxicam (Moss et al., 2014; Crofford, 2013).

Analgesics relieve pain associated with rheumatic diseases and most have no anti-inflammatory effect. They include tramadol, codeine, dextropropoxyphene and paracetamol. Lidocaine, an anaesthetic agent, and tricyclic antidepressants such as amitriptyline and imipramine are used in some cases to relief pain (da Silva and Woolf, 2010). Rubefacients are used as analgesics for relief of minor acute or chronic pain. Examples include capsaicin, menthol, methyl salicylate, camphor and isopropanol (Mózsik, 2014).

The glucocorticoids exert marked anti-inflammatory effect by inhibiting inflammatory mediator gene transcription. Examples include prednisone, methylprednisolone, hydrocortisone, triamcinolone and dexamethasone (Au et al., 2014; da Silva and Woolf, 2010). Colchicine and allopurinol are used for management of gouty arthritis.

2.2.3 Nonpharmacological modalities

Nonpharmacologic therapies are those that do not utilize drugs and they include surgery, exercise, rest, physiotherapy, occupational therapy, acupuncture, counseling and patient education. Surgical procedures such as synovectomy, total joint replacement, e.g., knee or hip joints and joint fusion may be done to either reduce pain or improve function. Exercise is advocated to help prevent or correct muscle atrophy as this can exert pain relief. Exercise also helps in weight reduction which may be of benefit. In severe pains and in some conditions, resting is usually encouraged. These include adequate sleep and avoiding fatigue (Moss et al., 2014).

Physiotherapy and occupational therapy help improve joint mobility and muscle strength, especially for patients with compromised activities of daily living (Lovell, 2008; Scalapino and Davis Jr., 2003). Acupuncture is a traditional technique that originated from China. This is where needles are inserted into inflamed and paining tissues to help alleviate these symptoms (Moss et al., 2014). Stressed patients are counseled on stress management as it helps improve

psychological well-being. Patients are also educated on techniques they can apply to reduce excessive loading on joints and these may involve use of assistive devices such as ambulation aids. This helps alleviate pain and inflammation as well as prevent disease progression (Zatarain, 2007).

2.2.4 Meloxicam

2.2.4.1 Description

Meloxicam is an NSAID and falls under the oxicams class (Xu et al., 2014). The international union of pure and applied chemistry (IUPAC) name of meloxicam is '4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H* -1, 2-benzothiazine-3-carboxamide 1, 1- dioxide'. It inhibits COX-2 more preferentially than COX-1, even though it is not exclusively a COX-2 selective inhibitor, and is used to manage mild to moderate pain as well as inflammation related to rheumatic diseases such as RA, OA, JRA and AS (Euller-Ziegler et al., 2001). The recommended oral daily dose of meloxicam is 7.5 – 15mg.

2.2.4.2 Physicochemical properties

The chemical formula of meloxicam is $C_{14}H_{13}N_3O_4S_2$ and its chemical structure is shown in *Figure 2.1*. The drug substance is pale yellow in colour, with a molecular weight of 373.377 g/mol and a water solubility of 7.15 mg/L which shows that it is poorly soluble in water. It complies with the Lipinski's rule of five since it has a molecular weight of less than 500, two hydrogen bond donors, five hydrogen bond acceptors and a Log P of 3.43 (Lipinski, 2000). It is a zwitterion with two predicted pKa of 4.47 and 0.47.
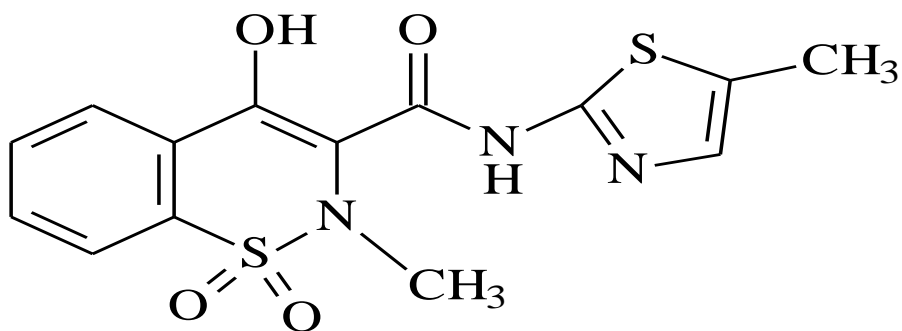
***Figure 2.1:* Chemical structure of meloxicam**.

2.2.4.3 Pharmacokinetics profile

Meloxicam is well absorbed upon oral administration and has an absolute oral bioavailability of 89%. Its volume of distribution is 10 L and it is usually 99% protein bound, primarily in albumin. In the body, meloxicam is practically fully metabolized by the cytochrome P450 (CYP450) isozymes into four inactive metabolites with 5'-carboxy meloxicam being the major metabolite. Meloxicam is mainly excreted in the urine and faeces, primarily in the form of inactive metabolites. Its half-life is between 15–20 h.

2.2.4.4 Pharmacodynamics profile

Meloxicam has analgesic, anti-inflammatory and antipyretic properties. These effects are thought to be due to its inhibition of COX (also called prostaglandin synthetase) enzymes at the site of pain and inflammation and thereby inhibiting prostaglandins biosynthesis. Prostaglandins contribute to joints inflammation and also sensitize pain receptors. Therefore, inhibition of their synthesis may be associated with reduced inflammation, analgesic and antipyretic properties.

2.2.4.5 Biopharmaceutical classification profile

The biopharmaceutical classification system (BCS) is a scheme that groups drugs into four categories primarily depending on their solubility and permeability parameters, and also takes into account pH and dissolution factors (Benet, 2013; Sachan et al., 2009; Amidon et al., 1995). The BCS correlates *in vitro* profiles of drug products to their *in vivo* bioavailability and thus help predict their *in vivo* performance. Drugs in BCS class I have both high solubility and permeability, drugs in BCS class II have low solubility and high permeability, drugs in BCS class III have high solubility and low permeability while drugs in BCS class IV have both low solubility and permeability (Charalabidis et al., 2019; Cardot et al., 2016; Amidon et al., 1995). Based on the BCS, meloxicam drug is classified in BCS class II since it has high permeability and low solubility.

2.2.4.6 Comparison with other NSAIDs

Meloxicam is more potent than most NSAIDs. A once daily dose of 7.5–15 mg per oral is sufficient to elicit desired effect in adults. In the case of ibuprofen for example, 400 – 600 mg three to four times a day is recommended. Its half-life of about 20 h is also long and therefore it has a longer duration of action (da Silva and Woolf, 2010).

In addition, meloxicam inhibits COX-2 isozymes more preferentially than COX-1 isozymes. Since inhibition of COX-1 enzymes is usually linked to most NSAIDs adverse effects,

meloxicam has less severe adverse effects and thus a better safety profile. A study by Hosie et al supports this (Hosie et al., 1996). The common adverse effects associated with oral intake of NSAIDs include GIT effects such as dyspepsia, gastro duodenal ulcers and GIT bleeding/ perforation, cardiovascular effects such as oedema, hypertension, stroke and myocardial infarction, and nephrotoxicity effects such as electrolyte imbalance, sodium retention and chronic kidney disease (Wongrakpanich et al., 2018). Oral meloxicam is a popular drug and was ranked number 35 in the top 200 drugs prescribed in the United States in 2018 (Fuentes et al., 2018).

2.2.5 Capsaicin

2.2.5.1 Description
Capsaicin is a rubefacient that naturally occurs in chili peppers. Its IUPAC name is '(6*E*)-*N*-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methylnon-6-enamide'. It is mostly used as a topical analgesic in conditions such as arthritis, backaches, muscle cramps, bruises and other painful musculoskeletal conditions.

2.2.5.2 Physicochemical properties
The chemical formula of capsaicin is $C_{18}H_{27}NO_3$ and its chemical structure is shown in *Figure 2.2*. Capsaicin is insoluble in cold water and its melting point is 65 °C while its boiling point is 210-220 °C. It also complies with the Lipinski's rule of five with a molecular weight of 305.41 g/mol, a predicted hydrogen acceptor count of three and donor count of two, and a predicted Log P of 3.75. Its two predicted pKa values are 9.93 and -0.52.
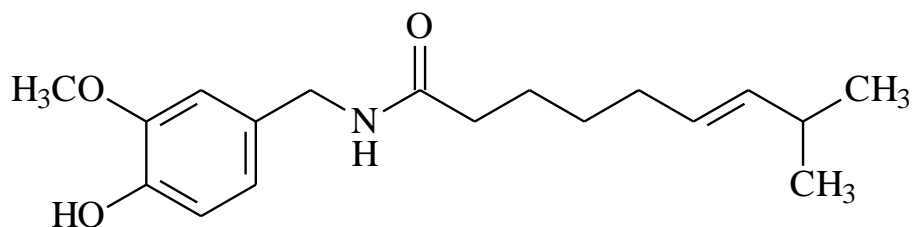
Figure 2.2: **Chemical structure of capsaicin**.

2.2.5.3 Pharmacokinetics profile
Oral and intravenous pharmacokinetics information of capsaicin in humans is scanty. Following topical application, it is well absorbed through the skin but it barely reaches systemic circulation. Information on its metabolism is also limited, but *in vitro* studies with human hepatic

microsomes suggest potential hepatic metabolism. Cytochrome P450 isozymes are believed to play a role. Topical capsaicin undergoes slow biotransformation and most of it remains unchanged. *In vivo* animal studies done suggest that capsaicin mainly undergoes renal excretion.

2.2.5.4 Pharmacodynamics profile

Capsaicin is an agonist of capsaicin receptors which are expressed in a subgroup of primary afferent nociceptive neurons (Mózsik, 2014). Capsaicin receptors are also known as 'transient receptor potential cation channel subfamily V member 1 (TRPV1) or vanilloid receptor 1'. TRPV1 is a receptor-ion channel complex that is usually stimulated by high temperatures above $43^{o}C$, pH of less than 6, endogenous lipids and also by other receptor agonists such as capsaicin which is able to cause persistent activation of these receptors (O'Neill et al., 2012). Their activation sends impulses to the central nervous system which bring about capsaicin effects that include warming, itching, stinging and burning (Jorge et al., 2010).

2.3 FORMULATED NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

2.3.1 Dosage forms available

Even though they exhibit their action in a similar mechanism of inhibiting prostaglandins synthesis, NSAIDs vary in their time to onset of effect, minimum effective concentration, duration of action, maximum tolerated dose, potency and severity of side effects. These parameters also fluctuate depending on the dosage form used. In addition, it has been noted that the efficacy and tolerance of different NSAIDs vary substantially from one person to another, although the mechanism is not clearly understood. It is for these reasons that patients are encouraged to test a few products in order to identify one that has the preferred benefit to risk ratio (da Silva and Woolf, 2010). Depending on the route of administration, NSAIDs dosage forms available in the market to date can be broadly classified into three: oral, parenteral and topical dosage forms (Crofford, 2013).

2.3.2 Oral dosage forms

Oral dosage forms are the most commonly used dosage forms of NSAIDs. Diclofenac, ibuprofen, indomethacin, naproxen, aspirin, piroxicam, meloxicam, nabumetone and celecoxib tablets or capsules are a few examples regularly found in the market. Their release from the drug product is either immediate or controlled. They cause amplified GIT and also systemic side effects especially cardiovascular and renal adverse effects. They also undergo first-pass hepatic metabolism (Wongrakpanich et al., 2018).

2.3.3 Parenteral dosage forms

Solutions for parenteral use dominate in this category. Examples include diclofenac and meloxicam solutions. They are commonly used in cases of acute pain or when a patient is not in a position to take oral medication, for example if unconscious. Like oral medication, they also cause systemic side effects. However, they do not undergo first-pass hepatic metabolism.

2.3.4 Topical dosage forms

Topical dosage forms of NSAIDs available are formulated as patches, suppositories, sprays, solutions, gels, emulsions (mostly creams), ointments and emulgels (Crofford, 2013; McPherson and Cimino, 2013; Jorge et al., 2010). Diclofenac patch is a good example of patches in the market. Patches are able to achieve local or systemic drug delivery and can allow for sustained release of the drug (Kumar et al., 2013). The most commonly used topical preparations are the semi-solid dosage forms (Chang et al., 2012), and the discussion on topical dosage forms has a bias towards them.

Emulsions are mixtures of two immiscible liquids that are thermodynamically unstable and are stabilized by addition of emulsifiers to exhibit acceptable shelf life at room temperature. One liquid, the internal or dispersed phase, is usually dispersed in another, the continuous phase. Where oil is dispersed in water, this is called an O/W emulsion and where water is dispersed in oil, a W/O emulsion is formed. Emulsions can contain both hydrophilic and hydrophobic drugs but are less stable (Arora et al., 2017). A gel is a two-component semi-solid system in which a liquid phase/system is constrained in a 3-dimensional polymeric matrix system. Gels are more stable and aesthetically appealing. Hydrogels are the most common but they can only contain hydrophilic drugs (Pednekar et al., 2015). Combining emulsions and gel dosage forms begets emulgels, which is a better formulation with numerous advantages of both a gel and an emulsion (Verma et al., 2018).

Compared to oral or parenteral forms, topical NSAIDs are associated with negligible GIT, and much reduced cardiovascular, renal and other systemic side effects. Their effects are mostly localized and at the peripherals since they scarcely get to systemic circulation. Moreover, their bioavailability is not affected by first pass hepatic metabolism and their safety and efficacy in relieving pain and inflammation has been proven (Crofford, 2013; Haroutiunian et al., 2010). They have an extra benefit of patients' involvement as they are required to apply on the localized part of their body that is in pain and this gives them a grander control over their condition (da

Silva and Woolf, 2010). Consequently, their use has brought about improved patient's compliance and no wonder their popularity in the market has been on the rise.

Topical NSAIDs are applied on the skin and have to penetrate through it to the target site in order to elicit desired effect. Their effects can be localized, systemic or both. It is therefore paramount to understand the skin anatomically and physiologically. Skin is the largest organ of the body and also the most easily accessible (Chen and Gao, 2016). It has four distinct layers, as shown in *Figure 2.3*. Stratum corneum (SC, also referred to as 'non-viable epidermis') is the outer most layer and forms the chief barrier to drug penetration. The drug also has to be transported through the viable epidermis, the dermis and the subcutaneous layers in order to be absorbed into systemic circulation or access the deeper tissues like bones and muscles (Singla et al., 2012). The SC is principally lipophilic and is better traversed by lipophilic and unionized drugs whereas viable epidermis is mostly hydrophilic and is better traversed by hydrophilic drugs. A drug with both lipophilic and hydrophilic properties is likely to achieve optimal penetration; otherwise it will be poorly absorbed unless its formulation is optimized (Haroutiunian et al., 2010).
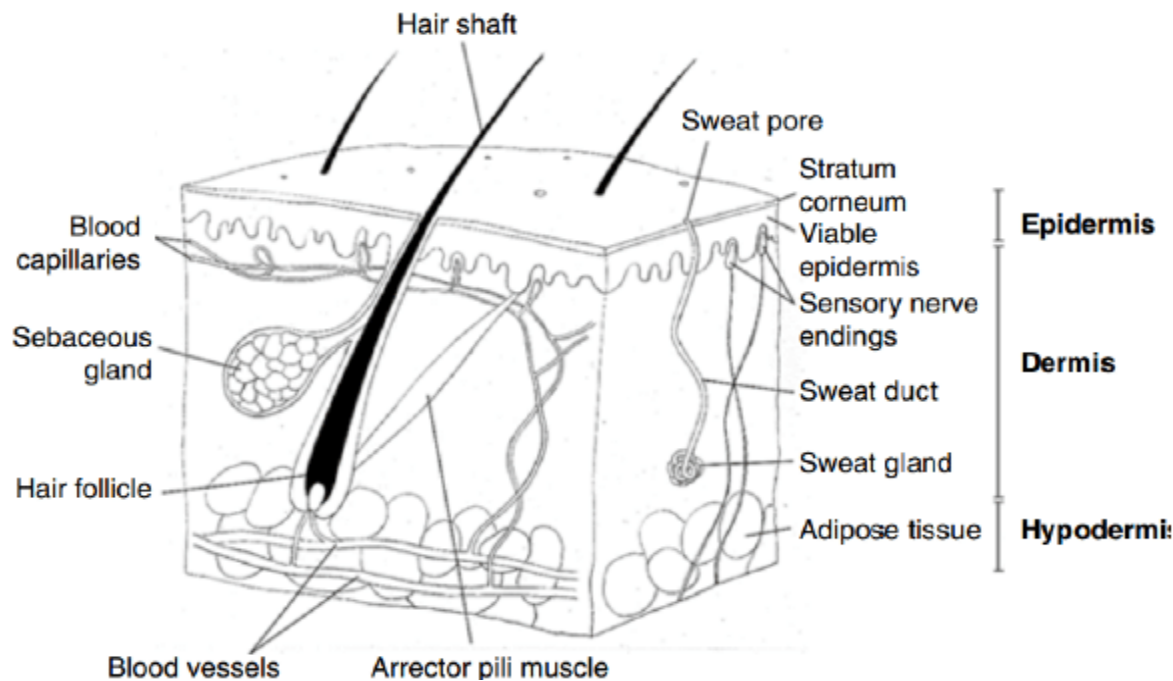
Figure 2.3: **An illustration of the skin layers**.

Other than lipophilicity of a drug, there are other properties that can affect its suitability for formulation as a topical agent. They include its molecular weight which should be reasonable

and preferably less than 500 Daltons, should exhibit a partition coefficient characterized by a log P of between 1.0–4.0, should not be locally irritating or sensitizing, should exhibit wide therapeutic index, low half-life preferably less than 10 h and low oral bioavailability (Kumar et al., 2013; Singla et al., 2012).

In summary, effectiveness of topical NSAIDs depends on the rate and extent of their penetration through the SC and their transportation through the other skin layers. This is influenced by several factors including the skin physiology, physicochemical characteristics of the API and excipients in addition to the dosage form design and fabrication.

2.4 FORMULATION AND EVALUATION OF EMULGELS

2.4.1 Preformulation studies
Preformulation refers to studies done on a drug molecule before formulation development and are useful in determining a suitable drug dosage form/ delivery system. Such studies include characterization of physicochemical, biopharmaceutical and pharmacokinetics profiles of the drug substance, in addition to compatibility studies of the drug with excipients. Preformulation is mostly applied for new chemical entities but also for existing molecules, for example when they need to be formulated in another dosage form (Williams et al., 2013; Kanig et al., 2008). Drugs diffuse through the skin in a similar manner to GIT and primarily depend on physical as well as chemical properties of the drug and factors such as skin physiology plus formulation properties are secondary. Therefore selection of a drug with appropriate properties such as solubility and lipophilicity is paramount (Kanig et al., 2008).

Taking meloxicam as an example, it is an existing drug substance that is formulated as an oral and parenteral dosage form. To be designed as a topical drug, preformulation studies should be conducted to test parameters such as its identity, solubility in selected solvents and also compatibility with excipients proposed to be used. Other known parameters need not be tested but they should be sought in relevant literature. From literature, meloxicam can be a good candidate for formulation as a topical emulgel because of its small oral dose, relatively low molecular weight and a good safety profile (Bachhav and Patravale, 2010).

2.4.2 Formulation considerations
Formulation of emulgels involves formulating the emulsion and gel forms separately before combining them. Emulsions are developed by preparing the oil and water phase separately before

mixing them together. The gel base is made by dissolving a gelling agent in water by vigorous stirring and adjusting the pH of the resulting phase by use of a strong base (Raj and Balakrishnan, 2016).

Most excipients used could have more than one function, depending on the concentrations used and they therefore have to be carefully selected to optimize their utility (Simões et al., 2018). Commonly used excipients in emulgel formulation include penetration enhancers, gelling agents, pH adjusters, vehicles, surfactants, solvents and preservatives. Penetration enhancers improve percutaneous absorption through various mechanisms which include altering the lipids in the SC e.g. by extracting them or disrupting their organization, modifying the proteins in SC to make it more permeable or promoting partition. Levomenthol, dimethyl sulfoxide, surfactants and oleic acid are a few examples of penetration enhancers (Osborne and Musakhanian, 2018; Chang et al., 2007).

Gelling agents are polymers that provide a three-dimensional gelled structure and also enhance the consistency of the emulgels. They can be classified as natural, semi-synthetic or synthetic. Xanthan gum, carboxy methyl cellulose and carbopol are examples of natural, semi-synthetic and synthetic gelling agents, respectively. Synthetic polymers are easier to handle and less prone to microbial attack (Arora et al., 2017; Gilbert et al., 2013). The pH Adjusters adjust the pH of the gel to a range that does not cause skin irritation and they also help thicken the formulation. Examples include sodium hydroxide, potassium hydroxide, triethanolamine and strong ammonia (Naga Sravan Kumar Varma et al., 2014). The oil phase vehicles commonly used include liquid paraffin (Khullar et al., 2012), vegetable oils, petrolatum and lanolin among others. Purified water is used in the water phase (Kapoor et al., 2014).

Surfactants are used in formulation of emulgels as emulsifiers to stabilize emulsions. Nonionic surfactants are preferred because of their low skin irritation and toxicity and are relatively cheaper. They are also less sensitive to changes in pH and electrolytes presence in the medium. Examples include span 20, 60 and 80, as well as tween 20, 60 and 80 (Kapadiya, 2016; Hyma et al., 2014). Solvents are used to facilitate dissolution of the drug substance. Examples include ethanol, isopropanol, dimethyl sulfoxide and propylene glycol (Naga Sravan Kumar Varma et al., 2014). Preservatives help prevent contamination and spoilage of the product by microorganisms throughout its shelf life. Methylparaben and propylparaben are frequently used (Drais and Hussein, 2017; Khullar et al., 2012).

