1. Introduction

1.1. Arthritis

World is facing a major global issue of arthritis which accounts for majority of cases of disability in many developed countries. There can be a number of causes for arthritis namely as those implications which may lead to any modification in the shape or change in the fundamental material of joints, or there may be detraction of support from the joint.(1) Diseases which may lead to alteration of joint constituents are crystal deposition disease, haemochromatosis or ochronosis, along with changes in the mechanical properties of the said tissue.(1, 2) In 2013, according to the Center for Disease Control and Prevention (CDC), it was estimated that approximately 54.4 million people were diagnosed with arthritis in the United States, which account for almost quarter of the population, along with an estimated future increase in cases in the coming years (78.4 million and 25.9% by 2040).(3)

Following classes of arthritis are few among the vast 200 types of arthritis or musculoskeletal conditions(2):

- Inflammatory arthritis (rheumatoid arthritis)
- Metabolic arthritis (gouty arthritis)
- Degenerative or mechanical arthritis
- Infectious arthritis
- Soft tissue musculoskeletal pain
- Connective tissue disease

Inflammatory arthritis and metabolic Arthritis have been selected for the present study from the above mentioned types given that these two classes of arthritis are highly prevalent.(1)

1.1.1. Rheumatoid Arthritis (RA)

RA being an autoimmune disease mainly affects joints which exhibits symptoms like systemic inflammation, autoantibodies and persistent synovitis. Arthritis results in swelling of joints which leads to stiffness, pain, and progressive loss of function.(4, 5) However, people suffering with RA may also show indications like fatigue, weight loss and low-grade fever. In majority cases, RA involves pair of joints which accounts for limbs but can also involve more than one joint, like small joints present in wrists and

hands.(4, 6) Eventually, various joints such as knees, shoulders, elbows, feet, and ankles can also be affected. In chronic conditions, inflammation caused by RA may lead to serious damage to joints. In few patients, this may cause to irreversible joint damage.(4) There is not much insight on the cause of rheumatoid arthritis but, it is believed that the etiology may be a combination of ecological and genetic factors. It is considered to be an autoimmune disease, wherein our body's immune system misinterprets own cells as invaders. Immune system attacks the synovium and membrane lining in the joints, which leads to swelling and solidifying of the joint capsule. Further, it also affects underlying cartilage and bone.(4, 6, 7)

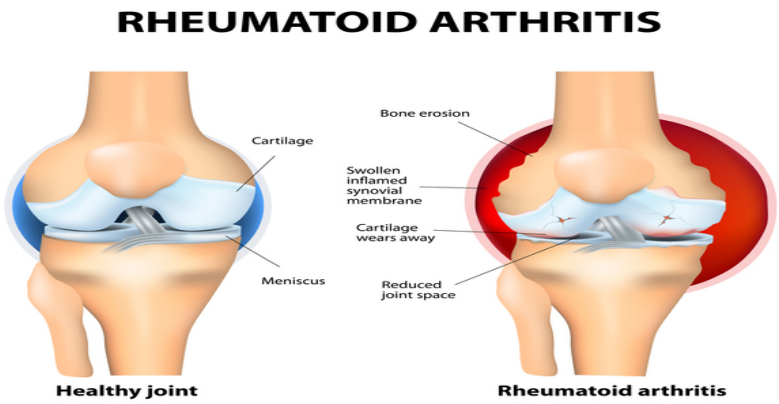

Figure 1.1: Pathophysiology of RA.(5)

1.1.1.1. Prevalence

In 2015, Worldwide prevalence of RA was accounted to be around 1% of population.(8) The prevalence is in the range of 0.5 and 1% in adults in the developed countries with 5 and 50 per 100,000 people freshly developing the disease each year.(6) Commencement of RA is mostly reported in the age range of around 30-50 years, and impacts women more as compared to men. Death toll due to RA went up from 28,000 in 1990 to 38,000 in 2013.(9)

