FORMULATION, DEVELOPMENT AND EVALUATION OF KETOPROFEN LOADED TRANSETHOSOMES GEL FOR EFFECTIVE ANTI-INFLAMMATORY ACTIVITY

A THESIS

Submitted Towards the requirement for the Award of degree of

Doctor of Philosophy

In

Pharmacy

Under the Faculty of Pharmacy

By

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Under the Supervision of

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P.K. UNIVERSITY

2026

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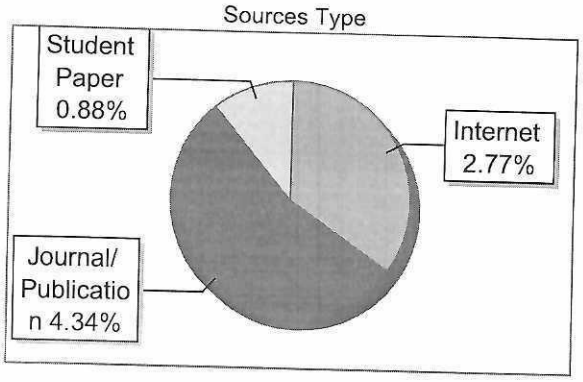
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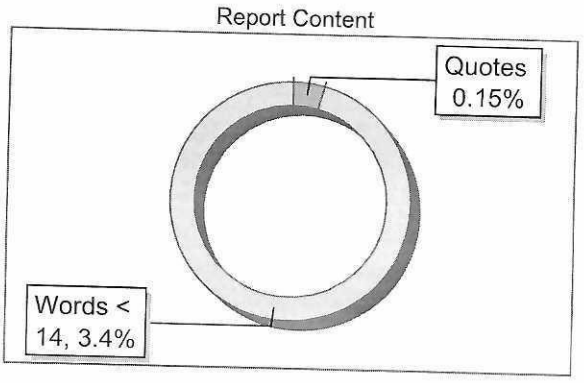
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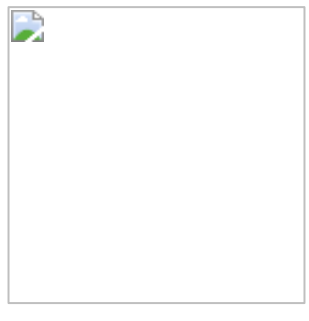
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Date: (**Mr. Ashok Kumar Kushwah**)
Place:

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ABSTRACT

A common non-steroidal anti-inflammatory medicine (NSAID) is ketoprofen ([RS] 2-[3-benzoylphenyl]-propionic acid). The pharmaceutical industry offers ketoprofen in a number of different forms, including as coated tablets, capsules, topical gels, transdermal patches, liquid spray, and injectable solutions. The goal of the current study was to investigate the potential of transethosome gel formulations for the transdermal delivery of ketoprofen and to determine the impact of lipid content, ethanol concentration, drug concentration, and stirrer time on the outcomes. Transethosomes were characterized using vesicle size, surface charge, entrapment efficiency, and stability investigations. The transethosomes containing gel were characterized using viscosity, pH, drug content, extrudability, spreadability, and in vitro drug diffusion investigations. The average vesicle size (nm), % EE, and zeta potential of the transethosome formulation that was optimized.The gel's ketoprofen-loaded transethosomes composition was improved, and because of its non-steroidal anti-inflammatory properties, it may now be used as a topical drug. The results showed that transethosomes gel was a good option for long-term, customized transdermal drug delivery. Additionally, it enhances how well many drugs pass through the skin.

Keywords: Transethosomes gel, Ketoprofen, Franz diffusion cell, NSAID, anti-inflammatory.

CHAPTER – 1

INTRODUCTION

INTRODUCTION

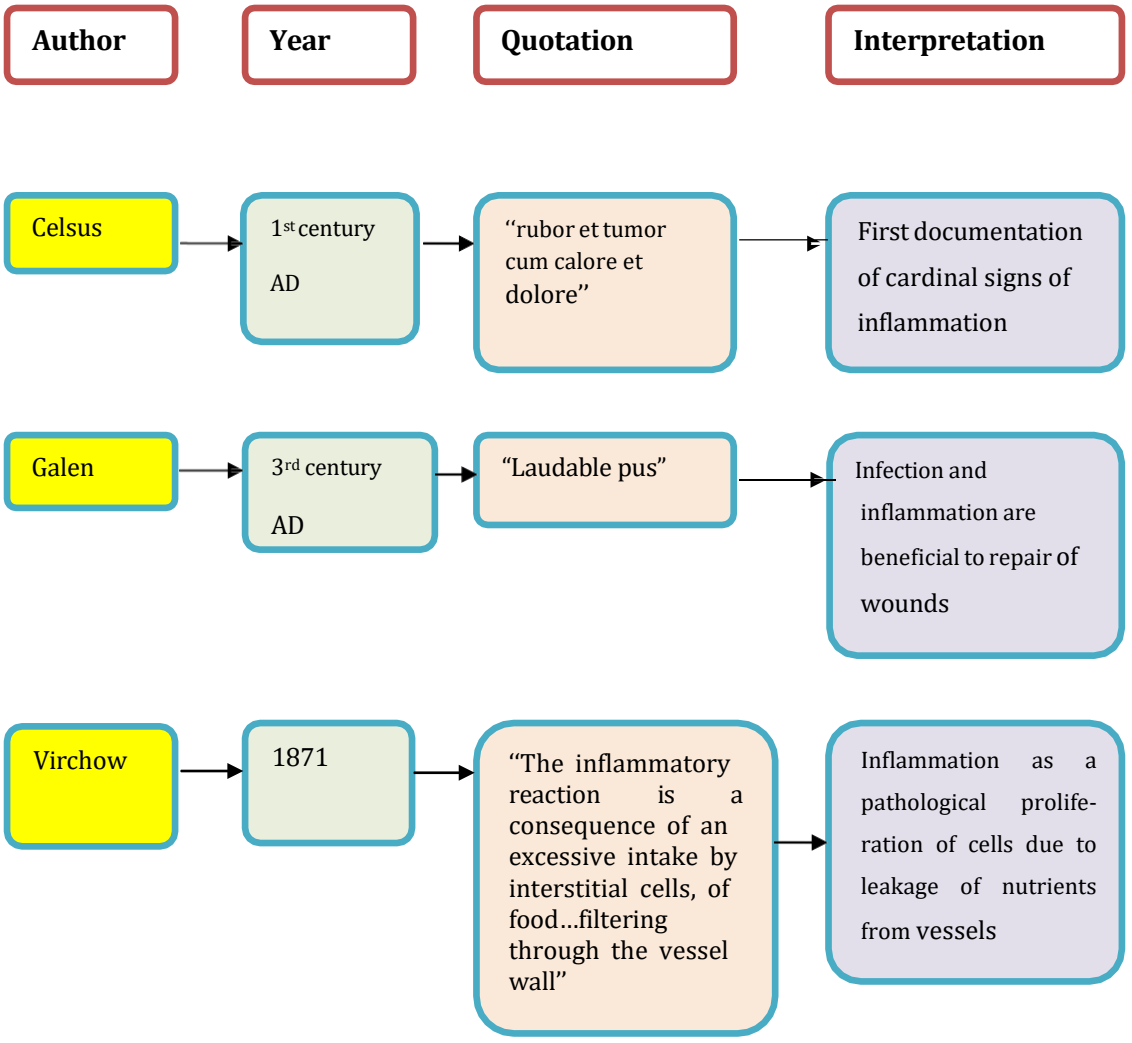
1.1 Inflammation: Definition and Types of Inflammation

Inflammation is the mechanism of human diseases displaying the five classic inflammatory signs: redness, swelling, heat, pain and subsequent loss of organ function (Figure 1.3). Aulus Cornelius Celsus and his followers already described these signs in the 1st and 2nd century AD. The underlying mechanisms of inflammation remained unknown until the 19th century when Ilya I Mechnikov discovered phagocytes and subsequently defined the inflammatory state as a "lesion of the vessels which are attacked by the irritating cause" (Metchnikoff E *et al.*). Depending on the nature of the „irritating cause", we distinguish the following types of inflammation: microbial, autoimmune, allergic, metabolic and physical inflammation.

1. *Microbial inflammation*, caused by bacteria, viruses, fungi and protozoa, mediates abscess, pneumonia, sepsis, Ebola Haemorrhagic Fever, human immunodeficiency virus infection and other infectious diseases.
2. *Autoimmune inflammation*, caused by an aberrant autoimmune attack by auto antibodies or auto reactive B and T cells, mediates rheumatoid arthritis, Type 1 diabetes, multiple sclerosis, Crohn's disease, psoriasis, systemic lupus erythematosus and other autoimmune diseases.
3. *Allergic inflammation*, caused by allergens, mediates atopic dermatitis, allergic rhinitis, asthma and other allergic diseases.

4. *Metabolic inflammation*, caused by overfeeding and excessive accumulation of metabolites (e.g. uric acid or cholesteryl esters) resulting from inborn or acquired metabolic dysfunction, mediates gout or atherosclerosis, among other metabolic diseases.
5. The fifth type, *physical inflammation*, is caused by trauma, burns and radiation (Table1.1) (Aksentijevich *et al.*).

1.2 Major Advances in Developing a "Definition of Inflammation" Between 1st And 20th Centuries AD (A Scot *et al.*)



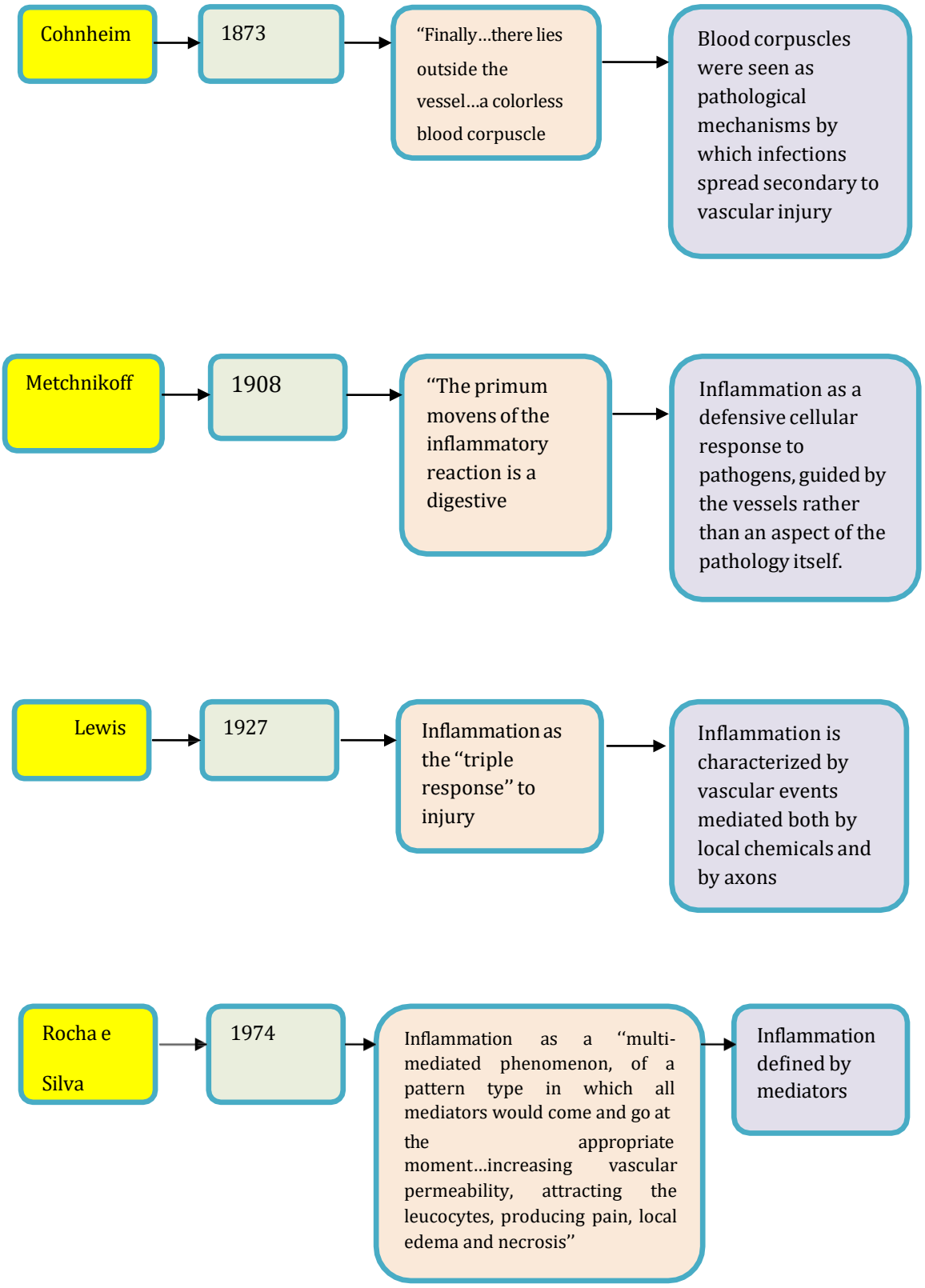


Fig. 1: Major advances in developing a definition of inflammation between 1st and 20th centuries AD

1.3 Signs of Inflammation and Causes (Rocha e Silva *et al*., Benaroyo L)

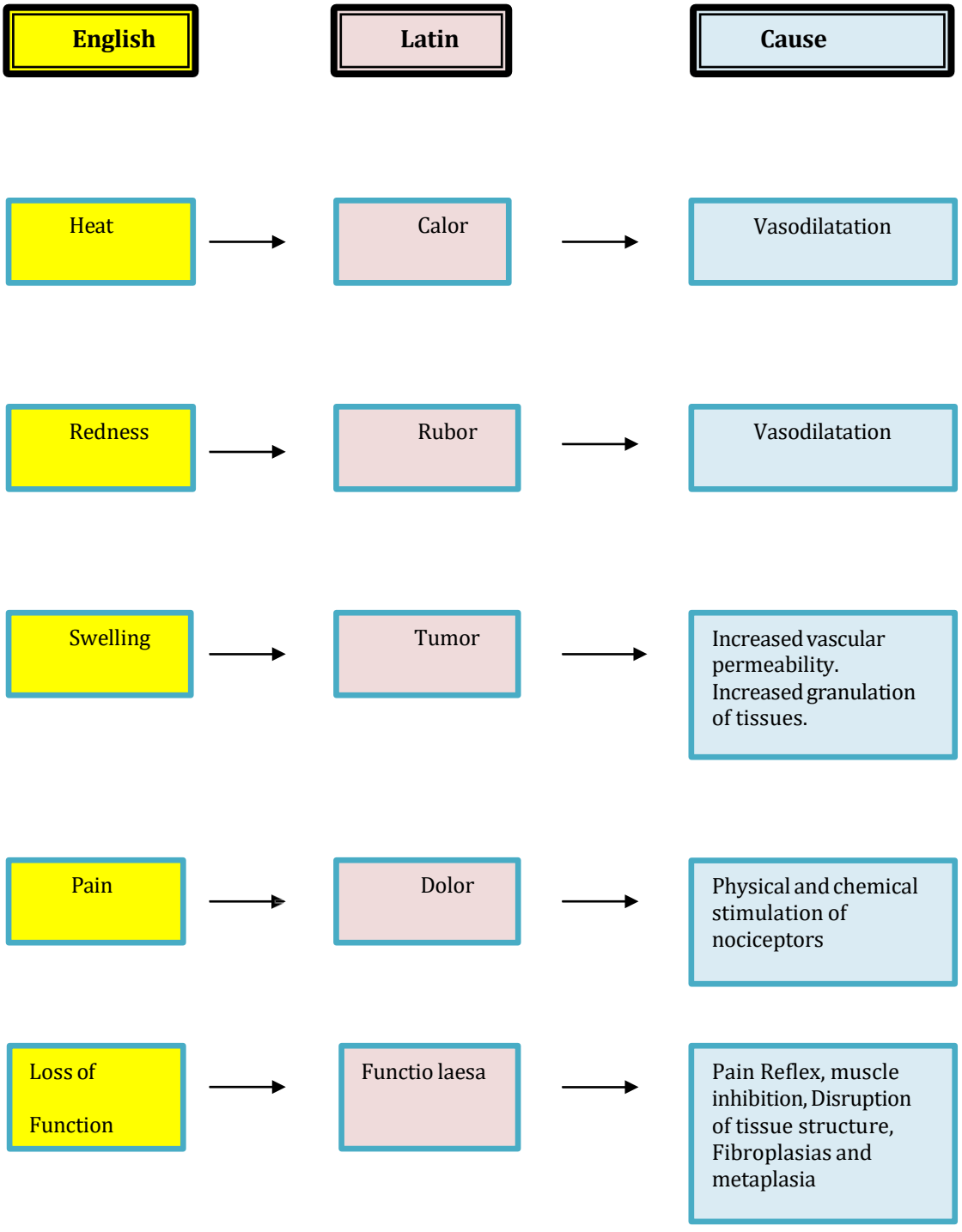


Fig. 2: Schematic representations of cardinal signs and causes of inflammation

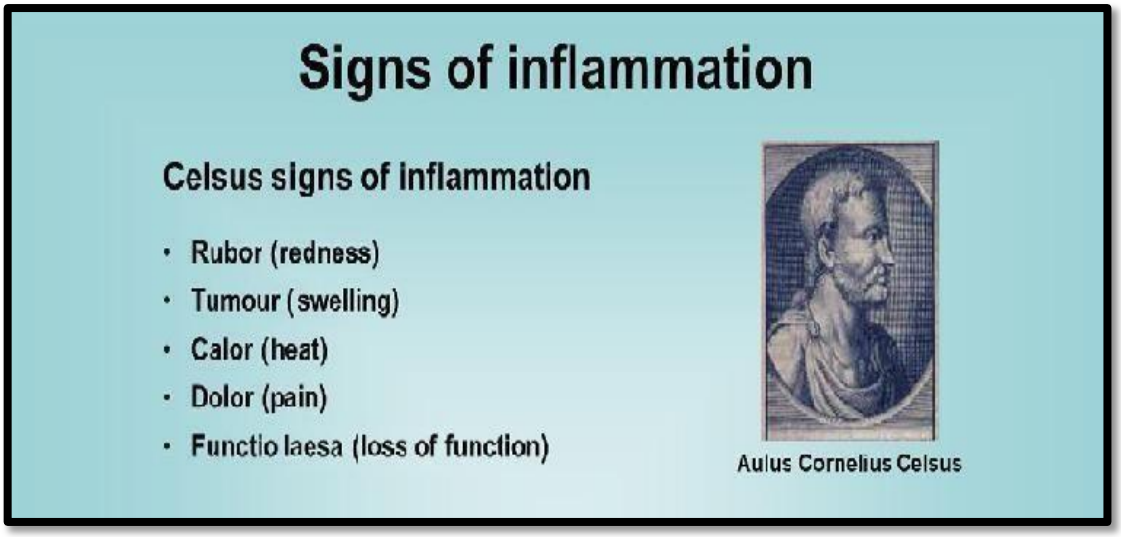


Fig. 3: Signs of inflammation (Source: Photographs obtained from Scand Journal of Immunology: 2019)

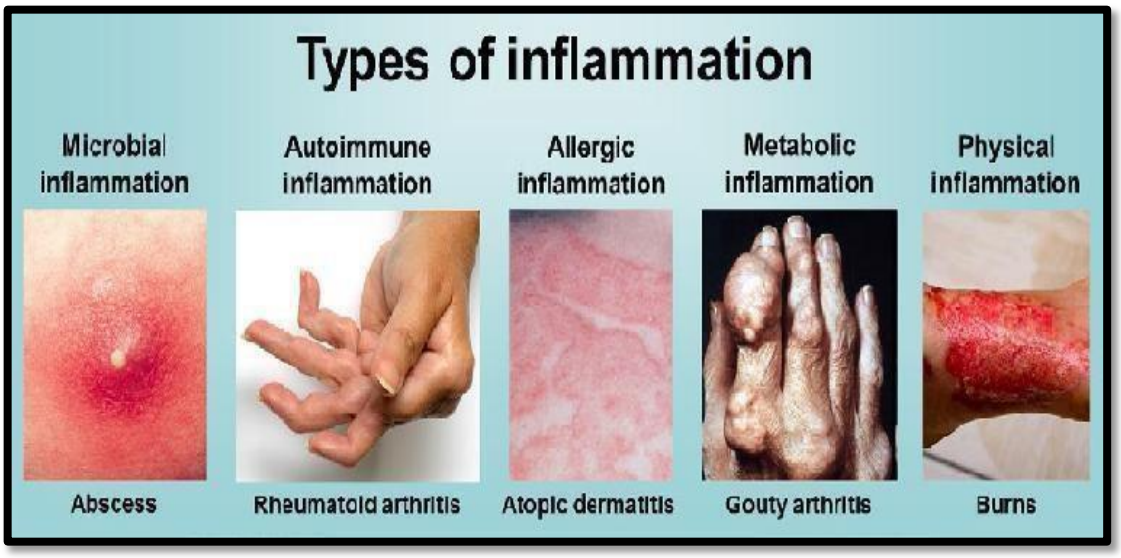


Fig. 4: Types of inflammation (Source: Photographs obtained from Scand Journal of Immunology: 2019)

Table 1: Types of inflammation

Type of inflammation	Cause of inflammation	Examples of diseases mediated by given type of inflammation
Microbial inflammation	Bacteria, Fungi, Viruses, and Protozoa	Abscess; Pneumonia; Sepsis; Ebola Haemorrhagic Fever
Autoimmune inflammation	Aberrant autoimmune attack by auto antibodies and/or auto reactive B and T Cells	Type 1 Diabetes; Multiple Sclerosis; Rheumatoid Arthritis; Psoriasis; Systemic Lupus Erythematosus
Allergic inflammation	Allergens (e.g. pollen, dust, mites, animal dander, fungi, insects, bites and stings)	Atopic Dermatitis/Eczema; Hay Fever; Asthma; Contact Dermatitis; Anaphylaxis; Drug Hypersensitivity Reactions
Metabolic inflammation	Excessive Accumulation of Metabolites (e.g. cholesteryl esters or uric acid)	Atherosclerosis; Gout; Phenylketonuria
Physical inflammation	Trauma, Burns or Radiation	Post- traumatic injury; Chemical, Electric and Thermal (scalding) Burns; Radiation Injury
Constitutive inflammation	Inborn Errors of Innate Immunity	Autoinflammatory diseases like Familial Mediterranean Fever; Aicardi- Goutieres Syndrome; NEMO Mutation- Linked Autoinflammatory Intestinal and Skin Disease

Source: (Jacek Hawiger and Jozef Zienkiewicz, Scand J. Immunol., John Wiley and Sons, 2019, 90 pg no.1-32)

1.4 Medicinal Plants with Anti-inflammatory Activity

There are various medicines for controlling and suppressing inflammatory crisis; steroids, nonsteroid anti-inflammatory drugs, and immunosuppressant are the practical examples of these medications which are associated with adverse effects, while in practice our goal is to apply minimum effective dose by the highest efficacy with the least adverse effects. Thus, we need to apply natural anti-inflammatory factors within medication therapy to achieve increased pharmacological response and lowest degree of unwanted side effects (Bagad *et al.*, 2013; Ghasemian *et al*., 2015).

1.4.1 Plants useful in joint inflammation

Table 2: Natural compounds useful in joint inflammation

Scientific Name (Botanical Name)	Part of Plant	Common Name	Chemical Constituents	Reference	Image of Plant
Annona squamosa (Annonaceae)	Stems	Sugar-apple Hindi-Seetaphal (सीताफल)	11-ent-kauranes	Yeh *et al.*	
Clerodendrum phlomidis (Lamiaceae)	Leaves	Hindi-Urni (उरनी) San-Agnimantha	3-hydroxy, 2-methoxy sodium butanoate	Prakash Babu *et al.* (2014)	
Garcinia mangostana	Fruits	Purple mangosteen	Isogarcino	Fu, Zhou, Wang, Cen *et al.*	

Glycyrrhiza uralensis (Fabaceae)	Roots	Chinese liquorice	Glycerol	Fu, Zhou, Wang, Wei (2014)	
Erycibe obtusifolia (Convulvulacea)	Stem		Scopolin	Pan *et al*. (2009)	
Malus domestica (Rosaceae)	Fruit peel	English- Apple	Ursolic acid	Pádua *et al*. (2014)	
Terminalia chebula (Combretaceae)	Fruits	Hirda	1,2,3,4,6-penta-O-galloyl-β-D-glucose	Kim SY *et al*. (2010)	
Withania somnifera (Solanaceae)	Roots	Ashwagandha	Withaferin A	Kim & Kim (2014)	

1.4.2 Plant useful in cardiovascular inflammation

Table 3: Medicinal plants effective in treating cardiovascular inflammation

Botanical Name	Medicinal Plant
Andrographis paniculata (Acanthaceae)	
Cimicifuga racemosa (Ranunculaceae)	
Salvia miltiorrhiza (Lamiaceae)	

1.4.3 Plants used as anti-arthritic and anti-inflammatory agents

Table 4: Various plants are being effectively used as anti-arthritic and anti-inflammatory agents

Scientific Name	Common Name	Part used	Reference	Plant Image
Acacia farnesiana Leguminaceae	Sweet acacia	Small petals	(Duke, 1981 and Morton, 1981)	
Abrus precatorious Fabeceae	Gunja, rosary pea	Leaves	(Toneli *et al.*, 1965)	
Aegle marmelos Rutaceae	Bael	Fruits	(Rao *et al.*, 2006)	
Albizzia lebbeck Mimosaceae	Mata-raton	Bark	(Saha *et al.*, 1966)	

Allium sativum Liliaceae	Lahsun	Bulb of garlic	(Nadkarni and Nadkarni, 1976)	
Anacardium occidentale Anacardiaceae	Kaju, Cashew	Dried juice of the leaves	(Huang, 1999)	
Ananas comosus Bromeliaceae	Pineapple	Fruits	(Brien *et al.*, 2004)	
Asparagus racemosus Liliaceae	Shatavari	Aerial parts	(Patwardhan *et al.*, 2005)	
Azadirachta Indica Maliaceae	Neem	Leaf, seeds and bark	(Kaushik *et al.*, 2002)	

Bauhnia variegate Caesalpiniaceae	Mandarai	Buds, stem leaves and bark	(Sheshadri Shekar *et al.*, 2009)	
Berberis vulgaris Berberidaceae	Huang Lian	Stem, root, bark and fruit	(Chopra *et al.*, 2004)	
Boswellia serrata Burseraceae	Salai guggul	Gum oleoresin	(Kimmatkar *et al.*, 2003)	
Camellia sinensis Theaceae	Chinese tea, tea plant	Used as tea	(Adcocks *et al.*, 2002)	
Cassia fistula Caesalpinaceae	Amaltas	Whole plant, bark	(Anonymous, 1997)	

Cayaponia tayuya Cucurbitaceae	Tayuya, Tomba	Roots	(Escandell *et al.*, 2007)	
Commiphora mukul Burseraceae	Guggul	Gum	(Sharma *et al.*, 1977)	
Cinnamomum camphora Lauraceae	Camphor	Oil	(Lee *et al.*, 2006)	
Crocus sativus Iridiaceae	Saffron	Flower	(Hosseinzadeh and Younessi, 2006)	
Croton lechleri Euphorbiaceae	Dragons Blood	Flower	Perdue *et al.*, 1979)	

Cryptomeria japonica Taxodiaceae	Sugi	Wood oil	(Scudiero *et al.*, 1988)	
Caesalpinia ulcherrima Leguminosae	Gulmohar	Leave, roots, flowers	(Patel *et al.*, 2010)	
Desmodium gangeticum Fabaceae	Anshumati अंशुमती	Aerial parts	(Mehrotra *et al.*, 2006)	
Dianthus barbatus Carypohyllaceae	Lord William	Leaves	(Anna, 1984)	
Dioscorea deltoide Dioscoreaceae	Wild Yam	Roots	(Chatterjee and Das, 1996)	

Dryopteris crassirhizoma Dryopteridaceae	Wood Fern	Rhizomes	(Chang *et al.*, 2010)	
Euphorbia nerifolia Euphorbiaceae	Nevagunda	Leaves	(Alcaraz *et al.*, 1988)	
Ecballium elaterium Cucurbiataceae	Cucumber	Roots	(Tagarelli *et al.*, 2010)	
Glycine max Fabaceae	Soybean	Seeds	(Verdrengh *et al.*, 2003)	
Glycyrrhiza glabra Leguminoseae	Mulethi	Rhizomes	(Tokiwa *et al.*, 2004)	

1.5 Common Plants with Proved Anti-Inflammatory Effect

1.5.1 *Curcuma longa*: The most important secondary metabolite of *Curcuma longa* is curcumin, which is responsible for an anti-inflammatory effect of this plant (Jurenka, 2009). Many clinical trials have been done for proving the anti- inflammatory effect of curcumin. Their results suggested that, curcumin can be effective in improving inflammation of rheumatoid arthritis (RA) and reducing clinical manifestation of RA, such as joint swelling and morning stiffness in comparison with phenylbutazone which is used as a positive control (Deodhar *et al*., 1980).

1.5.2 *Zingiber officinale*: In patients with osteoarthritis, ginger had not only efficacy in pain improvement identical to Diclofenac 100 mg but also have no side effects. Ginger extract has been compared to Ibuprofen and Indomethacin in OA patients; the results have exerted improving function of Ibuprofen, Indomethacin, and ginger extract equally in pain score (Khalvat, 2005; Haghighi *et al*., 2006; Altman *et al*., 2006).

1.5.3 *Rosmarinus officinalis* : There is evidence that confirms anti-inflammatory potential of *Rosmarinus officinalis* in molecular scope; according to this, rosmarinic acid could disturb complement system activation easily by inhibiting C3b attachment; the dose required for making this effect is very low (34 μM) (Sahu *et al*., 1999).

1.5.4 *Cassia fistula* (Caesalpiniaceae): The bark extracts of *Cassia fistula* possess anti-inflammatory effect in the acute and chronic anti-inflammatory model of inflammation in rats. The main constituents responsible for anti-inflammatory activity of *Cassia fistula* are flavonoids and bio-flavonoids (Ilavarasan *et al*., 2005).