15

2.4.3 Evaluation

The common quality parameters that are normally evaluated for emulgels are summarized in *Table 2.1* (Elmataeeshy et al., 2018; Osborne and Musakhanian, 2018; Simões et al., 2018; Arora et al., 2017).

Table 2.1: **Commonly evaluated quality parameters for emulgels**

Quality parameter	Description
Organoleptic properties	Properties such as color change, phase separation and odor are evaluated. They are qualitative indicators of chemical instability.
Spreadability	Refer to the ease with which emulgels spread over the skin.
Extrudability	Refer to the ease with which emulgels come out of a tube or pack.
Viscosity	Refer to a measure of resistance exhibited by an emulgel to flow at a given rate.
pH	A change in pH may indicate a chemical decomposition such as hydrolysis in the formulation.
Assay for drug content	These tests are quantitative indicators of instability.
***In vitro* drug permeation and release profile**	These tests employ *in vitro* techniques to study drug release and permeation through the skin. Franz diffusion cell or its modification is commonly used for these studies.
Irritation tests	They are done to test the irritancy level of a formulation. They can be done *in vitro* or *in vivo*. Draize skin test is an example of an *in vivo* test.
Microbial tests	They are normally done to test efficacy of preservatives used.
Efficacy tests	They are done to test the efficacy of the drug substance used in the formulation. For example, a topical NSAID emulgel would be subjected to anti-inflammatory test, either *in vitro* or *in vivo*.

CHAPTER THREE: MATERIALS AND METHODS

3.0 INTRODUCTION

Chapter Three gives details of the materials, equipment, methods and procedures that were used in conducting this study. It also describes the study design, study location and ethical considerations concerning the research.

3.1 STUDY DESIGN

The design of experiment (DoE) employed in this study was a laboratory based 3^2 factorial design that utilized both quantitative and qualitative approaches. A 3-level factorial design is one of the response surface methodologies of experimental design. Carbopol 934 and menthol were the two factors/ variables that were investigated and each factor had three levels/ concentrations that were studied. The API and all other excipients used in the formulation were kept constant. The Design Expert® software was used to randomly generate nine runs as displayed in *Table 3.1*, and these formed the basis of this study. Four responses/ variables were investigated, namely viscosity, spreadability, cumulative drug permeation at 1 h and cumulative drug permeation at 8 h.

Table 3.1: **Nine experimental runs generated using Design Expert® software**

	Run	1	2	3	4	5	6	7	8	9
Factor 1	Carbopol (%w/w)	1.5	1	0.5	1.5	1	1.5	0.5	1	0.5
Factor 2	Menthol (%w/w)	9	5	5	5	9	1	9	1	1

3.2 STUDY LOCATION

The study was conducted in the Department of Pharmaceutics and Pharmacy Practice laboratory, which is within the School of Pharmacy, University of Nairobi. The School of Pharmacy is located in Upper Hill area of Nairobi, Kenya, approximately 2 km from the Central Business District.

3.3 MATERIALS

All the materials used were of pharmaceutical grade. Meloxicam was the main API used. Capsaicin was also incorporated as an API in one of the optimized formulations. The excipients used were carbopol 934 (gelling agent), triethanolamine (pH adjuster and buffer), propylene glycol (preservative, humectant and solubilizer), liquid paraffin (oil phase vehicle), tween 20 and span 20 (emulsifying agents), menthol (penetration enhancer and rubefacient) and purified water.

The laboratory reagents used were of analytical grade and included methanol, sodium hydroxide, potassium bromide, potassium dihydrogen phosphate, phosphoric acid, ammonium acetate, glacial acetic acid and carrageenan.

3.4 EQUIPMENT

All the weights were weighed using analytical weighing balance (Sartorius®, Germany) while pH measurements were taken using digital pH meter (Jenway). Stability chamber (Binder®, Germany) was used to keep samples in optimum accelerated stability storage conditions whereas Fourier transform infrared (FTIR) spectrophotometer (Shimadzu, Japan) was used for conducting drug excipient compatibility tests. Dissolution testing apparatus (Erweka DT 6) was used to perform drug permeation studies, UV spectrophotometer (Genesys 10S UV-VIS) to analyze for drug content while viscometer (Cole-Parmer) was used for viscosity measurements. The water bath (Clifton) was used to heat and maintain phases in required temperature during emulsion formulation, the digital vernier caliper to measure the diameter size during spreadability test whereas the sonicator and the magnetic stirrer were used to enhance dissolution. High performance liquid chromatography (HPLC) (Shimadzu®, Japan) was used to orthogonally validate drug content results obtained following UV spectroscopy for meloxicam API and optimized formulations. The HPLC consisted of CTO-10AS VP column oven, Hitachi L-6200 intelligent pump, SPD-20A prominence UV-Vis detector, a manual sampler and a Gemini C18 column (250 mm × 4.6 mm, 5 µm).

3.5 PREFORMULATION STUDIES

3.5.1 Physical characteristics
Meloxicam API was evaluated for its organoleptic properties of colour, odour and texture.

3.5.2 Identification

Identification test of meloxicam API powder was performed using FTIR spectroscopy as per the British Pharmacopoeia (BP) 2017. This was further verified by comparing the retention time of the API sample with that of the USP meloxicam reference standard during assay evaluation that was done under similar experimental conditions.

3.5.3 Solubility studies

These studies helped in the selection of excipients to be used in the formulation. Solubility of meloxicam in liquid paraffin, propylene glycol, span 20, tween 20, isopropyl alcohol and water was determined qualitatively. About 20 mg of meloxicam was added to each volumetric flask that contained 20 mL of the selected solvent. After sealing, the mixtures were mechanically agitated for 2 h in a sonicator at room temperature and the dissolution of meloxicam visually observed.

3.5.4 Drug excipient compatibility tests

The drug excipient compatibility (DEC) studies of meloxicam and all excipients used was done using FTIR spectroscopy. The scanning was done over the 4000–500 cm^{-1} range using FTIR. Meloxicam powder and the standard were scanned separately before being scanned with each excipient in blends of 1:1 ratio (Kapadiya, 2016). The blends had earlier been stored in accelerated stability chamber for one month where the temperature was conserved at 40°C while the relative humidity was kept at 75%. The spectra obtained were visually examined for any variation that could infer a possibility of physicochemical incompatibility.

3.6 FORMULATION OF MELOXICAM EMULGELS

All the ingredients were weighed and prepared as illustrated in *Table 3.2*. Each emulgel was formulated in a three-steps process as explained below (Kapadiya, 2016; Kapoor et al., 2014).

Table 3.2: **Composition of different meloxicam emulgel formulations (%w/w)**

Ingredients	Formulation										
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Meloxicam	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Capsaicin	-	-	-	-	-	-	-	-	-	-	0.025
Carbopol 934	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5	0.5	0.5

Menthol	1	5	9	1	5	9	1	5	9	9	9
Triethanolamine	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs
Liquid paraffin	15	15	15	15	15	15	15	15	15	15	15
Propylene glycol	15	15	15	15	15	15	15	15	15	15	15
Tween-20	1	1	1	1	1	1	1	1	1	1	1
Span-20	2	2	2	2	2	2	2	2	2	2	2
Purified water	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs

3.6.1 Step 1: Formulation of the gel base

The gel phase was made by dissolving carbopol 934 in purified water with persistent mixing using a stirring rod. Triethanolamine was used to adjust the pH of the base to 5-7. Its composition is depicted in *Table 3.3*.

Table 3.3: **Composition of the gel base (% w/w)**

Ingredients	Formulations								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Carbopol 934	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
Triethanolamine	qs	qs	qs	qs	qs	qs	qs	qs	qs
Purified water	qs	qs	qs	qs	qs	qs	qs	qs	qs

3.6.2 Step 2: Formulation of oil-in-water emulsion

The O/W emulsion was prepared using the phase inversion method and the composition of the ingredients is illustrated in *Table 3.4*. The oil phase was made by dissolving span-20 emulsifier in liquid paraffin and the water phase was made by dissolving tween-20 emulsifier in water. Meloxicam and menthol were dissolved in propylene glycol and the preparation was mixed with the oil phase with consistent blending. Both phases were then warmed separately to a temperature of between 70°C and 80°C in a water bath. Upon reaching the optimum temperatures, the oil phase was added to the aqueous phase with perpetual blending. The mix was finally allowed to cool to room temperature for it to contour the desired o/w emulsion.

Table 3.4: **Composition of the emulsion base (% w/w)**

Ingredients	Formulations								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Oil phase									
Liquid paraffin	15	15	15	15	15	15	15	15	15
Span-20	2	2	2	2	2	2	2	2	2
Meloxicam	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol	15	15	15	15	15	15	15	15	15
Menthol	1	5	9	1	5	9	1	5	9
Water phase									
Tween-20	1	1	1	1	1	1	1	1	1
Purified water	qs	qs	qs	qs	qs	qs	qs	qs	qs

3.6.3 Step 3: Incorporation of gel base into emulsion base

With consistent and steady blending, the gel base was mixed into the emulsion base in a ratio of 1:1 in order to form the desired emulgel. The resulting formulation was then transferred into well labeled jars and its percentage yield calculated. This was repeated for all formulations. Appendix 3 shows a few pictures taken during this process.

3.7 EVALUATION OF THE FORMULATIONS

3.7.1 Physical examination

Each formulation was visually examined for homogeneity, clarity, grittiness, colour and potential phase separation.

3.7.2 pH measurement

A one-gram aliquot of the emulgel in one formulation was dissolved in distilled water and left to settle for about 2 h before measuring the pH using a digital pH meter (Panday et al., 2015). This was repeated for all the formulations. The acceptable pH range was 5-7 and this was necessary to avoid any skin irritation since pH of the human skin is usually within this range.

3.7.3 Viscosity measurement

A viscometer was used to determine the viscosity of all the formulations at room temperature. The torque readings were obtained between 15%−95% of the base scale. The L4 spindle type set at 10 rotations/min was used.

3.7.4 Spreadability studies

Spreadability was determined by placing 1 g of each emulgel within an already pre-marked circle of 1 cm diameter on a glass slab. Another pre-weighed glass slab was positioned on top and a weight that totalled to about 1 kg was put on the upper glass slab for 5 min. The resulting spread of the emulgel caused an increase in diameter which was measured using an electronic digital caliper (Shinde et al., 2019; Singh and Bedi, 2016; Bachhav and Patravale, 2010).

3.7.5 Phosphate buffer solution preparation

A phosphate buffer solution of pH 7.4 was prepared as per the BP 2017. A 250 mL aliquot of 0.2M potassium dihydrogen orthophosphate (KH_2PO_4) was added to 393.4 ml of 0.1M of sodium hydroxide (NaOH). The 0.1M NaOH was made by dissolving 4.2 g of NaOH in sufficient freshly distilled water to produce 1000 mL. A solution of 0.2M KH_2PO_4 was prepared by dissolving 27.22 g of KH_2PO_4 in sufficient distilled water to obtain 1000 mL. A fresh buffer solution was made every time it was needed for analysis and its pH was confirmed in each of those times. NaOH and phosphoric acid were used to adjust the pH whenever necessary.

3.7.6 Preparation of the standard graph of meloxicam

Ten milligrams of meloxicam powder (with a potency of 100.3%) was weighed in 100 mL volumetric flask and dissolved with freshly prepared phosphate buffer solution of pH 7.4 to produce a 100 µg/mL standard stock solution. The solution was sonicated for 30 min for meloxicam to dissolve fully. Working standards of 4, 8, 10, 12, 16 and 20 µg/mL were prepared by transferring 2, 4, 5, 6, 8 and 10 mL of the stock solution into 50 mL volumetric flasks and diluting to the mark using the buffer solution. To obtain the wavelength of maximum absorption, the 10 ug/mL working standard was scanned in the UV spectrophotometer in the $240 - 450$ nm range as depicted in Appendix 1. The wavelength of maximum absorption was noted. Absorbance values of the 6 working standard solutions at this wavelength were recorded and from this data, a standard curve of meloxicam was plotted.

3.7.7 Content uniformity determination

The meloxicam content in each formulation was evaluated in order to determine uniformity of meloxicam content in the formulations. A 1 g aliquot of each emulgel formulation (has approximately 5 mg of meloxicam) was dissolved in 100 mL freshly prepared phosphate buffer (pH 7.4) by means of sonication for about 2 h. The solution was then filtered with a *Whatman* filter paper and 10 mL of the filtrate was diluted to 50 mL with the buffer solution. UV-Vis

spectrophotometer was used to measure the absorbance at 362 nm and quantify the meloxicam content.

To rule out that other excipients did not absorb at the 362 nm analytical wavelength, a placebo product was formulated containing all ingredients used in the formulation except the API. A sample of the placebo was then prepared just like it was done for the other formulations and it was scanned in the UV spectrophotometer at a wavelength of between 240 – 450nm to determine its wavelength of maximum absorption. Absorbance value at 362 nm wavelength was also obtained.

3.7.8 *In vitro* drug permeation studies

These studies were conducted using a modified Franz diffusion (FD) cell. Cellulose nitrate membrane was soaked in freshly prepared phosphate buffer (pH of 7.4) for at least 24 h before use. One gram of each emulgel formulation was placed and smeared on the surface of the cellulose nitrate membrane which was fixed between donor and receptor compartments of the modified FD cell that had a diffusion area of 6.2 cm^2. The cell was then placed inside the dissolution vessel of the dissolution tester machine. The vessel functioned as the receptor compartment and it was filled with phosphate buffer (pH7.4) which was the dissolution medium. The temperature of the water bath was maintained at 37℃ by the circulating water jacket and the assembly was rotated using USP dissolution apparatus 2 at 50 rotations/ min (Mohamed et al., 2019; Farghaly et al., 2017; Fauzee et al., 2014). A 10 mL sample was drawn at suitable time interludes and replaced with equal amount of fresh dissolution medium to maintain a constant volume. The aliquots were collected and analyzed by UV-Vis spectroscopy at 362 nm wavelength and cumulative drug that permeated was calculated as a function of time for 8 h (Pednekar et al., 2015; Haneefa et al., 2013).

3.7.9 Optimization of the meloxicam emulgel

Design Expert® software was used to optimize meloxicam emulgel. There were two factors being studied and four responses under investigation as mentioned earlier. After formulation and evaluation of the nine emulgel formulations, the data obtained was fed into the software and was used to generate models that described the relationship between the factors and response variables. Based on the significant p values and correlation coefficients (R^2) in the individual models, the best models to explain the relationship between the variables were selected. Contour plots and response surface plots were generated to elucidate the relationships graphically.

Eventually the software was used to generate the optimized formulation with the objectives of keeping carbopol and menthol within the selected range, maximizing cumulative drug permeation at selected hours, maximizing spreadability and minimizing viscosity since it reduces both spreadability and drug release. The proposed formulation with the highest desirability was selected. To validate the proposed optimized formula, a final formulation was formulated and evaluated.

3.7.10 Drug release kinetics study

The *in vitro* drug permeation data obtained following the analysis of optimized formulations was used to analyze their drug release kinetics and mechanism. The data was converted to drug release data and with the use of DD Solver dissolution kinetic modeling software (Zhang et al., 2010), it was fitted into the subsequent kinetic equations (Siegel and Rathbone, 2012; Singhvi and Singh, 2011; Costa and Sousa Lobo, 2001).

A) Zero – order equation

$$Q_t = Q_0 + K_0 t$$

Where Q_t and Q_0 is the amount of drug released at time t and time zero, respectively, and K_0 is the zero-order release constant.

B) First – order equation

$$\ln Q_t = \ln Q_0 + k_1 t$$

Where Q_t and Q_0 is the amount of drug released at time t and time zero, respectively, and K_1 is the first-order release constant.

C) Higuchi's equation

$$Q = K_H \sqrt{t}$$

Where Q is the amount of drug released at time t and K_H is the higuchi diffusion rate constant.

D) Korsmeyer-Peppas equation

$$Mt/ M\infty = K_{KP} \times t^n$$

Where $Mt/ M\infty$ is the fraction of drug released at time t, K_{KP} is the Korsmeyer-Peppas release constant and n is the drug release exponent which describes drug release mechanism.

The model that fit best was selected by comparing R^2 values obtained from all the models.

3.7.11 *In vivo* anti-inflammatory studies

These studies were performed to test the efficacy of the optimized formulations using Winstar rats as animal models. Twenty rats were divided randomly into four equal groups. These were the standard/positive control (Voltaren® - diclofenac emulgel 1% w/w), negative control (untreated) and two test groups (formulations F10 and F11). Oedema was induced on the left hind paw of the rats by sub-plantar injection of 0.1 mL of freshly prepared 1% w/v solution of carrageenan lambda as previously described (Winter et al., 1962). The test formulations and the standard were applied 30 min before carrageenan was administered. The volume of the paw was measured at 0, 30, 60, 120, 180, 240 and 300 min using a modified plethysmometer by mercury displacement method (Khullar et al., 2012). Increase in paw volume in the test groups was compared with the control group and statistically analyzed to determine if there was any significant difference by use of analysis of variance (ANOVA) and student-*t* tests (Mondal et al., 2019; Tsai et al., 2015).

3.7.12 Assay for drug content

The optimized formulations contained 0.5% w/w meloxicam and they were assayed to determine the drug content and percentage label claim using UV spectroscopy and orthogonally validated using HPLC. The UV analysis was conducted in a similar manner with content uniformity determination method previously described for Formulations F1–F9.

The Shimadzu® HPLC equipment was used to assay both the Meloxicam API powder and the optimized emulgels using a modified literature method (Bachhav and Patravale, 2010). A system suitability test was done first, with acceptance conditions of a tailing factor of not more than (NMT) 2.0 and a relative standard deviation (RSD) of NMT 2.0%. Loss on drying test was also done at 105°C for 4 h and the acceptance criterion was NMT 0.5%. The mobile phase was made up of methanol/acetate buffer pH 4.5 (78:22, v/v); the flow rate was 0.5 mL/min whereas the injection volume was 20 µL. The column temperature was set at 40°C, the detection wavelength at 363 nm and the elution period at 8 min, since the retention time was about 5.6 min.

The samples were made by dissolving 10 mg of USP meloxicam reference standard, 10 mg of meloxicam API and 2 g of each optimized formulation (2 g has approximately 10 mg of meloxicam) using methanol/acetate buffer pH 4.5 (45:55, v/v) solution. They were dissolved in 50 mL volumetric flasks to make a concentration of about 0.2 mg/mL with the aid of a sonicator

for 15 – 30 min. They were then filtered using *Whatman* filter papers, stored in glass vials and refrigerated at $5 \pm 3°C$ as they waited to be assayed.

The percentage label claim of meloxicam in the samples taken was calculated using the following equation;

$$LC = (r_u/r_s) \text{ x } (C_s/C_u) \times 99.9\%$$

Where LC is the percentage label claim, r_u is the peak area of meloxicam from the sample, r_s is the peak area of meloxicam from the standard, C_s is the concentration of USP meloxicam standard in the standard preparation and C_u is the concentration of the sample preparation. The 99.9% value in the equation refers to the percentage potency of USP meloxicam standard. There were no compendial specifications that stipulate acceptance criteria for meloxicam emulgel since it was nonexistent in the market to date. Consequently, a targeted acceptance criterion was set by the researchers to be 90%−100%, based on USP 2015 acceptance criteria for meloxicam tablets and oral suspension as well as piroxicam cream.

3.7.13 Stability studies

Stability studies were performed according to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines. The optimized formulations were stored in a stability chamber at accelerated stability conditions of 40°C temperature and 75% relative humidity for one month. They were then physically examined and their spreadability as well as pH measured in addition to assay for drug content.

3.8 ETHICAL CONSIDERATIONS

Winstar rats are the animal subjects that were used to test the anti-inflammatory properties of the emulgels. Authority to use them was sought from the Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi. The animals were handled carefully and humanely throughout the test. Human subjects were not used in this study and therefore Ethics Review Board approval was not sought.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.0 INTRODUCTION

Chapter Four gives details of the findings of the preformulation, formulation and evaluation studies conducted. It also discusses results obtained and compares them with the existing literature.

4.1 PREFORMULATION STUDIES

4.1.1 Physical characteristics

Meloxicam was observed to be a pale-yellow powder of fine texture and had no discernible characteristic odour.