1.1.1.2. Signs and Symptoms

Swollen, painful and stiff joints, low fever, fatigue, anorexia, lump formation under the skin in hands or elbows, weight loss, dry eyes and dry mouth and over the time, decreased range of motion are reported to be the symptoms of RA.(5, 10, 11)

1.1.1.3. Treatment Options

After RA is diagnosed, commencement of the treatment is very crucial as soon as possible. Goal of the therapy is to reduce or stop subsequent joint damage, reduce pain and increase physical function etc. Change in lifestyle, surgery or medication are the treatment options that can be taken by patients.(6, 12) Antirheumatic drugs (DMARDs), Biological Response Modifiers (BRMs) and other Adjuvant drugs are included in the treatment. Apart from these, several NSAID (Non-steroidal Anti-inflammatory Drugs) like diclofenac, meloxicam, ibuprofen, piroxicam and selective COX-2 inhibitors (celecoxib, rofecoxib, valdecoxib) are also used for symptomatic relief.(13-15)

1.1.1.4. Immunosuppressants

As RA is reported to be an autoimmune disease, **immunosuppressant** are prescribed for slowing down the advancement of disease and improving the life of patients.(6, 16) Primary cause of RA is the high activity of the immune system so, immunosuppressant act by inhibiting the activation of T cell. Immunosuppressant also exhibit some anti-inflammatory effect.(6, 16)

1.1.1.5. Tacrolimus

Methotrexate is widely prescribed by the rheumatologists, but it is associated with serious adverse effects such as leukopenia, blood in urine or stools, jaundice, signs of liver problems, anemia etc.(17, 18) It was reported by Daniel E. Furst et al. that Tacrolimus (TAC) proved to improve disease activity in methotrexate intolerant of resistant patient suffering with RA. Optimal dose for RA suggested by them is > 1 mg but ≤ 3 mg/day.(19) Further, D.E Yocum et al. studies the long term efficacy of TAC in patients with RA and reported that TAC is safe and well tolerated and give therapeutic benefit for at least 12 months.(20)

TAC is an immunosuppressant which is indicated in organ rejection. TAC ointment is also available which is indicated for other autoimmune diseases such as psoriasis and eczema.(17) Moreover, there are reports suggesting its therapeutic benefits in RA and it also has a better adverse effect profile when compared to other immunosuppressants.(19, 21, 22) Thus, TAC is selected for the present project. TAC

is accessible in the market in various dosage forms which includes, Capsule (Olmis Cap: Unichem Laboratories, Tacloram: Wockhardt (Biotech), Tablet (Crolim-Ranbaxy Laboratories). Presently, oral dosage form is indicated for organ rejection and ointment is indicated for psoriasis. Tablets (1 mg, 2mg and 5mg) are administered depending on the requirement of the patient. Ointment (Olmis: Unichem Laboratories) is available in the market but drug absorption is incomplete. Oral bioavailability of TAC is 24%, Protein binding is ≥98.8%, Biological half-life is 11.3 hours for transplant patients (3.5-40.6 hr). It is primarily metabolized by the live microsomal enzyme and mostly excreted in faces.(17) Adverse effects like gastrointestinal disturbances such as diarrhea, nausea, vomiting, stomach pain etc. were caused by TAC and these adverse effects became severe in various cases which lead to the withdrawal of therapy.(23, 24) TAC has a limitation of poor oral bioavailability, food dependent absorption, gastrointestinal disturbances.