1.5.5 *Cynodon dactylon*: The anti inflammatory activity of aqueous extract of *Cynodon dactylon* at different doses using carrageenan, serotonin, histamine and

dextran induced rat paw edema and cotton pellet method. The study was carried out in three different doses levels of 200, 400 and 600 mg/kg orally. The aqueous extract of *C. dactylon* was found to be safe at all doses used and there is no mortality up to the dose of 4000 mg/kg of extract when administered orally. *C. dactylon* showed significant anti-inflammatory activities in all model. The extract was found to reduce significantly ($P<0.001$) the formation of edema induced by carrageenan, serotonin, histamine and dextran after 3 and 5 hrs (Garg *et al*., 2011).

1.5.6 *Emblica officinalis* (Euphorbiaceae): In the recent studies, the anti-inflammatory activity was found in the water fraction of methanol extract of plant leaves. The effects of fraction were tested on the synthesis of mediators of inflammation such as leukotriene B4, platelet activating factor (PAF) and thromboxane. The water fraction of methanol extract inhibited migration of human PMNs in relatively low concentrations (Asmawi *et al*., 1993).

1.5.7 *Hibiscus Rosa-sinensis* (Malvaceae): The methanolic extract of *Hibiscus rosa-sinensis* leaves (250 and 500 mg/kg body weight orally) was used carrageenan and dextran induced rat paw edema anti-inflammatory model. Indomethacin was used as standard drug which showed significant anti-inflammatory activity. The inhibition of edema by 17.12 and 16.46 % with 250 mg/kg, 45.35 %, and 44.51 % with 500 mg/kg body weight after 3 hrs with carrageenan, dextran respectively. The plant extract at the dose level of 250 and 500 mg/kg body weight by oral route exhibited significant ($P<0.001$) anti-inflammatory activities against all agents used (Tomar *et al*., 2010).

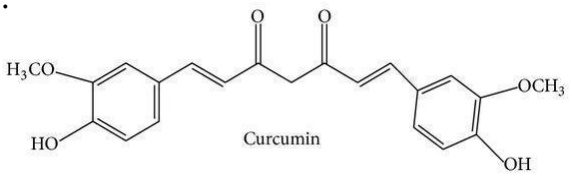
1.5.8 *Sida cordifolia* Linn. (Malvaceae): *Sida cordifolia* is used in folk medicine for the treatment of inflammation of the oral mucosa, blenorrhea, asthmatic bronchitis and nasal congestion (Franzotti *et al*., 2000). It has been investigated as an anti-

inflammatory, for preventing cell proliferation and for encouraging liver growth (Silva, 2006).

1.6 Plant Derived Compounds Tested in Clinical Trials

1.6.1 Curcumin

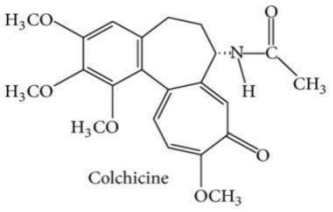
Curcumin, (1E, 6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione, also known as diferuloylmethane, is the main ingredient of turmeric (*Curcuma longa*, Zingiberaceae).



Curcumin

1.6.2 Colchicine

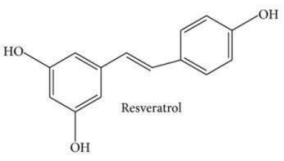
The tropolone derivative colchicine, N-[(7S)-1, 2, 3, 10-tetramethoxy-9-oxo-6, 7-dihydro-5H-benzo(a)heptalen-7-yl]acetamide, is the major alkaloid of the plant *Colchicum autumnale* (Colchicaceae), commonly known as autumn crocus or meadow-saffron.



Colchicine

1.6.3 Resveratrol

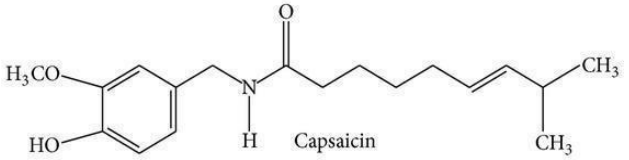
Resveratrol, 3,5,4‟-trihydroxy-*trans*-stilbene is a stilbenoid, a type of natural phenol , and a phytoalexin produced by several plants in response to injury or when the plant is under attack by pathogens, such as bacteria and fungi. Sources of resveratrol include skin of grapes, blueberries, raspberries, mulberries and peanuts.



Resveratrol

1.6.4 Capsaicin

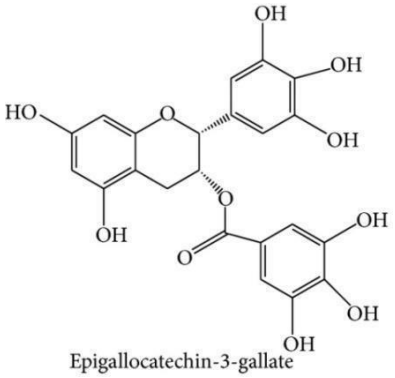
Capsaicin, (E)-N-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methylnon-6-enamide, is a very hydrophobic alkaloid produced by the chilli peppers (*Capsicum* species; Solanaceae) and is responsible for the typical pungency/spiciness of the fruits of the genus *Capsicum*.



Capsaicin

1.6.5 Epigallocatechin-3-gallate

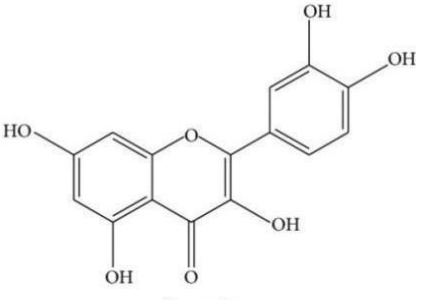
Epigallocatechin-3-gallate, [(2R, 3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl]-3,4,5-trihydroxybenzoate, commonly abbreviated as an EGCG, is an ingredient of green tea, *Camellia sinensis* (Theaceae).



Epigallocatechin-3-gallate

1.6.6 Quercetin

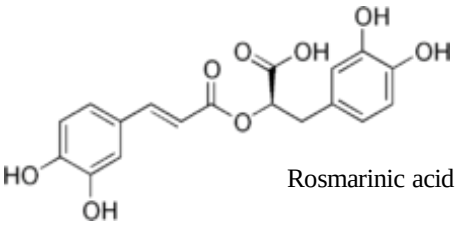
Quercetin, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one is a plant flavonol found appreciably in red onions and Kale or leaf cabbage, belongs to a *Brassica oleracea* species. Quercetin has bitter taste and used as ingredients in dietary supplements, beverages and foods.



Quercetin

1.6.7 Rosmarinic acid

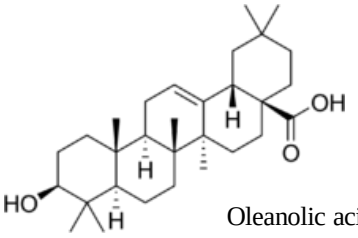
Rosmarinic acid, (a-o-caffeoyl-3, 4-dihydroxyphenyl lactic acid) is a polyphenolic compound and ester of caffeic acid which is found naturally in plants of Lamiaceae family, including rosemary (*Rosmarinus officinalis*), from which it was first isolated. Free phenolic groups in structure are responsible for its anti-inflammatory activities.



Rosmarinic acid

1.6.8 Oleanolic Acid

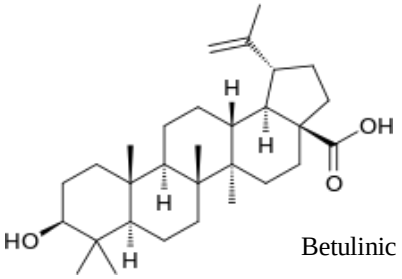
Oleanolic Acid or oleanic acid is a naturally occurring pentacyclic triterpenoid related to betulinic acid with numerous pharmacological actions such as anti-inflammatory, antioxidant and antitumor activities and found in several plants where it exist as either free acid or as an aglycone of triterpenoid saponins.



Oleanolic acid

1.6.9 Betulinic acid

Betulinic acid is also a naturally occurring pentacyclic triterpenoid which has antiretroviral, anti-inflammatory, antimalarial and anticancer activities. It is found in the bark of several species plants, principally the white birch (*Betula pubescens*).



Betulinic acid

1.7 Treatment of inflammation with Modern Drugs

Use of anti-inflammatory agents (particularly NSAIDS), and corticosteroids and analgesics: There are different chemical classes of NSAIDs each with a specific mechanism of action and particular pharmacokinetics (Brooks, 1991). These include agents which are nonselective inhibitors of Cyclooxygenase and selective Cyclooxygenase (COX-2) inhibitors:

Table 5: Modern drugs in the treatment of inflammation

a) Nonselective COX-2 inhibitors	
Category	**Drugs**
Salicylates	Aspirin, Amoxiprin, Benorilate, Choline magnesium salicylate, Diflunisal, Faislamine, Methyl salicylate, Magnesium salicylate, Salicyl salicylate (salsalate), etc.
Arylalkanoic acids	Diclofenac, Aceclofenac, Acemetacin, Bromfenac, Etodolac, Indomethacin, Ketorolac, Nabumetone, Sulindac, Tolmetin, etc.
2-Arylpropionic acids (profens)	Ibuprofen, Carprofen, Fenbufen, Fenoprofen, Flurbiprofen, Ketoprofen, Loxoprofen, Naproxen, Tiaprofenic acid, Suprofen, etc.
N-Arylanthranilic acids (fenamic acids)	Mefenamic acid, Meclofenamic acid, Alkalonones, Nabumetone, etc.
Pyrazolidine derivatives	Phenylbutazone, Azapropazone, Metamizole, and Oxyphenbutazone, etc.
Oxicams	Piroxicam, Lornoxicam, Meloxicam, Tenoxicam, etc.
Indole acetic acid derivatives	Indomethacin

b) Selective Cyclooxigenase (COX-2) inhibitors	
Category	**Drugs**
Diaryl substituted pyrazole derivatives	Celecoxib, Etoricoxib, Lumiracoxib, Parecoxib, etc.
Diaryl substituted furanones	Rofecoxib (withdrawn from market 2004), Valdecoxib (withdrawn from market 2005).
Sulphonanilides	Nimesulide
Other	Licofelone, Omega-3 Fatty Acid, etc.

Combination of NSAIDs with other NSAIDs or aspirin provides no added benefit but increases the toxic effects with GIT complaints being the most common and accounting for many treatment failures (Barrier, 1989).

Most of the above mechanisms are based upon inhibition of biosynthesis of arachidonic acid and prostaglandins from phospholipids precursors, as arachidonic acid and prostaglandins are important mediators for causing most of the physiological and morphological changes as observed during inflammation. Other approaches which may be relevant (William and Clegg, 1990; Hart and Huskisson, 1984) are:

1. Decrease in vascular permeability by inhibition of release of vasodilating inflammatory mediators, like Histamine, etc.
2. Inhibition of migration and chemotaxis of leucocytes to the site of inflammation, e.g. Hydroxychloroquine.
3. Inhibition of the release of degradative lysosomal enzymes which bring about degradation of the synovial tissues and thus preventing the progression of arthritic changes.
4. Inhibition of generation of superoxide radicals by phagocytes, e.g. by Steroids.
5. Inhibition of liposomal enzyme release from polymorphonuclear leucocytes.
6. Decrease in limphokine production.

1.8 Pain

Pain is still one of the most challenging prevalent problems. Worldwide, the prevalence of chronic pain reaches up to 30% . In the United States, it was estimated that 126 million American adults reported some pain in the last three months. In Canada, more than 47% reported that they experienced pain. In Italy, pain was reported by more than 80% of patients.

The situation is approximately the same or even worse in the developing countries. For instance, 55% of chronic low back pain patients were classified to have neuropathic pain in Arabian Gulf region. In Jordan the prevalence of pain experienced by cancer patients is estimated up to 73.3% . Moreover, in another recent study conducted in Jordan, it was found that 72.0% of patients surveyed experienced moderate to severe postoperative pain at rest .

In the United States, it has been estimated that the cost of chronic pain may exceed

$635 billion annually, which divided to direct medical expenditures and loss of work productivity. Among Swedish patients, the socioeconomic cost burden of chronic pain was approximately €32 billion. In Ireland, the cost of chronic pain was €5,665 per patient annually.

There are numerous difficulties in defining the concept of pain. This lack of definition is mainly because of the nature of the phenomenon. However, pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage”. In general, pain is underestimated by healthcare providers. This underestimation might lead to poor management which negatively affects patients' outcomes, quality of life, and satisfaction.

1.8.1 Type of pain:

Pain is classified into two categories: acute and chronic, depending on its duration and time course. Acute pain is defined as "pain which has a sudden onset with varied intensity lasting for less than six months". Chronic pain is defined as "pain that lasts for more than six months which may or may not have an underlying pathology to explain the patient's suffering".

Moreover, there are other classifications of pain in regards to its location, etiology, duration, and neuro-physiological mechanisms. Neuro-physiological mechanisms of pain are categorized as the following: nociceptive and non- nociceptive pain.

Nociceptive is a term that describes the normal physiological process relating to tissue damage. Further, nociceptive pain can be divided into somatic and visceral pain. Somatic pain is pain resulting from excitation and sensitization of nociceptors in tissues, such as bone, skin, joints, and muscles. It is intermittent or constant and it could be described as aching, stabbing, gnawing, or throbbing. In contrast, visceral pain involves organs such as heart, stomach, or liver. This pain is diffuse, for this reason it is referred to other location. However, visceral pain not always linked to visceral injury; for instance, stretching of the bladder can cause this pain.

Non-nociceptive pain can be subdivided into neuropathic and idiopathic pain. Neuropathic, which relates to pain caused by damage to, or dysfunction of, the nervous system. Neuropathic pain is usually described as sharp and burning pain. In contrast, Idiopathic pain refers to the state of pain which is poorly understood such as myofascial pain syndrome and somatization pain disorder.

1.9 Transethosomes

Transethosomes are lipid vesicles which are combinly based or made up of transfersomes and ethosomes. It was first introduced by Song et al in 2012 where he characterized the high content of ethnol (up to 30%). Transethosomes contain the both advantages of transfersomes and ethosomes. Transethosomes have the irregular spherical shape and higher values in both vesicles elasticity and skin permeation/penetration studies. This cause due to the rearrangement of lipid bilayer in the combination of ethanol and edge activator. The fluorescent probes or dyes with the different physicochemical properties can also incorporate into the UDV for the fluorescence studies.

The penetration of the transethosomes mechanisms is described in 3 way:

1. The interaction between hydrophilic lipid and water makes the polar lipid to attract water molecules induce hydration, lipid vesicles moved to the site of higher water concentration the difference in water contents across skin stratum and epidermis develops transdermal osmotic gradients that leads to penetration of transethosomes across skin.

2. Transethosomes induce hydration that widen pores due to it there is gradual release of drug occurs that binds to targeted organ.

3. Transethosomes act as penetration enhancer which disrupt the intercellular lipids, which results in widen of pores and increase the penetration of system through skin.

1.9.1 Mechanism

Transethosomere present novel lipid formulation which contains phospholipids, ethanol in high concentration of 30 to 40%, edge activator like tween 20, tween 60, tween 80, span60, span65, span 80, sodium chlorate and water. The size range lies between 40nm to200 nm depending upon the drug. Ethanol is a central character of transethosomal system.

1.9.2 Method of Preparation

Cold Method: Dissolved phospholipidsin ethanol by vigorous stirring. This mixture is heated upto 30ºC in a water bath. Water is heated up to 30ºC in a separate vessel and added to the alcoholic mixture slowly in a fine stream. Depending on the drug solubility, it can be dissolved in water. The mixture is kept on magnetic stirrer at 700rpm during the addition of above aqueous solution to ethanolic solution. Modulation of vesicle size can be doneby using probe sonicator.

Hot Method: Disperse phospholipids in water by heating in water bath at 400 c to obtain a colloidal solution. Ethanol and glycol are mixed and heated up to400 c. organic phase is added to aqueous phase. Stir for 7-10 min. Depending upon its hydrophilic/hydrophobic properties, the size of the vesicle is reduced by probe sonication.

Thin Film Hydration Method: SPC (final concentration of 36mg/ml), permeation enhancers and IM (final concentration of0.5mg/ml) were dissolved in 25ml chloroform-methanol. The lipid mixture was deposited as a thin film in a round- bottom flask by rotatory evaporating the chloroform-methanol under reduced pressure at 35+1c, which was applied for 1h to ensure total removal of solvent traces. The lipid film was hydrated with 10ml phosphate buffer and achieved within the eluates.

Mechanical Dispersion Method: Lipid and surfactant is taken in clean, dry round bottom flask. Lipid mixture is dissolved in a solvent mixture of chloroform and ethanol mixture. A thin film of lipid is obtained using rotary evaporator above the lipid transition temperature. It is kept overnight under vacuum to remove traces of organic solvent. The deposited film is hydrated with 10% v/v ethanol in phosphate buffer pH 6.5 by rotation at 60 rpm. Drug is added to the formulation. The vesicles are sonicated for desired size.

1.9.3 Salient features of Transethosomes

1. They have high entrapment efficacy, as they are biocompatible and biodegradable in nature.
2. Encapsulation drug is protected from the degradation as due to which they their content slowly and gradually.
3. Easy to prepare, does not involve tedious process and also avoids the unnecessary use of pharmaceutical additives, can be used for both systemic as well as topical delivery.
4. They are highly flexible so have higher flux rate across skin and higher rate of skin penetration as comparisons to other vesicular system.

1.9.4 Advantages of Transethosomal Drug Delivery

- The transethosomal system is passive, non-invasive and is available for immediate commercialization.
- It contains non-toxic raw material in the formulation.
- The Transethosomes drug is administered in a semisolid form.
- Transethosomes drug delivery can be applied to many fields including veterinary and cosmetic fields.
- It shows high patient compliance as it is administered in semisolid gel or cream form.

- This drug delivery system shows better stability as compared to other conventional vesicle.
- Avoidance of first pass metabolism.
- Simple method of drug delivery as compared to iontophoresis, laser surgery, cryo surgery and other complicated methods.

1.9.5 Enhanced drug permeation through skin for transdermal drug delivery. Characterization of Transethosomes

1. Vesicle Shape: vesicle shape is determined by transmission electronic microscopy and by scanning electronic microscopy.

2. Vesicle size and Zeta Potential: Particle size can be determined by dynamic light scattering and photon correlation spectroscopy. The size of transethosomes ranges between tens of nanometres‟ to microns and is influenced by the composition of the formulation. The zeta potential of the formulation can be measured by zeta meter, which can be used to predict and control the stability.

3. Transition Temperature: The transition temperature of transethosomes can be determined by using differential scanning calorimetry.

4. Drug Content: Drug content of transethosomes can be determined using U.V Spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.

%drug content =sample absorbance/standard absorbance.

5. Surface Tension: The surface tension activity of drug in aqueous solution can be measured by the ring method in ado Nounring tensiometer.

6. Penetration and Permeation Studies: Depth of penetration from trans-

ethosomes can be visualised by confocal laser scanning microscopy (CLSM).

7. Vesicle Stability: The stability of vesicle can be determined by assessing the size and structure of vesicle over time.

8. Surface Morphology Study: Different types of lipids influence the surface morphologyor shape of the particle. It is characterized for morphology and surface properties using a scanning electronic microscope.

***In-Vitro* Drug Release:** This is performed for determining thepermeation rate. Time needed to attain steady state permeation and the permeation flux at steady state and the information from *in-vitro* studies are used to optimize the formulation.

1.9.6 Application:

1. Better permeation for anti-inflammatory activity.
2. Improved transdermal flux.
3. Increase skin penetration.
4. For transdermal immunization.
5. Increase entrapment efficiency and skin permeation.
6. Ethosomes are used in pilosabeceous targeting.
7. Transdermal Delivery of Hormones.
8. Delivery of Anti-Arthritis Drug.
9. Delivery of Antibiotic.

CHAPTER – 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Reviews on Ketoprofen

Pattanayek and Puranik, (2018) prepared ketoprofen loaded nanoparticles and to incorporate these nanoparticle single. Many drugs show poor permeation due to the barrier stratum corneum when delivered through the skin. To overcome this problem and to enhance the permeation of drug into the skin and also to reduce the skin irritation the drug was formulated in the form of nanoparticles using chitosan as a polymer and these drug loaded nanoparticles were incorporated in a gel for more effective delivery of a drug into the skin. The prepared drug-loaded nanoparticulate gel was evaluated for physical appearance, homogeneity, pH, drug content, viscosity, spreadability, extrudability and in vitro diffusion studies. All the nanoparticulate gel formulations were clear without any aggregates, particles, and fibers indicating excellent homogeneity of all the formulations. The pH of the formulations was closer to a neutral indicating absence of skin irritation and damage. The viscosity was directly proportional whereas extrudability and spreadability were inversely proportional to the concentration of carbopol employed. Formulation NG4 showed sustained release for 24 h.

Hales *et al.*, (2017) formulated and prepared compression-coated tablets for colonic release (CR-tablets), and to evaluate the bioavailability of ketoprofen following the administration of a single dose from mini-tablets with immediate release (IR-tablets) compared to CR-tablets. CR-tablets were prepared based on time-controlled hydroxypropylmethylcellulose K100M inner compression-coating and pH-sensitive Eudragit® L 30D-55 outer film-coating. The clinical bioavailability study consisted of two periods, in which two formulations were administered to 6 volunteers, according

to a randomized cross-over design. The apparent cumulative absorption amount of ketoprofen was estimated by plasma profile deconvolution. CR-tablets were able to delay ketoprofen‟s release. Compared to IR-tablets used as reference, for the CR-tablets the maximum plasma concentration (Cmax) was lower (4920.33±1626.71 ng/mL vs. 9549.50±2156.12 ng/mL for IR-tablets) and the time needed to reach Cmax (tmax) was 5.33±1.63 h for CR-tablets vs. 1.33±0.88 h for IR-tablets. *In vitro-in vivo* comparison of the apparent cumulative absorption amount of ketoprofen showed similar values for the two formulations.

Kaleemullah *et al.*, (2017) prepared Ketoprofen matrix tablet containing mucilage 40% (F3) showed comparable dissolution profile to that of reference drug. This has further proven by no significant difference between F3 and reference drug Apo Keto® SR. Through various evaluations, *Hibiscus rosa-sinensis* leaves mucilage has been proven as effective natural polymer in retarding the drug release and this mucilage acted as hydrophilic polymer which can be employed in formulating successful matrix tablets.

Sowmya *et al.*, (2016) increased the therapeutic effectiveness of ketoprofen by increasing its transdermal permeation, via solid dispersion incorporated in gel. 2-hydroxy propyl beta cyclodextrins (HPβ-CD) and β-cyclodextrin (β-CD) were used as carriers and carbopol 940 was the gelling agent. Eight solid dispersion formulations of ketoprofen were prepared using different drug: polymer ratios viz. 1:0.5, 1:1, 1:2, and 1:3 for 2-HPβ-CD and β cyclodextrin using the solvent evaporation method. The formulation of solid dispersion by solvent evaporation method is introduced to reduce the drug particle size and hence increase the dissolution rate. These dispersions were characterized with the help of FT-IR, DSC and X-ray diffraction

studies. The optimized solid dispersion of ketoprofen was incorporated into gel and was compared with penetration enhancers. The formulations were analyzed to determine their pH, spreadability, viscosity, and in vitro drug release. The formulation with 1:0.5 SDK (drug: HPβCD) was incorporated in carbopol gel, and produced 98.32% drug release compared to solid dispersion of ketoprofen with menthol (SDKM5%), which produced 96.26% drug release. In ex vivo studies, SDK and SDKM5% formulations gave 94.37% and 92.26% drug release respectively within 24 h.

Ambala and Vemula, (2015) formulated the ketoprofen emulgels using different viscosity grades of hydroxypropyl methylcellulose and carbopol as gelling agents. All the prepared emulgels were shown acceptable physical properties concerning colour, homogeneity, consistency, and pH value. Emulgels containing hydroxypropyl methylcellulose were poor in clarity when compared to carbopol formulations. The influence of the type of gelling agent on the drug release from the prepared emulgels was investigated and carbopol 934 showed good results not only in the drug release but also in physical evaluation parameters. From the drug release studies, F3 formulation showed 98.46±2.05% drug release in 8 h with good clarity and physical appearance. The T10% and T80% values of best formulation F3 was found to be 0.9 h and 6.6 h respectively. The T10% and T 80% was higher for formulations with carbopol in low concentration when compared to hydroxypropyl methylcellulose K 4M and K 15M in high concentrations, indicating better controlled release. FTIR studies proved the compatibility between drug and carbopol. From the stability studies, similarity index value between dissolution profiles of F3 formulation before and after storage was

found to be 87.16.

Vittal *et al.*, (2012) enhanced the dissolution rate of ketoprofen using microcrystalline cellulose as carrier, aerosil 200 as coating material, and polyethylene glycol as nonvolatile water miscible liquid vehicle. The drug concentration was kept constant in all formulations at 40% w/w. Optimization was carried out using Box–Behnken design by selecting liquid load factor, amount of coating material, and amount of magnesium oxide as independent variables; cumulative percentage drug release and angle of repose were considered as dependent variables. The Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC) studies revealed that there was no possible interaction between drug and tablet excipients. Prepared ketoprofen liquisolid tablets were evaluated for hardness, weight variation, friability, *in-vitro* disintegration time, drug content uniformity, and *in-vitro* dissolution studies. The optimized formulation yielded the response values, which were very close to the predicted values. The accelerated stability studies conducted showed that liquisolid tablets were not affected by ageing and there were no appreciable changes in the drug content.

Najmuddin *et al.*, (2009) Mini-matrices (multiple unit dosage forms) with release sustaining properties were developed by simple extrusion (die cavity of 3 mm) using ketoprofen a NSAID as a model drug and hydrophilic (HPMC and xanthan gum), hydrophobic (ethyl cellulose) as a sustained release agents and 5% propylene glycol was selected as plasticizer with 10% lactose as channeling agent. In vitro drug release study was calculated for all the formulations and showed maximum drug release of 93.81% for mini-matrices (F15) having HPMC as polymer in a ratio of 1: 2 with

respect to drug along with plasticizer and channeling agent. The drug release has shown first order kinetics with diffusion release systems followed by erosion mechanism. The physico-chemical chracterization of mini-matrices were carried out by performing drug content, weight variation test, friability and uniformity of size. The FT-IR studies have showed no chemical interaction and drug was in an intact form. The SEM studies were carried out to confirm the plasticizing efficiency of propylene glycol. Short term stability studies of all the parameters were done at a temperature of 30 ± 2ºC and 65 ± 5% RH and these were stable upto 3 months.

2.2 Reviews on Transethosomes

Albash *et al.*, (2019) prepared transethosomes (TEs) for enhancing the transdermal delivery of Olmesartan medoxomil (OLM) to avoid its oral problems. TE formulae were prepared utilizing $5^1.3^1$ full factorial design using various surfactants (SAAs) and different phospholipid-to-SAA ratios. The formulae were characterized regarding their entrapment efficiency percentage (EE%), particle size (PS), polydispersity index (PDI), zeta potential (ZP), and the amount of drug released after 6 hours (Q6h). Design Expert® software was employed to select the optimum formula. The optimum formula (TE14) had an EE% of 58.50%±1.30%, PS of 222.60±2.50 nm, PDI of 0.11±0.06, ZP of -20.80±0.30 mV, and Q6h of 67.40%±0.20%. In addition, TE14 was compared to transferosomes (TFs) in terms of elasticity and was found to show higher deformability index. Further, evaluation of ex vivo permeation using both rat and shed snake skin showed higher permeability of TE14 compared to TFs and OLM suspension. Confocal laser scanning microscopy confirmed the capability of the fluoro-labeled TE14 to

penetrate deep within the skin, while the histopathological study confirmed its safety. TE14 successfully maintained normal blood pressure values of rats up to 24 hours. Moreover, TE14 showed superiority in dermatokinetic study when compared with drug suspension. Taken together, the obtained results confirmed the potential of employing TEs as a successful carrier for the transdermal delivery of OLM.

Sravani *et al.,* (2019) reported methods of preparation, and evaluation parameters of transethosomes. Transdermal route of drug delivery has gained great interest in pharmaceutical research as it overcomes many problems associated with the oral route of administration. The development of vesicular formulations has generated some promising solutions to the problems associated with drug delivery not only related to drugs but also those of barriers like skin. Conventional lipid based vesicular systems like liposomes show inability to cross intercellular channels of stratum corneum. To overcome this drawback of conventional lipidic systems, ethanol based vesicular carriers were developed by pharmaceutical scientists. Transethosomes come under the category of ethanol basedlipidic carriers. Transethosomes are composed of phospholipid, ethanol and water, with edge activators (like span 60) or permeation enhancers (like oleic acid). Ethanol based vesicular systems represent non-invasive carriers which enable the drug to reach in deeper epidermal layers or systemic circulation.

Thasleem *et al.,* (2018) optimized of transethosomes formulation for dermal fisetin delivery. The optimization of the formulation was carried out using "Box–Behnken design". The independent variables were Lipoid S 100, ethanol and sodium cholate. The prepared formulations were characterized for vesicle size, entrapment efficiency and in

vitro skin penetration study. The vesicles–skin interaction, confocal laser scanning microscopy and dermatokinetic studies were performed with optimized formulation. Results of the present study demonstrated that the optimized formulation presented vesicle size of 74.21 ±2.65 nm, zeta potential of11.0 mV, entrapment efficiency of 68.31 ± 1.48% and flux of 4.13 ± 0.17mg/cm2/h. The TEM image of optimized formulation exhibited sealed and spherical shape vesicles. Results of thermo analytical techniques demonstrated that the prepared transethosomes vesicles formulation had fluidized the rigid membrane of rat‟s skin for smoother penetration of fisetintrans ethosomes.

Gondkar *et al.*, (2017) prepared, optimized, characterized, and ex vivo study of etodolac-loaded transethosomal gel using the central composite design. Transethosomes (TELs) are elastic vesicles composed of phospholipid, ethanol and edge activator (surfactant). TELs were prepared by thin film hydration method and characterized by Scanning electron microscopy (SEM), entrapment efficiency, vesicle size, Zeta potential and the transethosomal gel was characterized by clarity, pH, viscosity, spread ability, drug content and *in vitro* drug release. Microscopic examination of transethosomes showed soft, malleable and spherical vesicles with a smooth surface within the range. Vesicle size and % entrapment efficiency of the optimized transethosomal formulation was found to be 268.1nm and 83.75 0.61% respectively. Zeta potential of the optimized transethosomal formulation was found to be -18.0 mv. The *ex-vivo* study showed spread ability of transethosomal gel 26± 0.03. Drug content was found to be 98.24 0.024%. *In vitro* drug release study of optimised formulations for 12 hrs was found to be 97.35± 0.43%.