4.1.2 Identification

Identification test of meloxicam powder by FTIR spectroscopy gave a spectrum that was concordant with the reference spectrum of meloxicam provided in the BP 2017. *Figures 4.1* and *4.2* illustrate these spectra. In addition, the retention times of meloxicam powder and USP meloxicam reference standards obtained during HPLC assay were similar at about 5.68 min. This confirmed positively that indeed the powder being used was meloxicam.
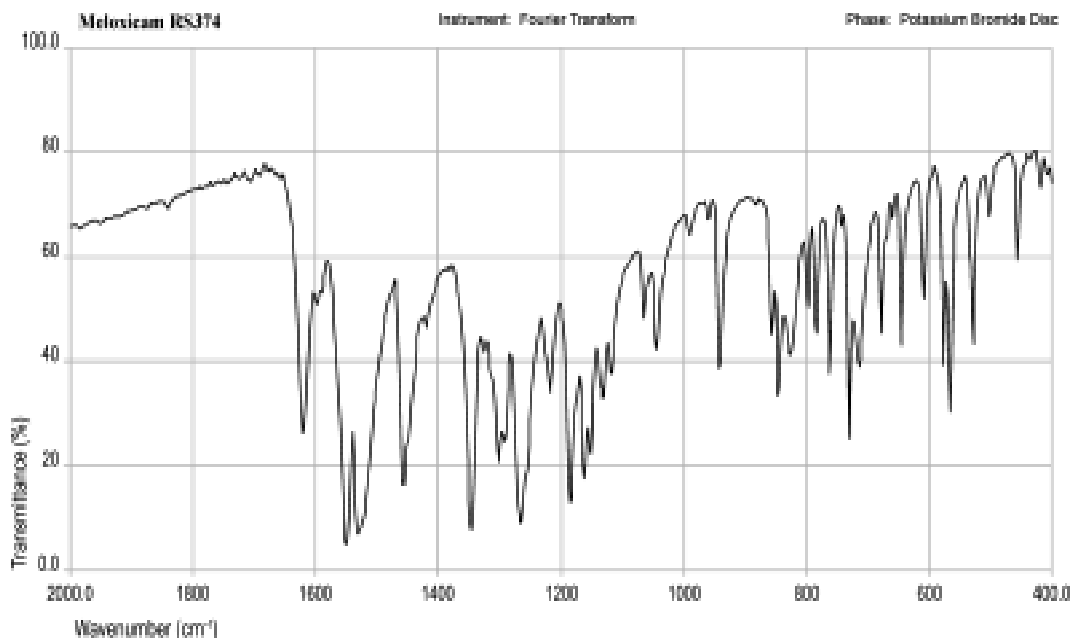

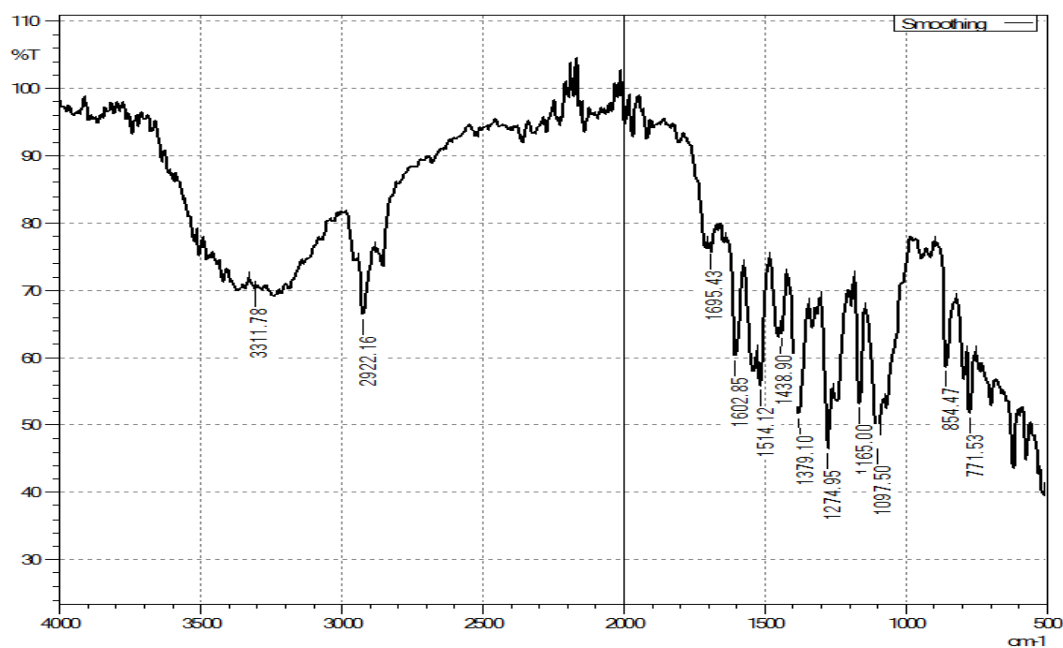*Figure 4.1:* **Infrared reference spectrum of meloxicam (BP 2017).**

Figure 4.2: **Infrared spectrum of meloxicam active pharmaceutical ingredient.**

4.1.3 Solubility studies

Meloxicam was found to be poorly soluble in water, very slightly soluble in isopropyl alcohol and slightly soluble in propylene glycol as well as liquid paraffin. It was soluble in span 20 and tween 20 surfactants. Following these results, propylene glycol and liquid paraffin were selected as the main solubilizing solvents. The surfactants were also chosen primarily as emulsifiers but also to enhance solubility of meloxicam.

4.1.4 Drug excipient compatibility tests

From examination of the FTIR spectra of the binary mixtures obtained, there was no observable variation or chemical group interaction between meloxicam API and each excipient. All the major peaks observed in meloxicam API spectrum were present in the spectra of the binary mixtures. A few minor changes observed in the spectra were attributed to overlying of the peaks of API and corresponding excipient. This predicts lack of drug and excipient interaction and can thus be said to be compatible with regards to their physicochemical properties. The spectrum of meloxicam API powder is shown in *Figure 4.2* whereas those of meloxicam USP standard and the binary mixtures with excipients are illustrated in the Appendix 2.

4.2 FORMULATION OF MELOXICAM EMULGELS

The meloxicam emulgels were formulated as earlier described and a few pictures captured during formulation process are shown in Appendix 3. The percentage yield of the formulations was calculated and the results are illustrated in *Table 4.1*. Formulation F7 had the lowest yield of 95.6% whereas F3 had the highest yield of 100.8%. The expected yield was 100% and the RSD was 1.8%. The minor deviation noted could be attributed to loss during transfer from the formulation container to the storage jars since a small amount was always left back. The small increase in the yield noted in F3 was as a result of addition of slightly excess water to *qs* during formulation.

Table 4.1: **Percentage yield of formulated emulgels**

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Yield (%)	96.5	97.0	100.8	96.7	97.1	96.6	95.6	99.6	98.6

4.3 EVALUATION OF THE FORMULATIONS

4.3.1 Physical examination

Upon visual examination, all formulations were found to be translucent, homogenous emulgels that looked like creams with no observable grittiness. Their colour was a shade of white (cream to off-white) and no phase separation was observed in all the formulations.

4.3.2 pH measurement

The pH of all the formulations was between the desired range of 5-7 as shown in *Table 4.2*. Formulation F7 had the lowest pH of 5.7 and 6.5 was the highest pH noted in F2. With the pH values obtained, no formulation could be anticipated to cause skin irritation.

Table 4.2: **pH of meloxicam emulgel formulations**

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
pH	6.4	6.5	6.4	6.1	6.0	6.1	5.7	5.8	5.9

4.3.3 Viscosity measurement

As shown in *Table 4.3* and *Figure 4.3*, viscosity of the formulations ranged between 20426 and 42336 mPa.s, the lowest and highest being exhibited by formulations F1 and F8 respectively. Emulgels having 0.5% w/w carbopol concentration had the lowest viscosity whereas those with 1.5% w/w concentration had the highest viscosity. This observation is in agreement with literature that as polymer concentration is increased in a formulation then viscosity increases, if other factors are held constant (Pednekar et al., 2015; Naga Sravan Kumar Varma et al., 2014).

Table 4.3: **Viscosity of the formulations**

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Viscosity (mPa.s)	20426	22961	21830	35752	35987	33140	41584	42336	40250
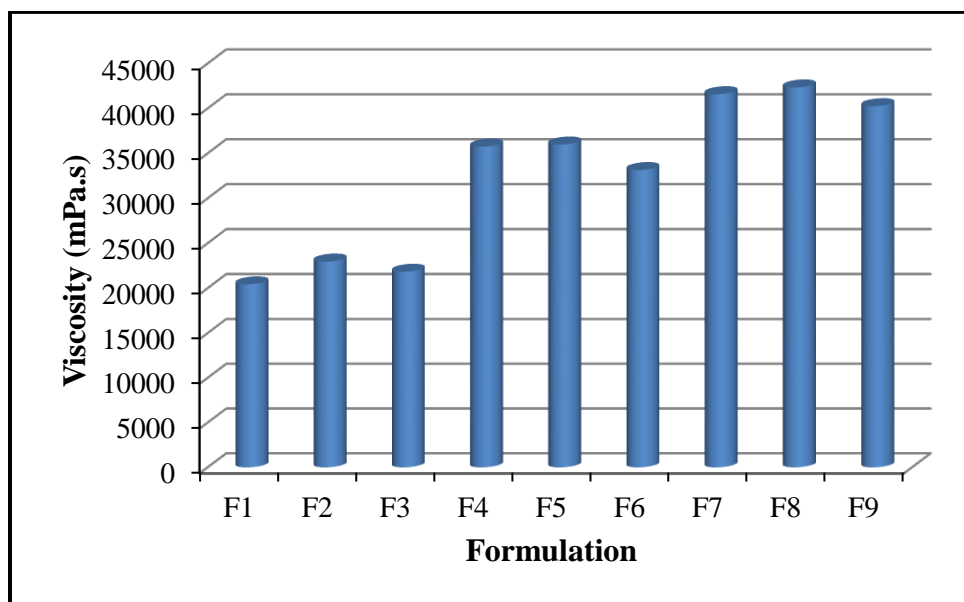
Figure 4.3: **Viscosity of the formulated emulgels.**

4.3.4 Spreadability studies

The spreadability of the formulated emulgels was denoted by their increase in diameter following the spreadability test and is illustrated in *Table 4.4* and *Figure 4.4*. Formulation F8 had the lowest spreadability of 7.0 cm while F2 had the highest at 8.5 cm. The spreadability of Formulations F1, F2 and F3 was above 8.0 cm and this can be attributed to the lowest polymer concentration of 0.5% w/w. Spreadability was found to be dependent on polymer concentration and viscosity. As polymer concentration increased in the formulations, viscosity increased and

consequently spreadability reduced (Pednekar et al., 2015). High spreadability of emulgels allows ease of application and this in turn increases the surface area available for drug permeation. Spreadability values above 7.5 cm imply good spreadability properties as was exhibited by Formulations F1 to F6 (Bachhav and Patravale, 2010).

Table 4.4: **Spreadability of the emulgel formulations**

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Diameter (cm)	8.4	8.5	8.3	7.6	7.8	7.9	7.2	7.0	7.1
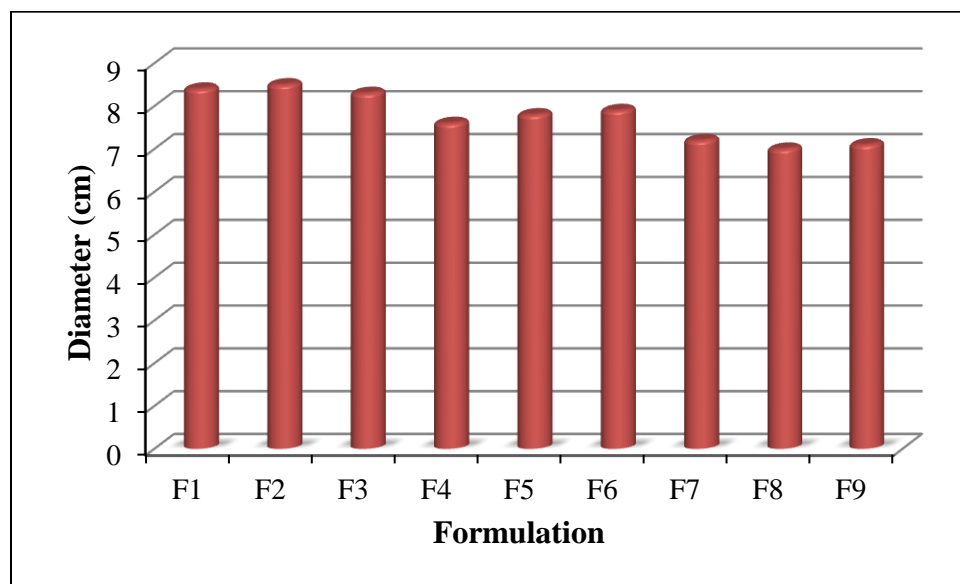
Figure 4.4: **Spreadability of the emulgel formulations.**

4.3.5 Preparation of the standard graph of meloxicam

The wavelength of maximum absorption obtained after the 10 ug/mL working standard of meloxicam was scanned in the UV spectrophotometer in the 240 – 450 nm range was 362 nm. Absorbance values of the 6 working standard solutions at this wavelength were recorded as shown in *Table 4.5* and from this data, a standard curve of meloxicam was plotted as illustrated in *Figure 4.5*.

Table 4.5: **Standard meloxicam concentrations and corresponding absorbance values (at 362 nm)**

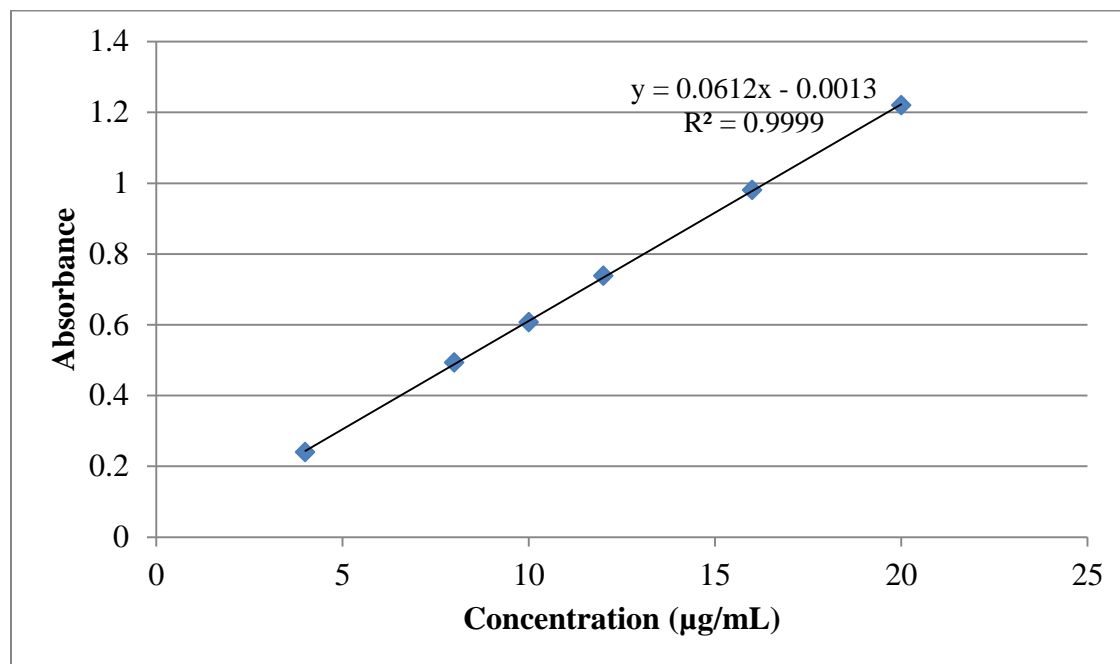| Concentration of standards (µg/mL)	4	8	10	12	16	20
Absorbance	0.240	0.493	0.607	0.738	0.980	1.220

Figure 4.5: Standard curve of meloxicam at 362 nm.

4.3.6 Content uniformity determination

Meloxicam drug content of the formulations was determined using UV spectroscopy as shown in *Table 4.6*. The percentage drug content was between 90.7% (for F7) and 109.9% (for F9) while the RSD was 6%. These parameters are an indication that the drug content was uniform.

Table 4.6: **Drug content of meloxicam emulgel formulations**

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug content (mg)	468.4	532.9	487.2	497.0	518.2	479.8	453.7	504.3	549.3
Drug content (%)	93.7	106.6	97.4	99.4	103.6	96.0	90.7	100.9	109.9

For the placebo formulation, the wavelength of maximum absorption was not between 240 – 450 nm but at a wavelength below 240 nm. Its absorption at 362 nm was 0.135, 0.184 at 240 nm and 0.124 at 450 nm.

4.3.7 *In vitro* drug permeation studies

The cumulative percentage of meloxicam drug that permeated following the study is depicted in *Table 4.7* and *Figure 4.6*. Based on cumulative drug permeation after 1 h and after 8 h, the order of permeation from the highest to the lowest was as follows; F3>F2>F1>F9>F6>F5 >F8>F4>F7 and F3>F2>F1>F6>F5>F9>F8>F4>F7, respectively. The first and the last three in both cases are similar. Formulation F3 which contained 0.5% w/w carbopol and 9% w/w menthol had the highest drug permeation of 37.1% after 8 h. Formulation F7 on the other hand had the lowest drug permeation of 9% after 8 h and it contained 1.5% w/w carbopol and 1% w/w menthol. For the formulations containing same amount of carbopol, drug permeation was highest in those with 9% w/w menthol, followed by those with 5% w/w menthol and lowest in those with 1% w/w menthol.

Table 4.7: **Cumulative percentage of drug permeated**

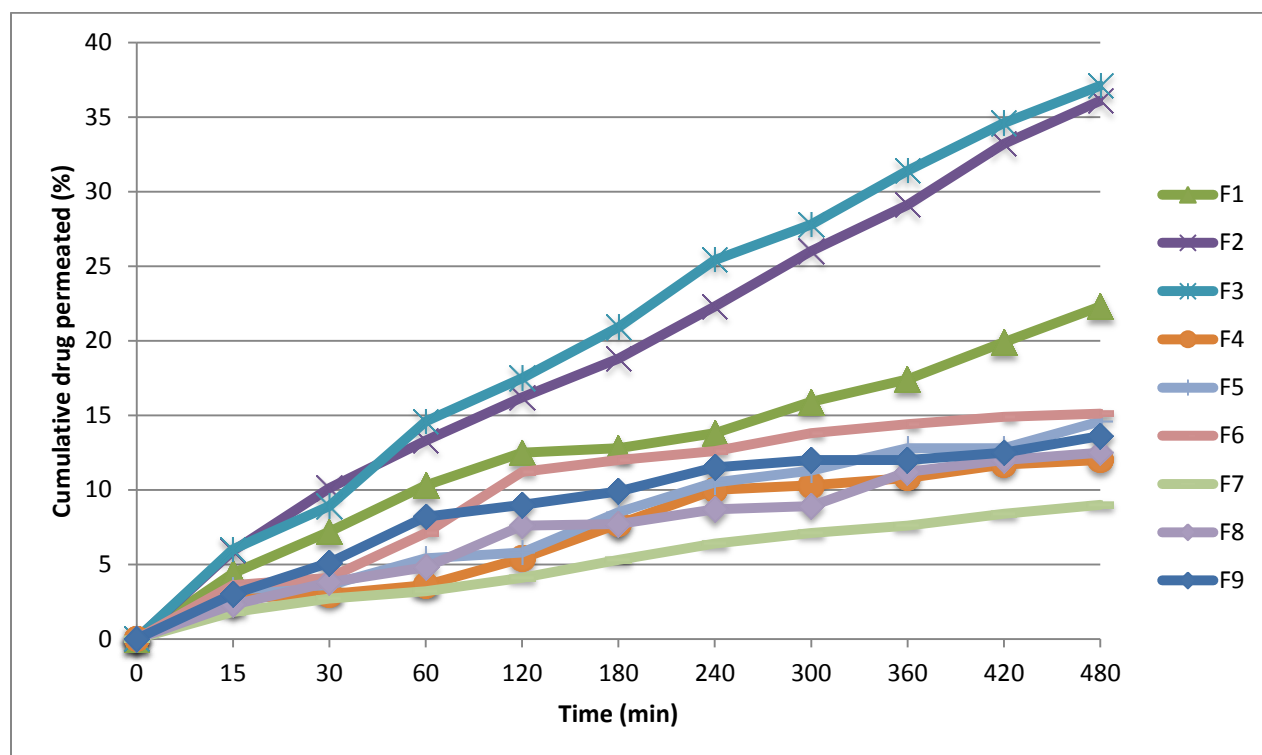| Time (min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
15	4.4	5.9	6.0	2.7	3.3	3.6	1.8	2.3	3.0
30	7.2	10.1	8.9	3.0	3.6	4.1	2.7	3.8	5.1
60	10.3	13.3	14.6	3.6	5.4	7.1	3.2	4.8	8.2
120	12.5	16.2	17.5	5.4	5.8	11.2	4.1	7.6	9.0
180	12.8	18.8	20.9	7.7	8.5	12.0	5.3	7.7	9.9
240	13.8	22.3	25.4	10.0	10.5	12.6	6.4	8.7	11.5
300	15.9	26.0	27.8	10.3	11.3	13.8	7.1	8.9	12.0
360	17.4	29.1	31.4	10.8	12.8	14.4	7.6	11.2	12.0
420	19.9	33.2	34.6	11.7	12.8	14.9	8.4	12.0	12.5
480	22.3	36.1	37.1	12.0	14.6	15.1	9.0	12.5	13.6

Figure 4.6: **Cumulative percentage drug permeated as a function of time.**

Both carbopol and menthol concentrations in a formulation contributed to meloxicam drug permeation. It was observed that the lower the carbopol concentration in a formulation, the higher the drug permeation. This is because formulations with low carbopol had low viscosity and thus a less resistance to flow (Hasçicek et al., 2009). The higher the menthol concentration in a formulation, the higher the amount of drug that permeated. This proved that menthol is a potent penetration enhancer (Patel et al., 2011; Jantharaprapap and Stagni, 2007; Chang et al., 2007). Its mechanism of enhancing penetration through the cellulose nitrate membrane used, however, was not established in this study.

4.3.8 Optimization of the meloxicam emulgel

A summary of the factor and response variables that were keyed in the Design Expert® software for analysis is shown in *Table 4.8*. Cumulative drug permeation at selected hours and spreadability of the formulations fit best in a linear model while quadratic model was the best to explain the relationship between the factors and viscosity. The contour and response surface plots that graphically elucidated these relationships are illustrated in Appendix 4.