1.1.2. Gout

Gout can be defined as a condition of uric acid disruption in the body. It is the utmost studied and scrutinized class of arthritis. When monosodium urate crystals (MSU) deposit in the tissues, it leads to the presentation of Gout. Uric acid crystals are formed when serum uric acid (SUA) increases over a specific limit and accounts for the main cause of deposition of uric acid crystals.(25, 26) Along with increase in SUA, it is believed that genetic predisposition play a part in the occurrence of gout.(25, 26) MSU crystals are majorly found in all the tissues present in and around the joints and this deposition of crystals is known as tophi.(25, 27) Gout can be diagnosed by identifying MSU crystals or by tophi aspirate method. Initial indication of gout is severe joint inflammation which can be rapidly treated by colchicine or NSAIDs. Delayed indications are renal stones and tophi.(25, 28) Main aim in the treatment of gout is the reduction of SUA levels below deposition threshold. This can be achieved by modification of diet or by using medication. This dissolve the MSU crystals and prevents further attacks.(29, 30)

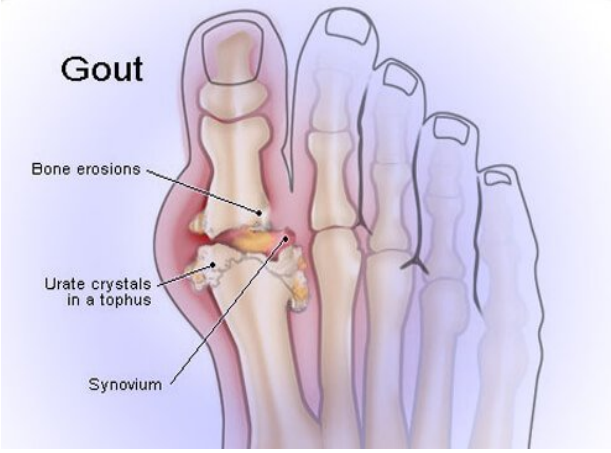

Figure 1.2: Pathophysiology of Gout(25)

1.1.2.1. Prevalence

Worldwide gout is found in 1-4% of the total population. In western countries, 3-6% men suffer from gout and occurrence in women is 1-2%. In some countries, an increase of 10% in the cases is also reported. Population in the age group of more than 80 years, around 10% men and 6% women suffer from gout. Yearly incidence of gout is reported to be 2.68 per 1000 people. The occurrence of gout is 2-6 folds greater in men when compared to women.(25) World has witnessed an increase in gout cases due to improper diet and sedentary lifestyle such as junk food, lack of physical activity, increased number of obesity cases.(25)

1.1.2.2. Signs and Symptoms

Characteristic indications of gout are: Sudden onset of joint pain, Joint swelling, stiffness and redness. However, heat in the affected area is also observed.(25, 31)

1.1.2.3. Treatment Option

Majorly management of gout is done by controlling the pain, minimizing joint damage and improvement in the quality of life. Treatment of gouty arthritis might involve: medications, non-pharmacologic therapies (Proper diet), physical therapy, splints or joint assistive aids and surgery.(14, 32) Widely used gouty arthritic medications include: Analgesics, Non-steroidal anti-inflammatory drugs (NSAIDs), Corticosteroids, Colchicine, Xanthine oxidase inhibitors, Uricosurics.(14, 32-34)

1.1.2.4. Xanthine Oxidase Inhibitors

A **xanthine oxidase inhibitor** is responsible for the inhibition of xanthine oxidase, an enzyme responsible for the metabolism of purine.(14) As reported in humans, inhibiting xanthine oxidase results in the reduction of uric acid production, and various drugs that constrain xanthine oxidase are employed for the management of hyperuricemia and other similar medical situations like gout. Therefore, xanthine oxidase inhibitors qualify as potential candidates (like Allopurinol and Febuxostat) and are always preferred for the treatment of chronic gout.(14, 25)