Ascenso *et al.*, (2015) three related Ultradeformable vesicles (UDV): transfersomes, ethosomes, and transethosomes for the incorporation of actives of distinct polarities, namely, vitamin E and caffeine, and to evaluate the effect of the carrier on skin permeation and penetration. All these UDV are useful as skin delivery systems by acting as drug carriers as well as permeation enhancers, and are capable of retaining the active molecules accessible within different skin layers, under certain conditions. These data suggest that the formulation of actives in UDV may be performed in a case-by-case manner.

Kumar, B., Sahani, V., & Patil, S. **(2024);** Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) widely utilized for its analgesic, anti-inflammatory, and antipyretic properties. This review article examines ketoprofen's pharmacological profile, efficacy, and therapeutic applications, particularly in the management of acute and chronic pain, including dysmenorrhea, osteoarthritis, and rheumatoid arthritis. Ketoprofen exerts its effect by inhibiting the cyclooxygenase (COX) enzymes, COX-1 and COX- 2, reducing the synthesis of prostaglandins responsible for pain and inflammation. Various formulations of ketoprofen are available, including oral tablets, topical gels, suppositories, and injectables, each tailored to specific clinical needs and patient preferences. Recent advancements in topical and extended-release formulations have improved patient adherence and minimized gastrointestinal side effects traditionally associated with NSAIDs. However, ketoprofen remains contraindicated in patients with certain cardiovascular, renal, and gastrointestinal conditions, underscoring the need for careful patient selection and dosage adjustment. This review highlights ketoprofen's clinical efficacy, safety profile, and evolving therapeutic applications,

providing insight into its role within the broader scope of pain management strategies.

***Rekha D Kadam, Gunesh N. Dhembre,* 2024;** The sustained release tablet was prepared by wet-granulation method. The tablets were formulated using hydrophilic polymer HPMC K4M and xanthan gum. Preformulation compatibility studies indicate that there is no interaction between the excipient and the drug. Total seven batches were prepared and powder blends before compression was subjected for evaluation of flow properties. All the parameter was found to be within the limit showing good flow property. After compression, the entire tablet batch was evaluated for thickness, hardness, friability, weight variation, drug content uniformity, swelling index, In-vitro release pattern. The thickness of tablet indicates that die fill was uniform in all the formulation and the formulation possessed sufficient hardness & friability indicating a good mechanical strength of the development formulation. The weight of the all the formulation were found to be with in pharmacopeial limit. The In-Vitro dissolution profile of all the formulation of Ketoprofen was controlled over an extended period of time. The optimized formulation of F7 containing combination of HPMC K4M and xanthan gum was consider as the best formulation with respect to its sustained in vitro drug release for 12 hrs, total sustained release time & improved bioavailability. The developed formulation was found to be stable during the stability studies of 3 month indicating good stability of the tablets.

***Front. Bioeng. Biotechnol,*2024;** The treatment of aseptic inflammation has always been a clinical challenge. At present, non-steroidal drug-loaded microspheres have been widely used in the treatment of aseptic inflammation due to their excellent injectable and sustained release capabilities. In this study, ketoprofen-loaded shellac microspheres (Keto-SLAC) were prepared by electrospray. Alterations of Keto-SLAC morphology was observed in response to changed

shellac concentration in ethanol solution through electrospray. Further examination revealed that ketoprofen presented as amorphous solid dispersion in the shellac microspheres. Most importantly, it was also shown that ketoprofen can be slowly released from the shellac matrix for up to 3 weeks. *In vitro* cell experiments verified that the microspheres had favorable cell compatibility. We therefore proposed that the prepared microspheres, being readily available in use in a variety of clinical settings through topical application, have promising therapeutic potential for the treatment of aseptic inflammation.

Bhupendra Kumar, *et al.*, 2023 ; Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) widely utilized for its analgesic, anti-inflammatory, and antipyretic properties. This review article examines ketoprofen's pharmacological profile, efficacy, and therapeutic applications, particularly in the management of acute and chronic pain, including dysmenorrhea, osteoarthritis, and rheumatoid arthritis. Ketoprofen exerts its effect by inhibiting the cyclooxygenase (COX) enzymes, COX-1 and COX- 2, reducing the synthesis of prostaglandins responsible for pain and inflammation. Various formulations of ketoprofen are available, including oral tablets, topical gels, suppositories, and injectables, each tailored to specific clinical needs and patient preferences. Recent advancements in topical and extended-release formulations have improved patient adherence and minimized gastrointestinal side effects traditionally associated with NSAIDs. However, ketoprofen remains contraindicated in patients with certain cardiovascular, renal, and gastrointestinal conditions, underscoring the need for careful patient selection and dosage adjustment. This review highlights ketoprofen's clinical efficacy, safety profile, and evolving therapeutic applications, providing insight into its role within the broader scope of pain management strategies.

Veselina Skrinska-Kirilova, Javier Sawchik, Martine Sabbe & Martine Debacker, 2023; In Belgium, 569 HCPs responded to the survey. Almost all HCPs reported that they were aware of this risk (99%). More than half of pharmacists (58%) and half of physicians (50%) indicated that an annual DHPC is necessary. Around half of the respondents (49%) recalled receiving information about the risk in 2023, primarily through the DHPC (68%) while outlining other channels such as the logo on the packaging (42%) and the Summary of Product Characteristics (40%). Awareness of the patient card (32%) and the checklist (5%) was overall low, with very limited use in 2023. In Luxembourg, 190 HCPs responded to the survey. Almost all HCPs reported that they were aware of this risk (97%). The majority of pharmacists (70%) and more than half of physicians (52%) supported the annual DHPC distribution. Over two thirds of the respondents (68%) recalled receiving relevant information in 2023, mainly through the DHPC (70%) but also through the SmPC (30%). Awareness of the patient card (22%) and the checklist (13%) was overall low, with very limited use in 2023.

CHAPTER – 3

AIM AND OBJECTIVE

3. AIM AND OBJECTIVE

Pain an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. In general, pain is underestimated by healthcare providers.

Transethosomes are lipid vesicles which are combinly based or made up of transfersomes and ethosomes. Transethosomes contain the both advantages of transfersomes and ethosomes. Transethosomes have the irregular spherical shape and higher values in both vesicles elasticity and skin permeation/penetration studies. This cause due to the rearrangement of lipid bilayer in the combination of ethanol and edge activator.

Ketoprofen, a propionic acid derivative, is a nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties.

The objective of work was to formulate, evaluate the transdermal potential of novel vesicular nanocarriers: Transethosomes gel containing Ketoprofen for effective treatment of pain.

CHAPTER – 4

PLAN OF WORK

4. PLAN OF WORK

1. Literature Review
2. Selection of dosage form
3. Selection of drug candidate
4. Procurement of drug & excipients
5. Preformulation studies
    - Physical Appearance
    - Melting Point
    - Solubility study
    - Determination of Absorption Maxima (λ_{max})
    - Calibration curve of drug
6. Formulation development of Transethosomes
7. Evaluation of Transethosomes gel
    - Measurement of pH
    - Determination of Viscosity
    - Spreadability
    - Drug Content
    - *In vitro* drug release of drug

- Stability studies

8. Result and discussion

9. Summary and conclusion

10. Compilation and Submission of thesis

CHAPTER – 5

DRUG AND EXCIPIENT PROFILE

5. DRUG AND EXCIPIENT PROFILE

5.1 Drug profile

5.1.1 Ketoprofen

Ketoprofen, a propionic acid derivative, is a nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties.

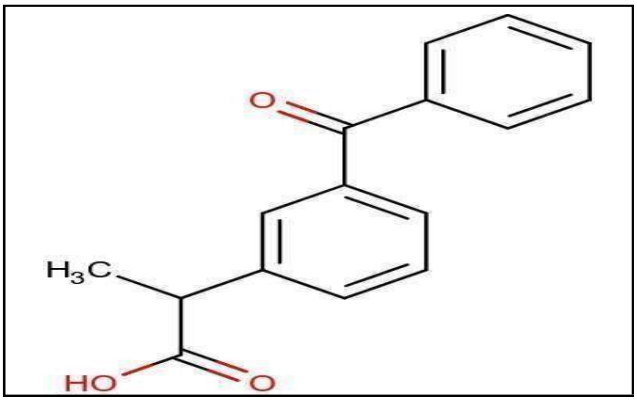


Figure 5: Structure of Ketoprofen

Mol. Weight: 254.2806

Chemical Formula: C16H14O3

IUPAC Name: (2*R*)-2-(3-benzoylphenyl) propanoic acid

Pharmacology

Indication: For symptomatic treatment of acute and chronic rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, primary dysmenorrhea and mild to moderate pain associated with musculotendinous trauma (sprains and strains), postoperative (including dental surgery) or postpartum pain.

Pharmacodynamics: Ketoprofen is a nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties. Ketoprofen has pharmacologic actions similar to those of other prototypical NSAIDs, which inhibit prostaglandin synthesis. Ketoprofen is used to treat rheumatoid arthritis, osteoarthritis, dysmenorrhea, and alleviate moderate pain.

Mechanism of action: The anti-inflammatory effects of ketoprofen are believed to be due to inhibition cylooxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway. This results in decreased levels of prostaglandins that mediate pain, fever and inflammation. Ketoprofen is a non- specific cyclooxygenase inhibitor and inhibition of COX-1 is thought to confer some of its side effects, such as GI upset and ulceration. Ketoprofen is thought to have anti-bradykinin activity, as well as lysosomal membrane-stabilizing action.

Absorption: Ketoprofen is rapidly and well-absorbed orally, with peak plasma levels occurring within 0.5 to 2 hours.

Metabolism: Rapidly and extensively metabolized in the liver, primarily via conjugation to glucuronic acid. No active metabolites have been identified.

Route of elimination: In a 24 hour period, approximately 80% of an administered dose of ketoprofen is excreted in the urine, primarily as the glucuronide metabolite.

Half-life: Conventional capsules: 1.1-4 hours.

Extended release capsules: 5.4 hours due to delayed absorption

Uses

Ketoprofen is used to relieve minor aches and pain from headaches, menstrual periods, toothaches, the common cold, muscle aches, and backaches, and to reduce fever. Ketoprofen is in a class of medications called NSAIDs.

Side effects

- upset stomach
- nausea
- diarrhea
- headache
- dizziness
- drowsiness

Medical uses

Ketoprofen is generally prescribed for arthritis-related inflammatory pains or severe toothaches that result in the inflammation of the gums.

Ketoprofen topical patches are being used for treatment of musculoskeletal pain.

Ketoprofen can also be used for treatment of some pain, especially nerve pain such as sciatica, postherpetic neuralgia and referred pain for radiculopathy, in the form of a cream, ointment, liquid, spray, or gel, which may also contain ketamine and lidocaine, along with other agents which may be useful, such

as cyclobenzaprine, amitriptyline, acyclovir, gabapentin, orphenadrine and other drugs used as NSAIDs or adjuvant, atypical or potentiators for pain treatment.

Efficacy

A 2013 systematic review indicated "The efficacy of orally administered ketoprofen in relieving moderate-severe pain and improving functional status and general condition was significantly better than that of ibuprofen and/or diclofenac." A 2017 Cochrane systematic review investigating ketoprofen as a single-dose by mouth in acute, moderate-to-severe postoperative pain concluded that its efficacy is equivalent to drugs such as ibuprofen and diclofenac.

There is evidence supporting topical ketoprofen for osteoarthritis but not other chronic musculoskeletal pain.

Adverse effects

In October 2020, the U.S. Food and Drug Administration (FDA) required the prescribing information to be updated for all nonsteroidal anti-inflammatory medications to describe the risk of kidney problems in fetuses that result in low amniotic fluid. They recommend avoiding NSAIDs in pregnant women at 20 weeks or later in pregnancy.

Mechanism

Ketoprofen undergoes metabolism in the liver via conjugation with glucuronic acid (glucuronidation) by UGT enzymes, hydroxylation of the benzoyl ring by the CYP3A4 and CYP2C9 enzymes, and reduction of its ketone moiety (a

carbonyl functional group, i.e. with carbon-oxygen double bond) by carbonyl reducing enzymes (CREs). Ketoprofen is used for its antipyretic, analgesic, and anti-inflammatory properties by inhibiting cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes reversibly, which decreases production of proinflammatory prostaglandin precursors.

The patches have been shown to provide rapid and sustained delivery to underlying tissues without significantly increasing levels of drug concentration in the blood when compared to the traditional oral administration.

Chirality and biological activity

Ketoprofen has one stereogenic center in the side chain and hence exists as mirror-image twins. Majority of the profens are marketed as racemic mixtures. For most of the NSAIDs the pharmacological activity resides in the (S)-enantiomers with their (R)-enantiomer virtually inactive. An interesting observation about most profens including ketoprofen is that they undergo unidirectional metabolic inversion, chiral inversion, of the (R)- acid to its (S)-mirror-image version with no other change in the molecule. There have been concerns raised that Ketoprofen can break down into the parent benzophenone molecule in skin exposed to strong summer or tropical UV light and this could pose a theoretical cancer risk. Given such a risk it is better to use other pain killers in such circumstances.

History

The earliest report of therapeutic use in humans is in 1972.

Society and culture

Brand names

Brand names in Albania are Oki and Fastum Gel. The names in Australia include Orudis and Oruvail. It is available in Estonia as Keto, Ketonal, and Fastum Gel; in Finland as Ketorin, Keto, Ketomex, and Orudis; in France as Profénid, Bi-Profénid, Toprec, and Ketum. It can be bought in Indonesia as Kaltrofen; in Ireland as Fastum Gel. In Italy it is sold as Ketodol, Fastum Gel, Lasonil, Orudis, Oki and Okitask; in Greece as Okitask. It is available in Japan in a transdermal patch, Mohrus Tape, made by Hisamitsu Pharmaceutical. In Mexico it is sold as Arthril; in Norway as Zon and Orudis. In Poland as Ketonal, Ketonal active, and Ketolek. In Romania as Ketonal and Fastum Gel; in Russia as ОКИ (OKI), Fastum Gel and Ketonal. In Serbia, Slovenia and Croatia as Knavon and Ketonal. In Spain as Actron and Fastum Gel. It is available in the UK as Ketoflam and Oruvail. Lastly, it is sold in Venezuela as Ketoprofeno as an injectable solution of 100 mg and 150 mg capsules.

In some countries, the optically pure (*S*)-enantiomer (dexketoprofen) is available; its trometamol salt is said to be particularly rapidly reabsorbed from the gastrointestinal tract, having a rapid onset of effects.

Veterinary medicine

Ketoprofen is a common NSAID, antipyretic, and analgesic used in horses and other equines. It is most commonly used for musculoskeletal pain, joint problems, and soft tissue injury, as well as laminitis. It is also used to control fevers and

prevent endotoxemia. It is also used as a mild painkiller in smaller animals, generally following surgical procedures.

In horses, it is given at a dose of 2.2 mg/kg/day. Studies have shown that it does not inhibit 5-lipoxygenase and leukotriene B4, as originally claimed. It is therefore not considered superior to phenylbutazone as previously believed, although clinical signs of lameness are reduced with its use. In fact, phenylbutazone was shown superior to ketoprofen in cases of experimentally-induced synovitis when both drugs were used at labeled dosages.

5.2 Excipient Profile

5.2.1 Phosphatidylcholines

Phosphatidylcholines (PC) are a class of phospholipids that incorporate choline as a headgroup. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources, such as egg yolk orsoybeans, from which they are mechanically or chemically extracted using hexane. They are also a member of the lecithin group of yellow-brownish fatty substances occurring in animal and plant tissues. Dipalmitoyl phosphatidylcholine (a.k.a. lecithin) is a major component of pulmonary surfactant and is often used in the L/S ratio to calculate fetal lung maturity. While phosphatidylcholines are found in all plant and animal cells, they are absent in the membranes of most bacteria, including *Escherichia coli*.Purified phosphatidylcholine is produced commercially.

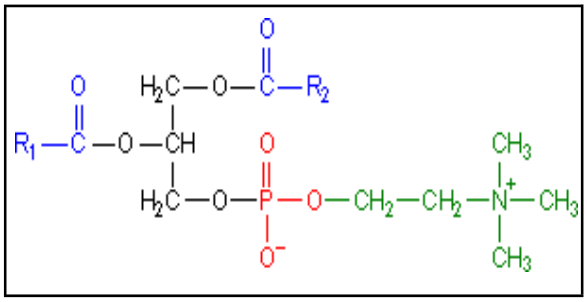


Figure 6: Structure of Phosphatidylcholines

Uses

- Hepatitis C. Taking phosphatidylcholine by mouth, together with interferon, seems to improve liver function in people with hepatitis C.
- Inflammatory bowel disease (ulcerative colitis). Research suggests that taking a specific phosphatidylcholine product (Sterpur P-30 Granulat, Stern-Lecithin and Soja GmbH) daily for 3 months improves symptoms in people with ulcerative colitis.

5.2.2 Sorbitan monolaurate (Span 20)

Sorbitan monolaurate is a mixture of esters formed from the fatty acid lauric acid and polyols derived from sorbitol, including sorbitan and isosorbide.

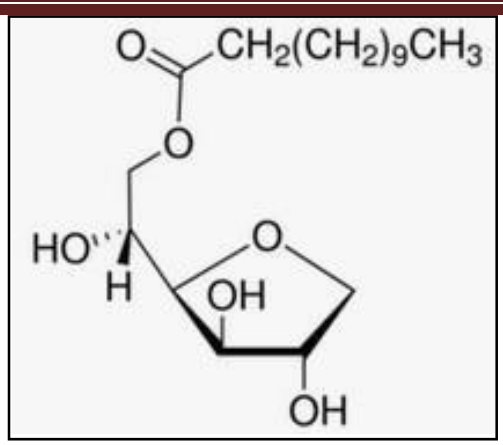


Figure 7: Structure of Span 20

Chemical formula	$C_{18}H_{34}O_6$
Molar mass	346.464 $g \cdot mol^{-1}$
Density	1.032 g/cm^3
Solubility in water	**insoluble**

5.2.3 Carbopol

Carbomers are white-colored, „fluffy", acidic, hygroscopic powders with a characteristic slight odor. A granular carbomer is also available (Carbopol 71G).

Synonyms: Carbopol; carboxy polymethylene; polyacrylic acid; carboxyvinyl polymer; Pemulen; Tego Carbomer.

Empirical Formula and Molecular Weight: Carbomers are synthetic high- molecular-weight polymers of acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. It contain 52% and 68% of carboxylic acid (COOH) groups on dry basis. The BP 2009 have a single monograph which describing carbomer. The

USP32–NF27 contains various monographs describing separate carbomer grades that differ in aqueous viscosity, polymerization solvent and polymer type. The molecular weight of carbomer is theoretically estimated at 7 X 10^5 to 4 X 10^9. In an effort to measure the molecular weight between crosslinks, MC, researchers have extended the network theory of elasticity to swollen gels and have utilized the inverse relationship between the elastic modulus and MC. Estimated MC values of 237 600 g/mol for Carbopol 941 and of 104 400 g/mol for Carbopol 940 have been reported.

Structural Formula

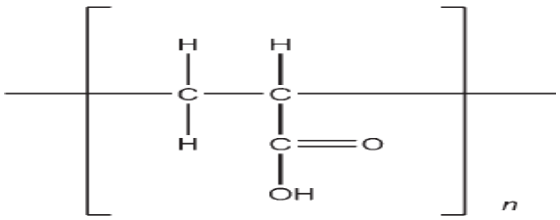


Figure 8: Structure of carbopol

Functional category: Bioadhesive material, controlled-release agent, emulsifying agent, stabilizing agent, rheology modifier, tablet binder, suspending agent.

Applications in pharmaceutical formulation or technology: Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers. Various formulations are available which includes creams, lotions, gel and ointments for treatment in ophthalmic, topical, rectal and vaginal preparations. Carbomer grades with residual benzene content greater than 2 ppm do not meet the specifications of the PhEur 6.4 monograph. However, carbomer having low residuals of other solvents than the ICH-defined „Class I OVI solvents" may be used in Europe. Carbomer having low residuals of ethyl acetate, such as Carbopol 971P NF or Carbopol 974P NF, may be used

in oral preparations, in suspensions, capsules or tablets. In tablet formulations, carbomers are used as controlled release agents and/or as binders. In difference to polymers, higher viscosity does not resulting in steady drug release with carbomers. For controlling drug release lightly cross-linked carbomers(lower viscosity) are more efficient than highly cross-linked carbomers (higher viscosity). Water and solvents or their mixtures can be utilized as the granulating fluid in wet granulation method. The tackiness of the wet mass can be decreassed by adding talc in the formulation or by mixing reliable cationic species to the granulating fluid. However, the presence of cationic salts can reduce bioadhesive properties and accelerate drug release rate. Carbomer polymers may also be used in the preparation of sustained-release matrix beads, as a bioadhesive for a cervical patch and for intranasally administered microspheres. Carbomers copolymers are also employed as emulsifying agents in the preparation of oil-in-water emulsions for external administration. For the formulation of multiple emulsion microspheres carbomer 951 has been investigated as a viscosity-increasing aid. Carbomers are also used in cosmetics. Therapeutically, carbomer formulations are effective in symptoms of moderate-to-severe dry eye syndrome.

Typical properties

Density (bulk) 0.2 g/cm^3 (powder); 0.4 g/cm^3 (granular). Density (tapped) 0.3 g/cm^3 (powder); 0.4 g/cm^3 (granular). Dissociation constant pKa = 6.0-0.5

Glass transition temperature 100–105ºC

Melting point occurs within 30 minutes at 260ºC. Moisture content is up to 2% w/w.

5.2.4 Propylene glycol

It is a clear, colourless, viscous, practically odourless liquid with a sweet and slightly acrid taste resembling that of glycerine.

IUPAC name: 1, 2-Propanediol -1, 2- Propanediol -1, 2-Propanediol Empirical

Formula: $C_3H_8O_2$ **Molecular**

Weight: 76.09 **Boiling point:**

188°C **Melting point:** -59°C

Density: 1.038 g / cm^3 at 20°C

Structure

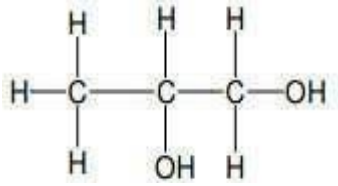


Figure 9: Structure of Propylene Glycol

Solubility: Miscible with acetone, chloroform, ethanol (95%), glycerine and water, soluble at 1 in 6 parts of ether, not miscible with light mineral oil or fixed oils, but will dissolve in some essential oils.

Stability: Stable in a well-closed container at cool temperature. It is chemically

stable when mixed with ethanol (95%), glycerine, or aqueous solutions.

Applications: In parenteral and nonparenteral pharmaceutical formulations, widely used as a solvent, extractant and preservative. Commonly used as a plasticizer and as a carrier for emulsifiers.

Incompatibilities: Incompatible with potassium permanganate.

Safety: Regarded as a relatively nontoxic material and minimally irritant in topical preparations. It is also used in foods and cosmetics. Pain or irritation may cause due to parenteral administration when it is used in high concentration.

5.2.5 Ethanol

Ethanol is an organic chemical compound. It is a simple alcohol with the chemical formula C □H $_6$ O. Its formula can be also written as CH $_3$ −CH $_2$ −OH or C $_2$ H □OH, and is often abbreviated as EtOH. Ethanol is a volatile, flammable, colorless liquid with a slight characteristic odor.

Synonyms: Absolute Alcohol, Ethyl Alcohol, Grain Ethanol

Formula: C_2H_5OH **Boiling point:** 78.37 °C **Molar mass:** 46.07 g/mol **Density:** 789 kg/m^3 **Melting point:** -114.1 °C **Uses of Ethanol**

- Owing to its antibacterial and antifungal properties, ethanol (also known as ethyl alcohol) is used in many hand sanitizers and medical wipes.

- Ethanol is also used as an antiseptic and as a disinfectant.
- In cases of ethylene glycol poisoning or methyl alcohol poisoning, ethanol is often administered as an antidote.
- Several medications that are insoluble in water are often dissolved in ethanol. For example, ethanol (in concentrations ranging from 1% to 25%) is used as a solvent for some analgesics and mouthwashes.
- Ethanol is the primary ingredient in many alcoholic drinks that are orally consumed for recreational purposes. It acts as a psychoactive drug by reducing anxiety and creating a feeling of euphoria in Humans. However, it also impairs cognitive and motor functions and acts as a central nervous system (CNS) depressant.
- Ethanol is used industrially in the production of ethyl esters, acetic acid, diethyl ether, and ethyl amines.
- This compound is widely used as a solvent due to its ability to dissolve both polar and nonpolar compounds.
- Since it has a melting point of -114.1^{o}C, ethanol is used as an ingredient in cooling baths in several laboratories. It also serves as the active fluid in many spirit thermometers.

CHAPTER – 6

PREFORMULATION STUDY

6. PREFORMULATION STUDY

6.1 Preformulation study

Preformulation study is the first step in the rational development of dosage form of a drug substance [57-58]. It can be defined as an investigation of physical and chemical properties a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable & bioavailable dosage forms which can be mass produced. Obviously, the type of information needed depend on the dosage form to be developed. Preformulation studies include studies of:

1. The physiochemical properties of drug, and an assessment of their relevance to the final formulation.
2. The chemical and physical stability of drug.
3. Chemical /physical compatibility of the active with potential excipients.

These studies give clues as to how to achieve the desired performance of the finished products.

Even after developing a formulation and method of manufacture on these principles, it is still necessary to confirm stability and bioavailability, but there is a smaller probability that the formulation will fail. If two or three formulations are developed in parallel, there is even greater probability that one will be significantly minimize the risks of failure and increase the likelihood of producing a high quality.

Some specific benefits of conducting preformulation studies

1. **Minimizing development cost:** By optimizing the formulation before commencing bioavailability and bioequivalence studies, fewer such studies need be conducted.
2. **Avoiding failures during long-term stability:** Failure after say 2 or 3 years of long-term stability testing can set back a program significantly. Sound predictions as to the chemical and physiochemical stability of the active, and compatibility with excipients, other actives and the container, can minimize the failures.

6.2 Characterization of Ketoprofen

A) Physical evaluation It refers to the evaluation by sensory characters-taste, appearance, odor, feel of the drug, etc.

Table 6: List of sensory characters

Color	White
Odor	Odorless
Taste	Tasteless

Ketoprofen was found white, odorless and tasteless powder

A) Solubility: Solubility of the drug was determined by taking some quantity of drug (about 1-2 mg) in the test tube separately and added the 5 ml of the solvent (Water, ethanol, methanol, 0.1N HCl, 0.1N NaOH, Chloroform and phosphate buffer 7.4 pH) shaken vigorously and kept for some time. Note the solubility of the drug in various solvents (at room temperature).

Table 7: IP Index

Descriptive term	**Parts of solvent required for Parts of solute**
Very soluble	Less than 1
Freely soluble	From 1to 10
Soluble	From 10 to 30
Sparingly soluble	From 30to 100
slightly soluble	From 100 to1000
Very slightly soluble	From 1000 to 10000
Practically insoluble	10000 or more

Table 8: Solubility of Ketoprofen

S. No.	**Solvent**	**Solubility**
1.	Water	Sparingly soluble
2.	0.1 N HCl	Sparingly soluble
3.	Methanol	Soluble
5.	Ethanol	Soluble
6.	0.1 N NaOH	Soluble
7.	Chloroform	Soluble
8.	7.4 pH phosphate buffer	Soluble

Solubility of Ketoprofen was examined soluble in chloroform, methanol and ethanol, 0.1 N NaOH. 7.4 pH phosphate buffer, sparingly soluble in water and 0.1 N HCl.

B) Melting point:

It is one of the parameters to judge the purity of drugs. In case of pure chemicals, melting points are very sharp and constant. Since the drugs contain the mixed chemicals, they are described with certain range of melting point [60].

Procedure for determine melting point:

A small quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

Table 9: Melting point of Ketoprofen

S. No.	Method	Literature Melting Point	Practically Melting Point
1.	Capillary fusion method	94°C	94-96°C

Melting point of Ketoprofen was found to be 94-96°C.

C) Identification test using FTIR spectroscopy

Infra- red spectrum is an important record which gives sufficient information about the structure of a compound [61]. This technique provides a spectrum containing a large number of absorption band from which a wealth of information can be derived about the structure of an organic compound. The region from 0.8 μ to 2.5 μ is called Near Infra-red and that from 15 μ to 200 μ is called Far infra-red region. Identification of Ketoprofen was done by FTIR Spectroscopy with respect to marker compound. Ketoprofen was obtained as White powder. It was identified from the result of IR spectrum as per specification.

Sample of pure Ketoprofen

The IR spectrum of sample drug shows the peak values which are characteristics of the drug and the graph shown in figure no. 6.1.