Table 4.8: **Summary of factors and responses under study**

Formulation	Run	Factor 1 Carbopol (% w/w)	Factor 2 Menthol (% w/w)	Response 1 Drug permeation 1 h (%)	Response 2 Drug permeation 8 h (%)	Response 3 Spreadability (cm)	Response 4 Viscosity (mPa.s)
F9	1	1.5	9	8.2	13.6	7.1	40250
F5	2	1	5	5.4	14.6	7.8	35987
F2	3	0.5	5	13.3	36.1	8.5	22961
F8	4	1.5	5	4.8	12.5	7	42336
F6	5	1	9	7.1	15.1	7.9	33140
F7	6	1.5	1	3.2	9	7.2	41584
F3	7	0.5	9	8.9	37.1	8.3	21830
F4	8	1	1	3.6	12	7.6	35752
F1	9	0.5	1	10.3	22.3	8.4	20426

To get an optimized formula, several parameters were set as shown in *Table 4.9*. Since carbopol concentration was found to increase viscosity which in turn retarded drug release and spreadability, it was preferred that it be minimized and still be kept within the initial range. Menthol had a linear relationship with drug permeation and preference was set to maximize its concentration and still keep it within the initial range. The best proposed solution had a desirability of 0.925 which recommended concentrations of 0.5% for carbopol and 9% for menthol. This resembled the composition of formulation F3. *Figure 4.7* shows the proposed solution ramps of the factors and predicted responses.

Table 4.9: **Summary of optimization parameters**

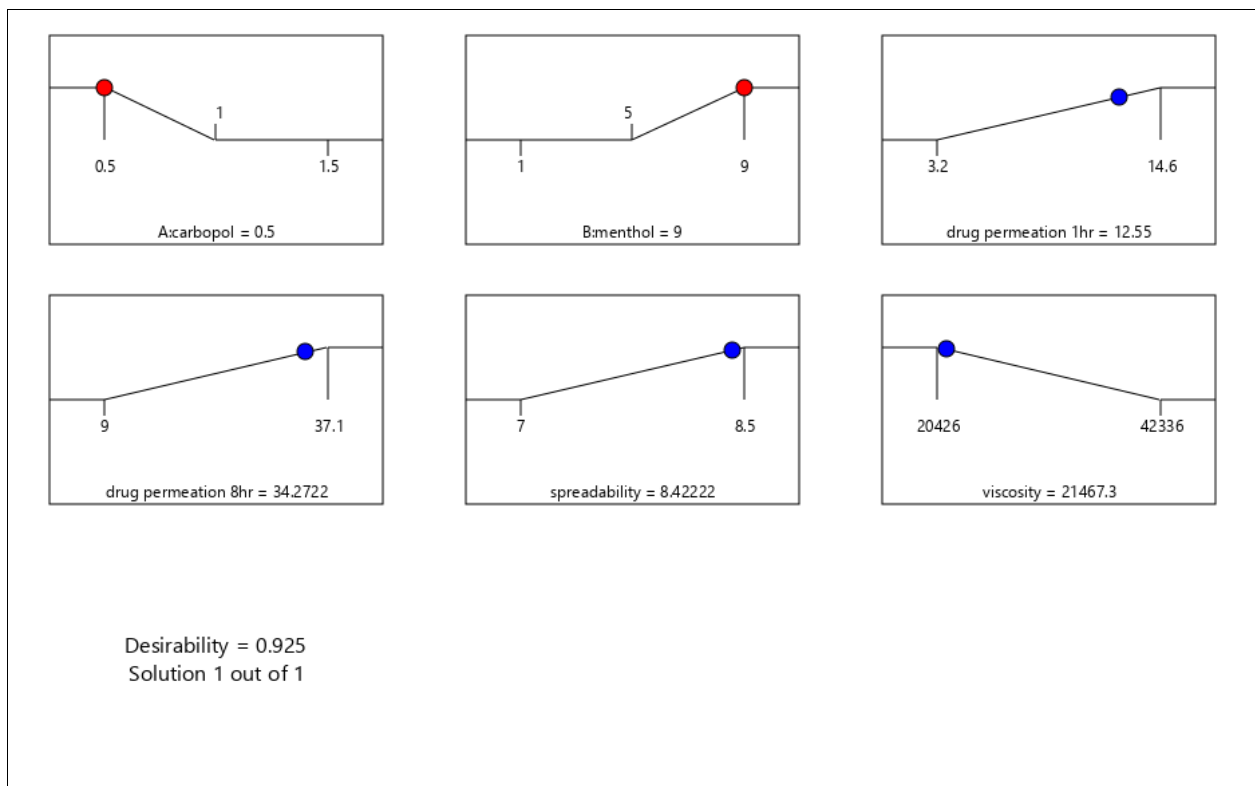| Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Carbopol	minimize	0.5	1	1	1	3
Menthol	maximize	5	9	1	1	3
Drug permeation 1 h	maximize	3.2	14.6	1	1	5
Drug permeation 8 h	maximize	9	37.1	1	1	5
Spreadability	maximize	7	8.5	1	1	5
Viscosity	minimize	20426	42336	1	1	5

Figure 4.7: **Proposed solution ramps of the factors and predicted responses.**

4.4 FORMULATION OF OPTIMIZED FORMULATIONS

To validate the proposed optimized formula, a final formulation (F10) was formulated and evaluated. Another formulation (F11) with similar composition to F10 and incorporating capsaicin was also formulated and evaluated. The final composition of Formulations F10 and F11 is illustrated in *Table 3.4*. Upon formulation, percentage yield was calculated and is shown in *Table 4.10*.

Table 4.10: **Percentage yield of optimized emulgels**

Formulation	F10	F11
Yield (%)	98.5	98.4

4.5 EVALUATION OF OPTIMIZED FORMULATIONS

4.5.1 Physical examination, pH, viscosity and spreadability

The two optimized formulations were examined visually and were found to be translucent, homogenous emulgels that looked like creams with no observable grittiness. Their colour was a shade of white (cream to off white) and no phase separation was observed both formulations. Their pH, viscosity and spreadability were evaluated and the results are shown in *Table 4.11*.

Table 4.11: **Evaluation results of optimized formulations**

Parameter	Expected	Formulation	
		F10	F11
pH	5 – 7	6.5	6.4
Viscosity (mPa.s)	20000 – 25000	23656	24524
Spreadability (cm)	8 – 10	9.9	9.5

4.5.2 *In vitro* drug permeation studies

The cumulative percentage of meloxicam drug that permeated as function of time following the study of optimized formulations is illustrated in *Table 4.12* and *Figure 4.8*. The cumulative percentage of drug that permeated after 1 h was 21.0% and 22.9% for Formulations F10 and F11

respectively, while after 8 h, 50.1% and 55.8% of the drug was permeated from the two formulations respectively. Both formulations recorded highest percentage of drug permeated when compared to formulations F1 – F9. Formulation F11 had a higher drug permeation profile than F10. This can be attributed to incorporation of capsaicin which has been found to have some penetration enhancing properties (Kim et al., 2014; Fang et al., 2001; Degim et al., 1999).

Table 4.12: **Cumulative percentage of drug permeated for optimized formulations**

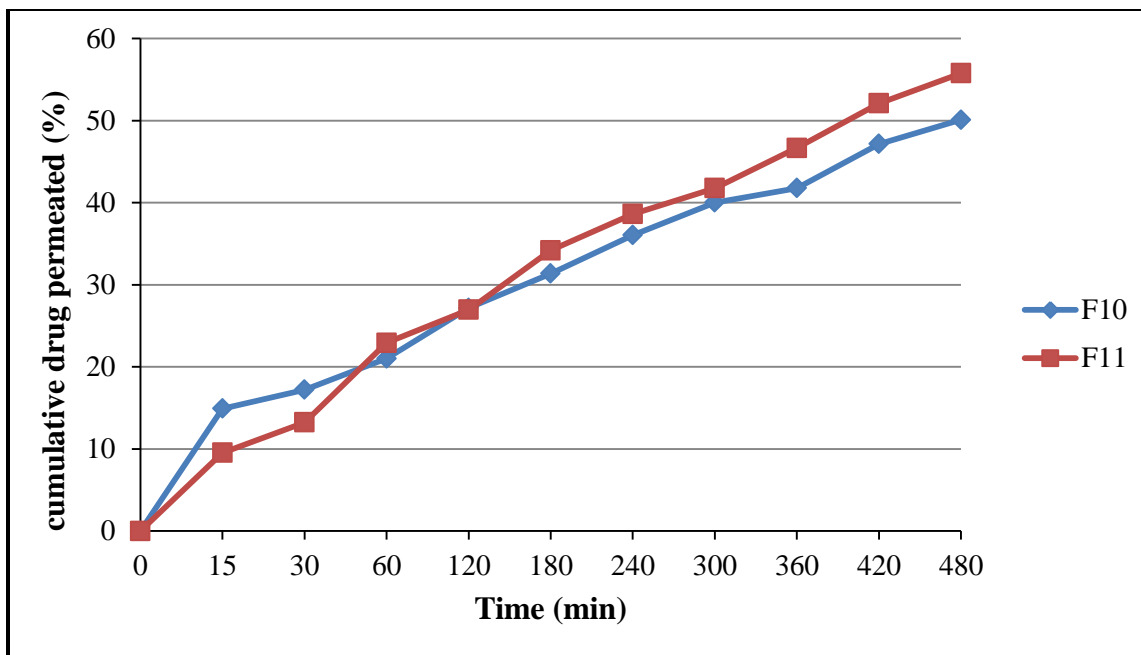| Formulation	Time (min)										
	0	15	30	60	120	180	240	300	360	420	480
F10	0	14.9	17.2	21.0	27.2	31.4	36.1	40.0	41.8	47.2	50.1
F11	0	9.5	13.2	22.9	27.0	34.2	38.6	41.8	46.7	52.1	55.8

Figure 4.8: **Cumulative percentage of drug permeated for optimized formulations.**

4.5.3 Drug release kinetics study

A summary of the kinetics modeling data obtained following the use of DD Solver dissolution kinetic modeling software is shown in *Table 4.13*.

Table 4.13: **Summary of the kinetics modeling data**

Formulation	Zero order		First Order		Higuchi		Korsmeyer-Peppas			Drug release mechanism
	K_0	R^2	K_1	R^2	K_H	R^2	k_{KP}	R^2	n	
F10	0.123	0.6019	0.002	0.7444	2.332	0.9689	4.338	0.9925	0.390	Fickian diffusion
F11	0.134	0.7825	0.002	0.8909	2.512	0.9946	2.682	0.9948	0.488	Fickian diffusion

The best fitting model was Korsmeyer-Peppas since it had the highest values of R^2, being 0.9925 and 0.9948 for formulations F10 and F11, respectively. The mechanism of drug release for both formulations is best described by Fickian diffusion. This is because n values were 0.390 and 0.488 for F10 and F11 respectively, values that are less than 0.5. When $n \leq 0.5$, this denotes Fickian diffusion as the leading drug release mechanism where drug is released predominantly through diffusion (Singhvi and Singh, 2011; Ritger and Peppas, 1987).

4.5.4 *In vivo* anti-inflammatory studies

The results of anti-inflammatory test are summarized in *Table 4.14* and graphically illustrated in *Figure 4.9*. After carrageenan injection, the paw volume in all the animals increased progressively, an indication of the inflammatory reaction, and reached its maximum at three hours. It was observed at 1 h, 2 h, 3 h and 4 h that Voltaren® emulgel and both Formulations F10 and F11 inhibited paw volume increase/oedema after carrageenan injection. Inhibition by Voltaren® emulgel and Formulation F11 at 2 h and 3 h after carrageenan injection was found to be statistically significant ($p < 0.05$).

Table 4.14: **Average paw volume (mL) after carrageenan injection**

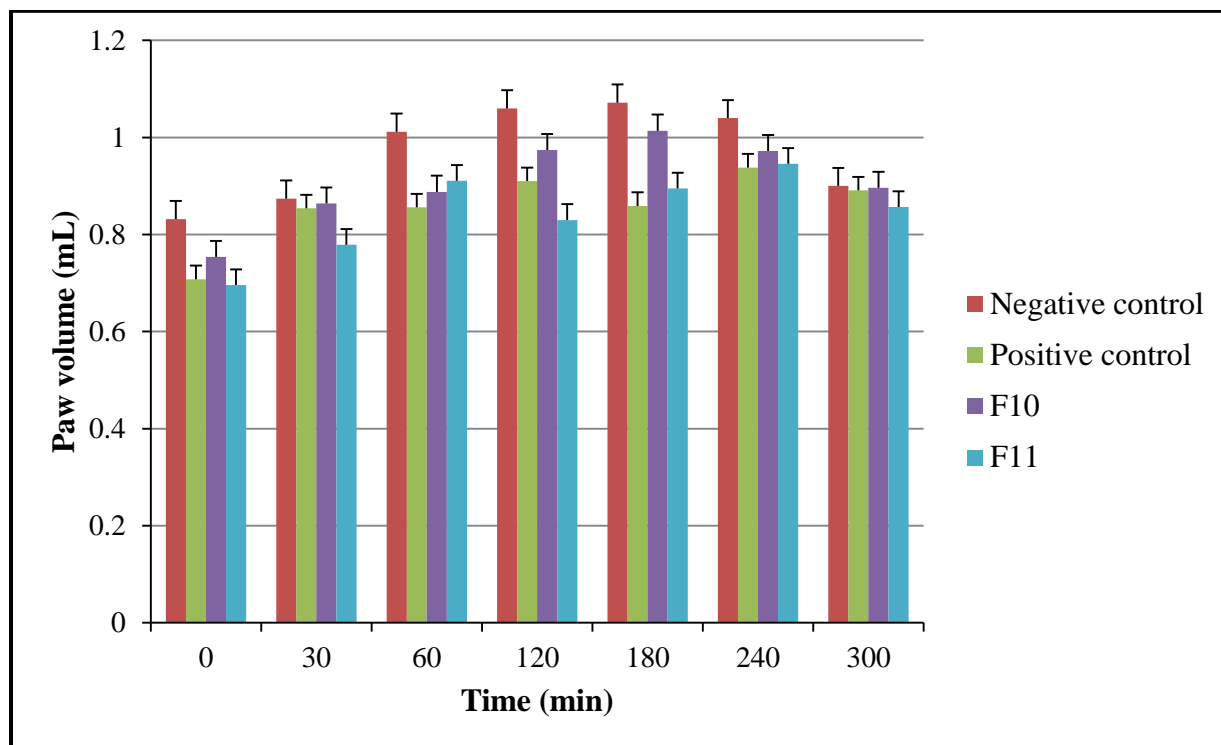| Sample	Time (min)						
	0	**30**	**60**	**120**	**180**	**240**	**300**
Negative control	0.832	0.874	1.012	1.060	1.072	1.040	0.900
Positive control	0.708	0.854	0.856	0.910	0.859	0.938	0.891
F10	0.754	0.864	0.888	0.974	1.014	0.972	0.896
F11	0.696	0.779	0.911	0.830	0.895	0.946	0.857

Figure 4.9: **Inflammation response after carrageenan injection.**

4.5.5 Assay for drug content

The HPLC system suitability test was found to be appropriate for the assay of meloxicam API powder, with a tailing factor of 1.7 and an RSD of 0.3%. The loss on drying of the API was 0.064%. The percentage drug content of the API powder was 100.3% and it complied with the USP 2015 compendial specifications that stipulate acceptance criteria of 99.0% − 100.5% on the dried basis. Drug content was first determined by UV spectroscopy and found to be 98.6% and 102.5% for Formulations F10 and F11, respectively, thus complying with the targeted acceptance criteria of 90%−100%. The results were validated using orthogonal HPLC analysis that gave the drug content in Formulations F10 and F11 as 90.4% and 92.9%, respectively. The chromatograms obtained are attached in Appendix 5.

4.5.6 Stability studies

Stability study results of the optimized formulations after storage in stability chamber for one month are summarized in *Table 4.15*. Upon physical examination, both formulations had a shade of white colour (cream to off-white) and were found to be translucent and homogenous with no observable grittiness or phase separation. Their appearance initially and after one month in accelerated stability conditions was not different.

Table 4.15: **Stability study results of optimized formulations**

Parameter	Expected	Formulation F10		Formulation F11	
		Time period		**Time period**	
		Initial	**1 month**	**Initial**	**1 month**
pH	5 – 7	6.5	6.5	6.4	6.4
Spreadability (cm)	8 – 10	9.9	9.8	9.5	9.3
Drug content (%)	90 – 110	98.6	96.8	102.5	100.5

These results imply that the formulated emulgels are stable after one month in accelerated stability chamber conditions.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.0 INTRODUCTION

This chapter draws conclusions and makes recommendations based on the study findings.

5.1 CONCLUSION

Meloxicam emulgels targeted to be applied topically were formulated using a 3^2 factorial design and also evaluated pharmaceutically and pharmacologically. The drug permeation, spreadability and viscosity results obtained after evaluation were analyzed using Design Expert® software to give optimized formulations. This was the purpose for the study and it was fully met.

The optimized formulations were translucent, homogenous, cream-like emulgels with acceptable pH and drug content. Their viscosity and spreadability was optimal to allow ease in application and increase the surface area for drug permeation. The drug release kinetics of these formulations was best explained by Korsmeyer-Peppas kinetic model and diffusion was the predominant mechanism of drug release. They were also found to have anti-inflammatory activity on topical application. One of the optimized formulations contained capsaicin rubefacient in addition to menthol which was primarily incorporated as a permeation enhancer.

This study showed that formulating and evaluating meloxicam emulgels as well as an emulgel containing both meloxicam and capsaicin for enhanced activity is achievable. If exploited further, the study findings could potentially provide a safe and efficacious alternative for symptomatic treatment of pain and inflammation associated with arthritis as well as other muscle pains. A better alternative can in turn lead to improved patients compliance to medication and thereby improve their quality of life.

5.2 STUDY LIMITATIONS

Because of lack of Franz diffusion cell equipment, permeation and release studies of meloxicam were done using a modified method that utilized a dissolution testing apparatus and a cellulose nitrate membrane.

5.3 RECOMMENDATIONS

It is recommended that *in vivo* drug permeation studies of the optimized emulgels be conducted as they will be more informative with regard to the permeability of drug. The studies should preferably be done using a Franz diffusion cell. There being no other meloxicam emulgel in the market currently, it is further recommended that its analytical method be developed and validated.

REFERENCES

Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420. https://doi.org/10.1023/a:1016212804288

Arora, R., Khan, R., Ojha, A., Upadhyaya, K., Chopra, H., 2017. Emulgel: A novel approach for hydrophobic drugs. Int. J. Pharm. Biol. Sci. 7, 18.

Au, S., Kim, N., Goldminz, A.M., Alkofide, M.A., Gottlieb, A.B., 2014. Psoriatic Arthritis: Clinical Review and Update, in: Weinberg, J.M., Lebwohl, M. (Eds.), Advances in Psoriasis: A Multisystemic Guide. Springer London, London, pp. 39–61. https://doi.org/10.1007/978-1-4471-4432-8_5

Bachhav, Y.G., Patravale, V.B., 2010. Formulation of meloxicam gel for topical application: In vitro and in vivo evaluation. Acta Pharm. Zagreb Croat. 60, 153–163. https://doi.org/10.2478/v10007-010-0020-0

Benet, L.Z., 2013. The Role of BCS (Biopharmaceutics Classification System) and BDDCS (Biopharmaceutics Drug Disposition Classification System) in Drug Development. J. Pharm. Sci. 102, 34–42. https://doi.org/10.1002/jps.23359

Cardot, J.-M., Garcia Arieta, A., Paixao, P., Tasevska, I., Davit, B., 2016. Implementing the Biopharmaceutics Classification System in Drug Development: Reconciling Similarities, Differences, and Shared Challenges in the EMA and US-FDA-Recommended Approaches. AAPS J. 18, 1039–1046. https://doi.org/10.1208/s12248-016-9915-0

Chang, J.-S., Tsai, Y.-H., Wu, P.-C., Huang, Y.-B., 2007. The effect of mixed-solvent and terpenes on percutaneous absorption of meloxicam gel. Drug Dev. Ind. Pharm. 33, 984–989. https://doi.org/10.1080/03639040601150294

Chang, R.-K., Raw, A., Lionberger, R., Yu, L., 2012. Generic Development of Topical Dermatologic Products: Formulation Development, Process Development, and Testing of Topical Dermatologic Products. AAPS J. 15, 41–52. https://doi.org/10.1208/s12248-012-9411-0

Charalabidis, A., Sfouni, M., Bergström, C., Macheras, P., 2019. The Biopharmaceutics Classification System (BCS) and the Biopharmaceutics Drug Disposition Classification System (BDDCS): Beyond guidelines. Int. J. Pharm. 566, 264–281. https://doi.org/10.1016/j.ijpharm.2019.05.041

Chen, J., Gao, Y., 2016. Strategies for meloxicam delivery to and across the skin: a review. Drug Deliv. 23, 3146–3156. https://doi.org/10.3109/10717544.2016.1157839

Coates, L.C., Helliwell, P.S., 2017. Psoriatic arthritis: state of the art review. Clin. Med. 17, 65–70. https://doi.org/10.7861/clinmedicine.17-1-65

Costa, P., Sousa Lobo, J.M., 2001. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13, 123–133. https://doi.org/10.1016/S0928-0987(01)00095-1

Crofford, L.J., 2013. Use of NSAIDs in treating patients with arthritis. Arthritis Res. Ther. 15, S2. https://doi.org/10.1186/ar4174

da Silva, J.A.P., Woolf, A.D., 2010. Drugs Commonly Used in Rheumatic Diseases, in: Pereira da Silva, J.A., Woolf, A.D. (Eds.), Rheumatology in Practice. Springer London, London, pp. 301–309. https://doi.org/10.1007/978-1-84882-581-9_30

Degim, I.T., Uslu, A., Hadgraft, J., Atay, T., Akay, C., Cevheroglu, S., 1999. The effects of Azone and capsaicin on the permeation of naproxen through human skin. Int. J. Pharm. 179, 21–25. https://doi.org/10.1016/S0378-5173(98)00353-6

Drais, H.K., Hussein, A.A., 2017. Formulation Characterization and Evaluation of Meloxicam Nanoemulgel to be Used Topically. Iraqi J. Pharm. Sci. 26, 9–16.