1.1.2.5. Febuxostat

FBX (Febuxostat) is a new, highly effective, non-purine selective xanthine oxidase inhibitor that is responsible for the inhibition of both oxidized and reduced forms of xanthine oxidase. It has been reported that (FBX) when compared to allopurinol is more efficient in the reduction and maintenance of serum urate levels. FBX is marketed as a 40, 80 and 120 mg tablet to be administered once a day and are marketed under the names Fabulas, Feboxa, Febuget, Febucip etc. Bioavailability achieved after oral administration of FBX is 38% and experiences food interaction. Oral bioavailability of FBX is reduced because of its low (< 15 μg/ml) aqueous solubility and enzymatic degradation in live and intestine. Furthermore, due to food interaction, C_{max} FBX in blood is reduced by 38–49%.(35) Shortcomings that come with FBX are poor bioavailability, food affected absorption, gut-wall metabolism, gastrointestinal disturbances (nausea, diarrhea, stomach pain, ulcers, vomiting).(36-38)

If we deliver these drugs by transdermal route instead of conventional oral route, then the above-mentioned problems associated with the marketed formulations of FBX and TAC can be overcome. Enhancement in bioavailability by avoiding first pass metabolism of a drug and prevention gastrointestinal disturbances can also be achieved because gastrointestinal route of drug delivery is sidestepped by using transdermal route of drug delivery.

1.1.3. Transdermal Drug Delivery System

Shortcomings associated with common route of drug delivery such as oral and parenteral can be overcome by the **transdermal route of drug delivery.** TAC and FBX face a number of limitations like poor oral bioavailability, food dependent absorption, gastrointestinal disturbances, and first pass metabolism and these problems can be avoided by using the transdermal route. Additional advantages which come with the transdermal route are ease of use, first-pass metabolism is by-passed, controlled drug delivery, painless technique, etc.(39-41) In spite of all these advantages, this route is not generally favored for drug delivery because of the presence of outermost nonviable layer of epidermis, stratum corneum which proves to be a barrier for delivery of drug through skin.(39, 42-44) Depending on the area of the body, thickness of stratum corneum may vary between ten to several hundred micrometers. This layer comprises of layers of flattened, dead keratinocytes encircled by a lipid matrix, which behave as a brick-and-mortar system and given to its structure becomes the most difficult layer to penetrate.(39, 45) Thus, diffusion of drugs through this layer is limited. Therefore, stratum corneum proves to be a barrier for around 90% of drug molecules. However, almost all molecules permeate this layer to a certain extent, they are unable to attain minimal effective plasma concentration and remain ineffective.(39, 46, 47) The viable epidermis layer lies below stratum corneum and it is around 10 times thick compared to stratum corneum. Nonetheless, diffusion of drugs through this layer is significantly higher because of the hydration of living cells of this epidermis layer.(47) Below the epidermis, dermis layer is found and shows a thickness of around 1 millimeter which is 100 times the thicker than stratum corneum. A dermis layer comprises of small blood vessels, responsible for the distribution of drugs to systemic circulation.(47) Thus, if it is possible for the drug to reach epidermis and dermis layer of skin than it can be made available for systemic circulation. So, to overcome this limitation several mechanical or chemical approaches are used, to achieve successful delivery of drug through skin.(44, 48, 49)

Some of these approaches are mentioned below(47, 50, 51):

A. Use of chemical penetration enhancers-surfactants, co-solvents eg. Dimethysulphoxide (DMSO), Ethanol, Decanol, Propylene Glycol, Azones (e.g. laurocapram), Sodium Lauryl Sulphate

B. Novel drug delivery systems such as Ethosomes, Liposomes, Ultra Deformable Vesicles, Niosomes, Pharmacosomes, Emulsomes, Cubosomes, Nanoparticles etc.

C. Mechanical approach for drug delivery involves Micro needles injection techniques, Iontophoresis, Sonophoresis, Laser radiation, Application of pressure, Radio-Frequency, Magnetophoresis, Thermophoresis, Skin stretching, Suction ablation, Chemical modification, and Skin abrasion.