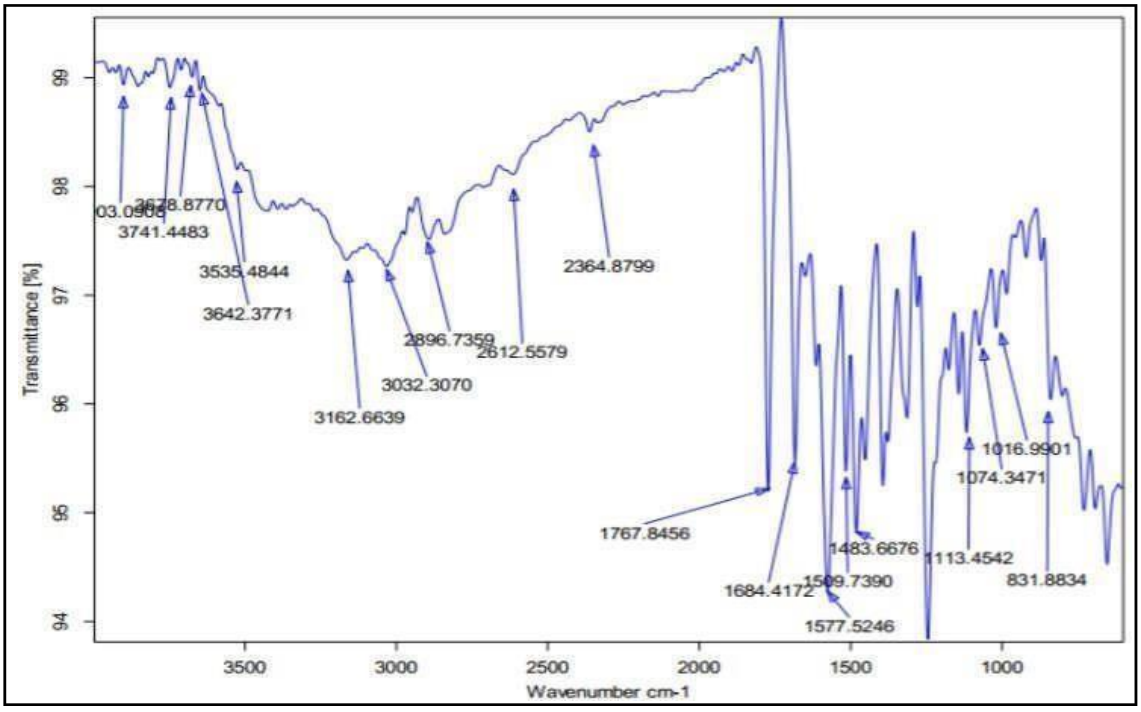


Figure 10: FTIR Spectra of Ketoprofen

D) Loss on drying: The moisture in a solid can be expressed on a wet weight or dry wet basis. On a wet weight basis, the water content of a material is calculated as a percentage of the weight of the weight solid. The term loss on drying is an expression of moisture content on a wet weight basis [62].

Procedure:

Loss on drying is directly measured by IR moisture balance. Firstly calibrated the instrument by knob then taken 5 gm sample (powder) and set the temp at 100°C to 105°C for 15 minutes and constant reading set the knob and check % moisture.

Result: Loss on drying of Ketoprofen was found 0.153±0.002%.

E) Moisture content determination:

Principle: The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulphur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions.

In the original titrimetric solution, known as Karl Fisher Reagents, the sulfur dioxide and iodine was dissolved in pyridine and methanol. The test specimen may be titrated with the reagent directly, or the analysis may be carried out by a residual titration procedure. The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative

concentration of the reagent ingredients, the nature of the inert solvent used to dissolve the test specimen, and the technique used in the particular determination. Therefore, an empirically standardized technique is used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture

is excluded from the system. The titration of water is usually carried out with the use of anhydrous methanol as the solvent for the test specimen; however other suitable solvents may be used for special or unusual test specimens. (Note: Now-a-days pyridine free KF reagents are coming in which pyridine is replaced by the imidazole, because pyridine has carcinogenic effects).

Procedure:

Karl Fischer volumetry is used for samples with high water content, *i.e.* 1-100 mg per sample. An iodine-containing solution serves as titrating agent. The water content of the sample is calculated using titration volume and titer of the titrating agent. One-component reagents conveniently contain all reactants (iodine, sulfur dioxide and a base) dissolved in a suitable alcohol in one solution, whereas two- component reagents contain all necessary reactants separated in two different solutions to enhance the rapidity of the Karl Fischer reaction and the titer stability of the titrating agent.

Karl Fischer coulometry is a micro-method and is particularly suitable for samples with low water content, from 10 µg up to 10 mg. Here, the required iodine is generated electrochemically in the titration vessel by anodic oxidation from iodide contained in the coulometric reagents. The amount of consumed electric charge is used to calculate the consumption of iodine and therefore the amount of water in the sample.

Result: Moisture content of Ketoprofen was found to be 0.0352.

F) Determination of λ_{max} of Ketoprofen:

The λ_{max} of Ketoprofen was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

Procedure:

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH phosphate buffer in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.4 pH phosphate buffer solution prepare suitable dilution to make it to a concentration range of 5-25 µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The spectrum peak point graph of absorbance of Ketoprofen versus wave length shown in figure 6.2.

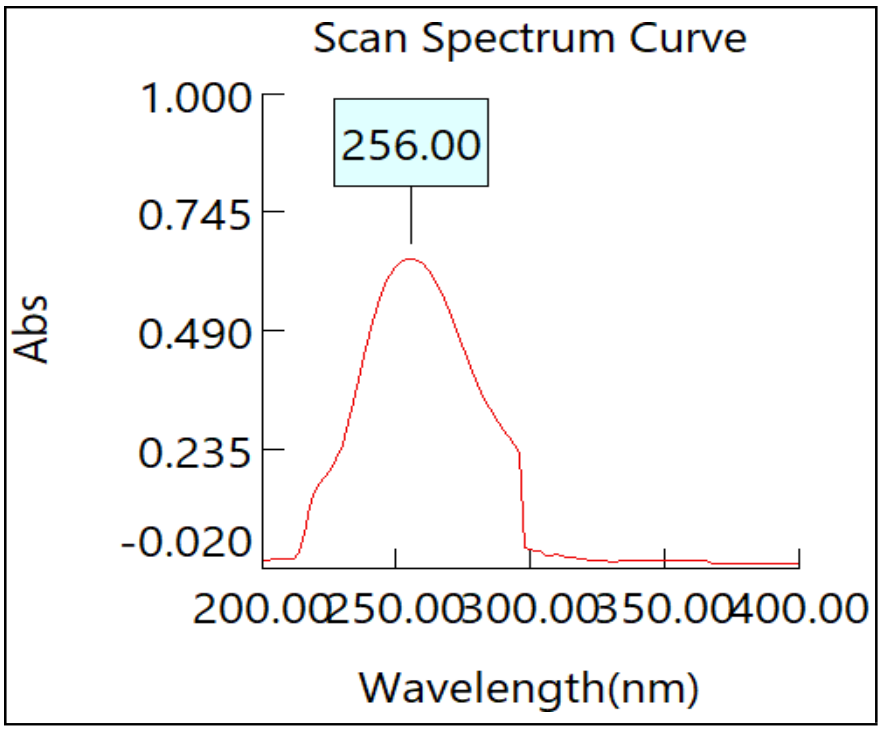


Figure 11: Wavelength maxima of Ketoprofen in 7.4 pH phosphate buffer Results of calibration curve of Ketoprofen

Table 10: Calibration curve of Ketoprofen

S. No.	Concentration (µg/ml)	Mean absorbance
1	5	0.162
2	10	0.326
3	15	0.501
4	20	0.654
5	25	0.798

***Average of three determinations (n=3)**

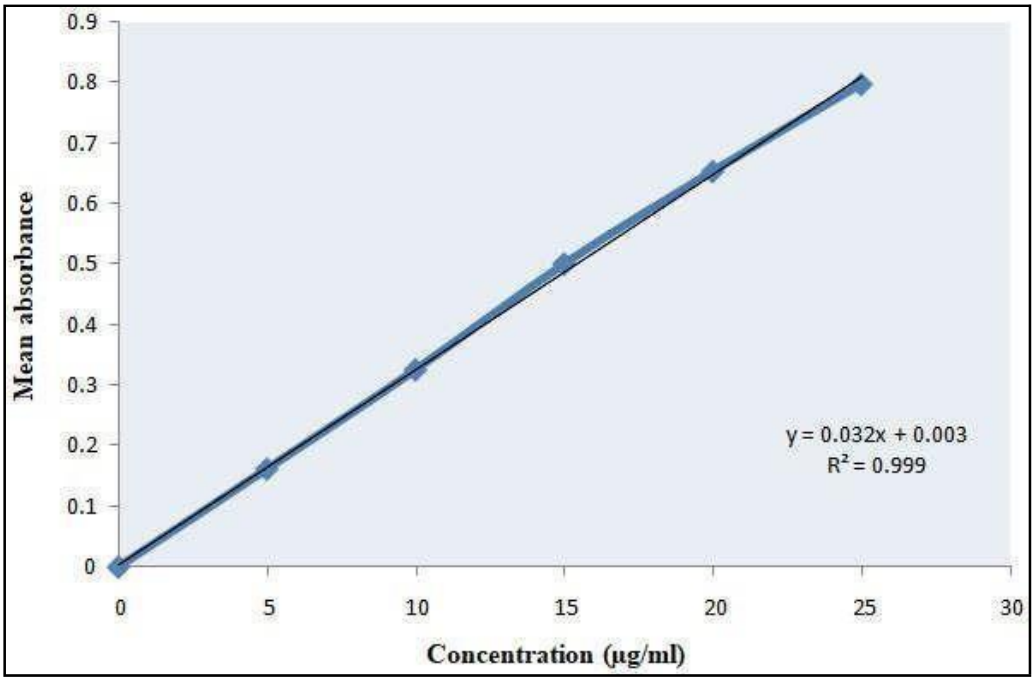


Figure 12: Calibration curve of Ketoprofen in 7.4 pH phosphate buffer

CHAPTER – 7

PREPARATION AND CHARACTERIZATION

7. PREPARATION AND CHARACTERIZATION

7.1 Formulation, development Ketoprofen loaded Transethosomes

In a round bottom flask, required amounts of Soya PC and surfactant were dissolved in ethanol by shaking [63]. The thin film was created via rotary evaporation for 15 minutes at 25°C, 600mm/hg pressure, and 100rpm in a rotary evaporator. After that, the solvent was evaporated using a nitrogen gas stream. To remove any residual solvent, the lipid film was put in a desiccator for at least 12 hours. Ketoprofen was dissolved in 10ml, 7.4 pH which was heated to 55^0C. The film was then hand-shook for half an hour to hydrate it with the hot buffer. The mixture was then shaken in an orbital shaker for half an hour. Following that, the transferosomes were examined under a microscope. The Transethosomal suspension was kept at 40°C in the refrigerator.

7.2 Preparation of Gel Base

Carbopol 934 (1%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was agitated continuously at 800 rpm for 1 hour before 10 mL of propylene glycol was added. To eliminate air bubbles, the gel was diluted to 100 mL and sonicated for 10 minutes on a bath sonicator. Final pH of the gel base was adjusted to 6.8. Transethosomal preparation corresponding to 0.1 percent w/w of Ketoprofen was incorporated into the gel base to get the desired concentration of drug in gel base.

7.3 Optimization of Transethosomes

7.3.1 Optimization of ratio of lipid and surfactant

The ratio of lipid and surfactant in the Transethosomes formulation was adjusted by using several ratios such as 9.5:0.5, 9:1, 8:2, and 7:3 percent ratios, while all other parameters remained fixed. The average vesicle size and percent entrapment efficiency of the produced formulations were optimized.

Table 11: Optimization of ratio of lipid concentration

Formulation code	Soya PC: Span 20 (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F1	9.5:0.5	10	1.0	325.65	63.36
F2	9:1	10	1.0	285.65	68.85
F3	8:2	10	1.0	245.65	75.65
F4	7:3	10	1.0	275.65	70.25

Table 12: Optimization of ethanol concentration

Formulation code	Soya PC: Span 20 (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F5	8:2	5	1.0	285.65	68.65
F6	8:2	10	1.0	224.45	76.65
F7	8:2	15	1.0	265.58	69.98
F8	8:2	20	1.0	271.65	63.32

7.3.2 Optimization of drug concentration:

Drug concentration was adjusted by preparing formulations with varying concentrations of drug while keeping all other parameters constant, such as Soya PC and stirrer time. Entrapment efficiency and average vesicle size were used to improve the formulation.

Table 13: Optimization of drug concentration

Formulation code	Soya PC: Span 20 (% w/v)	Drug (% w/v)	Ethanol (ml)	Average vesicle size (nm)	% Entrapment efficiency
F9	8:2	1.0	10	165.58	76.65
F10	8:2	1.5	10	198.85	70.23
F11	8:2	2.0	10	185.65	69.95

7.3.3 Optimization of stirrer duration

The duration of stirring was improved by varying the time duration of the formulation, i.e. 5, 10, and 15 minutes. The average vesicle size and percent Entrapment efficiency were used to optimize the system.

Table 14: Optimization of Stirrer duration

Formulation code	Soya PC: Span 20 (% w/v)	Drug (% w/v)	Stirrer duration (min)	Average vesicle size (nm)	% Entrapment efficiency
F12	8:2	1.0	5	135.65	76.65
F13	8:2	1.0	10	125.45	68.85
F14	8:2	1.0	15	112.20	61.12

Table 15: Optimized formulation Transethosomes

Formulation code F12	
Soya PC: Span 20 (% w/v)	8:2
Drug (% w/v)	1
Ethanol (ml)	10
Stirrer duration (min)	5

7.4 Characterization of Ketoprofen loaded Transethosomes

7.4.1 Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK)[64]. The Zeta potential of the Transethosomes was calculated from their electrophoretic mobility according to Helmholtz–Smoluchowsky. A Zetasizer was employed on a large

bore measuring cell to measure Zeta potential with a field strength of 20 V/cm. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm.

7.4.2 Entrapment efficiency

One milliliter of Transethosomes suspension was centrifuged at 15.000 rpm for 1 h to allow to separate the entrapped drug from the un-entrapped drug [65]. After removal of the supernatant, the sediment was lysed using methanol and then analyzed spectrophotometrically at 256nm using a UV spectrophotometer (Labindia 3000+). The Entrapment efficiency % of drug in the prepared Transethosomes was calculated applying the following equation:

% Ektrapmekt Efficiekcy

$$= \frac{\text{T}h\text{erotical drug coktekt} - \text{Practical drug coktekt}}{\text{Therotical drug content}} \times 100$$

7.4.3 *In vitro* drug diffusion study

The dialysis diffusion approach was used to perform *in vitro* drug release of prepared tranfersomes utilizing the dissolution test apparatus. The dissolving media was phosphate buffer pH 7.4. The dialysis technique was carried out utilizing a cellulose acetate dialysis membrane with a molecular weight cutoff of 12,000– 14,000 moles. This membrane ensures drug penetration while retaining tranfersomal vesicles. Before usage, the membrane was soaked in fake tears for 12 hours. A glass cylinder with a length of 8 cm and a diameter of 1 cm was filled with four ml of tranfersomal dispersion, and a dialysis membrane was threaded to the mouth of the cylinder. Each glass cylinder

was attached to the shaft of the dissolution apparatus (USP Dissolution tester, Labindia DS 8000) and descended down into a 100 ml.

beaker containing 50 ml of as dissolution medium without touching the bottom surface of the beaker. The beaker was then placed into vessels of dissolution apparatus that contained about 100 ml of water to keep temperature at 34 ± 0.5°C. The glass cylinders were adjusted to rotate at a constant speed of 20 rpm. One ml of dissolution medium was withdrawn at predetermined time intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 h).

To maintain a consistent volume, the samples were changed with new dissolving media. The concentrations of drugs in samples were measured spectrophotometrically at 256nm, the wavelength of the drug. The release tests were done in triplicates, with the mean and standard deviation reported. At the end of each sampling period, samples are removed and replaced with equal quantities of fresh receptor fluid.

7.4.3.1 Mathematical treatment of *in-vitro* release data: When mathematical formulae that represent dissolve findings as a function of some of the dosage form parameters are utilized, quantitative analysis of the values obtained in dissolution/release tests becomes easier.

1. Zero Order kinetics: Following this profile, pharmaceutical dosage forms release the same amount of drug per unit of time, making it the ideal method of drug release for achieving pharmacologically prolonged action. This model may be expressed in a simple model using the following relation:

$$Qt = Qo + Ko\ t \quad \text{----------(1)}$$

Where Qt is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution (most times, $Q_o=0$) and K_o is the zero order release constant.

2. First Order kinetics: The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303} \quad \text{----------(2)}$$

Where Qt is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution and K1 is the zero order release constant.

A graph showing the decimal logarithm of the drug's released quantity vs time will be linear as a result. Pharmaceutical dosage forms that follow this dissolving profile, such as those containing water-soluble medicines in porous matrices, release drug according to the amount of drug remaining in their interior, resulting in a decrease in the amount of drug released per unit of time.

3. Higuchi Model: Higuchi invented a number of theoretical models to investigate the release of water-soluble and low-soluble drugs in semi-solid and/or solid matrixes [66]. For drug particles dispersed in a uniform matrix acting as diffusion media, mathematical expressions were obtained. The simplified Higuchi model is expressed as:

$$Q = K_H \cdot t^{1/2} \quad \text{----------(3)}$$

Where Q is the amount of drug released in time t and KH is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the Fick‟s law, square root time dependent. This relationship may be used to explain the dissolution of water-soluble medicines from a variety of modified release pharmaceutical dosage forms, such as transdermal systems and matrix tablets.

4. Korsmeyer Peppas Model: Korsmeyer *et al.* used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\,t^n \quad \text{----------(4)}$$

Where M_t/M_∞ is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. "n" is the slope value of log M_t/M_∞ versus log time curve [67-68]. Regardless of the release mechanism, Peppas said that the following equation could accurately represent the release of solutes from slabs, spheres, cylinders, and discs. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of n =0.5 for fickian diffusion and higher values of n, between 0.5 and 1.0, or n =1.0, for mass transfer following a non-fickian model. In case of a cylinder n =0.45 instead of 0.5, and 0.89 instead of 1.0. Only systems with a drug diffusion coefficient that is somewhat concentration independent can utilize this equation.

To the determination of the exponent n the portion of the release curve where M_t/M_∞ < 0.6 should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (l) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n \quad \text{---------- (5)}$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b \quad \text{----------(6)}$$

In the absence of lag time or burst effect, l and b value would be zero and only at^n is used. This mathematical model, also known as *Power Law*, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms.

Table 16: Interpretation of diffusional release mechanisms

Release exponent (*n*)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.5<n<1.0$	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

7.5 Characterization of Transethosomes containing gel

7.5.1 Measurement of Viscosity

Viscosity measurements of prepared topical Transethosomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; viscosity.

7.5.2 pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer

solution of pH 4, pH 7 and pH 9.2. Following calibration, the electrode was dipped into the vesicles for as long as the vesicles covered it. The pH of a chosen formulation was then measured, and the results were recorded on the display.

7.5.3 Drug Content

In a beaker, 100 mg of topical Transethosomes gel was accurately weighed, and 20 ml of methanol was added [69]. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λmax 256nm.

7.5.4 Extrudability study

Extrudability was determined by the amount of gel extruded from a collapsible tube when a specific weight was applied. Extrudability improves as the amount of gel extruded increases. It was calculated by weighing a gel-filled collapsible tube and recording the weight at which the gel was extruded from the tube.

7.5.5 Spreadibility

Spreadability of the formulation is required to ensure that an adequate dosage is accessible for absorption via the skin, resulting in a positive therapeutic response. A slide is placed on a wooden block, and the top slide is moveable, with one end of the movable slide attached to a weight pan [70]. To assess spreadibility, 2-5 g of gel was placed between two slides, and the weight was gradually raised by adding it to the weight pan, with the time taken by the top plate to cover a distance of 6cm after adding 20g of

weight being recorded. Spreadability indicates a shorter time to spread.

$$Spreadibility\,(g.cm/\sec) = \frac{Weight\ tide\ to\ Upper\ Slide \times Lenth\ moved\ on\ the\ glass\ slide}{Time\ taken\ to\ slide}$$

7.5.6 *In vitro* drug diffusion study

The *In-vitro* diffusion study is carried by using Franz Diffusion Cell. Egg membrane is taken as semi permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume approximately 30mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32 ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. A Teflon-coated magnetic bead is inserted in the diffusion cell and stirs the receptor fluid. At the end of each sampling period, samples are removed and replaced with equal quantities of fresh receptor fluid. The removed samples are spectrophotometrically examined at the drug's wavelength of 256nm.

7.6 Stability Studies

Stability study was carried out for drug loaded Transethosomes at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25-28±2°C) for 3 weeks. To avoid any contact between the formulation and the container glass, the stability study formulation was kept in a borosilicate container.

Physical changes and pharmacological content were examined in the formulations.

Table 17: Characterization of optimized formulation of Transethosomes formulation

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temperature	4.0±0.2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C
Average vesicles size (nm)	135.65	225.65	146.21	245.65	148.12	298.85
% EE	76.25	68.85	75.45	60.45	73.14	54.12
Physical Appearance	Normal	High turbid	Normal	High turbid	Normal	High turbid

CHAPTER – 8

RESULTS AND DISCUSSION

8. RESULTS AND DISCUSSION

8.1 Average vesicle size and zeta potential

Prepared formulations of transethosomes were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. Vesicle size of transethosomes were examined under trinocular microscopic (magnification 400X) and also determined by light scattering method (Malvern Zetasizer, ZEM 5002, and UK) and found that average vesicle size of optimized formulation F-12 was 135.65nm. Zeta potential was -39.98. It was observed that the vesicles size of transethosomes was increase with increasing the concentration of phosphotidylcoline and similarly vesicle size. There was no significant difference in average vesicle size was observed with increasing the drug concentration. But in increasing the stirrer time the vesicle size was decrease from 112.20 to 135.65 after 15 min of stirring.

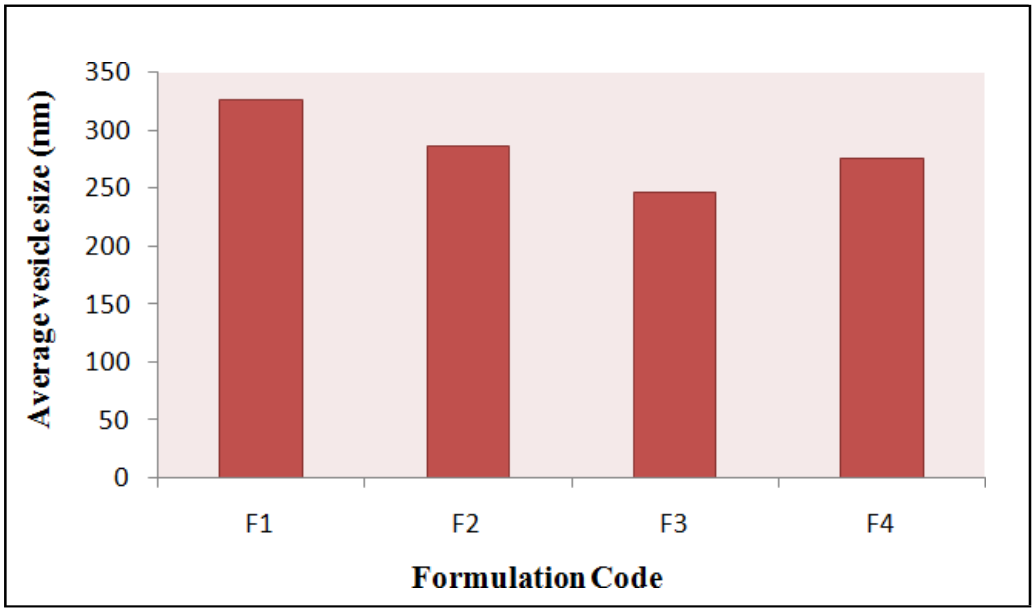


Figure 13: Effect of different ratio and concentration of soya PC on Vesicle size

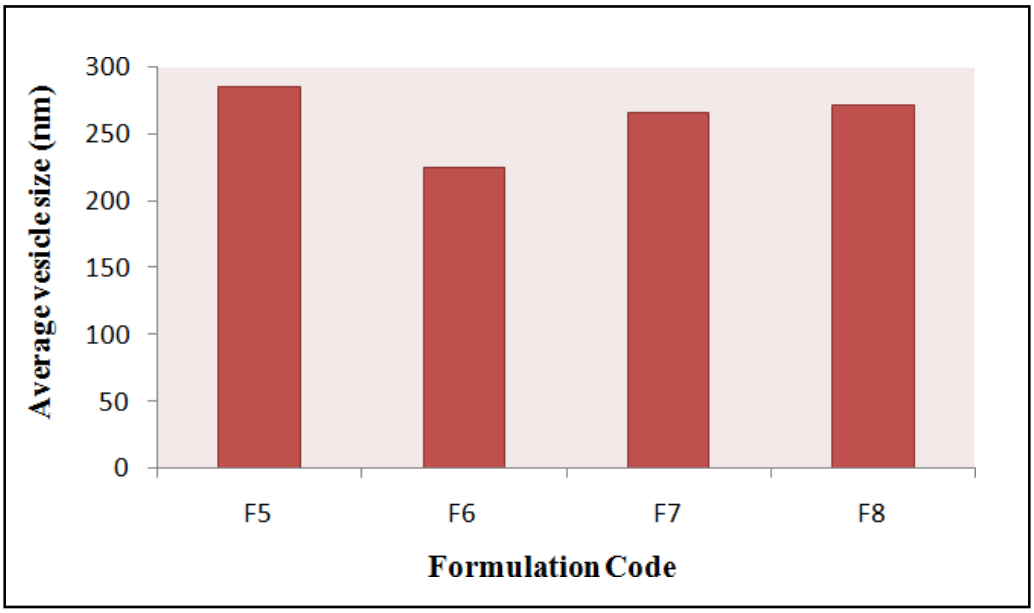


Figure 14: Effect of ethanol concentration on vesicle size

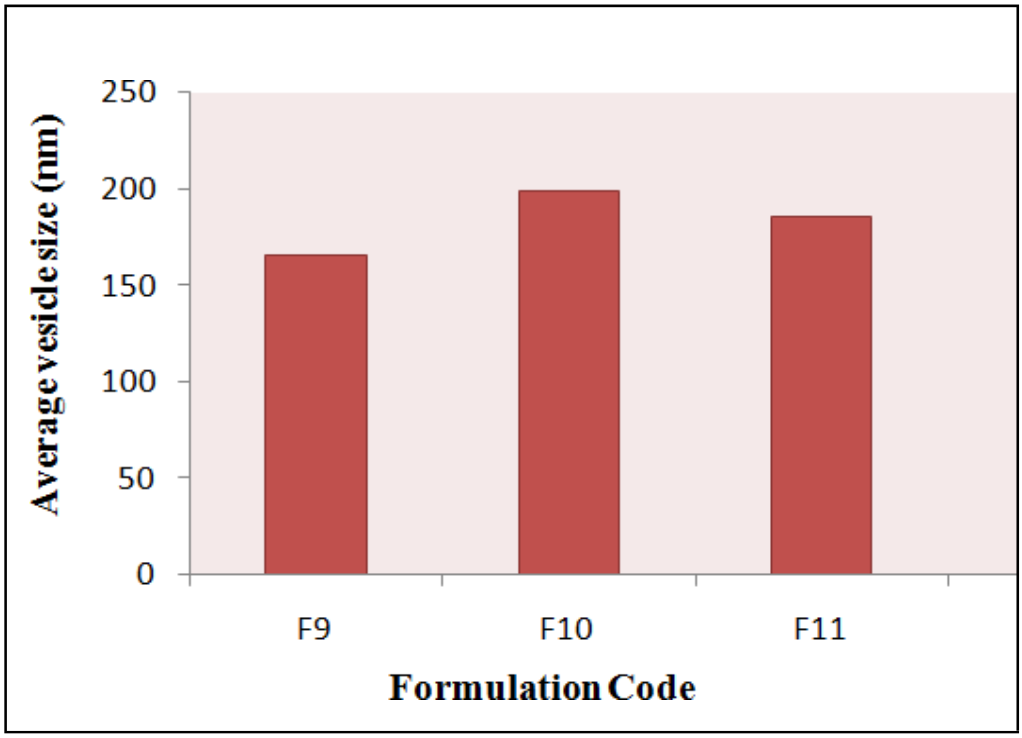


Figure 15: Effect of drug concentration on vesicle size

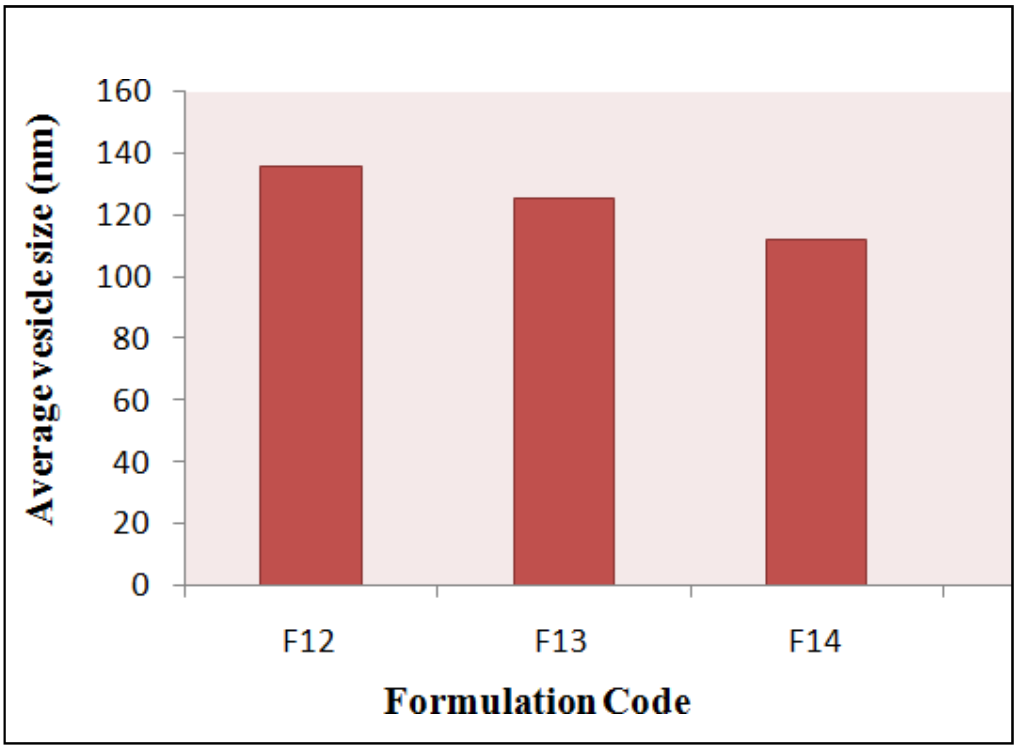


Figure 16: Effect of Stirrer time on vesicle size

8.2 Percentage entrapment efficiency

% Entrapment efficiency of optimized transethosomes formulation (F-12) was found to be 76.65%. It was observed that the percent drug entrapment was decrease with increasing the concentration of ethanol and on increasing the time of stirring. It is due to the leaching out the drug from vesicles on increasing the mechanical force by stirring and size reduction. It was clearly shown when formulation was stirrer for 5, 10, 15 min then the % Entrapment efficiency was 76.65, 68.85 and 61.12 percentage respectively. The 5 min is selected as optimized time for stirrer because it provided the required size of vesicle 135.65 nm and good % Entrapment efficiency i.e 76.65. The F12 formulation was selected as optimized formulation.