Euller-Ziegler, L., Velicitat, P., Bluhmki, E., Türck, D., Scheuerer, S., Combe, B., 2001. Meloxicam: a review of its pharmacokinetics, efficacy and tolerability following intramuscular administration. Inflamm. Res. 50, 5–9. https://doi.org/10.1007/PL00022377

Fang, J.-Y., Fang, C.-L., Hong, C.-T., Chen, H.-Y., Lin, T.-Y., Wei, H.-M., 2001. Capsaicin and nonivamide as novel skin permeation enhancers for indomethacin. Eur. J. Pharm. Sci. 12, 195–203. https://doi.org/10.1016/S0928-0987(00)00118-4

Farghaly, D.A., Aboelwafa, A.A., Hamza, M.Y., Mohamed, M.I., 2017. Topical delivery of fenoprofen calcium via elastic nano-vesicular spanlastics: optimization using experimental design and in vivo evaluation. AAPS PharmSciTech 18, 2898–2909.

Fauzee, A.F.B., Khamanga, S.M., Walker, R.B., 2014. The impact of manufacturing variables on in vitro release of clobetasol 17-propionate from pilot scale cream formulations. Drug Dev. Ind. Pharm. 40, 1683–1692.

Fuentes, A.V., Pineda, M.D., Venkata, K.C.N., 2018. Comprehension of Top 200 Prescribed Drugs in the US as a Resource for Pharmacy Teaching, Training and Practice. Pharm. J. Pharm. Educ. Pract. 6. https://doi.org/10.3390/pharmacy6020043

Gilbert, L., Picard, C., Savary, G., Grisel, M., 2013. Rheological and textural characterization of cosmetic emulsions containing natural and synthetic polymers: relationships between both data. Colloids Surf. Physicochem. Eng. Asp. 421, 150–163.

Haneefa, K.P.M., Easo, S., Hafsa, P.V., Mohanta, G.P., Nayar, C., 2013. Emulgel: An Advanced Review. J Pharm Sci 5.

Haroutiunian, S., Drennan, D.A., Lipman, A.G., 2010. Topical NSAID Therapy for Musculoskeletal Pain. Pain Med. 11, 535–549. https://doi.org/10.1111/j.1526-4637.2010.00809.x

Hasçicek, C., BediZ-Ölçer, A., Gönül, N., 2009. Preparation and evaluation of different gel formulations for transdermal delivery of meloxicam. Turk J Pharm Sci 6, 177–186.

Hosie, J., Distel, M., Bluhmki, E., 1996. Meloxicam in Osteoarthritis: A 6-Month, Double-Blind Comparison with Diclofenac Sodium. Rheumatology 35, 39–43. https://doi.org/10.1093/rheumatology/35.suppl_1.39

Hyma, P., Jahan, N., Raheemunissa, S.G., K, B., 2014. • Emulgel: a review. Int. J. Pharm. Arch. ISSN 2319-7226 3.

Jantharaprapap, R., Stagni, G., 2007. Effects of penetration enhancers on in vitro permeability of meloxicam gels. Int. J. Pharm. 343, 26–33. https://doi.org/10.1016/j.ijpharm.2007.04.011

Jorge, L.L., Feres, C.C., Teles, V.E., 2010. Topical preparations for pain relief: efficacy and patient adherence. J. Pain Res. 4, 11–24. https://doi.org/10.2147/JPR.S9492

Kanig, J.L., Lachman, L., Lieberman, H.A., 2008. The theory and practice of industrial pharmacy. Editorial Pueblo y Educación.

Kapadiya, B., 2016. Formulation and Evaluation of Spironolactone Loaded Emulgel for Topical Application. JPSBR 6, 740–752.

Kapoor, D., Vyas, R.B., Lad, C., Patel, M., Lal, B., Parmar, R., 2014. Formulation, Development and Characterization of Emulgel of a NSAID'S 8.

Khullar, R., Kumar, D., Seth, N., Saini, S., 2012. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharm. J. 20, 63–67. https://doi.org/10.1016/j.jsps.2011.08.001

Kim, J.H., Ko, J.A., Kim, J.T., Cha, D.S., Cho, J.H., Park, H.J., Shin, G.H., 2014. Preparation of a Capsaicin-Loaded Nanoemulsion for Improving Skin Penetration. J. Agric. Food Chem. 62, 725–732. https://doi.org/10.1021/jf404220n

Klippel, J.H., Stone, J.H., White, P.H., 2008. Primer on the rheumatic diseases. Springer Science & Business Media.

Kumar, S.V., Tarun, P., Kumar, T.A., 2013. Transdermal drug delivery system for non-steroidal anti inflammatory drugs: A review 3, 18.

Lipinski, C.A., 2000. Drug-like properties and the causes of poor solubility and poor permeability. J. Pharmacol. Toxicol. Methods, Current Directions in Drug Discovery:A Review of Modern Techniques 44, 235–249. https://doi.org/10.1016/S1056-8719(00)00107-6

Lovell, D.J., 2008. Juvenile Idiopathic Arthritis, in: Klippel, J.H., Stone, J.H., Crofford, L.J., White, P.H. (Eds.), Primer on the Rheumatic Diseases. Springer New York, New York, NY, pp. 142–169. https://doi.org/10.1007/978-0-387-68566-3_7

Maruotti, N., Corrado, A., Cantatore, F.P., 2014. Osteoporosis and rheumatic diseases. Reumatismo 125–135. https://doi.org/10.4081/reumatismo.2014.785

McPherson, M.L., Cimino, N.M., 2013. Topical NSAID Formulations. Pain Med. 14, S35–S39. https://doi.org/10.1111/pme.12288

Mohamed, M.I., Abdelbary, A.A., Kandil, S.M., Mahmoud, T.M., 2019. Preparation and evaluation of optimized zolmitriptan niosomal emulgel. Drug Dev. Ind. Pharm. 45, 1157–1167.

Mondal, A., Maity, T.K., Bishayee, A., 2019. Analgesic and Anti-Inflammatory Activities of Quercetin-3-methoxy-4′-glucosyl-7-glucoside Isolated from Indian Medicinal Plant Melothria heterophylla. Medicines 6, 59. https://doi.org/10.3390/medicines6020059

Moss, I.L., An, H.S., Shen, F.H., Li, Z., Andersson, G.B.J., Zhang, Y., 2014. The Nonsurgical Treatment of Back Pain, in: Shapiro, I.M., Risbud, M.V. (Eds.), The Intervertebral Disc: Molecular and Structural Studies of the Disc in Health and Disease. Springer Vienna, Vienna, pp. 247–259. https://doi.org/10.1007/978-3-7091-1535-0_15

Mózsik, G., 2014. Capsaicin as New Orally Applicable Gastroprotective and Therapeutic Drug Alone or in Combination with Nonsteroidal Anti-Inflammatory Drugs in Healthy Human Subjects and in Patients, in: Abdel-Salam, O.M.E. (Ed.), Capsaicin as a Therapeutic Molecule, Progress in Drug Research. Springer Basel, Basel, pp. 209–258. https://doi.org/10.1007/978-3-0348-0828-6_9

Naga Sravan Kumar Varma, V., Maheshwari, P.V., Navya, M., Reddy, S.C., Shivakumar, H.G., Gowda, D.V., 2014. Calcipotriol delivery into the skin as emulgel for effective permeation. Saudi Pharm. J. 22, 591–599. https://doi.org/10.1016/j.jsps.2014.02.007
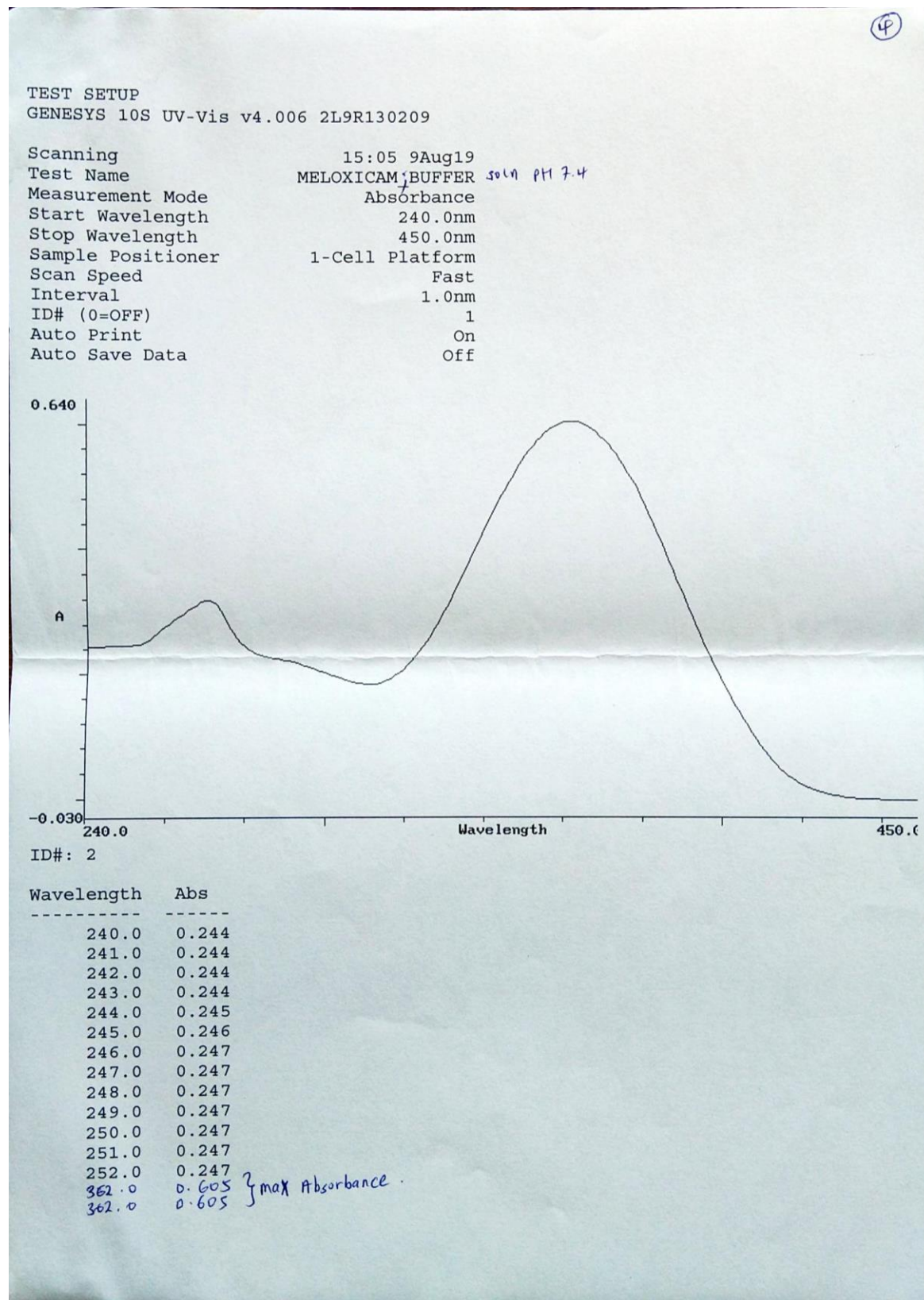
Nikumbh, K.V., Sevankar, S.G., Patil, M.P., 2015. Formulation development, in vitro and in vivo evaluation of microemulsion-based gel loaded with ketoprofen. Drug Deliv. 22, 509–515.

O'Neill, J., Brock, C., Olesen, A.E., Andresen, T., Nilsson, M., Dickenson, A.H., 2012. Unravelling the Mystery of Capsaicin: A Tool to Understand and Treat Pain. Pharmacol. Rev. 64, 939–971. https://doi.org/10.1124/pr.112.006163

Osborne, D.W., Musakhanian, J., 2018. Skin Penetration and Permeation Properties of Transcutol®—Neat or Diluted Mixtures. AAPS PharmSciTech 19, 3512–3533. https://doi.org/10.1208/s12249-018-1196-8

Panday, P., Shukla, N., Sisodiya, D., Jain, V., Mahajan, S., 2015. Design and characterization of microsponge loaded controlled release epicutaneous gel of lornoxicam. Appl. Med. Res. 1, 16–21.

Patel, M., Joshi, A., Hassanzadeth, H., Juluru, R., Stagni, G., 2011. Quantification of dermal and transdermal delivery of meloxicam gels in rabbits. Drug Dev. Ind. Pharm. 37, 613–617. https://doi.org/10.3109/03639045.2010.534098

Pednekar, A., Dandagi, P., Gadad, A., Mastiholimath, V., 2015. Formulation and characterisation of meloxicam loaded emulgel for topical application. Int. J. Pharm. Pharm. Sci. 7, 216–222.

Raj, E.L.P.S., Balakrishnan, S., 2016. Short Review - Emulgel. J. Compr. Pharm. Volume-3, Issue-1.

Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. J. Controlled Release 5, 23–36. https://doi.org/10.1016/0168-3659(87)90034-4

Sachan, N., Bhattacharya, A., Pushkar, S., Mishra, A., 2009. Biopharmaceutical classification system: A strategic tool for oral drug delivery technology. Asian J. Pharm. 3, 76. https://doi.org/10.4103/0973-8398.55042

Scalapino, K.J., Davis Jr., J.C., 2003. The treatment of ankylosing spondylitis. Clin. Exp. Med. 2, 159–165. https://doi.org/10.1007/s102380300000

Shinde, U.A., Parmar, S.J., Easwaran, S., 2019. Metronidazole-loaded nanostructured lipid carriers to improve skin deposition and retention in the treatment of rosacea. Drug Dev. Ind. Pharm. 45, 1039–1051.

Siegel, R.A., Rathbone, M.J., 2012. Overview of Controlled Release Mechanisms, in: Siepmann, J., Siegel, R.A., Rathbone, M.J. (Eds.), Fundamentals and Applications of Controlled Release Drug Delivery, Advances in Delivery Science and Technology. Springer US, Boston, MA, pp. 19–43. https://doi.org/10.1007/978-1-4614-0881-9_2

Simões, A., Veiga, F., Vitorino, C., Figueiras, A., 2018. A Tutorial for Developing a Topical Cream Formulation Based on the Quality by Design Approach. J. Pharm. Sci. 107, 2653–2662. https://doi.org/10.1016/j.xphs.2018.06.010

Singh, D., Bedi, N., 2016. Microemulsion Based Hydrogel of Tacrolimus for the Treatment of Atopic Dermatitis. Pharm. Nanotechnol. 4, 136–154.

Singhvi, G., Singh, M., 2011. Review: in-vitro drug release characterization models. Int. J. Pharm. Stud. Res. II, 77–84.

Singla, V., Saini, S., Joshi, B., Rana, A.C., 2012. Emulgel: a new platform for topical drug delivery. Int. J. Pharma Bio Sci. 3, 485–498.

Syngle, A., 2006. Arthritis and its Treatment, in: Rattan, S.I.S., Kassem, M. (Eds.), Prevention and Treatment of Age-Related Diseases. Springer Netherlands, Dordrecht, pp. 105–132. https://doi.org/10.1007/1-4020-5058-5_7

Tsai, D.-S., Huang, M.-H., Tsai, J.-C., Chang, Y.-S., Chiu, Y.-J., Lin, Y.-C., Wu, L.-Y., Peng, W.-H., 2015. Analgesic and Anti-Inflammatory Activities of Rosa taiwanensis Nakai in Mice. J. Med. Food 18, 592–600. https://doi.org/10.1089/jmf.2014.3197

Van der Heijde, D., 2008. Ankylosing Spondylitis, in: Klippel, J.H., Stone, J.H., Crofford, L.J., White, P.H. (Eds.), Primer on the Rheumatic Diseases. Springer New York, New York, NY, pp. 193–216. https://doi.org/10.1007/978-0-387-68566-3_9

Verma, A., Jain, A., Tiwari, A., Jain, S.K., 2018. Emulgels: Application Potential in Drug Delivery, in: Thakur, V.K., Thakur, M.K. (Eds.), Functional Biopolymers, Springer Series on Polymer and Composite Materials. Springer International Publishing, Cham, pp. 343–371. https://doi.org/10.1007/978-3-319-66417-0_11

Williams, H.D., Trevaskis, N.L., Charman, S.A., Shanker, R.M., Charman, W.N., Pouton, C.W., Porter, C.J., 2013. Strategies to address low drug solubility in discovery and development. Pharmacol. Rev. 65, 315–499.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547. https://doi.org/10.3181/00379727-111-27849

Wongrakpanich, S., Wongrakpanich, A., Melhado, K., Rangaswami, J., 2018. A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. Aging Dis. 9, 143–150. https://doi.org/10.14336/AD.2017.0306

Xu, S., Rouzer, C.A., Marnett, L.J., 2014. Oxicams, a Class of NSAIDs and beyond. IUBMB Life 66, 803–811. https://doi.org/10.1002/iub.1334

Zatarain, E., 2007. Osteoarthritis, in: Nuovo, J. (Ed.), Chronic Disease Management. Springer New York, New York, NY, pp. 307–326. https://doi.org/10.1007/978-0-387-49369-5_10

Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C., Xie, S., 2010. DDSolver: An Add-In Program for Modeling and Comparison of Drug Dissolution Profiles. AAPS J. 12, 263–271. https://doi.org/10.1208/s12248-010-9185-1

APPENDICES

APPENDIX 1: UV spectra


```
TEST SETUP
GENESYS 10S UV-Vis v4.006 2L9R130209

Scanning                      15:05 9Aug19
Test Name            MELOXICAM;BUFFER soln PH 7.4
Measurement Mode           Absorbance
Start Wavelength              240.0nm
Stop Wavelength              450.0nm
Sample Positioner    1-Cell Platform
Scan Speed                     Fast
Interval                      1.0nm
ID# (0=OFF)                       1
Auto Print                       On
Auto Save Data                  Off
```

```
ID#: 2

Wavelength    Abs
----------   ------
   240.0    0.244
   241.0    0.244
   242.0    0.244
   243.0    0.244
   244.0    0.245
   245.0    0.246
   246.0    0.247
   247.0    0.247
   248.0    0.247
   249.0    0.247
   250.0    0.247
   251.0    0.247
   252.0    0.247
   362.0    0.605   } max Absorbance.
   362.0    0.605
```