With prolonged used, it is reported that chemical penetration enhancers cause skin toxicity and irritation. Thus, for present work, various nanocarriers were employed for enhancement of transdermal permeation. For example, Wei Lei et al. prepared transdermal gel loaded with transfersomes containing TAC and noted that transfersomes-gel loaded TAC has higher effective concentration in epidermis and dermis layer when compared to traditional-gel and marketed ointment. Moreover they established that TAC loaded transfersomes have enhanced penetration of drug in skin and enhanced therapeutic efficacy improvement by performing in-vivo study.(52) Sanju Singh et al. prepared the niosomal gel of FBX and proved the transdermal potential and anti-gout efficacy of developed formulation.(53)

1.1.4. Novel Drug Delivery System (Cubosomes)

In the present day, many novel drug delivery systems are available for transdermal delivery of drug due to their merit of deeper penetration in skin like liposome(54), niosome(55), ethosome(56), ultra-deformable vesicles(57), nanoparticles(58), cubosomes(59, 60) etc. Among the mentioned novel dosage forms, after a thorough literature search and study, cubosomes are selected for the present study due to its significant advantages over other drug delivery systems like biocompatibility, GRAS status, cheaper raw materials, bioadhesiveness, high drug loading, thermodynamic stability, and entrapment, ease of manufacturing, deeper skin penetration etc.(61-63)

“Cubosomes” are the “sub-micron, discrete, nanostructured particles of the bicontinuous cubic liquid crystalline phase in aqueous phase. The term "bicontinuous"

used in the definition of cubosomes describes two individual hydrophilic areas which is separated by the bilayer.(64, 65) The structure of bicontinuous cubic crystalline materials is reported to be suitable for controlled release formulation making it a popular area of research. Cubosomes are colloidal dispersions having size range of 100 to 300 nm which can be prepared by dispersing the bicontinuous cubic liquid crystalline structures in aqueous medium having surface active agents.(64-66) As research on cubosomes is increasing day by day, it has been reported that cubosomes when used as nanocarrier have a promising future in the applications of drug industry. Various classes of drugs like hydrophilic, lipophilic and amphiphilic drugs can be encapsulated by cubosomes. Total seven types of cubic phases have been identified till the present day: *Ia3d* (Q^{230}), *Pn3m* (Q^{224}), *Im3m* (Q^{229}), *Fm3m* (Q^{225}), *Pm3n* (Q^{223}), *Fd3m* (Q^{227}), *P6$_3$Immc* and *P4$_3$32*. Q^{223} and Q^{227} discontinuous in nature while other cubic phases are bicontinuous.(67)

The structure cubosomes is distinctive and comprises of curved bicontinuous lipid bilayer expanded in three dimensions with two interpenetrating hydrophilic water channel. However, distinctive nanochannels have interfacial area of around 400 m^2/g. A lipid bilayer is around 3 nm thick whereas the approximate diameter of swollen aqueous nanochannels is 5 nm.(68-70) Phytantriol and Glyceryl monooleate are mostly employed as amphiphilic lipid for preparing cubosomes.(64, 66, 71) Cubosomes can entrap lipophilic, amphiphilic and hydrophilic guest molecules due to its compartmental like structure. In this structure, lipophilic drugs entrap into lipid bilayer of cubosomes, while hydrophilic molecules can be situated in the water channels or near the polar portion of emulsifier, and amphiphilic molecules are positioned at the interface.(72)

1.1.5. Mechanical Approach for Drug Delivery (Microneedles)

Majority of the times, such nanocarriers are not able to sufficiently enhance the transdermal permeation and achieve desired plasma concentration, and there comes the need of use of some physical penetration enhancement techniques. It was reported by Eneko Larraneta et al. that the parallel working of nanomedicine and microneedle technology strategies have been possible due to the rapid progression of new scientific/fabrication/manufacturing technologies. Few of the nanomedicines are being delivered by microneedles such as lipidic vesicle, lipid nanoparticles, polymeric nanoparticle and microparticle, microemulsion, metallic and mineral nanoparticles