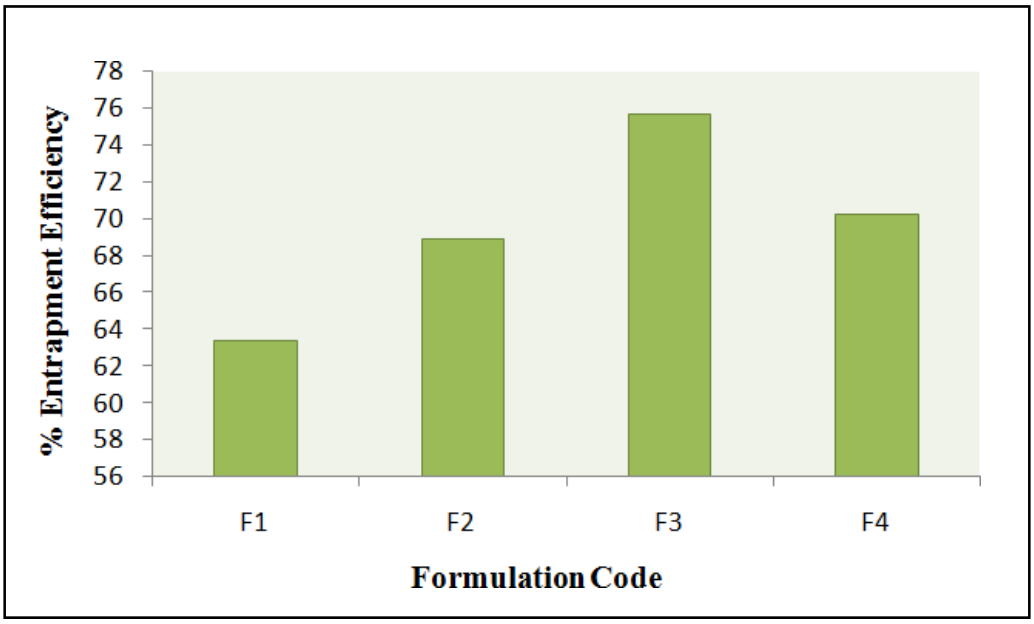


Figure 17: Effect of different ratio and concentration of soya PC on %EE

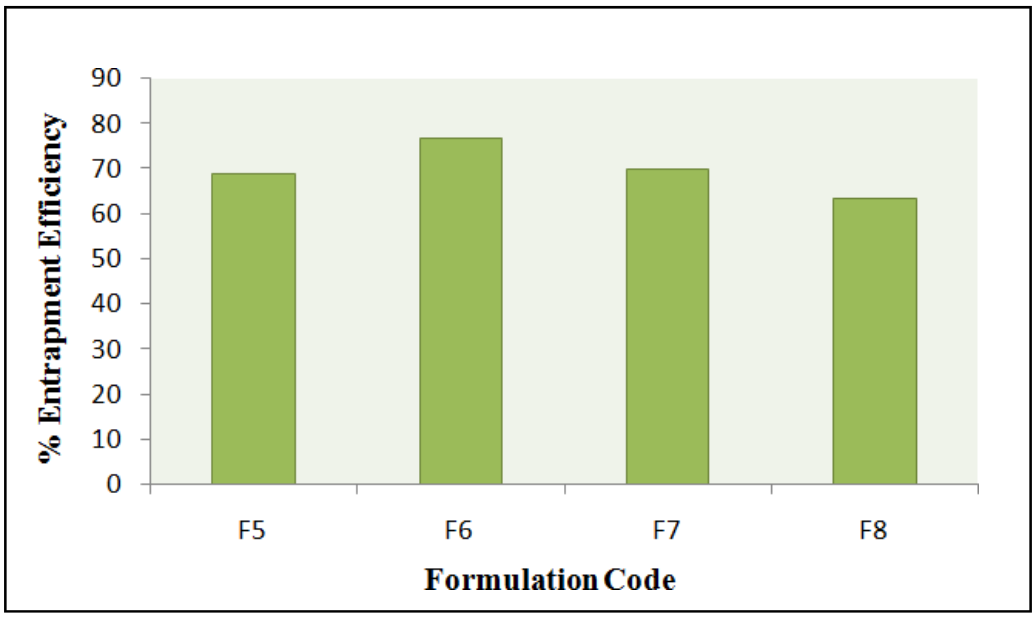


Figure 18: Effect of ethanol concentration of drug on % EE

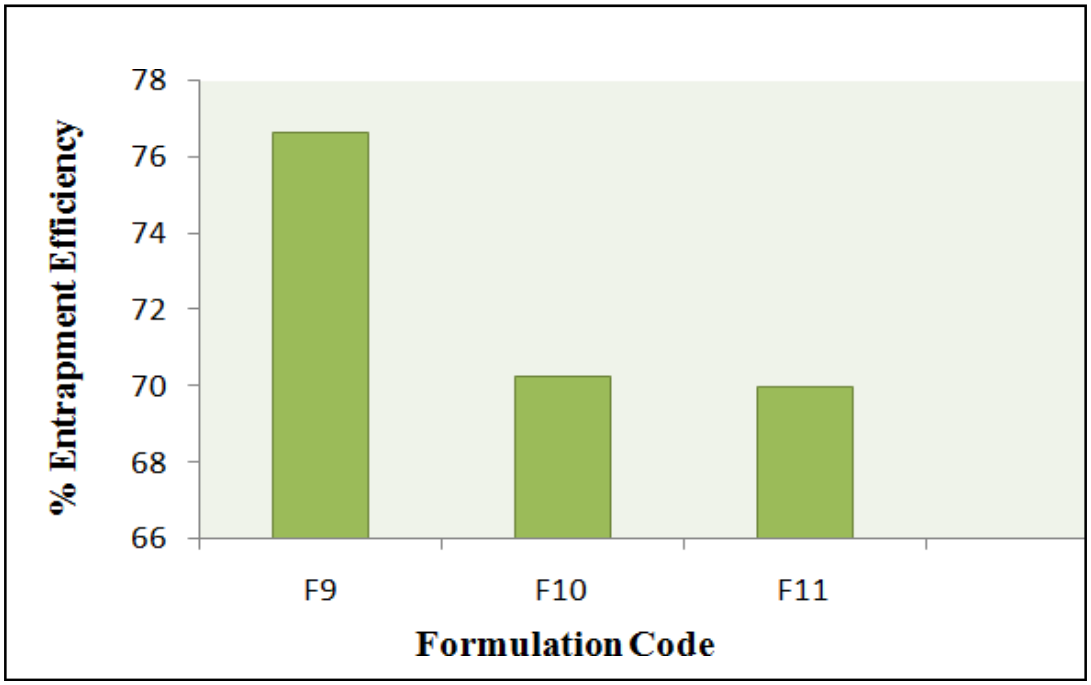


Figure 19: Effect of drug concentration of drug on % EE

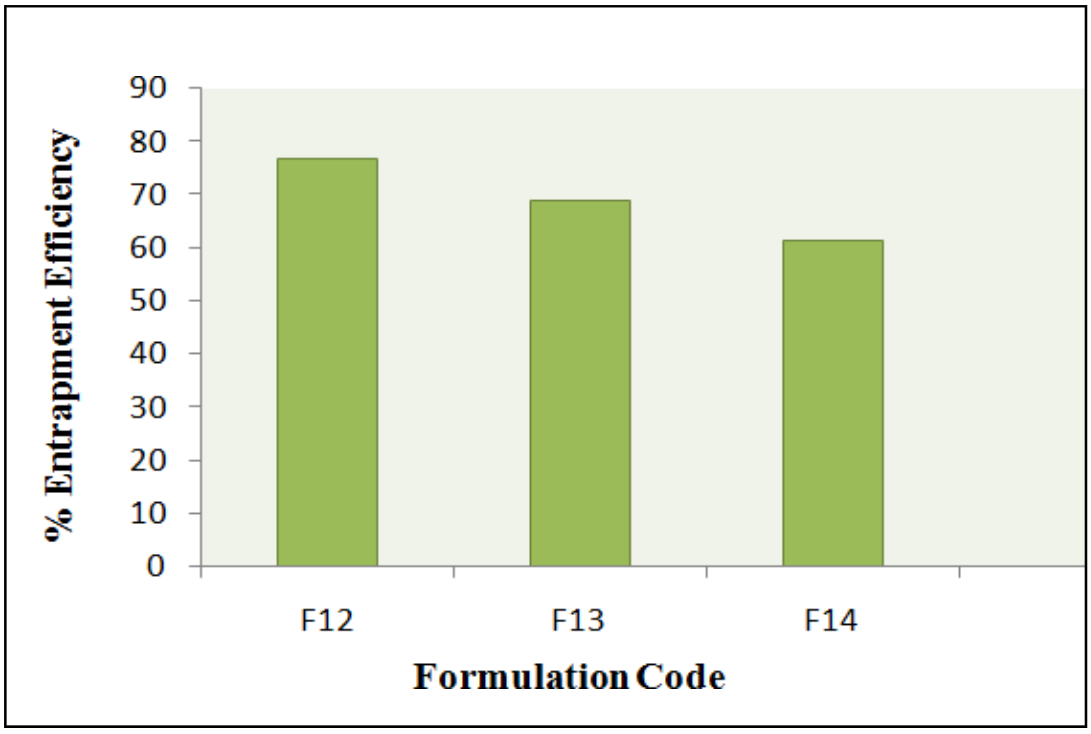


Figure 20: Effect of time on % EE

8.3 Results of characterization of optimized formulation of transethosomes

Table 18: Characterization of Optimized formulation of transethosomes

Characterization	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F12	135.65	76.65	-39.98

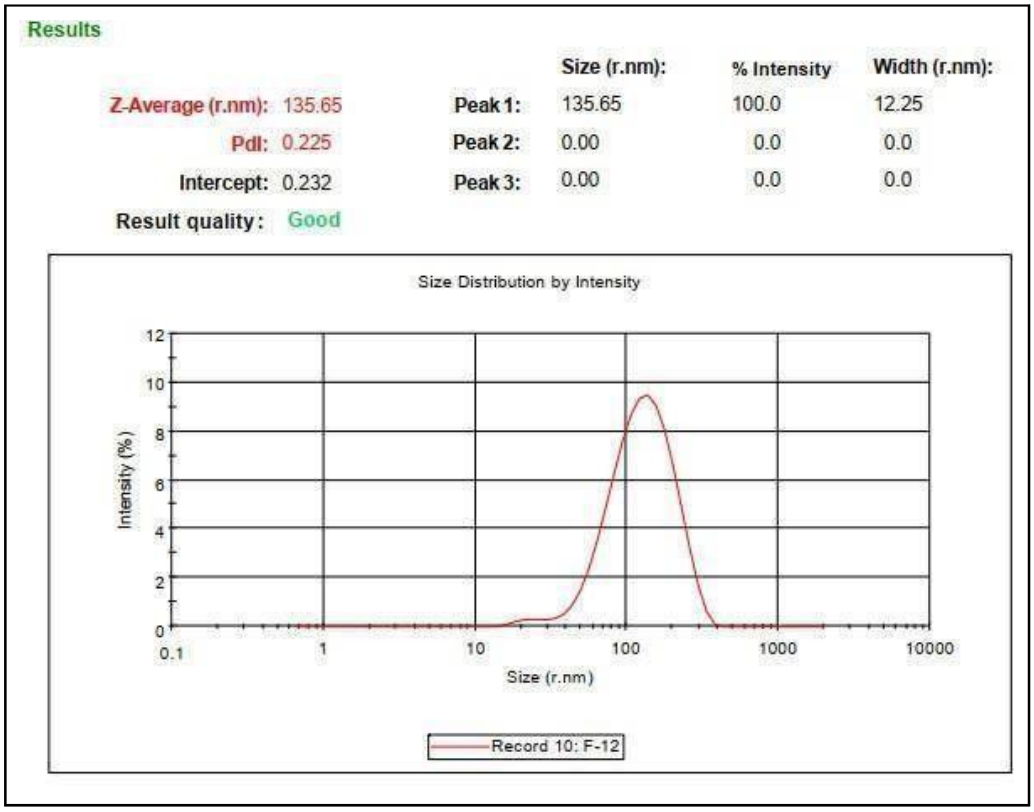


Figure 21: Image of Average vesicle size of optimized formulation F12

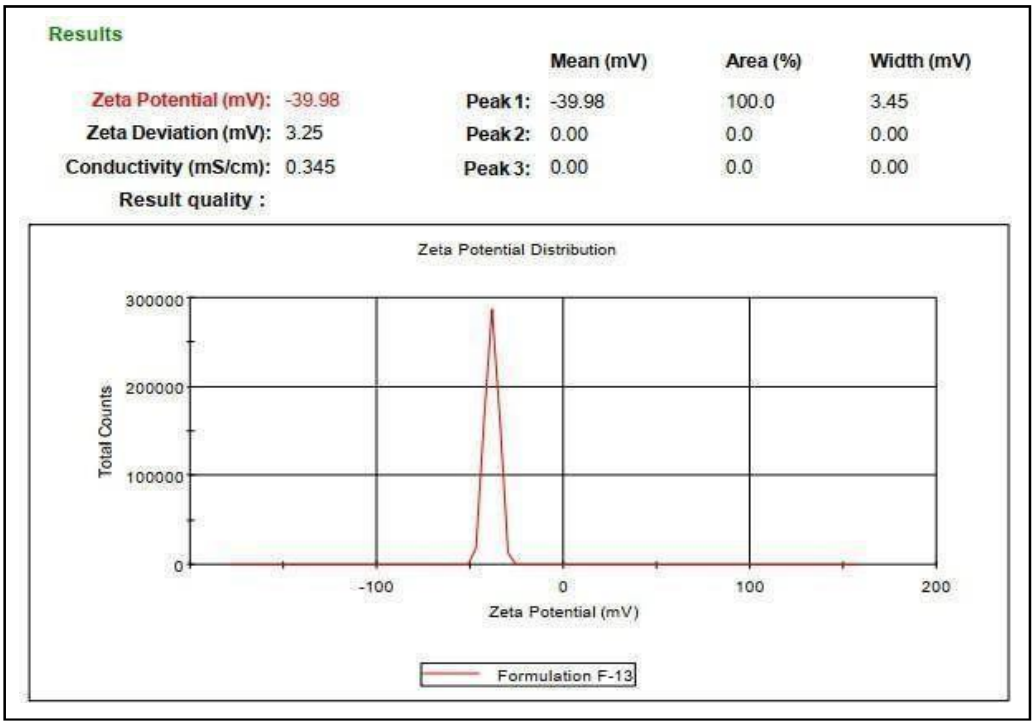


Figure 22: Image of Zeta Potential of optimized formulation F12

8.4 Stability Study

Stability study data was revealed that the optimized formulation (F12) stable after 3 month of storage at 4ºC while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remain, average vesicles size and physical appearance.

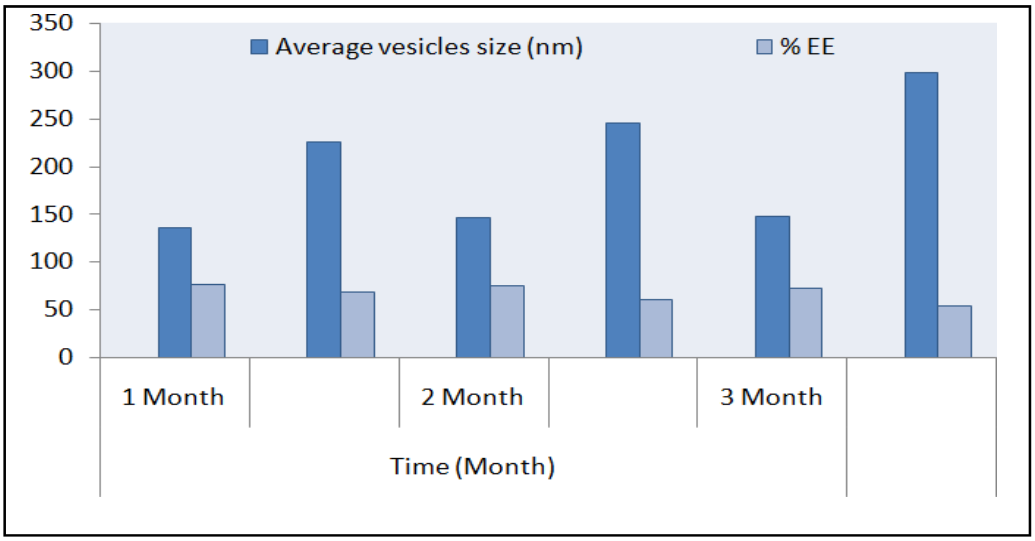


Figure 23: Average vesicle size of after storage at different temperature for three month

Table 19: Characterization of gel based formulation of prepared gel containing Ketoprofen loaded Transethosomes

Formulation	Viscosity* (cps)	Assay* (%)	Extrudability* (g)	Spreadability* (g.cm/sec)
Gel formulation	3540±15	99.05±0.45	175±0.25	13.65±0.35

***Average of three determination**

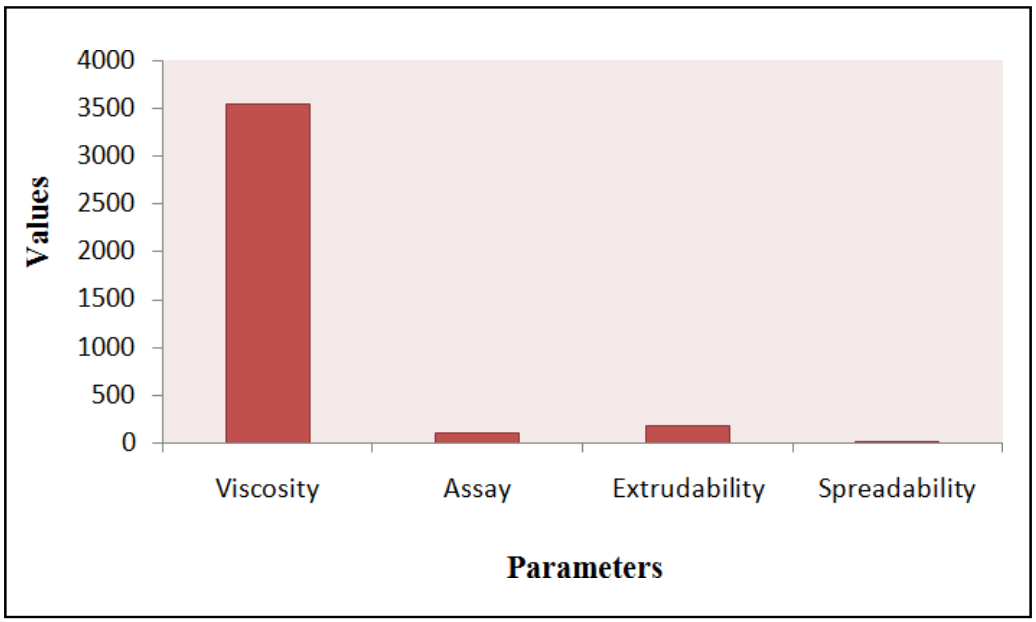


Figure 24: Characterization of gel based formulation of prepared gel containing Ketoprofen loaded Transethosomes

Prepared gel evaluate for viscosity, % assay, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel was 3540±15cps, % assay was 99.05±0.45, Extrudability was 175±0.25g and Spreadibility (g.cm/sec) was found that 13.65±0.35 (g.cm/sec) respectively. *In vitro* drug release from Transethosomes gel was carried out using Franz diffusion cell method and found 98.12% in 10 hr. In first 30 min it was 182±4 drug release which slightly high. It was due to the release of free drug present in bag after leaching from Transethosomes gel. Drug release from transethosomes formulation was found in very sustained and controlled manner.

Table 20: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	11.12
2	1	26.65
3	1.5	39.98
4	2	46.65
5	4	59.98
6	6	76.65
7	8	89.98
8	10	99.12

*Average of three determination

Table 21: *In-vitro* drug release data for gel formulation

Time (h)	Square Root of Time(h)1/2	Log Time	Cumulative * % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative% Drug Remaining
0.5	0.707	-0.301	11.12	1.195	84.35	1.926
1	1.000	0.000	26.65	1.426	73.35	1.865
1.5	1.225	0.176	39.98	1.589	61.15	1.786
2	1.414	0.301	46.65	1.669	53.35	1.727
4	2.000	0.602	59.98	1.778	40.02	1.602
6	2.449	0.778	76.65	1.859	27.77	1.444
8	2.828	0.903	89.98	1.949	11.02	1.042
10	3.162	1.000	99.12	1.992	1.88	0.274

***Average of three reading (mean ±SD)**

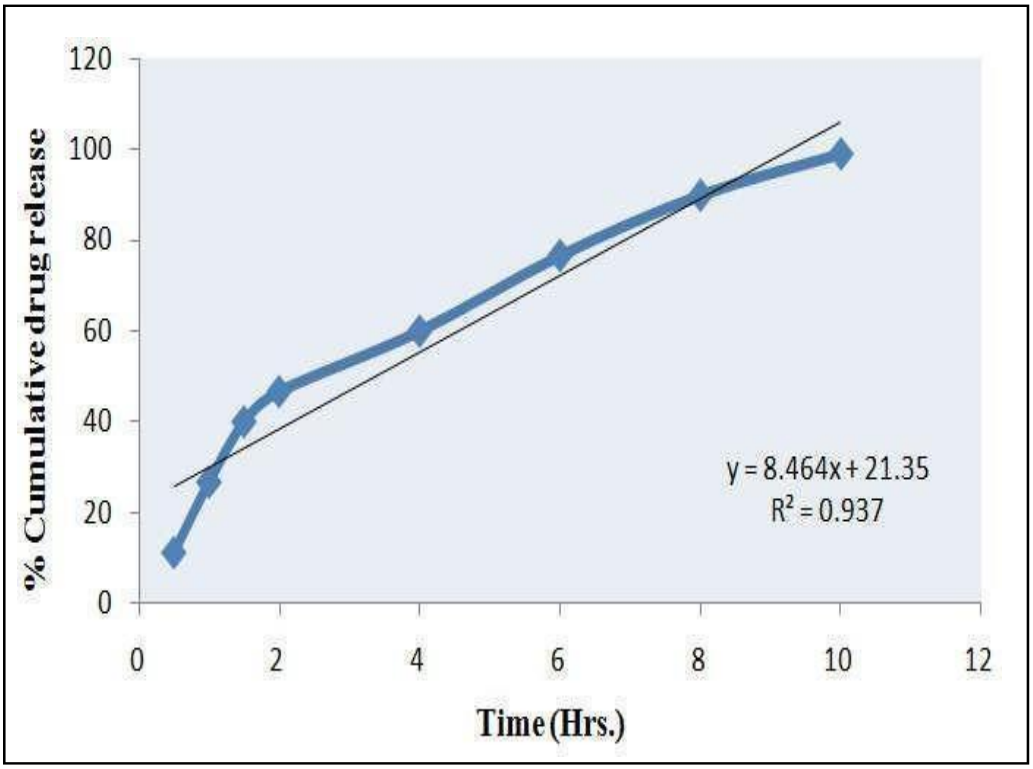


Figure 25: Cumulative % drug released *Vs* Time

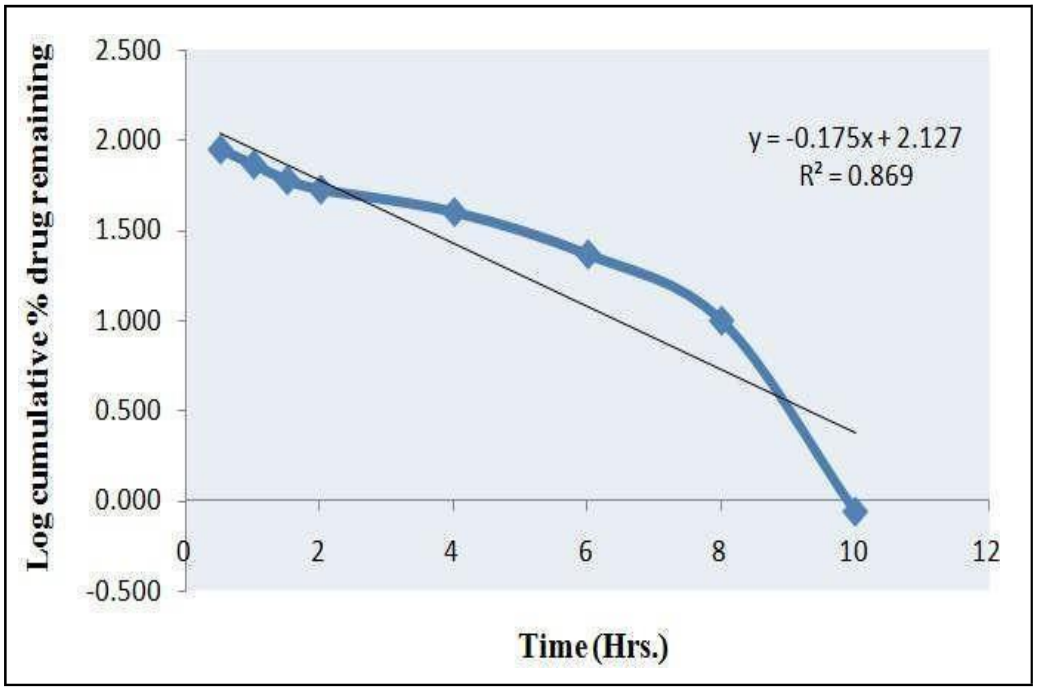


Figure 26: Log cumulative % drug remaining *Vs* Time

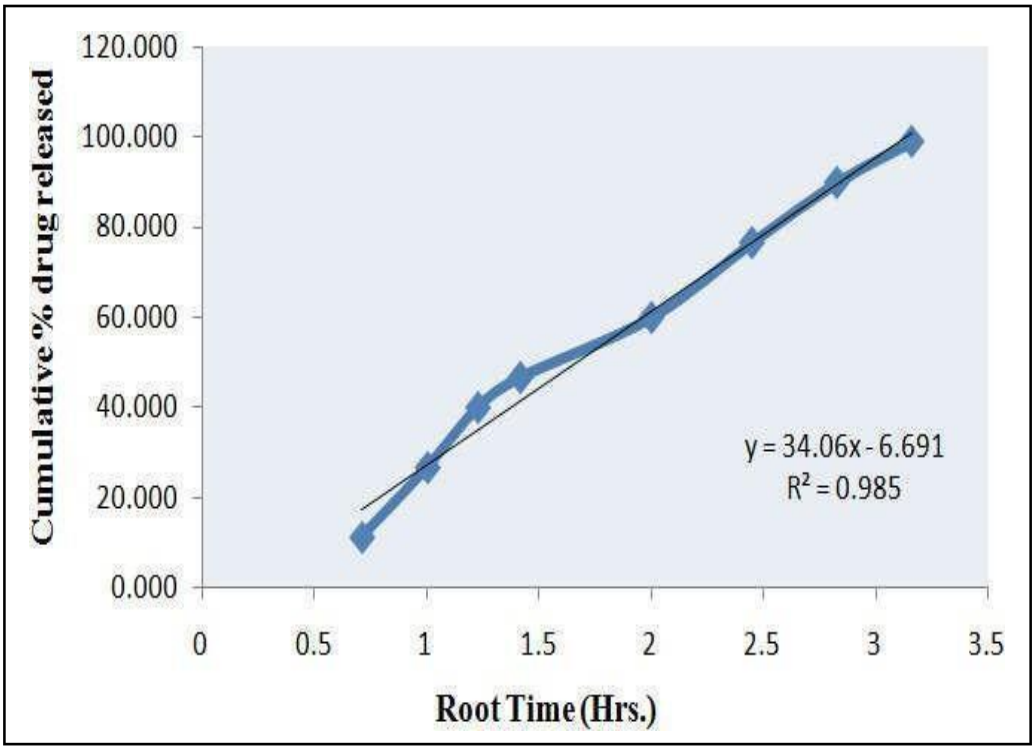


Figure 27: Cumulative % drug released *Vs* Square root of Time

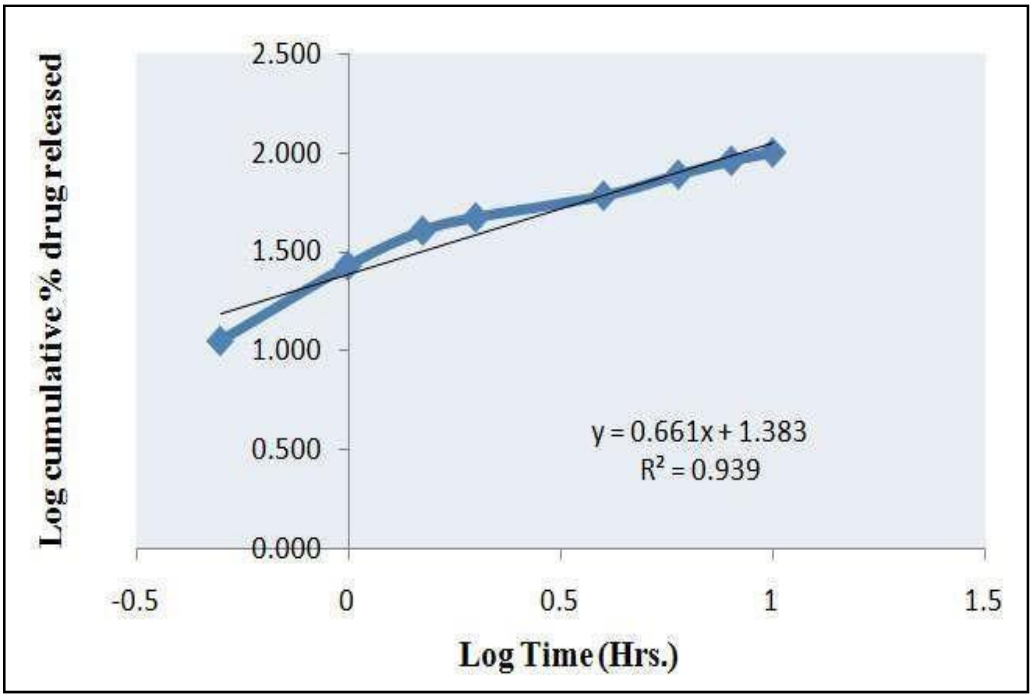


Figure 28: Log cumulative % drug released *Vs* log Time

Table 22: Regression analysis data of transethosomes gel formulation

Batch	Zero Order	First Order	Higuchi's Model	Kerseymeres Peppa's Equation
	R^2			
Optimized gel formulation	0.937	0.869	0.985	0.939

CHAPTER – 9

SUMMARY AND CONCLUSION

9. SUMMARY AND CONCLUSION

A preformulation study of Ketoprofen powder was performed. The result was found as below:- Ketoprofen powder was found to be white powder, all most odorless with tasteless. Solubility of Ketoprofen was examined soluble in chloroform, methanol and ethanol, 0.1 N NaOH. 7.4 pH phosphate buffer, sparingly soluble in water and 0.1 N HCl. Melting point of Ketoprofen was found to be 94-96°C. Loss on drying of Ketoprofen was found 0.153±0.002%. Moisture content of Ketoprofen was found to be 0.0352.