UV spectrum of Meloxicam using phosphate buffer solution pH 7.4 as the solvent

I

APPENDIX 2: FTIR spectra
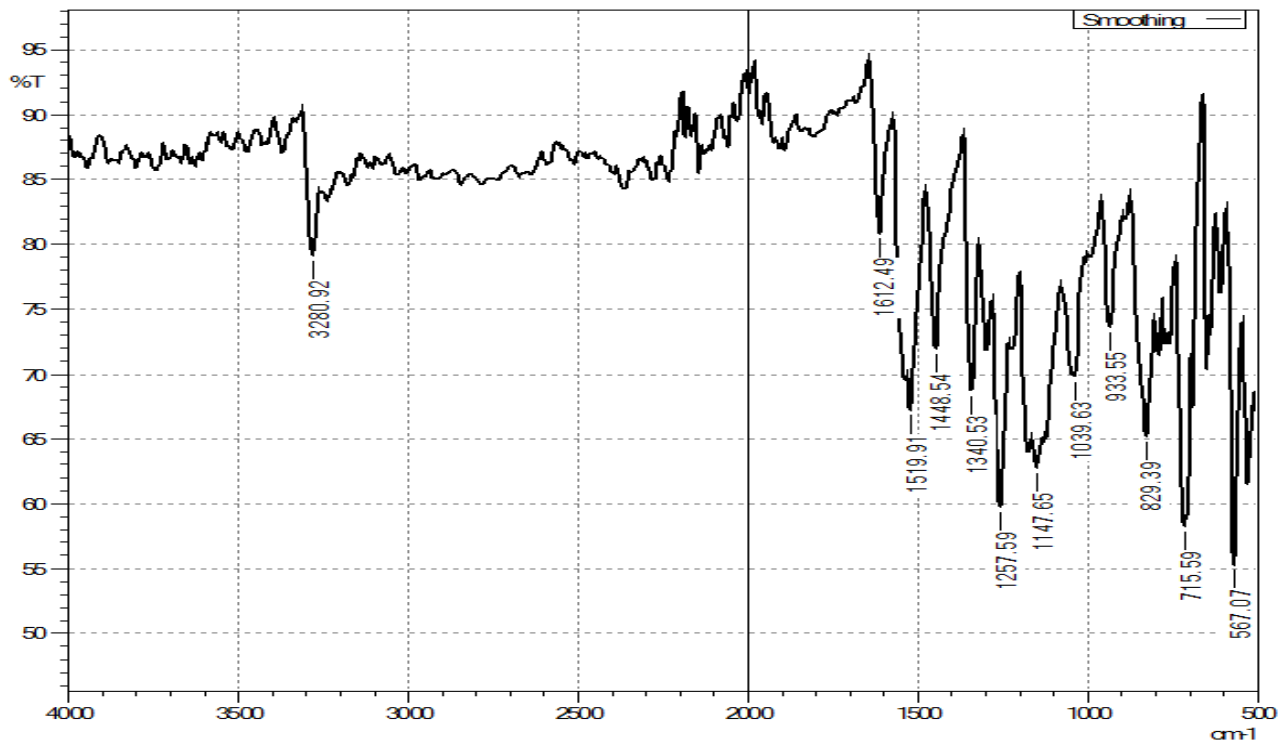
FTIR Spectrum of USP Meloxicam Standard
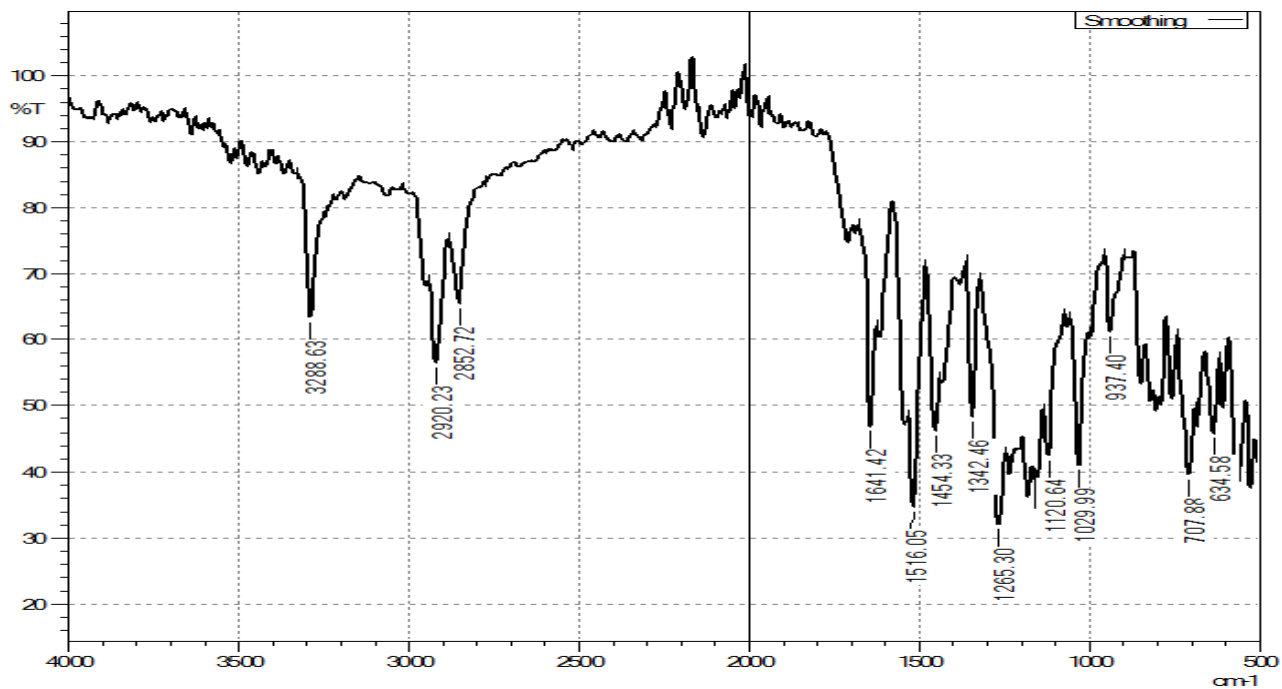
FTIR Spectrum of Meloxicam API and Capsaicin binary mixture
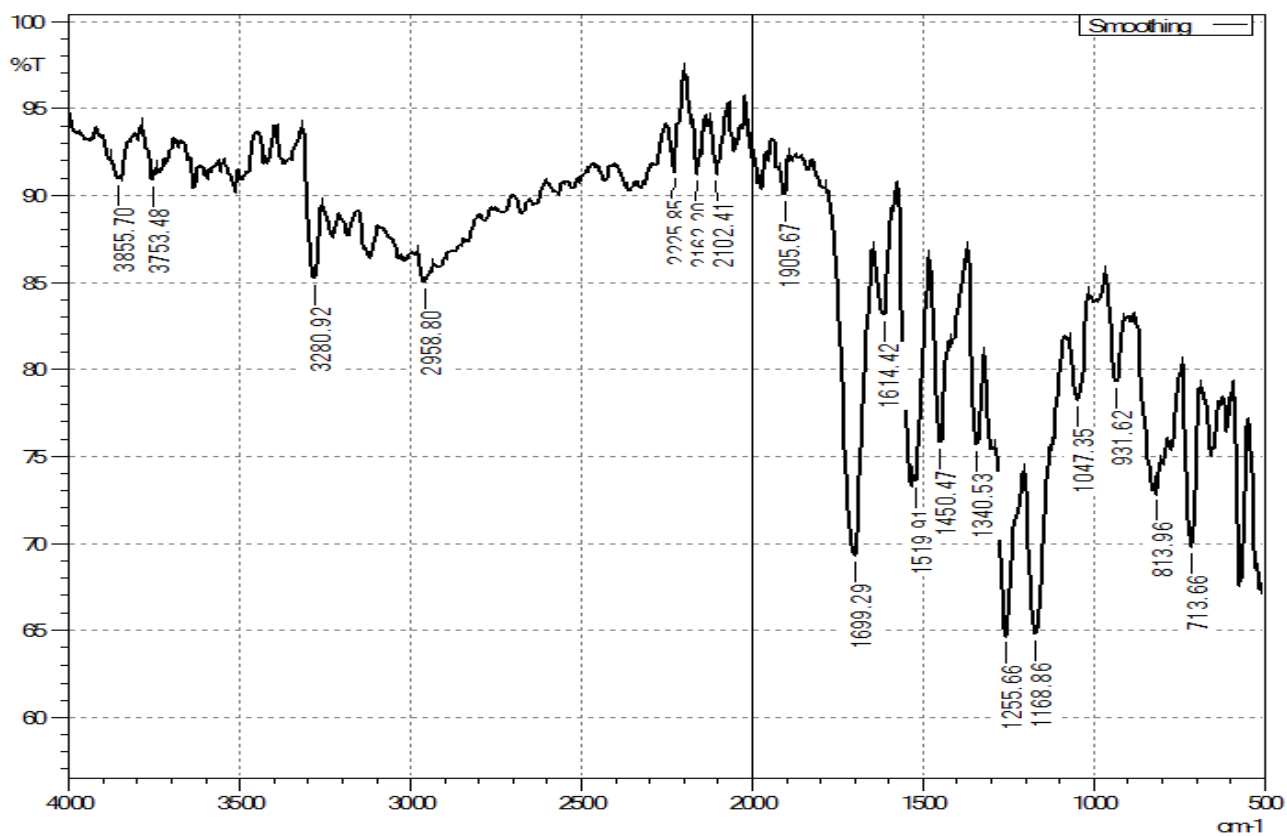
FTIR Spectrum of Meloxicam API and Carbopol 934 binary mixture
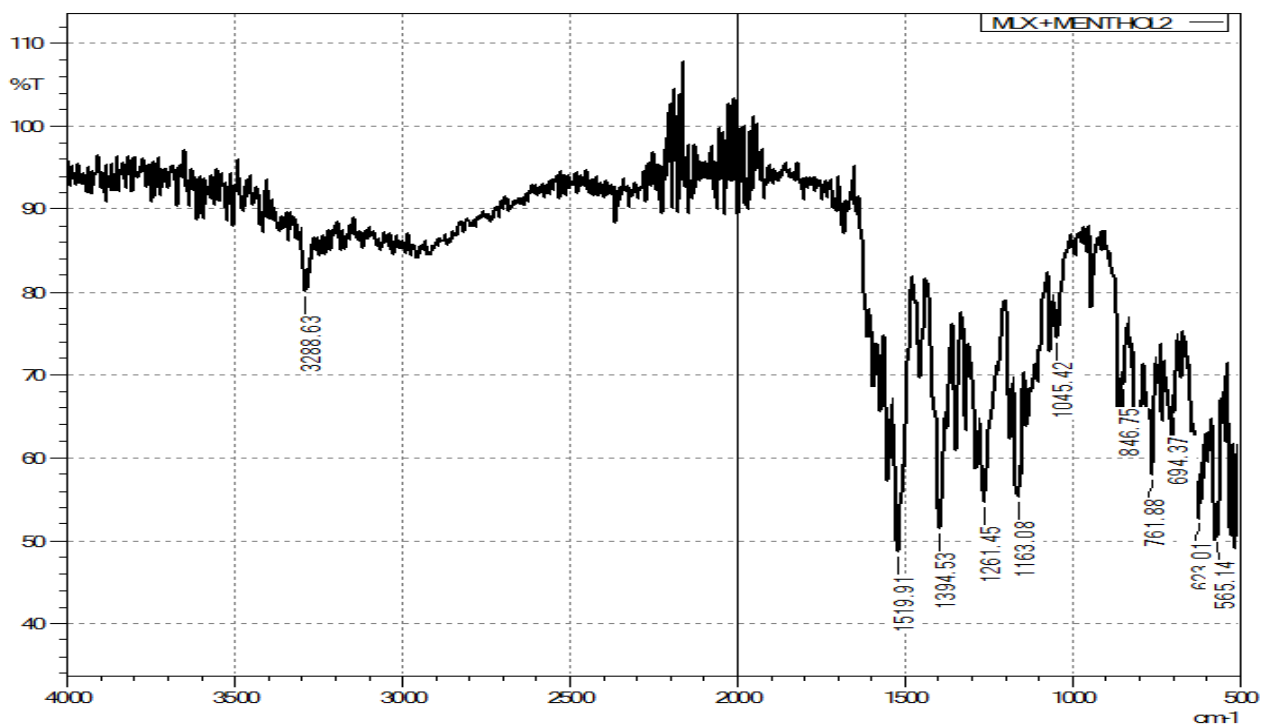
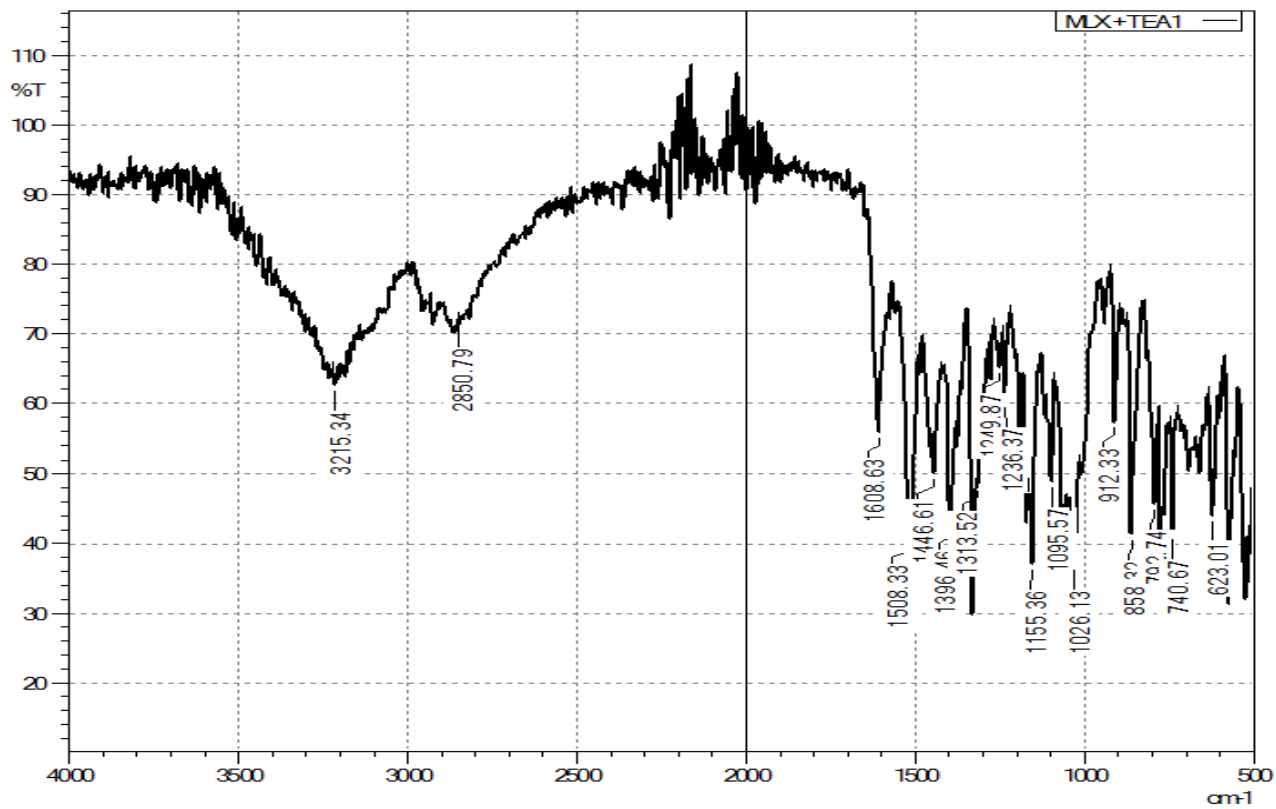FTIR Spectrum of Meloxicam API and Menthol binary mixture

III
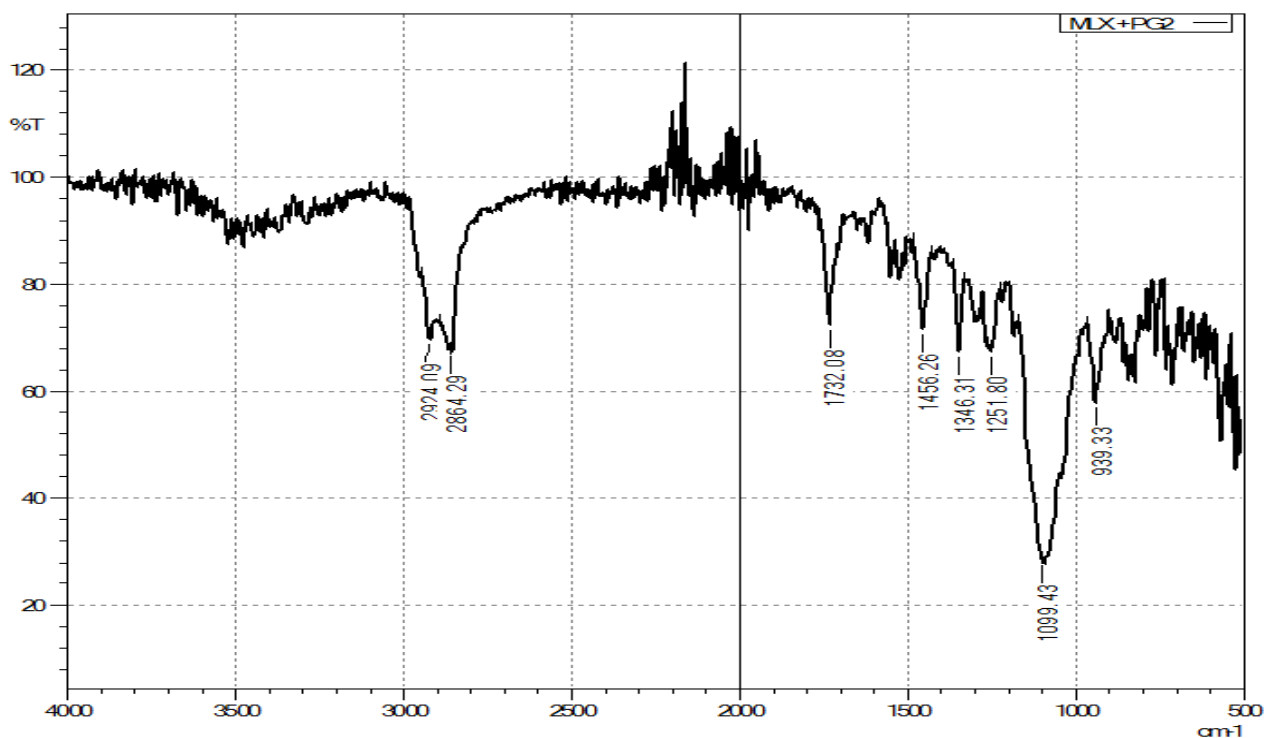
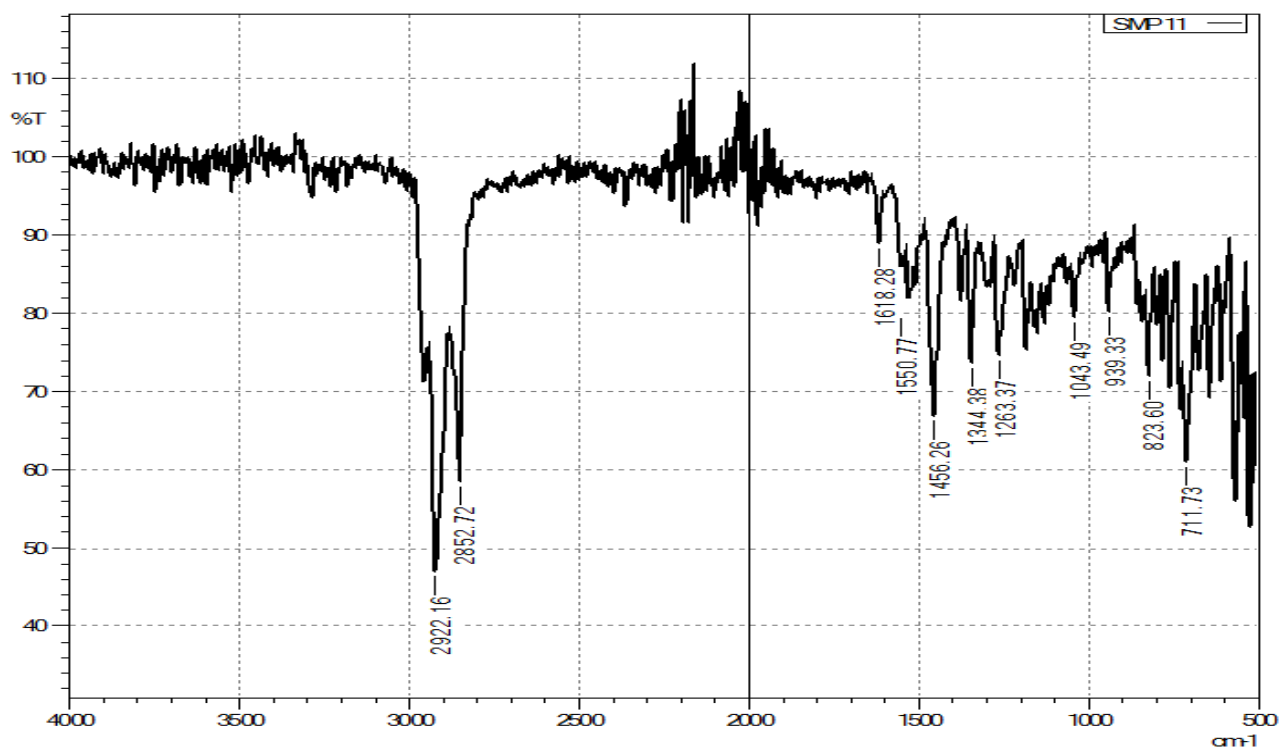FTIR Spectrum of Meloxicam API and Propylene Glycol binary mixture
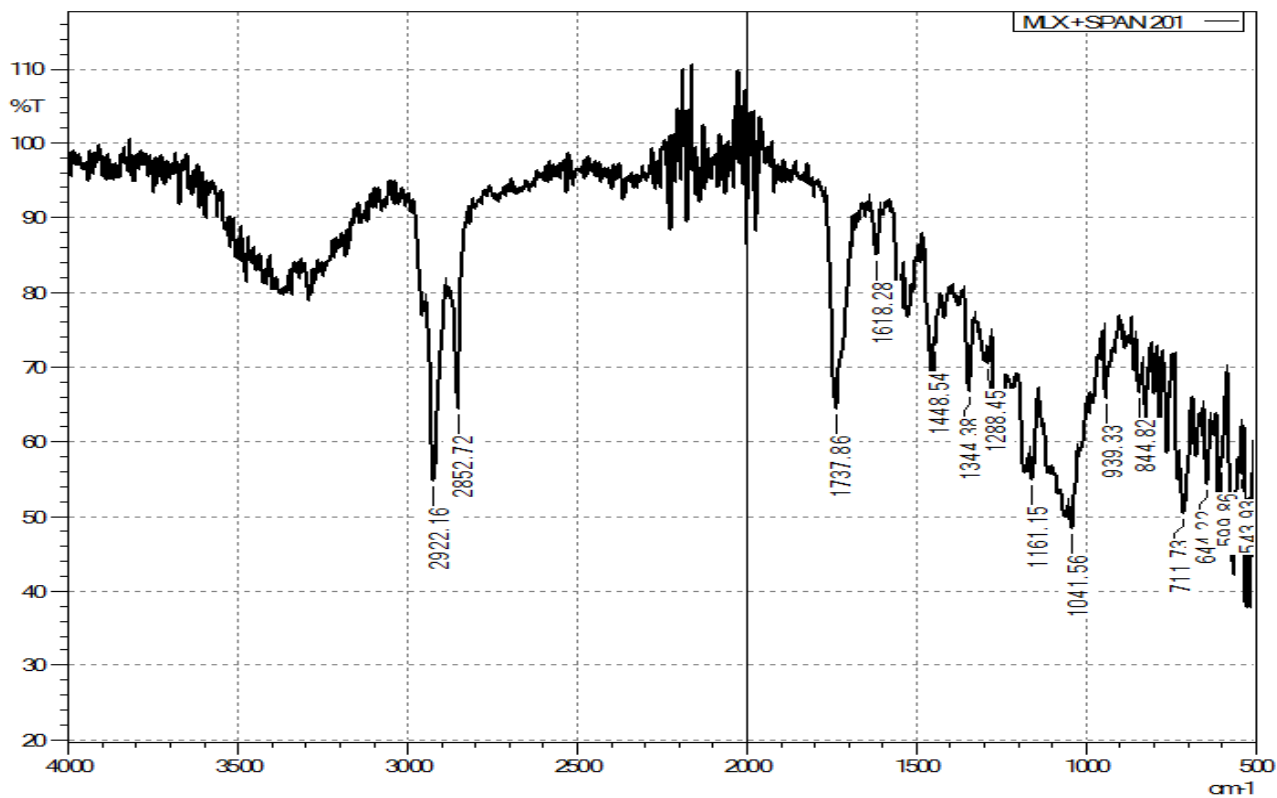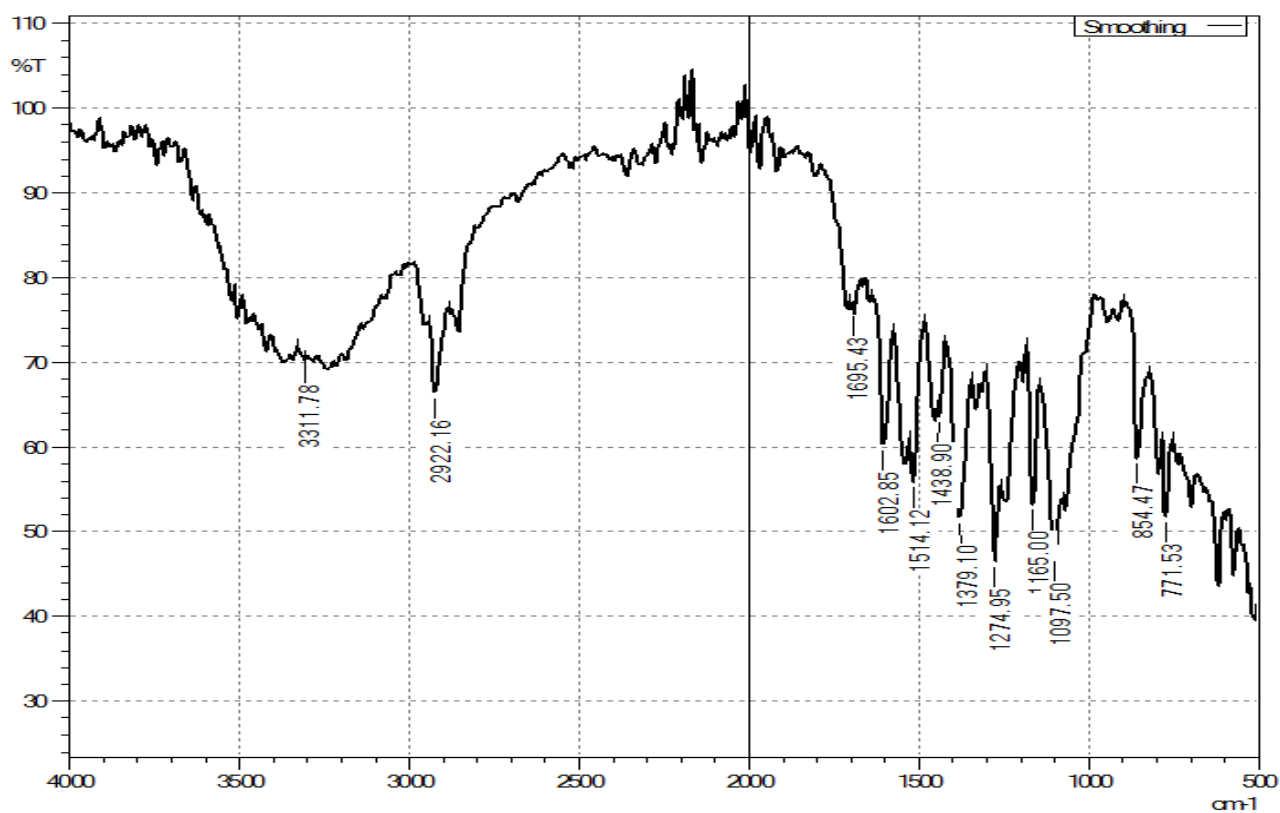