etc.(44) Among the many techniques described above, application of microneedles proves to be advantageous as it facilitates the permeation across the toughest barrier, stratum corneum. Another advantage of microneedles is that, they are very short length and have narrow diameter, and so are incompetent to reach the nerve endings and are thus painless.(46) Essential advantages of microneedles are: lack of pain, delivery of large molecules efficiently, patient compliance, avoidance of first pass metabolism, target based delivery, minimal potential for tissue trauma from an injection.(39, 41) Microneedles are also a potential choice for vaccine delivery because of the presence of antigen-producing cells such as the Langerhans cells and the dendritic cells in the skin and thus microneedles give better immune response as compared to intramuscular or subcutaneous injection.(73, 74) Other advantages include improvement of enhanced shelf life of drugs stored as dry coating in solid-coated microneedles even at room temperature, useful for systemic delivery of drug, even facilitates controlled delivery of drug, hollow microneedles could be used for the extraction of fluid from body for diagnostic purpose such as blood glucose measurement (42, 43, 73, 75, 76)

Various materials can be used in the fabrication of microneedles such as metal, silicon, titanium, glass or maltose.(39, 77) They can be either biodegradable or non-biodegradable. Biodegradable or soluble microneedles are prepared using materials such as amylopectin, polyvinyl alcohol-poly vinyl pyrrolidone, poly lactic acid, PLGA etc.(46) They are preferred over the non-biodegradable ones as the accidental breakage of the later during insertion may lead to complications like sepsis due to the broken part of microneedles.(46, 78) Thus, for the present project, microneedles were fabricated using hydrophilic biodegradable polymers. A dissolving microneedle can be prepared using water soluble materials or polymers likes polyvinyl alcohol 600, sucrose, lactose, maltose etc. It will dissolve in skin due to hydration of dermis layer and release the entrapped drug to the dermis layer of the skin.(79, 80) Different types of microneedles are available like solid microneedle, coated microneedle, hollow microneedle, biodegradable microneedle, hydrogel microneedle etc.(44) Microneedle can be applied using various techniques such as coat and poke technique, poke with patch technique, poke and release technique, poke and flow and so on. Microneedle have been prepared in different size and shape like arrow shaped, triangular, rounded etc. with or without bore, for various

application.(46) Length of a typical microneedle is 150-1500 µm, while width base diameter is 50-250 µm and tip diameter is 1-25 µm and they have the capacity to make microchannels into the skin to facilitate the passage of the molecule.(44) With advancement in technology various type of microneedle can be prepared. Critical factors which affect the transdermal permeation of microneedle are depth of permeation, coating strategies, microneedle geometry and force of penetration.(44, 46, 75, 81)

1.1.6. Aim and Objectives

Objective of the current study is to formulate and optimize cubosomes of FBX (Xanthine Oxidase Inhibitors) and TAC (immunosuppressant) and to further incorporate them in to fast dissolving polymeric microneedles patch and to evaluate their potential for transdermal application via ex-vivo and in-vivo studies with following objectives:

- Enhancement in permeation of drug(s)
- Sustained drug release for prolonged period
- Improvement of bioavailability
- Sidestepping gastro-intestinal disturbances associated with selected drugs
- Reduction in dose and dosing frequency
- Improvement in patience compliance
- Effective treatment or management of both types of arthritis

1.1.7. Plan of Work

- Review of literature
- Procurement of drugs and excipients
- Authentication of drug samples and drug-excipients compatibility study
- Development of analytical methods and their validation (UV/HPLC)
- Development and statistical optimization of FBX loaded Cubosomes
- Development and statistical optimization of TAC loaded Cubosomes
- Development and statistical optimization of fast dissolving polymeric microneedle patch
- Loading of optimized cubosomal formulation of FBX/TAC in fast dissolving polymeric microneedle patch
- In-vitro characterization of developed formulations

- Ex-vivo permeability and safety evaluations of developed formulations
- Short term stability studies as per ICH guidelines
- In vivo pharmacokinetic and pharmacodynamic studies

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