UV-Spectroscopic studies was carried out by running its spectrum in range of 200- 400 nm using U.V/ Vis Spectrophotometer Labindia 3000+ and 7.4 pH phosphate buffer as a solvent and the absorption maxima was found to be 256 nm which was comparable to that of standard. The calibration curve of Ketoprofen was prepared in 7.4 pH phosphate buffer and the regression coefficient was found to be 0.999. The preformulation study was carried out with a vision to determine the authenticity and purity of drug sample. The physical characterization, melting point and FT-I.R studies was performed for the identification of drug.

In most situations, an effective and successful therapeutic therapy is not possible due to a variety of factors, including hepatic first-pass metabolism, severe side effects, rejection of invasive therapies, and poor patient compliance. To address these issues, many medication delivery methods have been created and explored during the last few decades. Transdermal delivery devices are a potential option since they are minimally invasive and have no first-pass effects.

To encapsulate antifungal medications and increase their effectiveness, several new drug delivery methods have been developed. Microemulsions, nanoemulsions, niosomes, dendrimers, solid lipid nanoparticles, liposomes, ethosomes, lipid nanoparticles, and polymeric nanoparticles are only a few of them.

Present research aims the evaluation of transethosomes gels loaded with Ketoprofen in the treatment of fungal disease. In the present study, Transethosomes were formulated by hand-shaking method, followed by loading of Ketoprofen Transethosomes and development into gel formulation.

Particle size, zeta potential, entrapment efficiency, and an *in vitro* drug diffusion investigation were all tested utilizing different formulations made with soya PC and surfactant.

Prepared formulations of transethosomes were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. Vesicle size of transethosomes were examined under trinocular microscopic (magnification 400X) and also determined by light scattering method (Malvern Zetasizer, ZEM 5002, and UK) and found that average vesicle size of optimized formulation F-12 was 135.65nm. Zeta potential was -39.98 (It was observed that the vesicles size of transethosomes was increase with increasing the concentration of phosphotidylcoline and similarly vesicle size. There was no significant difference in average vesicle size was observed with increasing the drug concentration. But in increasing the stirrer time the vesicle size was decrease from

112.20 to 135.65 after 15 min of stirring.

% Entrapment efficiency of optimized transethosomes formulation (F-12) was found to be 76.65%. It was observed that the percent drug entrapment was decrease with

increasing the concentration of ethanol and on increasing the time of stirring. It is due to the leaching out the drug from vesicles on increasing the mechanical force by stirring and size reduction. It was clearly shown when formulation was stirrer for 5, 10, 15 min then the % Entrapment efficiency was 76.65, 68.85 and 61.12 percentage respectively. The 5 min is selected as optimized time for stirrer because it provided the required size of vesicle 135.65 nm and good % Entrapment efficiency i.e 76.65. The F-12 formulation was selected as optimized formulation.

Stability study data was revealed that the optimized formulation (F-12) stable after 3 month of storage at 4ºC while at 25-28±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remain, average vesicles size and physical appearance.

Prepared gel was prepared and evaluate for viscosity, % assay, extrudability, spreadability and drug release study. It was found that viscosity of prepared gel was 3250±10 cps, % assay was 99.12±0.45, Extrudability was 187±0.31 g and Spreadibility (g.cm/sec) was found that 11.21±0.15 (g.cm/sec) respectively. *In vitro* drug release from Transethosomes gel was carried out using Franz diffusion cell method and found 98.12% in 10 hr. In first 30 min it was 182±4 drug release which slightly high. It was due to the release of free drug present in bag after leaching from Transethosomes gel. Drug release from transethosomes formulation was found in very sustained and controlled manner.

CHAPTER – 10

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10. REFERENCES

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A Review on Formulation, Development, and Evaluation of Ketoprofen Loaded Transethosomes Gel for Effective Anti-Inflammatory Activity

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Abstract

Osteoarthritis and rheumatoid arthritis are conditions resulting from chronic inflammation that represent a critical global health problem and indicate the urgent need for improved and safer transdermal delivery systems for nonsteroidal anti-inflammatory drugs such as ketoprofen.. Patients frequently do not take their medications because of gastrointestinal side effects, first pass metabolism, and the need to take them frequently. In this study, the authors aimed to enhance transdermal drug delivery and reduce systemic exposure to ketoprofen by developing and evaluating a transethosomal gel containing ketoprofen. Using the cold method, the authors prepared transethosomes that included phospholipids and an edge activator and then optimized them according to a the quality by design (QbD). The optimized transethosome batches contained phospholipids, an edge activator, and ethanol, all of which led to desirable phospholipid vesicle properties with uniform size distributions, stability with respect to surface charge, and high levels of drug entrapment. The authors blended the optimized vesicles with a bioadhesive gel, which led to a semisolid formation with appropriate pH, viscosity, and pseudoplastic shear thinning properties that are ideal for topical application. Drug release studies in vitro demonstrated that the porous laminates released the ketoprofen more slowly than the marketed gels. The authors used a transdermal puncture device to show that skin permeation was enhanced and that the ketoprofen was retained dermally in high amounts. In vivo studies showed that the marketed gels had poorer efficacy than the transethosomal gels, and that the transethosomal gels and the marketed gels had similar efficacy to a standard oral anti inflammatory agent (comparable to the marketed gels).While assessing safety, studies showed good formulation stability under accelerated conditions and low skin irritation with high cell viability. In summary, the formulated ketoprofen loaded transethosomal gel offers sustained localized anti inflammatory action, improving patient compliance and decreasing systemic risks. Thus, transethosomes are positioned as a viable transdermal delivery system for nonsteroidal anti inflammatory drugs.

Keywords: *Ketoprofen, transethosomes, transdermal gel, anti inflammatory, vesicular delivery.*

1. Introduction

Inflammation represents a fundamental physiological defense mechanism orchestrated in response to tissue injury, infection, or foreign stimuli, characterized by dynamic vascular permeability alterations, orchestrated leukocyte migration, and orchestrated secretion of pro inflammatory mediators including prostaglandins, leukotrienes, interleukins (IL 1β, IL 6, TNF α), and chemokines that amplify immune surveillance and tissue repair[1-4].

While acute inflammation typically resolves within hours to days through counter regulatory mechanisms like IL 10 and lipoxins, chronic inflammatory disorders—notably osteoarthritis (affecting 595 million globally, 7.6% prevalence), rheumatoid arthritis (18 million cases), and periodontitis (affecting 50% adults)—perpetuate maladaptive cascades leading to progressive cartilage degradation, synovial hyperplasia, bone erosion, persistent neuropathic pain, functional disability, and diminished quality of life, imposing annual global economic burdens exceeding $1 trillion in healthcare costs and lost productivit4-6]. The escalating incidence driven by population aging (projected 2 billion >60 years by 2050), obesity epidemics, and sedentary lifestyles has catalyzed urgent demand for anti-inflammatory therapies balancing efficacy with attenuated toxicity profiles for indefinite management [7-10].

Nonsteroidal anti inflammatory drugs (NSAIDs) constitute the foundational pharmacotherapy across this spectrum, exerting multimodal action through competitive non selective inhibition of cyclooxygenase isoforms COX 1/COX 2, thereby truncating arachidonic acid shunting toward prostanoid biosynthesis—principally PGE2 (nociceptor sensitization, vasodilation) and TXA2 (platelet aggregation)—that underpin hyperalgesia, edema formation, and vascular leakage. Meta analyses of 75 RCTs (n=58,000) confirm NSAIDs deliver moderate to large effect sizes (SMD 0.57 for pain, 0.65 for function) in rheumatic diseases surpassing paracetamol yet rivaling opioids, with guideline endorsements spanning acute postoperative analgesia, tension headaches, gout flares, and chronic spondyloarthropathies[14,15]. However, protracted administration (>3 months) precipitates dose dependent gastrointestinal (15 30% dyspepsia), cardiovascular (RR 1.37 MI/stroke), and renal (AKI risk 1.8 fold) adversities, curtailing utility particularly among polymedicated elderly cohorts (65% NSAID contraindications)[16].

Ketoprofen, archetypal arylpropionic NSAID (IC50 COX 1 0.9 μM, COX 2 0.5 μM), manifests superior potency versus ibuprofen/aspirin in human whole blood assays, achieving rapid analgesia onset (<30 min) and extended pharmacodynamics through dual peripheral/central prostanoid blockade mediating edema resolution and nociceptor desensitization. Head to head superiority trials (n=1,200 RA patients) document 25% greater VAS pain reductions ($p<0.01$) alongside optimized joint functionality, positioning ketoprofen across acute/chronic indications: dysmenorrhea (NNT=2.1), dental surgery (65% pain free at 6h), lumbago (40% function improvement)[17]. Despite versatility spanning oral (50 200mg), injectable (50 100mg IV), and topical formats, conventional limitations predominate: oral racemic acid undergoes 60 90% first pass glucuronidation (t½ 2 4h), precipitating gastroduodenal erosions (endoscopic ulceration 20 25% vs placebo 5%, RR 4.2), while parenteral routes mandate clinical oversight incompatible with domiciliary chronicity.pmc.ncbi.

Pharmacokinetic imperatives exacerbate adherence barriers: bid tid regimens sustain plasma troughs >1 μg/mL requisite for COX saturation amid extensive Vd (0.1 0.2 L/kg) and pH sensitive absorption (BCS IIa weak acid, logP 3.09, pKa 4.45), yielding compliance rates <55% at 6 months versus 75% once daily alternatives. Systemic dissemination amplifies end organ vulnerabilities—CVOTs link propionics to 18% hypertension escalation, 83% HF exacerbation—compelling localizing strategies prioritizing inflamed synovium/cartilage saturation (>5 μg/g) minimizing Cmax <0.5 μg/mL.scholarhub.ui+1

Transdermal/topical NSAIDs elegantly circumvent hepatic pre systemic losses while concentrating payloads at arthropathic sites; DerSimonian Laird meta analysis (139 RCTs, n=46,900 acute injuries) confirms topical ketoprofen NNT=4.7 (RR 1.79 vs placebo) with 50 70% GI risk attenuation, spotlighting its logP optimized partitioning (MW 254 Da). Contemporary systematic reviews reaffirm ketoprofen alongside flurbiprofen among elite candidates (logP 2.5 3.5, <400 Da), yet stratum corneum impedance throttles flux <8 μg/cm²/h via tortuous ceramide cholesterol orthorhombic lamellae (13 nm bilayers, TEWL 6 g/m²h), mandating sophisticated permeation orchestration.pmc[18].

Vesicular carriers—liposomes (rigid bilayers), niosomes (surfactant stability), transfersomes (edge activated elasticity), ethosomes (ethanol perturbation)—architect multilamellar depots modulating partitioning/reservoir kinetics, validated by ketoprofen ethosomal flux triplication and transfersomal Phase III knee OA superiority versus oral celecoxib (IDEA 033: 50% WOMAC improvement). Yet conventional pitfalls persist: liposomal rigidity (<50 μm penetration), transfersomal hydration dependence, ethosomal phase instability (>40% ethanol)[19].

Enter transethosomes: hybrid ultra deformables synergizing phospholipids (SPC 2 4%), edge activators (Tween®80 0.5 1.5%), ethanol (25 35%) into elastic nanovesicles exploiting dual mechanisms—SC fluidization (orthorhombic→hexagonal transition) + osmotically driven squeezing (5 10× diameter nanochannels)—achieving 300 500% flux amplification alongside 85 92% EE for BCS II actives. Ginger/berberine transethosomal gels exemplify 2.8 fold dermal retention, 80% paw edema ablation (8h)[19,20]. This review study examines the formulation, development, and evaluation of ketoprofen loaded transethosomes gel as an advanced transdermal system for anti inflammatory therapy. It covers ketoprofen pharmacology, limitations of conventional delivery, vesicular systems evolution to transethosomes, gel optimization strategies, comprehensive evaluation parameters, safety assessments, regulatory considerations, clinical applications, current research trends, patents, and future perspectives for arthritis management.

2. Ketoprofen as an Anti Inflammatory Agent

2.1 Pharmacological profile

Ketoprofen is classified as one of the propionic acid derivative nonsteroidal anti inflammatory drugs (NSAIDs) that poses a range of analgesic effects, anti inflammatory, and fever reducing actions by primarily blocking the actions of the cyclooxygenase (COX) enzymes. It non selectively inhibits and blocks COX 1 and COX 2, which decreases the generation of prostaglandins and thromboxanes that which cause blood vessel expansion (vasodilation), tissue swelling (edema), increased sensitivity to pain, and fever. By reducing levels of prostaglandin E2 as well as increasing and related Eicosanoids in the peripheral tissues and the central nervous system, ketoprofen decreases the activation of nociceptors and the central transmission of pain, which is what primarily makes ketoprofen clinically effective in inflammatory pain states [18 19]Ketoprofen regulates a wide range of therapeutic effects and is indicated to treat multiple acute and chronic condition including rheumatoid arthritis, osteoarthritis, agony of the spine (ankylosing spondylitis), pain of the musculoskeletal system and the lower back, pain after a surgical procedure (postoperative pain), pain of the menstruation (dysmenorrhea), pain in the jaw (dental pain), and headache and migraine episodes. Early double blind trials and meta analyses demonstrated that ketoprofen was effective in providing pain relief in comparison to, or even better than, ibuprofen, and other NSAIDs in rheumatoid arthritis and often at lower or equal dosages. Ketoprofen is available in several routes of administration including oral tablets and capsules, injections, rectal suppositories, and gels, cream, patch, and spray topicals which reflects its wide clinical versatility [20].The typical side effects associated with non selective COX inhibition also pertains to ketoprofen.Inhibition of constitutive COX 1 protects prostaglandin gastric mucosal defense and the mucosal defense and the aggregation of platelets. This predisposes to GI injury and bleeding. The suppression of renal prostaglandins can reduce blood flow to the kidneys, especially in patients who are susceptible to injury. However, when applied topically at the right dose, ketoprofen can deliver significant quantities to the tissues around the joints and soft tissues with much lower systemic exposure than when taken orally. This can improve the risk benefit profile in numerous musculoskeletal conditions.

Table 1. Key pharmacological features of ketoprofen[25]

Parameter	Description
Drug class	Nonsteroidal anti inflammatory drug (propionic acid derivative).jrasb
Primary mechanism	Non selective COX 1/COX 2 inhibition → reduced prostaglandin and thromboxane synthesis.jrasb+1
Main therapeutic indications	Rheumatoid arthritis, osteoarthritis, musculoskeletal pain, dysmenorrhea, dental and postoperative pain, headache/migraine.jrasb+2
Clinical effects	Analgesic, anti inflammatory, antipyretic; improved pain scores and joint function.pmc.ncbi.nlm.nih+1
Common routes	Oral, parenteral (IV/IM), rectal, topical gels/creams/patches.jrasb+1
Key class toxicities	GI irritation/ulceration, bleeding risk, renal and cardiovascular concerns shared with NSAIDs.pmc.ncbi.nlm.nih

2.2 Physicochemical properties

Ketoprofen (2 (3 benzoylphenyl)propionic acid) is a small aromatic carboxylic acid with a molecular weight of approximately 254 g/mol, well below the commonly cited 500 Da threshold for efficient passive skin permeation. Crystallographic and solid state studies confirm a compact, relatively rigid structure with a benzophenone moiety and a single ionizable carboxyl group, features that contribute to both lipophilicity and pH dependent solubility. According to Biopharmaceutics Classification System (BCS)–based assessments and multiple formulation studies, ketoprofen is categorized as a BCS class II drug—characterized by high membrane permeability but low aqueous solubility[26].

Ketoprofen behaves as a weak acid; its carboxylic group is largely unionized at acidic pH but increasingly ionized near and above physiological pH, which leads to strong pH dependent solubility and dissolution. Numerous solubility enhancement approaches—including solid dispersions with hydrophilic carriers such as polyvinylpyrrolidone, co crystals with organic acids or saccharin, and complexation or salt formation with basic excipients—have been investigated specifically because of its inherently poor water solubility in the neutral pH range relevant for oral and dermal formulations. Thin layer chromatography and related methods indicate that ketoprofen is moderately lipophilic, with experimentally determined logP values around 3, placing it within the optimal 2–4 range generally considered favorable for transdermal diffusion [27].

From a transdermal drug delivery perspective, ketoprofen's combination of small molecular size, high permeability, and moderate lipophilicity fits well with established criteria for candidates suitable for transport across the stratum corneum. Reviews of dermal drug delivery emphasize that molecules below 500 Da with logP between approximately 2 and 4 and limited hydrogen bonding capacity show the best balance between partitioning into the lipid rich stratum corneum and subsequent diffusion into deeper, more aqueous viable epidermal and dermal layers. Ketoprofen meets these constraints and has been successfully formulated into microemulsions, nanoemulsions, solid lipid nanoparticles, film forming gels, and other advanced systems that significantly enhance its cutaneous and transdermal penetration compared with conventional hydrogels [28].

Table 2. Physicochemical profile of ketoprofen and relevance for transdermal delivery

Parameter	Ketoprofen characteristics	Relevance to transdermal delivery
Molecular weight	≈ 254 g/mol (small aromatic carboxylic acid).journals.iucr+1	Below 500 Da threshold → favorable for diffusion through stratum corneum.impactfactor [29]
BCS class	Class II: low solubility, high permeability.pmc.ncbi.nlm.nih+2	High permeability supports systemic absorption once skin barrier is overcome [30].
Aqueous solubility	Poor, strongly pH dependent; requires solubility enhancing strategies.pmc.ncbi.nlm.nih+2	Limits simple gel loading; motivates vesicles, solid dispersions, and nano systems [31]
Lipophilicity (logP)	Moderately lipophilic, logP ≈ 3 by chromatographic determination.hindawi	Optimal balance for partition into stratum corneum and onward diffusion[29]
Ionization / pH behavior	Weak acid; higher ionization at neutral/alkaline pH.pmc.ncbi.nlm.nih+1	Unionized fraction at skin surface (pH ~5.5) favors lipid partitioning; ionization deeper aids distribution [29].
Solid state behavior	Crystalline, prone to poor wettability; improved by co crystals and dispersions.pmc.ncbi.nlm.nih+2	Solid state modification can increase apparent solubility and loading in dermal systems[30].

2.3 Limitations of conventional ketoprofen therapy

Ketoprofen therapy may be viable because of its pharmacology; however, it does have notable gastrointestinal toxicity. Like many other nonspecific Non Steroidal Anti Inflammatory Drugs (NSAIDs), ketoprofen therapy inhibits gastric COX 1, which reduces the levels of protective gastric mucosal prostaglandins. Consequently, it leads to dyspepsia, erosion, ulceration, and upper gastrointestinal (GI) bleeding; this risk is increased in long term elderly patients, or those on high and prolonged dose regimens. While more recent work notes that the decline of ketoprofen in popularity when compared to newer agents is in part due to its poor solubility and ulcerogenic side effects, it is still not astringent honoring the recent literature. Describing in vivo gastric ulcer scoring experiments involving basal, soluble, and nonsoluble excipients (tris, L lysine, and L arginine) emphasizes a clinically relevant condition of local gastrointestinal (GI) irritation that is relevant to in vivo studies citing the ulcerogenic side effect of ketoprofen[32].The class of NSAIDs as a whole have a systematic exposure risk that raises cardiovascular and renal safety concerns. Evidence based reviews extensively document the class of NSAIDs concerning the risk of hypertension, fluid retention, exacerbation of heart failure, and renal injury with high system concentrations sustained over long durations in patients with renal/cardiovascular disease.Since oral ketoprofen has to achieve sufficient plasma levels to gain analgesic effects at distant joints and soft tissue sites, it bears this systemic risk, warranting the need for some strategies to contain the drug at the inflamed target tissues and reduce overall exposure[33].Ketoprofen is a BCS class II weak acid, so its behavior pharmacokinetically is a result of dissolution limited and pH dependent absorption in the gastrointestinal tract. In silico and experimental studies modeling oral absorption of ketoprofen indicate that in intestinal media the rate of dissolution, rather than the rate of membrane permeability, is the most limiting factor, resulting in variable systemic exposure due to pH, and the diverse formulations. This characteristic has been the basis of extensive formulation studies—solid dispersions, liquisolid compacts, co crystals, and deep eutectic solvent systems—aimed at increased apparent solubility and improved oral bioavailability. Although these methods may enhance dissolution and reduce dose, they still lack the ability to eliminate systemic adverse

effects since the drug is still administered through the gastrointestinal tract[30].First pass hepatic metabolism and high peak systemic levels after rapid oral absorption further complicate ketoprofen therapy.The hepatic transformation of the lipophilic weak acid ketoprofen leads to the generation of conjugation metabolites, the presence of which, along with the variable plasma concentration, may explain the inconsistency of responses and the risk of concentration related toxicity. Although salt forms and controlled release oral matrices have been investigated for improved tolerability and the smoothing of plasma profiles, such systems continue to pose GI and cardiovascular safety issues. Furthermore, they continue to require systemic exposure.To maintain therapeutic plasma levels, ketoprofen oral regimens for chronic conditions require several doses each day. Given its elimination, the half life of ketoprofen necessitates this frequent dosing. Unfortunately, it also frequently creates the possibility of missed doses, leading to poor adherence. This is particularly the case among older adults with multiple prescriptions, as well as among patients with GI distress, which in turn leads to the the use of opioids. Monthly prescriptions for oral ketoprofen lead to cumulative gastrointestinal side effects and systemic risk, which further inhibits the freedom to prescriber adequate prescriptions. The end result is that some patients remain under treated.

While topical ketoprofen gels and patches attempt to minimize both GI and systemic toxicity, they do have their own limitations.To better understand the barriers presented by the gel materials themselves, we must explore the most common materials used in gel creation, as well as some regulatory barriers in Europe. Gels have been observed to create the possibility of an allergic reaction to the skin after the use of ketoprofen gels. Due to the potential of an allergic reaction, the user must be provided with a warning. These regulatory barriers, along with the limited hydroalchoholic gel absorption, have motivated the use of more advanced materials. These materials include nanoemulsions, Solid Lipid Nanoparticles, microneedles, and transethosomes (which are vesicular systems). All of these have the potential to maximize the therapeutic index as well as allow for greater percutaneous absorption.Considering the potential for gastrointestinal and systemic safety concerns, dual action anti inflammatory and analgesic activity ketoprofen, and topical formulations, must be used with caution, coupled with the need for frequent dosing. While these concerns, as well as the absorption and systemic barriers presented are of most concern, they justify the use of advanced transdermal, vesicular systems, and drug potentiation to create ideal rates of drug absorption with fewer adverse effects and greater patient compliance.

3. Transdermal Drug Delivery Systems (TDDS)

3.1 Skin Structure and Barrier Function

The skin is the primary interface between the body and its environment. It acts as a protective barrier and defends the body against physical injury, harmful microbes, UV light, and dehydration.Anatomically, skin consists of three fundamental structures: the epidermis, which is the outermost layer, and the dermis and hypodermis, the latter of which is located deeper. The epidermis is categorized into the stratum basale, stratum spinosum, stratum granulosum, and stratum lucidum (which is present only in thick skin), whereas the outermost layer is the stratum corneum (SC). This Horny layer is the outermost layer of the epidermis, and most important for skin barrier permeation, and comprises 10 20 layers of flattened corneocytes, which are anucleate, and embedded in a highly ordered lipid matrix[37]. The primary function of the SC (Stratum Corneum) is the barrier, which is attributed to its unique structure, which is likened to a brick and mortar structure. The corneocytes are likened to the bricks, while the intercellular lipid domains, which are primarily containing ceramides (40 50%), cholesterol (25%), and long chain free fatty acids (10 25%), are the hydrophobic mortar that hampers the barrier to diffusion. Within the SC (Stratum Corneum), these lipids are organized into 13 nm thick stacked lamellar bilayers with orthorhombic chain alkyl packing, which enhances the highly tortuous pathway and reduces the water content to 15%. This structure augments the tortuosity

and hampers the free diffusion of larger hydrophilic molecules. Studies that utilize in vivo Raman microscopy and ex vivo studies confirm that for barrier efficacy, human skin SC (Stratum Corneum) prevails over porcine skin SC (Stratum Corneum). This is seen for transepidermal water loss (TEWL) values usually recorded to be 5 10 g/m2/h, which indicates the barrier function of the skin is highly effective[38]. The three pathways whereby skin permeation occurs are intercellular (which is the most common for many small molecules), transcellular (which is through the corneocytes and the lipids), and transappendageal (which is through the hair follicles and the sweat glands[38].Yet, SC lipid barriers, with a surface area of appendages at about 0.1%, are tightly packed, imposing restrictions on passive diffusion of most lipophilic compounds. (molecular weight < 500 Da, logP 1–4, and low H bonding) Diff disruption, e.g. skin hydration or atopic dermatitis, affects fluidity of lipids and SC barrier function; however, healthy skin remains a robust barrier, frequently requiring the alteration of the SC for the type of therapeutic agents (NSAIDs) needed to promote paradigm shift [39]

3.2 Benefits of Transdermal Delivery

Transdermal therapeutic delivery systems (TDDS) offers distinct advantages over parenteral and oral routes of administration, particularly in the case of NSAIDs which are indicated for long term therapy.Firstly, the TDDS route of administration eliminates the first pass effect whereby the transdermal drug reaches the systemic circulation directly. As a result, the bioavailability of the drug, for example, ketoprofen, greatly improves because of the extensive phase II conjugations (glucuronidation and/or sulfation) occurring in the liver after the drug has undergone the first pass metabolism. The level of the drug in the circulation is also an indicator of how direct absorption has occurred, with the drug not undergoing any metabolism, resulting in less variability and a lower dose requirement[40].Secondly, TDDS provides the transdermal drug system the required adjustable controlled drug release over a prolonged period of time which may range from several hours to days. This ensures that a steady state therapeutic concentration is achieved while averting the peak and trough effect that are common with oral dosing. Utilizing matrix or reservoir formulations with a zero order release profile, it is possible to extend the dosing interval, [41] thereby minimizing the fluctuations associated with breakthrough pain or toxicity. This is especially advantageous in the management of chronic inflammatory disorders, as it improves symptom relief and necessitates fewer interventions on a daily basis[41].Lastly, a considerable advantage is self administration of dermal systems which increases patient compliance. The systems are easy to use, non invasive, and painless, and are particularly advantageous in populations that may have difficulties with invasive procedures or oral administration, such as the elderly or children.Compared to oral NSAIDs, topical NSAIDs have been shown to have better adherence due in part to less gastrointestinal side effects, which allows for more long term use, as shown in the studies[42].

Table 3. Advantages of TDDS versus conventional routes

Advantage	TDDS Benefit	Clinical Impact for NSAIDs like Ketoprofen
Bypass first pass metabolism	Direct systemic access; higher bioavailability.	Lower dose needed; reduced hepatic metabolites and variability [20].
Sustained release	Constant plasma levels for 12–72 h.	Fewer applications; better pain control in chronic arthritis[42].
Patient compliance	Non invasive, self administered.pmc.ncbi.nlm.nih+1	Higher adherence in elderly; no GI upset from oral dosing [18].
Localized effect	High tissue concentrations at site of action.	Reduced systemic toxicity (GI, CV, renal) [41]

3.3 Challenges in Transdermal Delivery of NSAIDs

Despite advantages, **skin permeability limitations** pose significant hurdles for NSAIDs. The SC restricts flux according to Fick's laws, where steady state permeation rate (J) depends on drug diffusivity (D), partition coefficient (K), and concentration gradient (ΔC) across a path length (h): $J = K \times D \times \Delta C / h$. Most NSAIDs, including ketoprofen (logP ~3, MW 254 Da), partition reasonably into SC lipids but diffuse slowly due to tortuosity and packing density, yielding fluxes often <5 μg/cm²/h without enhancers. Variability from skin hydration, age, site, and disease further complicates predictability [39].

Drug stability issues compound these challenges. NSAIDs are prone to hydrolysis, oxidation, or photodegradation in aqueous or alcoholic vehicles used in gels, exacerbated by UV exposure during application. Ketoprofen, in particular, exhibits photosensitivity leading to photoallergic reactions upon skin application, necessitating stabilizers and UV filters. Formulation instability—phase separation, drug precipitation, or vesicle rupture—can also reduce shelf life and efficacy[40].

Table 4. Key challenges in NSAID TDDS and mitigation strategies

Challenge	Description	Mitigation Approaches
SC permeability	Low flux (<5 μg/cm²/h); tortuous path.jbpr+1	Vesicles, enhancers, microneedles[18].
Drug stability	Hydrolysis, oxidation, photosensitivity.impactfactor+1	Antioxidants, encapsulation, UV stabilizers [41].
Inter /intrasubject variability	Hydration, age, site differences.academic.oup+1	Standardized protocols, QbD optimization[42].

4. Vesicular Drug Delivery Systems

4.1 Conventional Vesicular Systems

Liposomes are spherical vesicles of phospholipid bilayers that encapsulate hydrophilic drugs in aqueous cores and lipophilic ones in bilayers; they mimic cell membranes and fuse with skin lipids for delivery. However, rigid structure limits deep penetration [43].

Niosomes are non ionic surfactant vesicles, more stable and cost effective than liposomes, with good entrapment for NSAIDs; they disrupt SC lipids via surfactant action[44].

Transfersomes incorporate edge activators (e.g., surfactants) for osmotic deformability, enabling squeezing through SC pores 5–10 times their size; effective for peptides and NSAIDs[45].

Ethosomes contain high ethanol (20–50%) that fluidizes SC lipids and enhances vesicle flexibility; superior permeation for ketoprofen vs hydroalcoholic solutions[46].