FTIR Spectrum of Meloxicam API and Span 20 binary mixture

IV
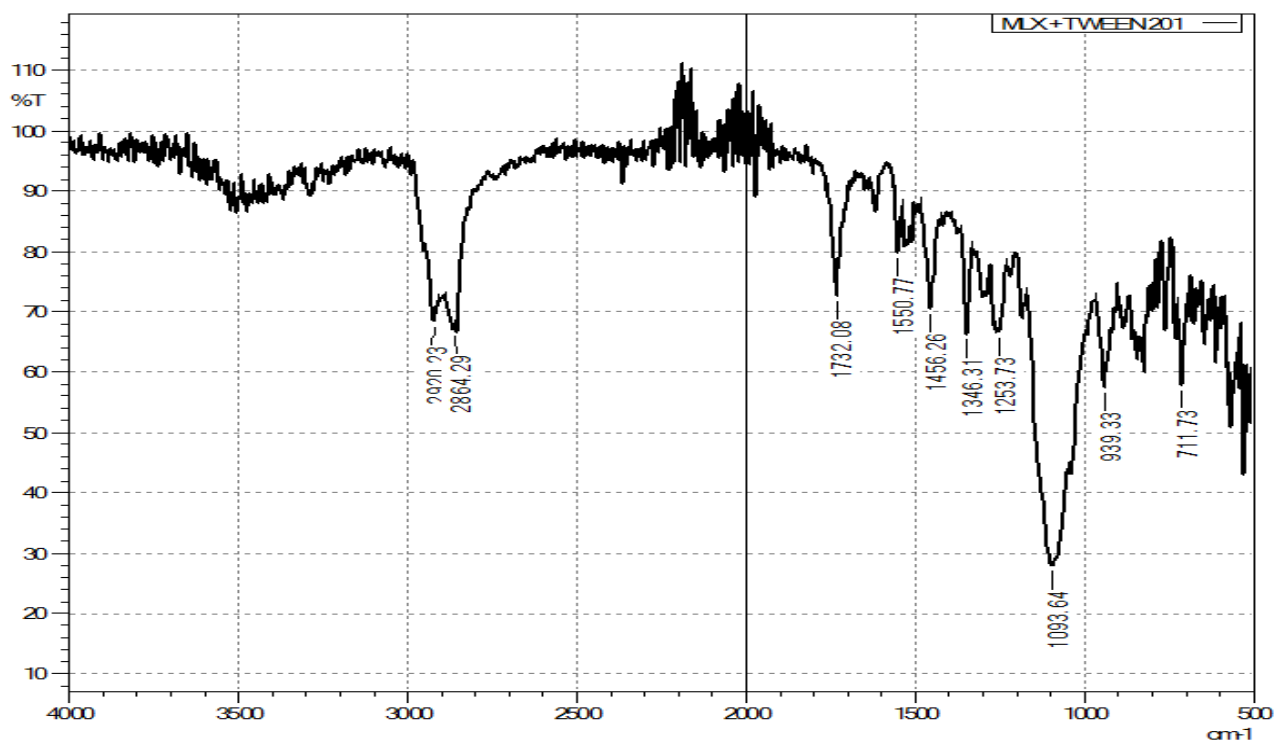
FTIR Spectrum of Meloxicam API and Tween 20 binary mixture

FTIR Spectrum of Meloxicam API and Triethanolamine binary mixture

FTIR Spectrum of Meloxicam API and Liquid paraffin binary mixture

FTIR Spectrum of Meloxicam API and all excipients used binary mixture

VI

APPENDIX 3: Pictures captured during formulation of emulgels

Mixing process during emulgel formulation

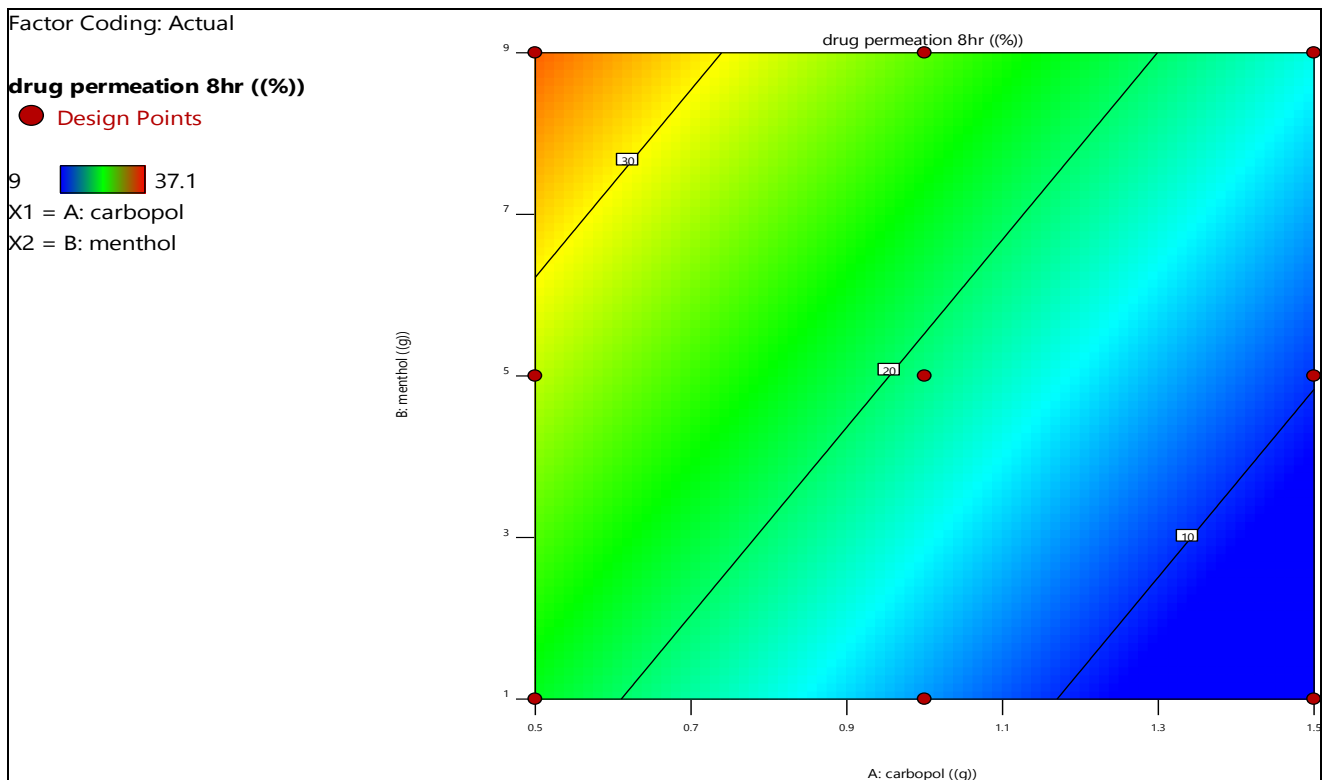Formulated emulgels packed in well labeled containers

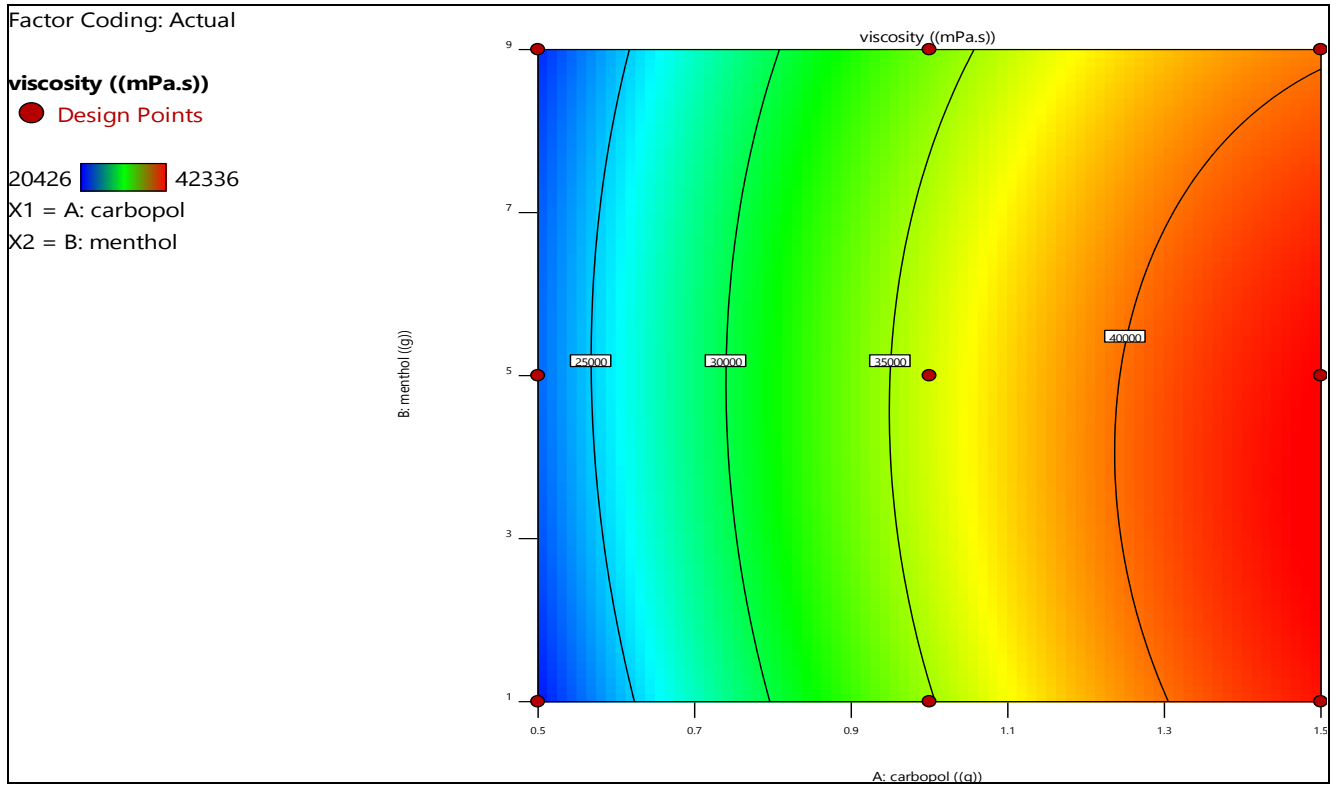Formulations F10 and F11 after formulation

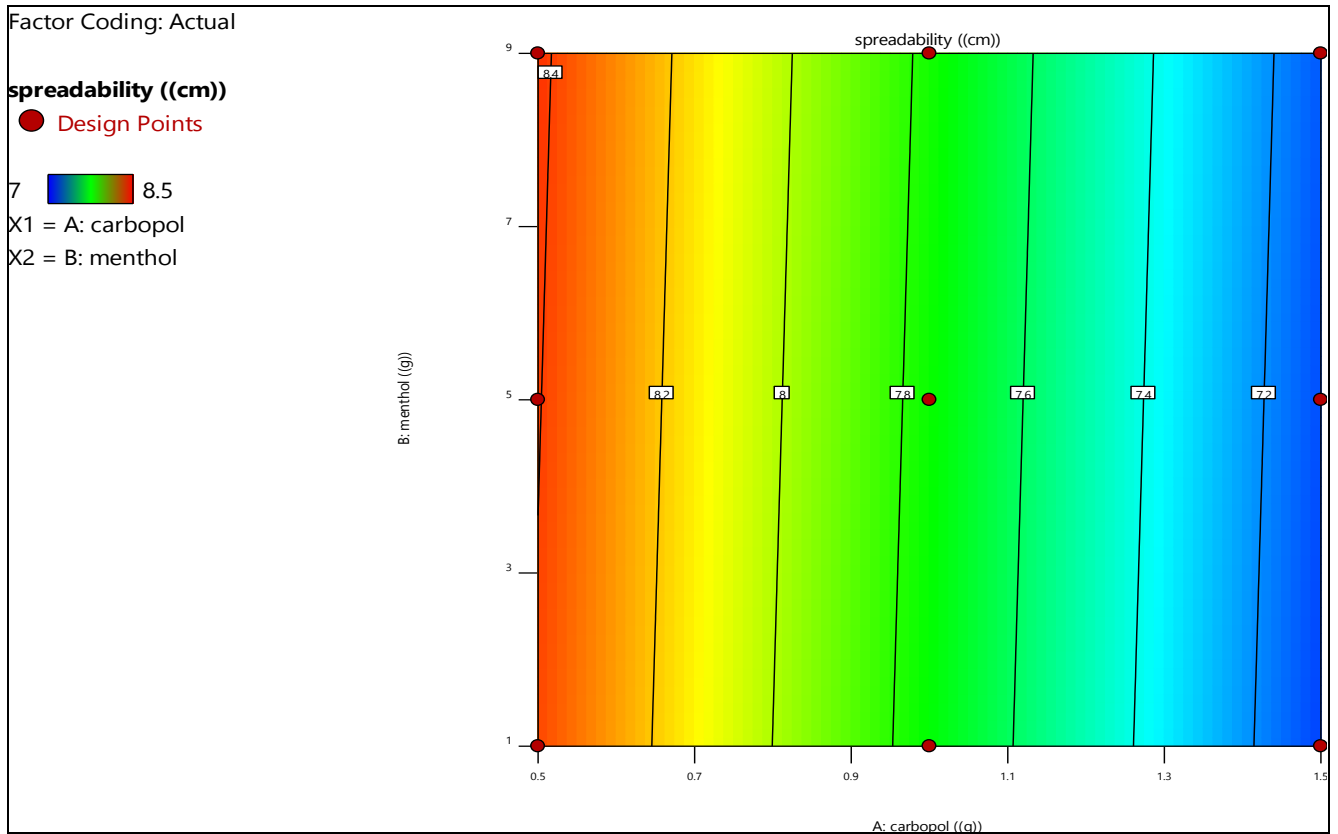# APPENDIX 4: Contour and response surface plots
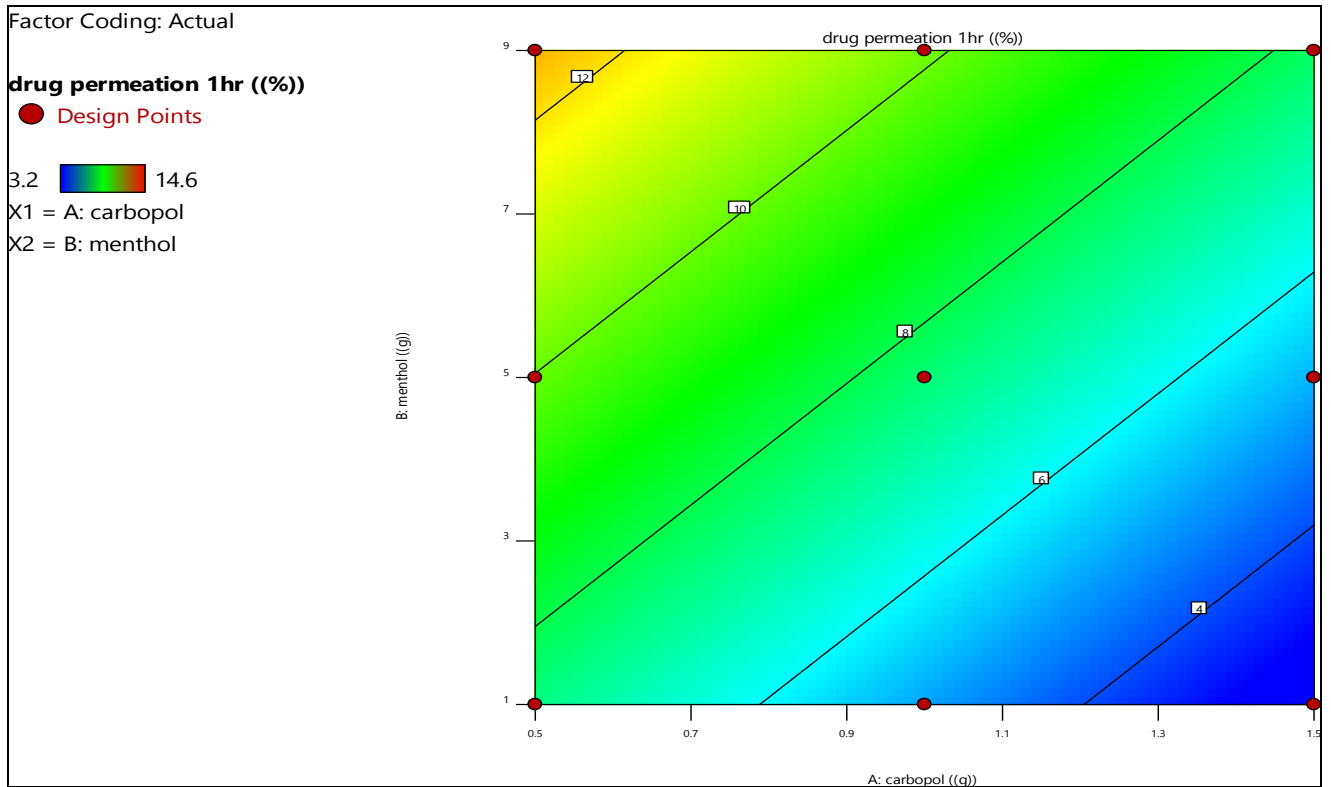
Contour plot showing effect of carbopol 934 and menthol on cumulative drug release after 1 hr
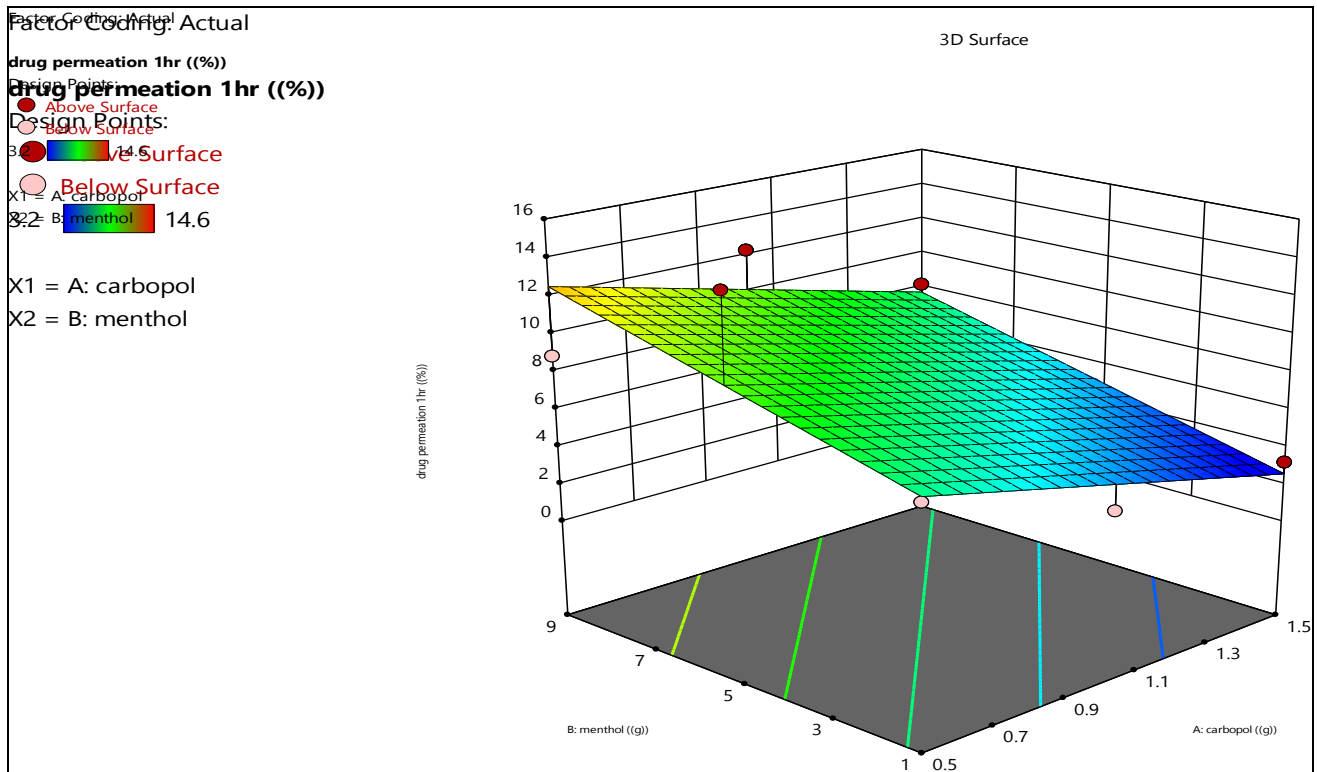

Contour plot showing effect of carbopol 934 and menthol on cumulative drug release after 8 hrs
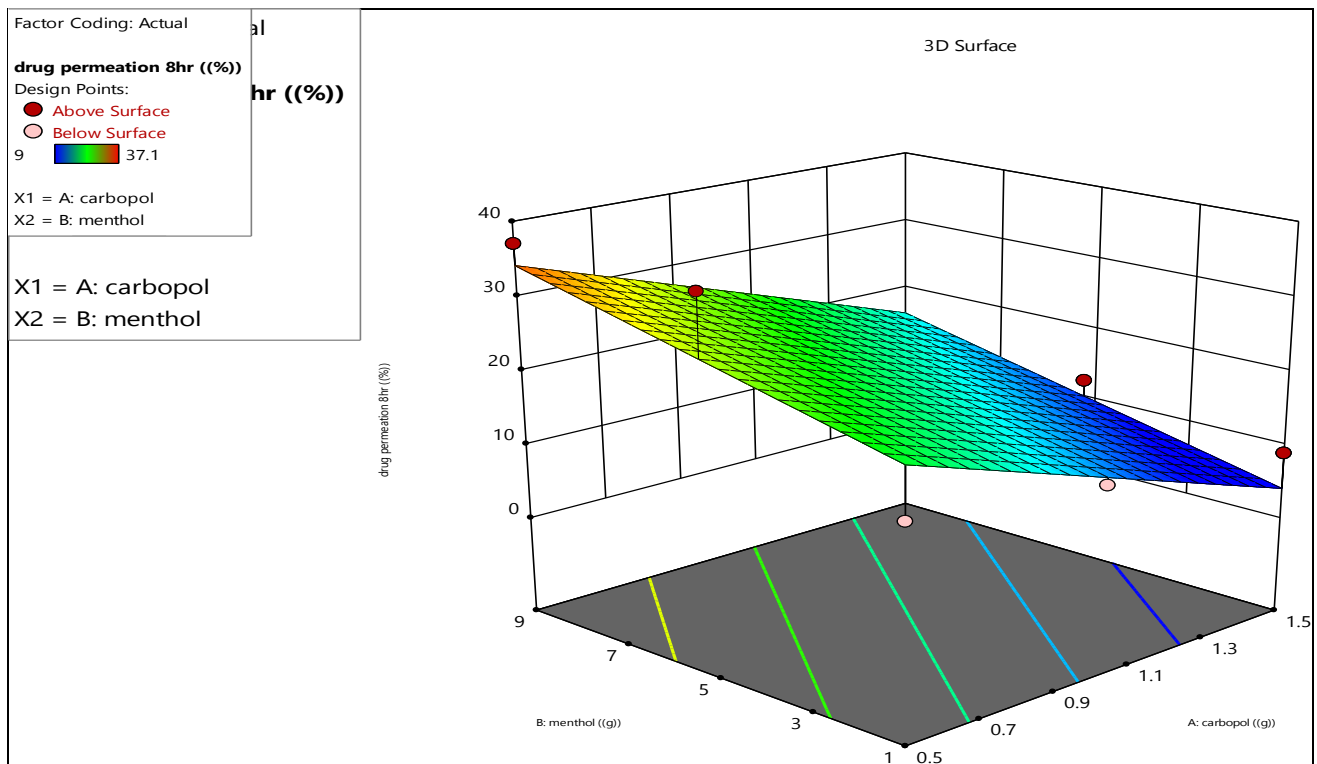
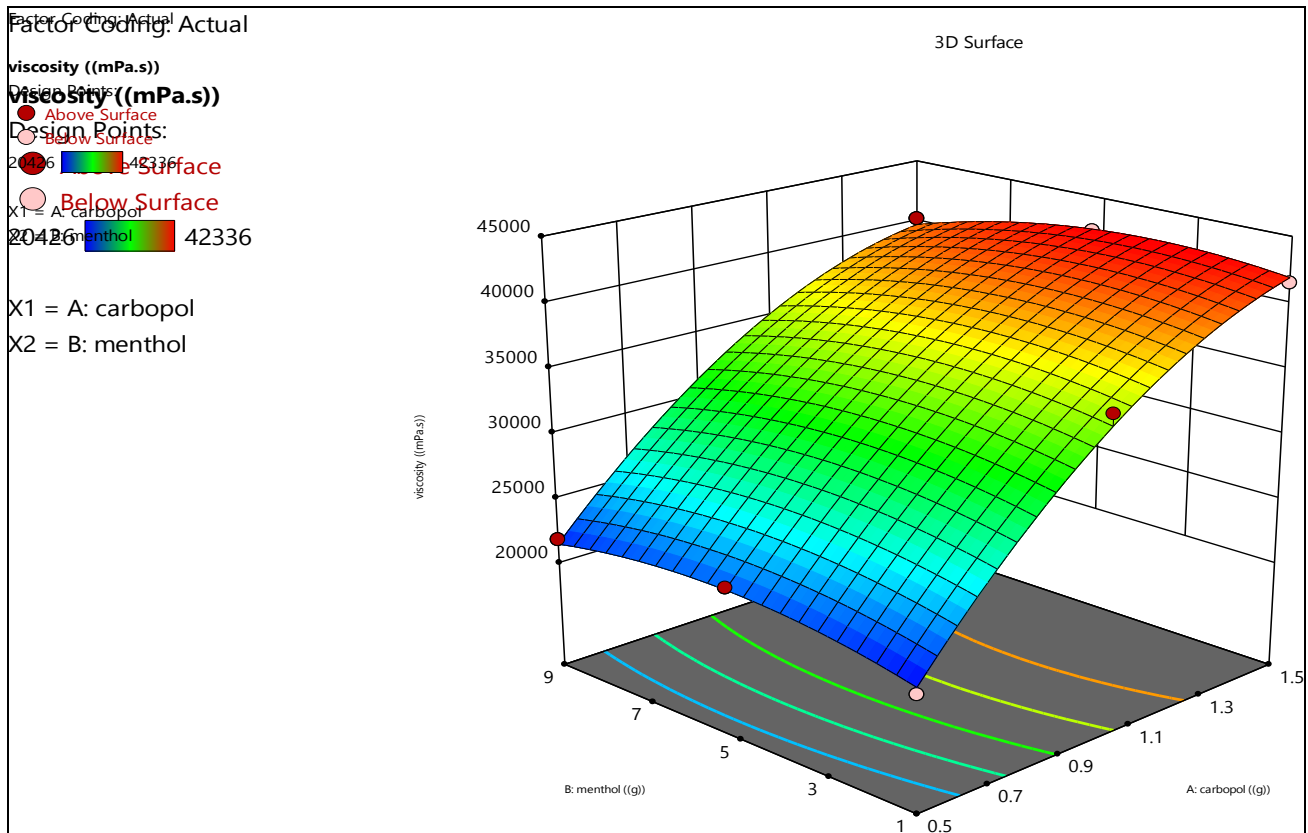Contour plot showing effect of carbopol 934 and menthol on viscosity

Contour plot showing effect of carbopol 934 and menthol on spreadability
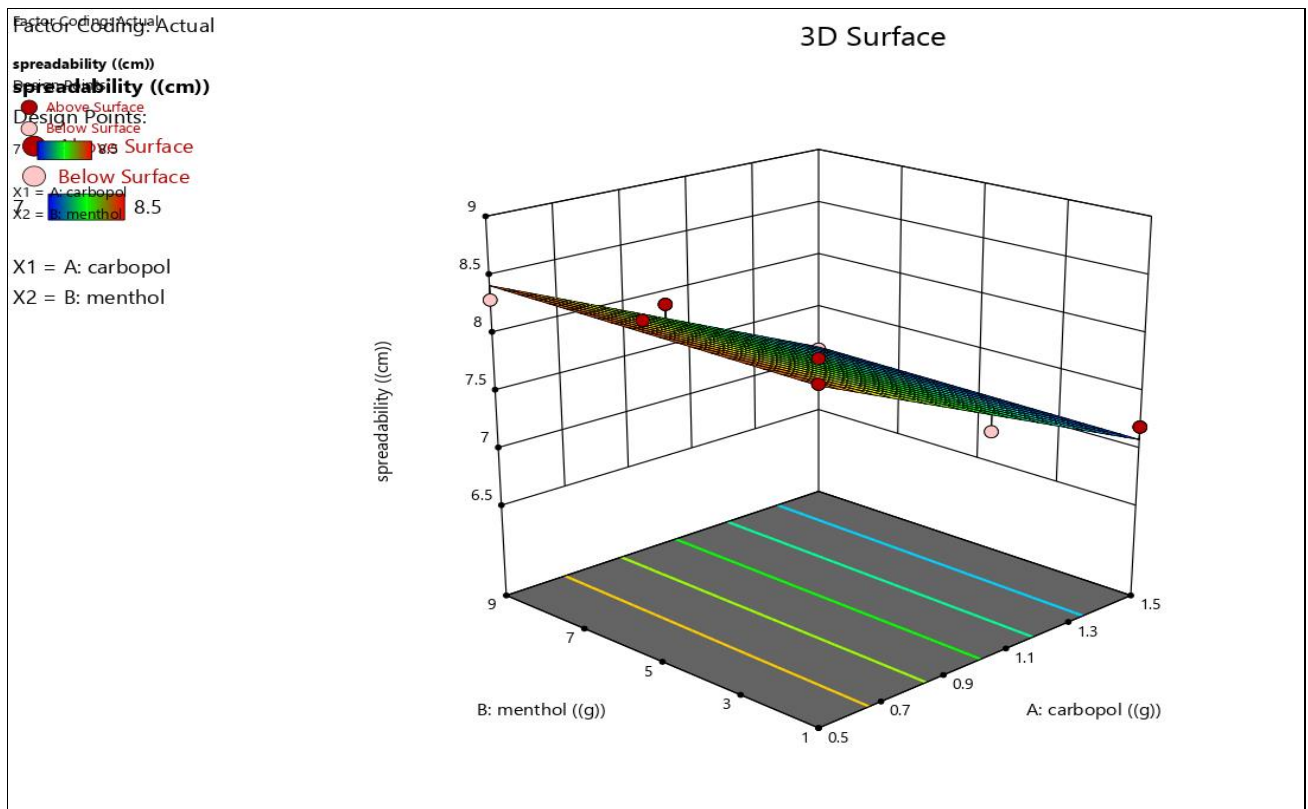
X

Response surface plot showing effect of carbopol 934 and menthol on cumulative drug release after 1 hr

Response surface plot showing effect of carbopol 934 and menthol on cumulative drug release after 8 hrs
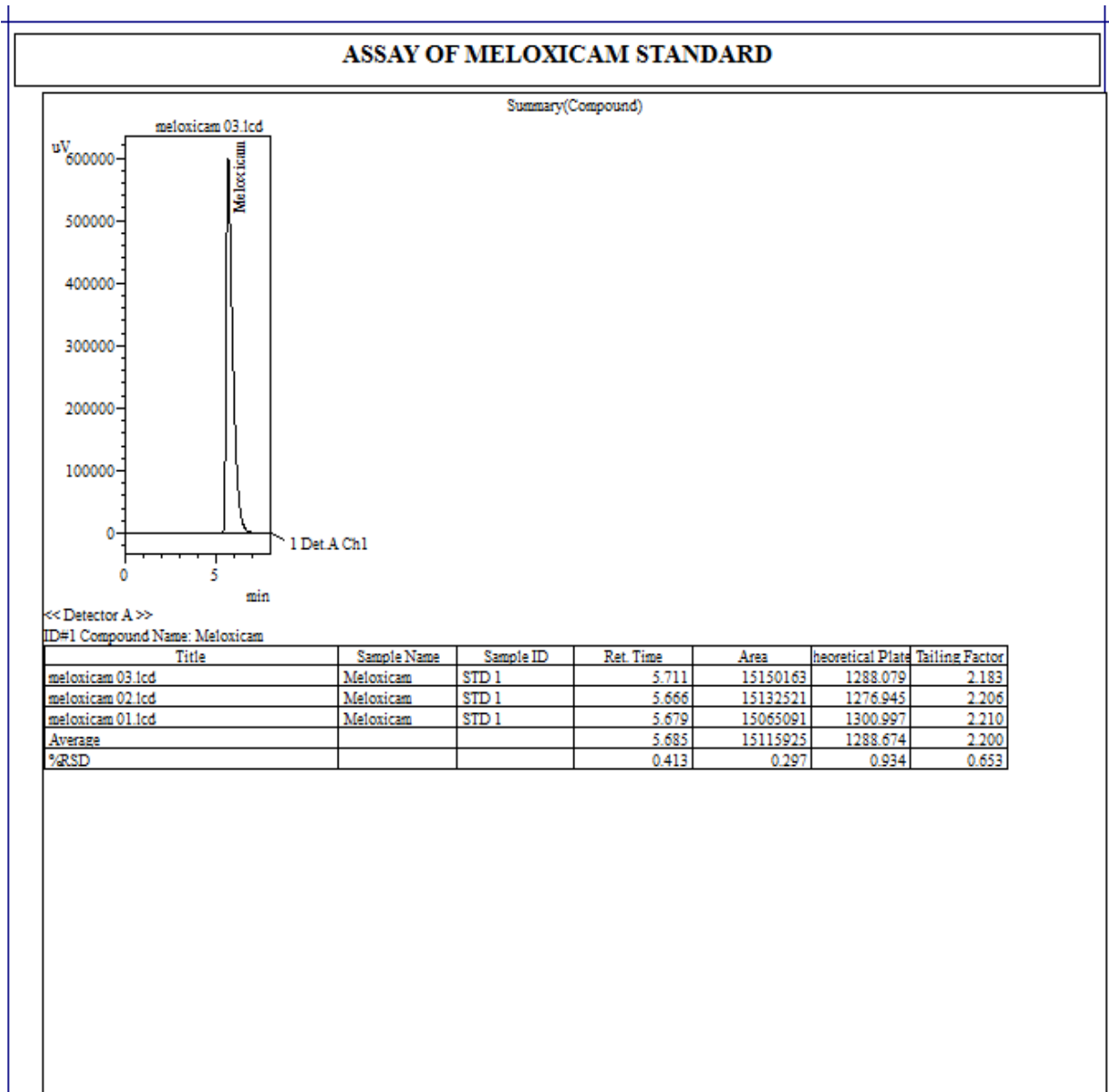
Response surface plot showing effect of carbopol 934 and menthol on viscosity

Response surface plot showing effect of carbopol 934 and menthol on spreadability

XII

APPENDIX 5: Chromatograms

ASSAY OF MELOXICAM STANDARD

Summary(Compound)

meloxicam 03.lcd

<< Detector A >>
ID#1 Compound Name: Meloxicam

Title	Sample Name	Sample ID	Ret. Time	Area	Theoretical Plate	Tailing Factor
meloxicam 03.lcd	Meloxicam	STD 1	5.711	15150163	1288.079	2.183
meloxicam 02.lcd	Meloxicam	STD 1	5.666	15132521	1276.945	2.206
meloxicam 01.lcd	Meloxicam	STD 1	5.679	15065091	1300.997	2.210
Average			5.685	15115925	1288.674	2.200
%RSD			0.413	0.297	0.934	0.653

Chromatogram of USP meloxicam Standard

ASSAY OF MELOXICAM POWDER

Summary(Compound)
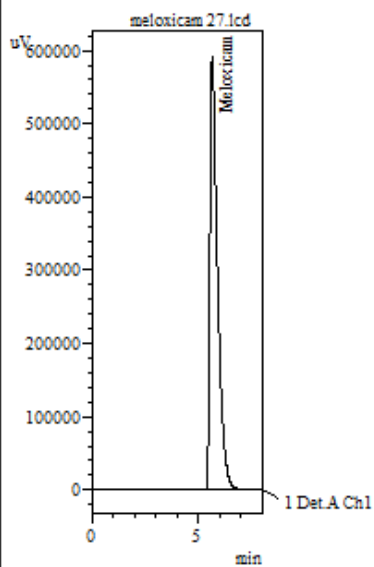
meloxicam 27.lcd

<< Detector A >>
ID#1 Compound Name: Meloxicam

Title	Sample Name	Sample ID	Ret. Time	Area	Theoretical Plate	Tailing Factor
meloxicam 27.lcd	Meloxicam powde	Sample 2	5.668	14928584	1272.463	2.151
meloxicam 26.lcd	Meloxicam powde	Sample 2	5.680	14928053	1272.638	2.140
meloxicam 25.lcd	Meloxicam powde	Sample 2	5.694	14926134	1273.108	2.155
Average			5.681	14927590	1272.736	2.148
%RSD			0.227	0.009	0.026	0.343

Chromatogram of Meloxicam API powder

ASSAY OF MELOXICAM GEL F10

Summary(Compound)
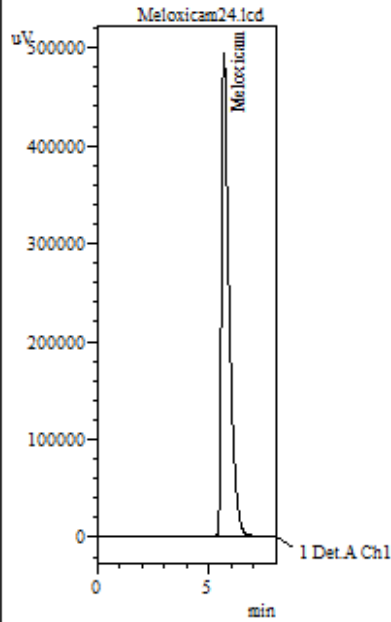
Meloxicam24.lcd

1 Det.A Ch1

<< Detector A >>
ID#1 Compound Name: Meloxicam

Title	Sample Name	Sample ID	Ret. Time	Area	Theoretical Plate	Tailing Factor
Meloxicam24.lcd	Meloxicam gel	F10 - 1 rpt	5.688	11940536	1402.515	2.097
Meloxicam23.lcd	Meloxicam gel	F10 - 1	5.691	12190088	1360.905	2.149
Meloxicam22.lcd	Meloxicam gel	F10 - 1	5.696	12202940	1367.409	2.174
Average			5.692	12111188	1376.943	2.140
%RSD			0.075	1.221	1.626	1.837

Chromatogram of meloxicam emulgel formulation F10

ASSAY OF MELOXICAM GEL F11

Summary(Compound)
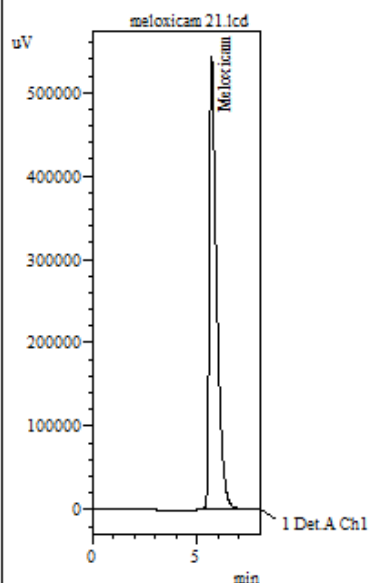
meloxicam 21.1cd

<< Detector A >>
ID#1 Compound Name: Meloxicam

Title	Sample Name	Sample ID	Ret. Time	Area	Theoretical Plate	Tailing Factor
meloxicam 21.1cd	Meloxicam gel F11	Sample 3	5.711	13282568	1357.693	2.093
meloxicam 20.1cd	Meloxicam gel F11	Sample 3	5.706	13412880	1368.324	2.096
meloxicam 19.1cd	Meloxicam gel F11	Sample 3	5.679	13282634	1351.573	2.098
Average			5.698	13326027	1359.197	2.096
%RSD			0.295	0.564	0.624	0.122

Chromatogram of meloxicam emulgel formulation F11