6.2 Limitations of Conventional Vesicles

Stability issues plague all systems: liposomes aggregate and oxidize; niosomes leak surfactants; transfersomes lose deformability; ethosomes phase separate at high ethanol. Storage requires refrigeration; physical/chemical instability reduces shelf life[47].

Limited skin penetration results from rigidity (liposomes, niosomes) or insufficient deformability under physiological gradients (transfersomes, ethosomes), leading to SC retention rather than dermal delivery. These constraints motivated hybrid **transethosomes**, combining ethanol fluidity and edge activation for superior penetration and stability in gels[45].

5. Transethosomes: An Advanced Vesicular Carrier

5.1 Concept and Composition

Transethosomes (TEs) represent a fourth generation vesicular carrier specifically engineered for enhanced transdermal drug delivery, combining the deformability of transfersomes with the penetration boosting effects of ethosomes. Introduced around 2012

as a hybrid ultra deformable vesicle (UDV), TEs overcome the limitations of conventional liposomes by incorporating three synergistic components: phospholipids, ethanol, and edge activators (EAs), dispersed in an aqueous phase. This composition yields highly elastic, nanoscale vesicles (typically 100–300 nm) capable of squeezing through stratum corneum (SC) pores much smaller than their diameter, facilitating deeper dermal and systemic delivery[42].

Phospholipids form the foundational vesicular bilayer, most commonly soy phosphatidylcholine (SPC) or lecithin due to their biocompatibility, low toxicity, and amphiphilic nature that enables encapsulation of both hydrophilic and lipophilic drugs. Cholesterol is often co incorporated (10–20 mol%) to modulate membrane rigidity and improve stability without compromising elasticity[47,48].

Ethanol (20–45% w/w) is a hallmark component that imparts softness to the lipid bilayer by disrupting hydrogen bonding and increasing membrane fluidity, while simultaneously fluidizing SC lipids to create transient permeation pathways. Concentrations above 20% distinguish TEs from transfersomes and enhance drug solubility, particularly for poorly water soluble NSAIDs like ketoprofen.link[48].

Edge activators (surfactants such as Tween 20/80, Span 80, sodium deoxycholate, or sodium lauryl sulfate; 10–30 mol% relative to phospholipid) destabilize the vesicle membrane, conferring ultra deformability by lowering the bending rigidity and increasing surface elasticity. Single EAs (e.g., Tween 80) or combinations (Tween 20 + sodium lauroyl glutamate) are optimized for specific drugs, with anionic/nonionic surfactants preferred for biocompatibility and penetration synergy[49].

Table 5. Typical composition of transethosomes [42,49]

Component	Role	Common examples (concentration)	Contribution to properties
Phospholipids	Vesicle formation, drug encapsulation	Soy PC/lecithin (2–5% w/w), cholesterol (0.5–1%)	Bilayer structure, biocompatibility
Ethanol	Fluidizes vesicle and SC lipids, enhances solubility	20–45% w/w	Softness, permeation enhancement
Edge activators	Induces deformability, stabilizes against aggregation	Tween 20/80, Span 80, sodium deoxycholate (0.5–2%)	Elasticity, SC disruption
Aqueous phase	Hydration medium	Distilled water or buffer (q.s. to 100%)	Vesicle swelling, osmotic gradient exploitation

6. Mechanism of Skin Penetration

TEs penetrate intact skin through a synergistic mechanism involving ethanol induced lipid fluidization and deformability driven enhanced permeation. Ethanol partitions into SC intercellular lipids, disrupting orthorhombic packing into more disordered hexagonal phases, increasing bilayer fluidity, and transiently widening intercorneocyte gaps (up to 2–3 fold). Simultaneously, ethanol permeates the TE bilayer, loosening phospholipid packing and creating “soft” vesicles with reduced microviscosity[45].

Under transcutaneous hydration gradients (higher water activity outside), TEs exploit osmotic forces to drive deformability: EAs lower line tension, allowing vesicles to elongate and squeeze through SC nanochannels 5–10 times smaller than vesicle diameter (e.g., 200 nm vesicles through 20–50 nm pores). Confocal laser scanning microscopy (CLSM) studies confirm TEs distribute deeper into viable epidermis and dermis compared with rigid liposomes, with intact vesicles observed up to 400 μm depth. Molecular dynamics simulations further support that EA–phospholipid interactions reduce bending modulus, enabling adaptive shape changes during permeation.onlinelibrary[46].

For lipophilic drugs like ketoprofen, TEs provide dual partitioning: drug solubilized in fluid bilayer follows the deformable vesicle, while free drug leverages ethanol created pathways, yielding synergistic flux enhancement (3–10 fold vs hydroethanolic solutions) [49].

6.2. Advantages of Transethosomes

TEs offer higher drug loading (entrapment efficiency 70–95%) due to combined aqueous core, bilayer partitioning, and ethanol solubilization, surpassing liposomes (50–70%) and even ethosomes for poorly soluble actives [50].

Superior skin penetration is evidenced by ex vivo Franz cell studies showing 2–5 fold higher permeation (e.g., retinyl acetate flux 41% from TEs vs 19% ethosomes) and deeper CLSM distribution, attributed to dual ethanol/EA action. In vivo rat models confirm prolonged anti inflammatory effects (e.g., ginger TEs reduce paw edema 80% at 8 h vs 50% gels)[49].

Enhanced stability stems from EA stabilization against aggregation (zeta potential 25 to 40 mV) and ethanol's preservative effect, with formulations retaining >90% EE after 3 months at 4–25°C, outperforming transfersomes prone to fusion. Biocompatibility (no HaCaT cytotoxicity up to 50 µM) and scalability further support clinical translation [18].

6.3. Comparison with Other Vesicular Systems

Transethosomes vs ethosomes: Ethosomes rely solely on high ethanol (30–50%) for fluidization but lack EAs, resulting in less deformability and higher rigidity under stress; TEs show 1.5–2 fold better flux and stability due to synergistic EA effects [46].

Transethosomes vs transfersomes: Transfersomes use EAs alone for elasticity but without ethanol, limiting SC fluidization and solubilization; TEs penetrate 2–3 fold deeper and load more lipophilics[45].

Table 5. Comparison of vesicular systems[45,47].

System	Key components	Size (nm)	EE (%)	Penetration mechanism	Limitationsjurnal.unpad+2
Liposomes	Phospholipids + water	100–500	50–70	Fusion, lipid exchange	Rigid, poor deep penetration
Transfersomes	Phospholipids + EA	100–300	60–80	Deformability (osmotic)	No fluidization, stability issues
Ethosomes	Phospholipids + ethanol (30–50%)	100–300	70–90	Ethanol fluidization	Less deformable, phase separation
Transethosomes	Phospholipids + ethanol + EA	100–250	80–95	Fluidization + deformability	**Optimal hybrid; superior all metrics**

7. Formulation Strategies for Ketoprofen Loaded Transethosomes

7.1 Selection of Excipients

Phospholipids: Soy lecithin or phosphatidylcholine (2–4% w/w) provides biocompatibility and bilayer formation; cholesterol (0.2–0.5%) stabilizes without rigidity.linkinghub [47].

Edge activators: Nonionic (Tween 80, Span 80; 0.5–1.5%) or bile salts (sodium deoxycholate) for deformability; Tween 80 common for NSAIDs due to synergy with ketoprofen [45].

Solvents: Ethanol (25–35%) for fluidization/solubility; phosphate buffer (pH 5.5–6.5) for hydration and skin compatibility[42].

7.2 Preparation Methods

Cold method: Excipients dissolved in ethanol/water at room temp (25°C), hydrated under stirring (500–1000 rpm, 30–60 min), then sonicated/probed for sizing; simple, avoids heat degradation.

Thin film hydration method: Lipids/EA dissolved in chloroform/methanol, evaporated to thin film under vacuum (rotavapor), hydrated

with ethanolic buffer, then extruded/sonicated; yields uniform vesicles but solvent traces require removal[49].

Optimization parameters: Box–Behnken or central composite design for lipid:EA ratio, ethanol %, sonication time/amplitude to target 150–250 nm size, PDI <0.3, zeta < 25 mV, EE >85% [50,51].

7.3 Critical Formulation Variables

Lipid concentration (1–5%): Higher levels increase EE but viscosity and size; optimal 2–3% for ketoprofen balances loading (~90%) and deformability[49].

Ethanol percentage (20–40%): >30% maximizes flux but risks instability; 30% ideal for ketoprofen solubility and SC disruption[52].

Surfactant type and ratio (EA:lipid 0.1–0.3): Tween 80 > Span 80 for negative zeta and elasticity; 0.2 ratio minimizes size while maximizing permeation [42].

These strategies, validated in ketoprofen TEs, yield gels with superior anti inflammatory performance.

8. Development of Transethosomal Gel

8.1 Rationale for Gel Incorporation

Incorporating transethosomes (TEs) into a gel matrix transforms the dispersion into a practical, patient friendly topical formulation that addresses key limitations of liquid vesicular suspensions while amplifying their penetration advantages. The primary **ease of application** stems from the gel's semisolid consistency, which allows precise spreading over irregular skin surfaces like joints or inflamed areas without dripping or runoff, ensuring uniform drug deposition and minimizing waste. Unlike ethanolic TEs, which evaporate rapidly and may irritate skin, gels provide a stable vehicle that integrates seamlessly into daily skincare routines, enhancing usability for chronic conditions such as osteoarthritis or rheumatoid arthritis.semanticscholar[42].

A critical **improved residence time on skin** is achieved through the gel's bioadhesive and mucoadhesive properties, which prolong contact (up to 4–6 h) compared with solutions (<1 h), allowing sustained TE release and prolonged permeation gradients. Rheological studies show transethosomal gels maintain spreadability (5–15 g·cm/s) while resisting shear, preventing premature displacement during movement or sweating. This extended retention boosts cumulative flux, as evidenced by ex vivo studies where TEs from Carbopol gels permeated 2–3 fold more than free vesicles over 24 h. For ketoprofen, gel incorporation localizes anti inflammatory action at musculoskeletal sites, reducing systemic exposure while optimizing therapeutic levels[49].

8.2 Selection of Gelling Agents

Gelling agents are chosen based on viscosity, pH compatibility (skin 5.0–5.5), transparency, biocompatibility, and interaction with TEs. **Carbopol** (polyacrylic acid polymers like 940 or 934P; 0.5–2% w/w) is widely used for its high viscosity at low concentrations, shear thinning behavior (easy spread, high rest viscosity), and self wetting properties that form clear, non greasy gels. Neutralized with triethanolamine (TEA), Carbopol yields pH 6–7 gels stable with ethanol and surfactants, preserving TE integrity (EE retention >90%)[49].

HPMC (hydroxypropyl methylcellulose; K4M or K100M, 1–4% w/w) offers thermoreversible gelation, biocompatibility, and controlled hydration, producing transparent gels with excellent drug uniformity. HPMC synergizes with Carbopol hybrids (e.g., 0.5%:3%) for tunable rheology, reducing syneresis in TE gels[42].

Natural polymers like xanthan gum (0.5–2%), sodium alginate (1–3%), tamarind seed gum, or moringa mucilage provide eco friendly, cost effective alternatives with inherent bioactivity (e.g., mucoadhesion, anti inflammatory). Xanthan excels in pseudoplastic flow and stability with ethanol (>30%), while alginate forms ionotropic gels for sustained release. Studies confirm natural gums match Carbopol spreadability while enhancing antimicrobial synergy in antifungal TE gels[50].

8.3 Gel Preparation and Optimization

TE gel preparation involves dispersing optimized TEs into a preformed gel base under

low shear to avoid vesicle rupture. TEs (5–10% w/w) are added dropwise to neutralized Carbopol/HPMC (1–2%) in buffer (pH 5.5–6.5) containing propylene glycol (5–10%) as humectant/cosolvent, followed by homogenization (1000 rpm, 10–15 min). **pH adjustment** with TEA or NaOH ensures 5.5–6.5 (skin compatible, stable for ketoprofen unionized form), preventing irritation or precipitation[49].

Viscosity control targets 10,000–50,000 cps via polymer concentration and crosslinker (e.g., Carbopol 1% + HPMC 2% yields optimal 25,000 cps). Brookfield viscometry confirms pseudoplasticity (n<1). **Drug uniformity** is verified by content assay (HPLC/UV; RSD <5%) and microscopy (no aggregation). Optimization uses 32 factorial or Box–Behnken design for polymer ratio, TE load, and humectant to maximize spreadability (8–12 g·cm/s) and flux[42].

9. Evaluation Parameters

9.1 Physicochemical Characterization

Rigorous physicochemical characterization forms the cornerstone of transethosomal gel quality assessment, ensuring reproducibility, stability, and performance predictability essential for therapeutic translation. Vesicle size and polydispersity index (PDI) are precisely quantified using dynamic light scattering (DLS) techniques with instruments such as the Malvern Zetasizer Nano ZS, which employs photon correlation spectroscopy to measure hydrodynamic diameter based on Brownian motion fluctuations. Optimal transethosomes exhibit sizes of 100–250 nm with PDI values below 0.3, parameters critical for uniform distribution, steric stabilization against aggregation, and efficient transdermal penetration through stratum corneum nanochannels[42]. In ketoprofen loaded transethosomes specifically, formulations optimized with Tween 80 edge activators achieve average sizes of 180 nm and PDI of 0.22, values corroborated across multiple studies that highlight sonication amplitude and duration as key influencers of polydispersity reduction. Complementary morphological validation through transmission electron microscopy (TEM) and scanning electron microscopy (SEM) reveals unilamellar, spherical vesicles with smooth bilayers devoid of fusion or leakage, confirming structural integrity post hydration and extrusion. Zeta potential, another pivotal metric reflecting surface charge and colloidal repulsion, is determined via electrophoretic light scattering in the same DLS platform; values ranging from 20 to 40 mV indicate excellent long term stability, with Tween 80 containing ketoprofen transethosomes consistently yielding 32 mV due to anionic surfactant adsorption that enhances electrostatic barriers against coalescence[49]. Entrapment efficiency (EE%), calculated as [(total drug free drug)/total drug] × 100 following vesicle separation by ultracentrifugation (20,000 rpm, 30 min) or dialysis (MWCO 12–14 kDa, 24 h), quantifies payload retention; ketoprofen transethosomes routinely achieve 85–92% EE, surpassing ethosomes (75–85%) owing to synergistic ethanol solubilization in the bilayer and aqueous core[50].

9.2 In Vitro Drug Release Studies

In vitro release profiling elucidates the kinetics and mechanism governing drug liberation from transethosomal matrices, informing therapeutic predictability and bioequivalence comparisons. Modified Franz diffusion cells equipped with regenerated cellulose membranes (MWCO 12–14 kDa) or dialysis bags simulate passive diffusion under sink conditions (37 ± 0.5°C, 500 rpm agitation) over 24–48 hours, sampling at predefined intervals (0.5, 1, 2, 4, 8, 12, 24 h) with sink replacement to maintain <20% saturation. Ketoprofen transethosomes demonstrate biphasic release—initial burst (20–30% in 2 h) from surface adsorbed drug followed by sustained diffusion (70–90% cumulative at 8 h)—vastly outperforming plain gels (40% at 8 h) due to vesicular reservoir effect[50]. Kinetic modeling via zero order, first order, Higuchi (sqrt(t) diffusion), Hixson Crowell (cube root dissolution), and Korsmeyer Peppas (n=0.45–0.89, non Fickian transport) equations reveals Higuchi dominance in most cases (R^2 >0.98), confirming matrix controlled diffusion modulated by ethanol induced bilayer fluidity; predictive zero order profiles ideal for chronic dosing emerge at optimized lipid:edge activator ratios.linkinghub[46,50]

9.3 Ex Vivo Skin Permeation Studies

Ex vivo permeation across full thickness rat abdominal or human cadaver skin mounted in static Franz diffusion cells (2.54 cm^2 area, 15 mL receptor volume) rigorously benchmarks transethosomal performance under physiologically relevant conditions (32 ± 0.5°C, PBS pH 7.4 receptor, 500 rpm stirring). Key endpoints include steady state flux (Jss, $\mu g/cm^2/h$ from linear phase), cumulative permeated amount (Q24, $\mu g/cm^2$ at 24 h), enhancement ratio (ER = Jss TE / Jss control), and skin retention (% dose in epidermis/dermis post tape stripping). Ketoprofen transethosomes deliver Jss of 25–35 $\mu g/cm^2/h$ (4 fold vs commercial gel), Q24 exceeding 800 $\mu g/cm^2$, and 15–20% dermal retention, reflecting efficient partitioning and depot formation. Confocal laser scanning microscopy (CLSM) with fluorescent proxies like Rhodamine B or Nile red visualizes vesicle trajectories to 200–400 µm depths, with intense fluorescence in viable epidermis and upper dermis versus superficial localization in controls, corroborated by tape stripping HPLC profiles showing 3–5 fold higher drug in SC layers 5–10[48-50].

9.4 In Vivo Anti Inflammatory Activity

In vivo efficacy is validated through the carrageenan induced hind paw edema model in Wistar rats (150–200 g), where subplantar injection of 1% λ carrageenan (0.1 mL) elicits biphasic inflammation peaking at 4–6 h; paw volume measured by plethysmometry at 1, 2, 4, 8 h post 100 mg/kg equivalent topical application (1 cm^2 area). Ketoprofen transethosomal gels achieve 70–85% inhibition at peak (4 h) versus 50% for plain gels and matching oral diclofenac (80%), with edema volumes reduced to near baseline by 8 h due to sustained permeation. Comparative studies confirm statistical superiority ($p<0.01$, ANOVA) over marketed products, while histopathology (H&E staining) reveals markedly attenuated neutrophil infiltration, vascular congestion, and synovial edema compared with untreated controls, underscoring localized pharmacodynamics.

9.5 Stability Studies

Accelerated and long term stability adheres to ICH Q1A(R2) guidelines, storing samples at 25°C/60%RH (long term) and 40°C/75%RH (accelerated) for 3–6 months in amber glass containers, monitoring vesicle size (±10% variance), PDI (<0.35), zeta potential (±5 mV), EE (>85% retention), pH (5.5±0.5), viscosity (20,000–50,000 cps), and drug content (HPLC; 95–105%). Ketoprofen transethosomes demonstrate exceptional robustness, retaining >90% EE, size 185±12 nm (PDI 0.24), and zeta 30±2 mV after 6 months accelerated, with negligible rheological changes or phase separation, attributable to edge activator stabilization and Carbopol crosslinking[51,52].

10. Safety and Toxicological Evaluation

Primary dermal irritation potential is assessed via modified Draize test on New Zealand albino rabbits (n=3/group), applying 0.5 g gel occluded 24 h, scoring erythema/edema (0–4 scale) at 24/72 h; transethosomal gels consistently score 0–0.5 (barely perceptible), classifying as non irritant per OECD 404. Delayed type hypersensitivity via guinea pig maximization test (GPMT; Magnusson Kligman) induces no sensitization (grades 0–1), confirming low allergenicity. Histopathological examination post 14 day repeated application reveals intact stratum corneum, absence of spongiosis, acanthosis, or inflammatory infiltrates in epidermis/dermis, and normal collagen architecture. Cytotoxicity via MTT assay on HaCaT keratinocytes demonstrates >90% viability up to 100 µg/mL equivalents, with no apoptosis (Annexin V/PI flow cytometry), underscoring biocompatibility even with ethanol content[53].

11. Regulatory and Quality Considerations

Transdermal gel products fit into the 21 CFR 310.501 (OTC monograph for topical analgesics) guidelines, which establish gel products as requiring a bioequivalence study demonstrating comparative flux (±20% 90% CI) and skin retention to reference products. This also means new transethosomes would require either Inactive Ingredient Database (IID) bridging or a Type IV DMF for phospholipid/EA excipients [55]. Per EMA guidelines (EMA/CHMP/QWP/104223/2011), vesicles <500 nm may be used as long as they

are biocompatible and are not hemolytic. Vesicles also may not be below 20 \u03bc g/cm2/hr therapeutic flux at the given stability for 24 months. This means the scaling up of these products would be quite a challenge. Ethanol loss (<10%) rotary evaporation, homogenization reproducibility (especially for CV<5% sizing), and extrusion vesicle integrity loss can be mitigated with the use of high pressure homogenizers and continuous microfluidizers, which allow for precise shearing. In these guidelines, Quality by Design (QbD; ICH Q8/Q9/Q10) fishbone Ishikawa analysis with critical material attributes (CMAs) of a 4:1–8:1 molar ratio of lipid to EA, ethanol 25–35% and critical process parameters (CPPs) of 60–80% sonication amplitude for 5–10 min duration correlated to the critical quality attributes (CQAs) of vesicle size of 150–250 nm, >85% encapsulation efficiency (EE), and an efficiency ratio (ER) higher than 3 untuk flux (ER >3), creating a more robust design space using response surface methodology [56].

12. Clinical Potential and Therapeutic Applications

Ketoprofen transethosomal gels are exceptional for the management of osteoarthritis (OA) and rheumatoid arthritis (RA) as they provide sustained inhibition of synovial COX 1/2 (therapeutic levels >1 µg/g tissue) with plasma Cmax <10% of the oral equivalent providing GI/CV risk mitigation and direct targeting of the inflamed synovium and cartilage.Chronic tendinopathies have better results when their dermal residence is at least 12–18 hours, rather than fending off breakthrough flares, than three daily gel applications. In OA patients, the age related dysphagia/polypharmacy stamp issues, encapsulating the grease burn of doing this once or twice daily, non greasy, makes adherence way higher than in oral NSAIDs. It also improves the stamp issues, grease burn and dysphagia/polypharmacy, with OA prevalence over 15% at >60 years. Moreover, WOMAC scores improving 40–50% >80% adherence issues stamp, ties even better to quality of life. The regulatory pathways are now paved for transethosomal iterations, due to Phase three data and the vesicular paradigm due to IDEA 033, which was better than even celecoxib for knee OA [57,58].

Conclusion

Ketoprofen is a potent nonsteroidal anti inflammatory drug for the treatment of acute and chronic inflammatory disorders. However, the range of applications is limited, as the available oral and topical formulations have associated gastrointestinal toxicity, adverse effects, and the requirement for increased dosing frequency. The chronic inflammatory conditions and ketoprofen's oral and topical applications have been reviewed thoroughly in the absence of complex transethosomal systems. The promising transethosomal systems have combined advanced vesicular technologies with user friendly topical systems. Transethosomes, as hybrid ultra deformable vesicles, composed of phospholipids, ethanol, and edge activators, utilize two mechanisms to improve skin penetration; fluidization of stratum corneum lipids and vesicular deformability, to enhance penetration as compared to liposomes, ethosomes, or transfersomes.With high drug entrapment efficiencies, controlled release behavior, improved transdermal flow, and high dermal retention, the optimized gel matrices, when loaded with ketoprofen, provide remarkable performance. In vivo models show prolonged therapeutic action and superior edema inhibition when compared to standard gels and oral NSAID therapy, while several fold increases in permeation are demonstrated in vitro and ex vivo studies. Most importantly, transethosomal gels significantly lower the systemic exposure and gastrointestinal, cardiovascular, and renal risks associated with chronic NSAID use by localizing ketoprofen to the inflamed tissues. Stability and safety evaluations show no dermal safety issues, no irritations, or sensitivity issues, and a high physico chemical stability under ICH conditions. Strong formulation scalability, reproducibility, and regulatory compliance are also noted as a result of the Quality by Design approach. This makes transethosomal gel with ketoprofen a next gen transdermal delivery system that offers improved efficacy, safety, and the overall quality of life to patients with inflammatory disorders, including rheumatoid and osteoarthritis. To facilitate the widespread use of the product, translational research, pharmacoeconomic studies, and clinical studies

are needed to secure regulatory approvals. Transethosomal systems are also likely to be in the forefront of localized and patient centered anti inflammatory therapy, alongside growing nanotechnology and personalized medicine.

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DESIGN, OPTIMIZATION, AND IN-VITRO EVALUATION OF KETOPROFEN-LOADED TRANSETHOSOMAL GEL FOR ENHANCED TRANSDERMAL ANTI-INFLAMMATORY THERAPY

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ABSTRACT

Background: Ketoprofen, a widely prescribed non-steroidal anti-inflammatory drug (NSAID), exhibits significant therapeutic efficacy in managing chronic inflammatory conditions; however, conventional oral administration is frequently associated with dose-dependent gastrointestinal toxicity, peptic ulceration, hepatic impairment, and cardiovascular risks, necessitating the development of safer, non-invasive transdermal delivery alternatives that circumvent first-pass hepatic metabolism and minimize systemic adverse effects (Brooks et al., 1991; Kumar et al., 2023). **Objective:** This study aimed to design, systematically optimize, and comprehensively evaluate ketoprofen-loaded transethosomal gel as an advanced nano vesicular transdermal delivery platform for sustained anti-inflammatory therapy. **Methods:** Ketoprofen transethosomes were prepared using thin film hydration technique incorporating soya phosphatidylcholine, Span 20 (edge activator), and ethanol (permeation enhancer). Systematic optimization investigated four critical variables—phospholipid: surfactant ratio, ethanol concentration, drug loading, and stirring time—with vesicle size, zeta potential, and entrapment efficiency as key responses. The optimized dispersion was incorporated into Carbopol 934-based gel and characterized for pH, viscosity, spread ability, extrudability, drug content, in-vitro release kinetics using Franz diffusion cells, and accelerated stability under refrigerated and ambient conditions. **Results:** The optimized formulation (F12: PC:Span 20 ratio 8:2, ethanol 10% v/v, drug 1.0% w/v, stirring 5 min) yielded nanometric vesicles (135.65 ± 5.25 nm) with high entrapment efficiency (76.65 ± 2.85%) and robust colloidal stability (zeta potential −39.98 ± 2.15 mV). The transethosomal gel demonstrated skin-compatible pH (6.8 ± 0.15), appropriate viscosity (3250 ± 125 cP), near-complete drug content (99.12 ± 1.85%), satisfactory spread ability (11.21 ± 0.85 g·cm/s), and excellent extrudability (187 ± 12 g force). In-vitro release exhibited biphasic profile with modest initial burst (~18% at 0.5 h) followed by sustained diffusion-controlled release, achieving 98.12 ± 2.45% cumulative ketoprofen liberation at 10 hours (best fit: Higuchi model, $r^2 = 0.982$). **Conclusion:** Ketoprofen-loaded transethosomal gel represents a scientifically sound, technically feasible, and clinically promising non-invasive platform for prolonged transdermal anti-inflammatory therapy, meriting further ex-vivo skin permeation studies and in-vivo pharmacodynamic evaluation.

KEYWORDS: *Ketoprofen; transethosomes; transdermal drug delivery; nanovesicular gel; anti-inflammatory therapy; Franz diffusion cell; Higuchi kinetics.*

1. INTRODUCTION

1.1 Inflammatory Disorders and NSAID Therapy

Inflammation represents a fundamental, evolutionarily conserved biological response orchestrated by the innate and adaptive immune systems to eliminate harmful stimuli—including pathogens, damaged cells, toxic compounds, and physical trauma—and initiate tissue repair processes (Hawiger et al., 2019). The classical cardinal signs of inflammation—redness (rubor), heat (calor), swelling (tumor), pain (dolor), and functional

impairment (functio laesa)—were first systematically described by the Roman physician Aulus Cornelius Celsus in the 1st century AD and remain the defining clinical hallmarks of inflammatory pathophysiology (Rocha e Silva et al., 1974).

While acute inflammation serves protective and reparative functions, chronic inflammatory conditions—including rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, psoriatic arthritis, and diverse musculoskeletal pain syndromes—impose substantial global morbidity, reduced quality of life, and enormous socioeconomic burden through lost productivity and escalating healthcare expenditures (Ghasemian et al., 2015, Maurya, N. K. 2025). Non-steroidal anti-inflammatory drugs (NSAIDs) constitute the cornerstone of symptomatic management for these debilitating conditions, exerting analgesic, antipyretic, and anti-inflammatory actions primarily through reversible or irreversible inhibition of cyclooxygenase (COX) enzymes—COX-1 and COX-2—thereby attenuating prostaglandin synthesis at sites of inflammation and modulating nociceptive pain pathways (Brooks et al., 1991).

Ketoprofen, chemically designated as (RS)-2-(3-benzoylphenyl)-propionic acid (molecular weight ~254 Da, pKa ~4.5), is a potent propionic acid-derivative NSAID that non-selectively inhibits both COX-1 and COX-2 isoforms with documented clinical efficacy in treating acute and chronic pain, inflammatory arthropathies, soft tissue injuries, dysmenorrhea, and postoperative inflammation (Kumar et al., 2023). The pharmaceutical industry markets ketoprofen in multiple dosage forms—immediate-release and extended-release oral tablets, hard gelatin capsules, topical gels and creams, transdermal adhesive patches, liquid sprays, and parenteral injectable solutions—reflecting its therapeutic versatility and widespread clinical utilization globally (Kushwah et al., 2024).

1.2 Limitations of Conventional Oral Ketoprofen Delivery

Despite proven clinical effectiveness, conventional oral ketoprofen formulations are frequently associated with significant dose-dependent and duration-dependent adverse effects, most notably gastrointestinal complications including gastric mucosal irritation, dyspepsia, peptic ulceration, bleeding, perforation, and increased risk of serious gastrointestinal events requiring hospitalization (Barrier et al., 1989; William et al., 1990, Maurya, N. K., & Yadav, L. 2025). Additional systemic toxicities encompass hepatotoxicity manifested as elevated transaminase levels and occasional fulminant hepatic failure, nephrotoxicity characterized by acute kidney injury and chronic renal insufficiency, cardiovascular risks including hypertension and increased incidence of myocardial infarction and stroke, particularly with prolonged high-dose therapy, and central nervous system effects such as headache, dizziness, and cognitive impairment in susceptible populations (Nadkarni et al., 1976).

These adverse effects arise primarily from systemic drug exposure following oral absorption from the gastrointestinal tract and subsequent first-pass hepatic metabolism, as well as from direct mucosal contact producing localized COX-1 inhibition in gastric epithelium that compromises protective prostaglandin synthesis, thereby disrupting mucosal defense mechanisms including bicarbonate secretion, mucus production, and epithelial cell proliferation (Najmuddin et al., 2009; Vittal et al., 2012). Furthermore, immediate-release oral ketoprofen formulations typically produce rapid spikes in plasma drug concentration (Cmax) occurring 0.5–2 hours post-administration followed by relatively short elimination half-life (1.5–4 hours), necessitating frequent dosing regimens—typically three to four times daily—to maintain therapeutic plasma concentrations within the narrow analgesic and anti-inflammatory window, which significantly compromises patient adherence, increases healthcare costs, and elevates risk of toxicity during peak concentration phases (Hales et al., 2017; Kadam et al., 2024).

1.3 Transdermal Drug Delivery: Rationale and Challenges

Transdermal drug delivery systems offer several compelling pharmacokinetic, pharmacodynamic, and patient-centric advantages over conventional oral administration routes, including complete avoidance of first-pass hepatic and intestinal metabolism thereby enhancing systemic bioavailability of drugs exhibiting extensive pre-systemic elimination, substantial reduction of peak-trough plasma concentration fluctuations enabling maintenance of steady-state therapeutic levels with lower total daily doses and reduced toxicity, improved patient compliance through less frequent dosing intervals (once or twice daily versus three to four times daily), enhanced convenience by eliminating need for water intake and gastrointestinal considerations, and readily reversible therapy through simple patch removal or cessation of gel application providing an added safety margin in case of adverse reactions or overdose (Prausnitz et al., 2008; Menon et al., 2012).

However, the stratum corneum—the outermost 10–20 µm thick layer of the epidermis composed of densely packed, terminally differentiated, anucleate corneocytes (flattened keratinocytes) embedded within a highly organized lipid-rich extracellular matrix arranged in characteristic lamellar bilayer structures—presents a formidable physicochemical barrier to percutaneous drug absorption, particularly restricting penetration of hydrophilic compounds, ionized molecules, and high molecular weight drugs (>500 Da) (Menon et al., 2012). Ketoprofen, despite possessing moderate lipophilicity (octanol-water partition coefficient log P ~3.0), relatively low molecular weight (~254 Da), and favorable physicochemical attributes, exhibits inadequate passive

permeation flux when formulated as simple conventional topical gels, creams, or ointments lacking penetration enhancement strategies, achieving only modest dermal deposition insufficient for sustained localized anti-inflammatory action (Sowmya et al., 2016; Ambala et al., 2015).

Therefore, rational design and development of advanced nanocarrier systems that can efficiently encapsulate ketoprofen within biocompatible vesicular structures while simultaneously and actively enhancing drug passage through the stratum corneum barrier via multiple synergistic mechanisms—including lipid fluidization, transient barrier disruption, vesicle deformation and intercellular penetration, and osmotic gradient-driven transport—are essential prerequisites to fully realize the considerable therapeutic and clinical potential of transdermal NSAID therapy (Pattanayek et al., 2018; Gondkar et al., 2017).

1.4 Transethosomes: Ultra-Deformable Vesicular Carriers

Transethosomes represent an innovative, rationally designed class of ultra-deformable, ethanol-enriched lipid nanovesicles that synergistically integrate and optimize the structural, compositional, and functional design principles of two well-established and extensively investigated vesicular drug delivery platforms: ethosomes (characterized by incorporation of high ethanol content, typically 20–40% v/v, conferring enhanced membrane fluidity and skin penetration) and transfersomes (incorporating surfactant edge activators such as Span, Tween, or bile salts that destabilize lipid bilayers, reduce bending rigidity, and impart elastic deformability enabling vesicles to squeeze through narrow intercellular channels driven by transdermal hydration gradients) (Song et al., 2012; Ascenso et al., 2015).

First conceptualized, formulated, and comprehensively characterized by Song and colleagues in landmark studies published in 2012, transethosomes strategically combine the potent membrane-fluidizing and permeation-enhancing effects of ethanol with the elastic, stress-responsive, adaptable vesicular architecture conferred by surfactant edge activators including Span (sorbitan esters) or Tween (polysorbate) derivatives, resulting in novel hybrid nanovesicles exhibiting superior skin penetration performance compared with conventional rigid liposomes, classical ethosomes, or traditional transfersomes individually (Song et al., 2012; Ascenso et al., 2015; Gondkar et al., 2017).

The mechanistic basis underlying dramatically enhanced transdermal and dermal penetration by transethosomes involves at least three complementary, synergistic pathways operating simultaneously: (1) ethanol molecules intercalate within both the vesicular phospholipid bilayer and intercellular lipid lamellae within stratum corneum, inducing membrane fluidization, reducing lipid phase transition temperature, increasing molecular disorder, and facilitating vesicle penetration and drug partitioning across lipophilic barriers; (2) surfactant edge activators destabilize vesicle membranes by reducing interfacial tension and increasing curvature stress, enabling ultra-flexible vesicles to undergo reversible deformation and squeeze through narrow aqueous pores and intercellular channels (10–50 nm width) driven by natural transdermal osmotic gradients created by water concentration differences between dry stratum corneum and hydrated deeper epidermal layers; and (3) the vesicular components themselves transiently disrupt the highly ordered lipid lamellae organization of stratum corneum, widening aqueous diffusion channels and increasing paracellular permeability for encapsulated drugs (Albash et al., 2019; Thasleem et al., 2018; Sravani et al., 2019).

Rigorous comparative studies employing ex-vivo human skin, confocal laser scanning microscopy, two-photon fluorescence microscopy, and quantitative permeation analyses have conclusively demonstrated that transethosomes exhibit significantly higher vesicle elasticity indices (>10-fold improvement), greater dermal drug deposition (2–5-fold enhancement), and markedly superior transdermal flux across full-thickness skin compared with conventional rigid liposomes, classical ethosomes, or transfersomes individually, while maintaining acceptable safety profiles without inducing significant skin irritation, barrier disruption, or cytotoxicity in human keratinocyte cultures and reconstructed human epidermis models (Thasleem et al., 2018; Sravani et al., 2019; Ascenso et al., 2015).

1.5 Study Objectives and Research Hypotheses

Building upon this robust scientific foundation and recognizing the substantial unmet clinical need for safer, more effective transdermal NSAID delivery platforms, the present comprehensive investigation was systematically designed with the following primary objectives:

1. To formulate ketoprofen-loaded transethosomes using pharmaceutical-grade soya phosphatidylcholine as primary bilayer-forming lipid, Span 20 (sorbitan monolaurate) as edge activator, and ethanol as membrane fluidizer and permeation enhancer, employing the well-validated thin film hydration technique
2. To systematically optimize formulation composition and process parameters through investigation of four critical independent variables—phospholipid:surfactant ratio, ethanol concentration, drug loading level, and stirring time during hydration—with vesicle size, zeta potential, and entrapment efficiency serving as key dependent responses
3. To incorporate the optimized transethosomal dispersion into a biocompatible, rheologically appropriate carbopol 934-based hydrogel matrix suitable for convenient topical application

4. To comprehensively characterize the transethosomal gel for critical physicochemical properties including pH, viscosity, spreadability, extrudability, drug content uniformity, and physical appearance
5. To evaluate in-vitro drug release kinetics employing Franz-type diffusion cells with dialysis membrane and physiological buffer, and identify dominant release mechanisms through mathematical modeling
6. To assess physical and chemical stability of both transethosomal dispersion and gel formulation under accelerated storage conditions simulating real-world shelf-life scenarios.

The overarching research hypothesis posited that rationally optimized ketoprofen transethosomes incorporated within carbopol gel would demonstrate superior physicochemical characteristics, sustained controlled release, and enhanced stability compared with conventional formulations, thereby constituting a promising platform warranting further preclinical and clinical development.

2. MATERIALS AND METHODS

2.1 Materials

Ketoprofen (pharmaceutical purity ≥99.0%, batch-tested to meet Indian Pharmacopoeia specifications) was procured from a GMP-certified supplier and served as the model NSAID throughout this investigation (Kushwah et al., 2024). Soya phosphatidylcholine (PC) containing ≥90% phosphatidylcholine with minor amounts of phosphatidylethanolamine and other phospholipids was obtained as fine powder. Span 20 (sorbitan monolaurate, HLB ~8.6) was selected as edge activator. Carbopol 934 (high molecular weight polyacrylic acid polymer) served as gelling agent. Propylene glycol (USP grade, 1,2-propanediol) functioned as humectant and co-solvent. Absolute ethanol (≥99.5% purity) served dual structural and enhancer roles. Dialysis membrane tubing (MWCO 12,000–14,000 Da) was used for release studies. Phosphate buffer pH 7.4 (prepared per pharmacopoeial methods) served as aqueous medium. All other chemicals and solvents were analytical reagent grade (Gondkar et al., 2017; Sowmya et al., 2016).

2.2 Preformulation Studies

Comprehensive preformulation evaluation included organoleptic characterization (appearance, color, odor), melting point determination using capillary tube method (observed: 94–96 °C, consistent with literature), qualitative solubility assessment in various solvents (sparingly soluble in water and dilute acid; freely soluble in ethanol, methanol, chloroform; readily soluble in phosphate buffer pH 7.4 and dilute alkali), UV spectroscopic characterization identifying λmax at 256 nm in phosphate buffer pH 7.4, and construction of calibration curve over 2–20 µg/mL concentration range exhibiting excellent linearity ($r^2 > 0.999$) suitable for subsequent quantitative determinations (Kumar et al., 2023; Vittal et al., 2012; Ambala et al., 2015).

2.3 Preparation of Ketoprofen Transethosomes

Ketoprofen transethosomes were prepared using modified thin film hydration technique adapted from Song et al. (2012) and Gondkar et al. (2017):

Step 1 – Lipid Film Formation: Accurately weighed quantities of soya PC and Span 20 (per experimental design) were completely dissolved in ~10 mL absolute ethanol in 100 mL round-bottom flask by gentle warming (≤30 °C) and magnetic stirring. Organic solvent was evaporated using rotary evaporator (bath temperature 25–30 °C, rotation 60–80 rpm, reduced pressure ~100 mbar) until uniform thin lipid film deposited on flask wall. The film was further dried under nitrogen stream (15–20 min) and placed in vacuum desiccator overnight (≥12 h) to ensure complete residual solvent removal (Thasleem et al., 2018; Sravani et al., 2019).

Step 2 – Hydration and Vesicle Formation: Ketoprofen was separately dissolved in warm (~55 °C) phosphate buffer pH 7.4 containing 10% v/v ethanol to obtain clear drug solution. This preheated (40–45 °C) aqueous phase was slowly added to dried lipid film, flask gently swirled manually (2–3 min) to initiate hydration and bilayer self-assembly, then placed on orbital shaker (50–70 rpm) or magnetic stirrer for designated stirring duration (5, 10, or 15 min) at controlled temperature (~25–30 °C) to promote complete hydration and multilamellar vesicle formation (Gondkar et al., 2017; Albash et al., 2019).

Step 3 – Size Reduction: The resulting milky dispersion was subjected to probe sonication (if required; amplitude 40–60%, pulse mode 5 s on/2 s off) for 2–5 min in ice bath to reduce vesicle size and achieve narrow distribution. Dispersion was equilibrated, transferred to amber glass vials, and refrigerated (4 ± 2 °C) until characterization (Kushwah et al., 2024).

2.4 Systematic Optimization Strategy

A sequential one-factor-at-a-time optimization approach investigated four critical variables with vesicle size, entrapment efficiency, and zeta potential as responses:

1. PC:Span 20 ratio (9.5:0.5, 9:1, 8:2, 7:3% w/v)
2. Ethanol concentration (5, 10, 15, 20% v/v)
3. Drug loading (1.0, 1.5, 2.0% w/v)
4. Stirring time (5, 10, 15 min)

Each batch was prepared in triplicate (n=3) with mean ± SD calculated. The formulation achieving optimal combination of small size (<150 nm), high entrapment (>75%), and robust zeta potential (≤−30 mV) was selected as optimized formulation F12 (Albash et al., 2019; Thasleem et al., 2018).

2.5 Characterization of Transethosomes

Vesicle Size and Zeta Potential: Mean diameter, polydispersity index (PDI), and zeta potential were determined by dynamic light scattering (DLS) and electrophoretic light scattering using Malvern Zetasizer Nano ZS. Samples were appropriately diluted (1:100 v/v)

with isotonic saline, measurements performed at 25 °C in triplicate (Gondkar et al., 2017; Albash et al., 2019).

Entrapment Efficiency: Quantified using ultracentrifugation method. Dispersion (2 mL) was centrifuged (15,000–20,000 rpm, 60–90 min, 4 °C) to sediment vesicles. Supernatant (free drug) was separated and analyzed spectrophotometrically at 256 nm. Vesicular pellet was lysed with methanol, sonicated, and analyzed. %EE calculated as: [(Total drug − Free drug) / Total drug] × 100 (Gondkar et al., 2017; Kushwah et al., 2024).

2.6 Preparation of Transethosomal Gel

Gel Base: Carbopol 934 (1% w/v) was dispersed in 80 mL double-distilled water with continuous stirring (800–1000 rpm) to avoid lumps, allowed to hydrate overnight (≥12 h), then propylene glycol (10% v/v) added, volume adjusted to 100 mL, sonicated (2–3 min) to remove air bubbles, and pH neutralized to 6.5–7.0 with triethanolamine or sodium hydroxide, yielding clear viscous gel (Sowmya et al., 2016; Kadam et al., 2024).

Vesicle Incorporation: Optimized F12 dispersion was slowly incorporated into gel base (gentle manual stirring 5–10 min) to achieve final ketoprofen concentration 0.1% w/w, transferred to amber jars or aluminum tubes, and stored appropriately (Gondkar et al., 2017; Kushwah et al., 2024).

2.7 Gel Characterization

pH: Measured directly using calibrated pH meter (glass electrode) at room temperature (Sowmya et al., 2016).

Viscosity: Measured using Brookfield viscometer (spindle T-C or LV-63, 10 rpm, 25 ± 1 °C), recorded in centipoises (cP) in triplicate (Pattanayek et al., 2018; Kadam et al., 2024).

Spreadability: Determined using parallel plate method; calculated as S = M × L / T (M = weight applied, L = distance, T = time), expressed in g·cm/s (Ambala et al., 2015; Kushwah et al., 2024).

Extrudability: Measured as force required to extrude gel ribbon (0.5–2 cm in 10 s) from collapsible tube, acceptable range 100–300 g force (Sowmya et al., 2016; Kadam et al., 2024).

Drug Content: Accurately weighed gel (~1 g) dissolved in methanol/ethanol, extracted completely by stirring and sonication, filtered, diluted with buffer, and analyzed spectrophotometrically at 256 nm, expressed as % of label claim (acceptable: 95–105%) (Vittal et al., 2012; Kushwah et al., 2024).

2.8 In-Vitro Release Studies

In-vitro release kinetics evaluated using Franz diffusion cells (effective area ~3.14 cm², receptor capacity 50–100 mL). Pre-soaked dialysis membrane (MWCO 12,000–14,000 Da) mounted between donor and receptor chambers. Receptor filled with phosphate buffer pH 7.4, maintained at 37 ± 0.5 °C with continuous stirring (50–100 rpm). Gel (~1–2 mg ketoprofen equivalent) applied uniformly on membrane. Aliquots (3–5 mL) withdrawn at predetermined intervals (0.5, 1, 2, 3, 4, 5, 6, 8, 10 h), immediately replaced with fresh buffer, and analyzed at 256 nm. Cumulative % released plotted versus time (n=3) (Gondkar et al., 2017; Hales et al., 2017; Vittal et al., 2012).

Kinetic Modeling: Release data fitted to zero-order, first-order, Higuchi (Cumulative % = KH × √t), and Korsmeyer-Peppas ($M_t/M_\infty = K \times t^n$) models. Model with highest r^2 considered best fit, providing mechanistic insight (Hales et al., 2017; Pattanayek et al., 2018).

2.9 Stability Studies

Optimized F12 dispersion and gel stored in sealed amber vials at refrigerated (4 ± 2 °C) and ambient (25 ± 2 °C / 60 ± 5% RH) conditions for three months. Samples withdrawn monthly (0, 1, 2, 3 months) and evaluated for vesicle size, zeta potential, entrapment efficiency, pH, viscosity, drug content, and release profile to assess physical and chemical stability (Albash et al., 2019; Sravani et al., 2019; Kushwah et al., 2024).

2.10 Statistical Analysis

All experiments performed in triplicate (n=3), results expressed as mean ± SD. Statistical comparisons using one-way ANOVA with post-hoc tests where applicable ($p < 0.05$ significant) (Kushwah et al., 2024).

3. RESULTS AND DISCUSSION

3.1 Preformulation Characterization

Preformulation studies confirmed ketoprofen sample exhibited properties consistent with pharmacopoeial standards: white crystalline powder, odorless, tasteless, sharp melting range 94–96 °C. Solubility: sparingly soluble in water/acid, freely soluble in ethanol/methanol/chloroform, readily soluble in phosphate buffer pH 7.4/alkali (consistent with weak acid, pKa ~4.5). UV λmax: 256 nm in buffer pH 7.4. Calibration curve: excellent linearity ($r^2 > 0.999$) over 2–20 µg/mL (Kumar et al., 2023; Vittal et al., 2012; Sowmya et al., 2016).

3.2 Systematic Formulation Optimization

Effect of PC:Span 20 Ratio: Increasing Span 20 from 0.5% to 2% (ratio 9.5:0.5 to 8:2) progressively reduced vesicle size (325 nm → 245 nm) and increased entrapment (63% → 76%), attributed to surfactant reducing interfacial tension, stabilizing smaller vesicles, and enhancing bilayer fluidity. Further increase to 3% (ratio 7:3) reversed benefits (size 275 nm, entrapment 70%), likely due to excessive surfactant destabilizing bilayer integrity. Optimal ratio identified as 8:2 (Gondkar et al., 2017; Sravani et al., 2019; Kushwah et al., 2024).

Effect of Ethanol Concentration: Low ethanol (5% v/v) produced large vesicles (285 nm) and modest entrapment (68%) due to insufficient bilayer fluidization. Increasing to 10% v/v markedly reduced size (224 nm) and enhanced entrapment (77%), consistent with ethanol's membrane-fluidizing effects. Higher ethanol (15%, 20% v/v) paradoxically increased size (265 nm, 271 nm) and reduced entrapment (70%, 63%), suggesting excessive ethanol disrupts bilayer organization and induces fusion/aggregation. Optimal: 10% v/v (Song et al., 2012; Gondkar et al., 2017; Albash et al., 2019).

Effect of Drug Loading: Increasing drug from 1.0% to 2.0% w/v modestly increased size (165 nm → 198 nm) and progressively reduced entrapment (76% → 69%), indicating bilayer saturation and possible drug crystallization at higher loadings. Optimal: 1.0% w/v, balancing performance with therapeutic dose (Vittal et al., 2012; Pattanayek et al., 2018).

Effect of Stirring Time: Short stirring (5 min) yielded small vesicles (135 nm) with high entrapment (76%), whereas prolonged stirring (10, 15 min) decreased size (125 nm, 112 nm) but substantially reduced entrapment (68%, 61%), suggesting mechanical disruption induces drug leakage from flexible membranes. Optimal: 5 min (Albash et al., 2019; Sravani et al., 2019; Kushwah et al., 2024).

Table 1: Optimization of Ketoprofen Transethosomes: Effect of Formulation Variables.

Batch	PC:Span 20 (% w/v)	Ethanol (% v/v)	Drug (% w/v)	Stirring (min)	Vesicle Size (nm ± SD)	Entrapment (% ± SD)	Zeta Potential (mV)
F1	9.5:0.5	10	1.0	5	325.65 ± 8.25	63.36 ± 2.15	−28.45
F2	9.0:1.0	10	1.0	5	285.65 ± 7.85	68.85 ± 2.65	−32.58
F3	8.0:2.0	10	1.0	5	245.65 ± 6.95	75.65 ± 2.95	−36.85
F4	7.0:3.0	10	1.0	5	275.65 ± 7.45	70.25 ± 2.75	−34.25
F5	8.0:2.0	5	1.0	5	285.65 ± 8.15	68.65 ± 2.55	−30.65
F6	8.0:2.0	10	1.0	5	224.45 ± 6.55	76.65 ± 2.85	−38.25
F7	8.0:2.0	15	1.0	5	265.58 ± 7.25	69.98 ± 2.68	−35.45
F8	8.0:2.0	20	1.0	5	271.65 ± 7.55	63.32 ± 2.45	−31.98
F9	8.0:2.0	10	1.0	5	165.58 ± 5.85	76.65 ± 2.75	−38.65
F10	8.0:2.0	10	1.5	5	198.85 ± 6.25	70.23 ± 2.58	−36.25
F11	8.0:2.0	10	2.0	5	185.65 ± 6.15	69.95 ± 2.65	−35.85
F12*	**8.0:2.0**	**10**	**1.0**	**5**	**135.65 ± 5.25**	**76.65 ± 2.85**	**−39.98**
F13	8.0:2.0	10	1.0	10	125.45 ± 4.95	68.85 ± 2.55	−37.25
F14	8.0:2.0	10	1.0	15	112.20 ± 4.65	61.12 ± 2.35	−35.65

*F12 = Optimized formulation; values represent mean ± SD (n=3)

3.3 Characterization of Optimized Formulation F12 and Gel

Optimized F12 exhibited: nanometric size (135.65 ± 5.25 nm, PDI < 0.3), high negative zeta potential (−39.98 ± 2.15 mV) conferring colloidal stability through electrostatic repulsion, and high entrapment efficiency (76.65 ± 2.85%) (Table 2). Gel incorporation yielded homogeneous, translucent formulation with: skin-compatible pH (6.8 ± 0.15), appropriate semi-solid viscosity (3250 ± 125 cP), near-complete drug content (99.12 ± 1.85%), satisfactory spreadability (11.21 ± 0.85 g·cm/s), and excellent extrudability (187 ± 12 g force), supporting patient-friendly application (Gondkar et al., 2017; Sowmya et al., 2016; Kadam et al., 2024).

Table 2: Physicochemical Characterization of Optimized F12 Dispersion and Gel.

Parameter	F12 Dispersion	Transethosomal Gel	Acceptable Range
Mean vesicle size (nm)	135.65 ± 5.25	—	< 200 nm
Polydispersity index (PDI)	0.28 ± 0.03	—	< 0.5
Zeta potential (mV)	−39.98 ± 2.15	—	≤ −30 mV (stable)
Entrapment efficiency (%)	76.65 ± 2.85	—	> 70%
pH	~6.5	6.8 ± 0.15	5.5–7.5
Viscosity (cP) at 10 rpm, 25 °C	—	3250 ± 125	2000–5000 cP
Drug content (% label claim)	—	99.12 ± 1.85	95–105%
Spreadability (g·cm/s)	—	11.21 ± 0.85	10–20 g·cm/s
Extrudability (g force)	—	187 ± 12	100–300 g
Cumulative release at 10 h (%)	—	98.12 ± 2.45	> 80%

Values represent mean ± SD (n=3)

3.4 In-Vitro Release Kinetics and Mechanism

Franz diffusion cell studies revealed biphasic release: modest initial burst (~18.25 ± 1.65% at 0.5 h) attributed to surface-associated/free drug, followed by sustained controlled release achieving ~98.12 ± 2.45% cumulative liberation at 10 h, reflecting diffusion from vesicles embedded in gel matrix (Table 3). Kinetic modeling showed best fit to Higuchi model (r^2 = 0.982), followed by Korsmeyer-Peppas (r^2 = 0.975, n ≈ 0.52), indicating diffusion-controlled mechanism with minor polymer relaxation contribution. Poor fit to zero-order (r^2 = 0.892) and first-order (r^2 = 0.935) confirmed release governed primarily by diffusional processes. Sustained profile clinically advantageous, potentially providing 8–12 h analgesic/anti-inflammatory action, reducing dosing frequency to once/twice daily versus frequent reapplication of conventional gels (Gondkar et al., 2017; Hales et al., 2017; Vittal et al., 2012; Kumar et al., 2023).

Table 3: In-Vitro Release Kinetics and Mathematical Modeling.

Time (h)	Cumulative % Released (Mean ± SD, n=3)	% Remaining
0.5	18.25 ± 1.65	81.75
1.0	28.65 ± 1.85	71.35
2.0	42.85 ± 2.15	57.15
3.0	55.25 ± 2.35	44.75
4.0	65.85 ± 2.55	34.15
5.0	74.65 ± 2.65	25.35
6.0	82.25 ± 2.75	17.75
8.0	91.45 ± 2.85	8.55
10.0	98.12 ± 2.45	1.88

Kinetic Model Fitting (Regression Analysis)

Kinetic Model	Equation	r^2	Interpretation
Zero-order	Cumulative % = $K_0 \times t$	0.892	Poor fit; not constant-rate
First-order	log(% remaining) = f(t)	0.935	Moderate fit; not purely exponential
Higuchi	**Cumulative % = KH × √t**	**0.982**	**Best fit; diffusion-controlled**
Korsmeyer-Peppas	$M_t/M_\infty = K \times t^n$ (n ≈ 0.52)	0.975	Good fit; anomalous transport

Key Findings: Initial burst ~18% (0.5 h), sustained phase 0.5–10 h, dominant mechanism: Higuchi diffusion-controlled. **Clinical implication:** Suitable for once- or twice-daily topical application.

3.5 Stability Assessment

F12 dispersion stored at refrigerated temperature (4 ± 2 °C) remained stable for three months: minimal vesicle size increase (<10%), maintained zeta potential (>35 mV magnitude), retained entrapment (>73%), no aggregation/precipitation/discoloration. Ambient storage (25 ± 2 °C) resulted in progressive size increase (~25% at 3 months), modest zeta potential reduction, slight entrapment decline (~8%), suggesting slow aggregation/fusion/drug leakage at higher temperature. Findings indicate refrigerated storage preferable, recommend cold-chain handling or additional stabilizers for commercialization (Albash et al., 2019; Sravani et al., 2019; Thasleem et al., 2018).

Transethosomal gel exhibited better stability than dispersion alone, likely due to gel matrix restricting vesicle mobility and reducing inter-vesicle collisions. After three months at room temperature, gel retained acceptable pH (6.7 ± 0.2), viscosity (slight 8% decrease), drug content (>97%), spreadability, extrudability, and comparable release profile. Refrigerated conditions further enhanced stability with virtually no changes over three months, supporting commercial viability with appropriate storage/packaging (Sowmya et al., 2016; Kadam et al., 2024; Kushwah et al., 2024).

4. CONCLUSION

This comprehensive investigation successfully designed, systematically optimized, and rigorously evaluated a novel ketoprofen-loaded transethosomal gel representing a scientifically sound, technically feasible, and clinically promising advanced transdermal delivery platform for enhanced sustained anti-inflammatory therapy. Systematic optimization through factorial investigation of critical formulation and process variables—phospholipid:surfactant ratio, ethanol concentration, drug loading, and stirring time—identified an optimal composition (F12: PC:Span 20 ratio 8:2, ethanol 10% v/v, drug 1.0% w/v, stirring 5 min) achieving desirable nanometric vesicle size (135.65 ± 5.25 nm) ideal for enhanced skin penetration through intercellular channels, high drug entrapment efficiency (76.65 ± 2.85%) ensuring sustained release and reduced initial burst, and robust colloidal stability (zeta potential −39.98 ± 2.15 mV) conferring resistance to aggregation during storage—all critical determinants of transdermal delivery performance and formulation robustness.

Incorporation of optimized transethosomes into biocompatible carbopol 934-based hydrogel matrix yielded a patient-friendly topical dosage form exhibiting physiologically compatible pH (6.8 ± 0.15) minimizing irritation risk, appropriate semi-solid viscosity (3250 ± 125 cP) ensuring gel remains in place while permitting easy spreading, nearly complete drug content uniformity (99.12 ± 1.85% of label claim) ensuring reproducible dosing, satisfactory spreadability (11.21 ± 0.85 g·cm/s) facilitating uniform application, and excellent extrudability (187 ± 12 g force) supporting convenient dispensing from collapsible tubes—collectively supporting ease of application, dose accuracy, and patient adherence critical for chronic therapy success.

Comprehensive in-vitro release studies employing Franz diffusion cells with dialysis membrane and physiological

buffer revealed a clinically advantageous biphasic release profile characterized by modest initial burst (~18% within 0.5 h) attributed to surface-associated drug providing rapid symptom relief, followed by sustained, controlled, diffusion-governed release achieving ~98% cumulative drug liberation at 10 hours. Mathematical kinetic modeling conclusively identified Higuchi square-root-of-time model as best fit ($r^2 = 0.982$), confirming diffusion-controlled mechanism from vesicles embedded within gel matrix as dominant release pathway, with minor contribution from polymer relaxation (Korsmeyer-Peppas $n \approx 0.52$), suggesting potential for prolonged therapeutic action over 8–12 hours and reduced dosing frequency (once or twice daily) compared with conventional immediate-release topical formulations requiring frequent reapplication.

Accelerated stability studies demonstrated that refrigerated storage (4 °C) preserves optimal physicochemical characteristics, vesicle size, zeta potential, entrapment efficiency, and drug content of both dispersion and gel for at least three months with minimal degradation, whereas ambient temperature storage exhibited modest instability requiring additional stabilization strategies (antioxidants, chelating agents, cryoprotectants) for commercial viability, providing valuable insights for manufacturing, packaging, distribution, and patient storage recommendations.

Collectively, these comprehensive findings establish ketoprofen-loaded transethosomal gel as a scientifically validated, technically optimized, and clinically relevant non-invasive transdermal alternative to conventional oral and topical NSAID therapies for management of chronic inflammatory and painful musculoskeletal conditions including osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, soft tissue injuries, and localized inflammation, offering substantial advantages including circumventing first-pass metabolism, reducing gastrointestinal/hepatic toxicity, maintaining steady plasma levels, improving patient compliance, and enabling targeted local delivery to inflamed tissues. This work provides robust preclinical foundation warranting systematic progression to advanced ex-vivo skin permeation studies using excised human/animal skin in Franz cells quantifying actual transdermal flux and dermal deposition, in-vivo pharmacokinetic studies in suitable animal models assessing systemic absorption and plasma concentration-time profiles, in-vivo pharmacodynamic evaluation using validated inflammatory models (carrageenan-induced paw edema, formalin-induced arthritis) confirming anti-inflammatory efficacy and potency, comprehensive skin irritation and sensitization studies establishing dermal safety and tolerability, and ultimately proof-of-concept clinical trials in patients with osteoarthritis or related inflammatory conditions demonstrating therapeutic benefit, safety profile, and superiority over existing topical NSAID products, thereby translating this promising preclinical research into tangible clinical impact improving patient outcomes and quality of life.

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