Three

Objectives and Work plan
3.1 RATIONALE AND OBJECTIVES OF THE STUDY

Osteo-arthritis is the most common of the rheumatic diseases and is the principal source of pain and disability in the elderly. The prevalence of this disease increases with age. 10-20% of the population over the age of 65 suffer from this disease. Currently over 50% of oral NSAIDs are prescribed for osteoarthritis. NSAIDs (Non Steroidal Anti-inflammatory Drugs) are amongst the most widely used of all therapeutic agents. They are frequently prescribed for rheumatic musculoskeletal complaints. They are the drugs of first choice for the management of a variety of acute and chronic inflammation and chronic degenerative orthopathies. The major drawback to anti-inflammatory drug use is the preponderance of gastrointestinal side effects encountered with majority of agents. These are generally recognized to be due to interference by the drug with the biosynthesis of prostaglandins (PGs) and other arachidonic acid metabolites in the gastric mucosa. These side effects can reduce patient compliance and discourage physician from prescribing them. The most common GI adverse effects include GI perforations, ulcerations and bleeding, which may require hospitalization. There is, therefore a need for a delivery system for NSAIDs with improved GI tolerability, which retains its efficiency. The NSAID mediated toxicity is often dose related. Thus reduction in serum concentration should also lessen the risk of potentially serious systemic adverse effects secondary to NSAID induced prostaglandin inhibition viz. acute renal insufficiency, nephritic syndrome, NSAID gastropathy, prolonged bleeding time and fluid retention. This leads the need of an alternative route of administration, which can bypass the gastrohepatic metabolism of the drug. Transdermal route is an alternative choice of route of administration for such drugs. This route is a self-contained discrete system which when applied to the intact skin, delivers the drugs through the skin at a controlled rate to the systemic circulation. In case of application of ointments, solutions and lotions on to the dermal tissues, it is difficult to expect their effects for a significant period of time because -wetting, temperature, movement etc. easily remove them. New formulations with better permeation which show sustained effects are required. Thus, it is anticipated that transdermal delivery of NSAIDs will result in the release
of drug at appropriate rate to maintain suitable plasma drug levels for the therapeutic efficacy by using skin as the port of the entry of drugs.

It is also anticipated that developed formulation will offer the following advantages:

[i] Sustained anti arthritic, anti-inflammatory and analgesic activity leading to improvement in overall therapy of the anti arthritic condition and better management of pain and inflammation.

[ii] Better patient compliance and good tolerability after prolonged treatment in the elderly patients.

[iii] Suitable for drugs which undergo hepatic "first-pass" metabolism.

[iv] Suitable for effective therapy overnight.

[v] Easy termination of medication leading to better patient compliance.

The aim of the study was to develop a low dose transdermal dosage form for NSAID drugs, which undergo hepatic first-pass metabolism and show low bioavailability. We plan to design our formulation in such a way that it provides the delivery of drug at a controlled rate across intact skin to achieve a therapeutic effective drug level for a longer period of time. Since this formulation would be delivered by transdermal route, it would by-pass hepatic first pass metabolism and hence would provide higher bioavailability compared to conventional dosage forms. With this background the main objectives of the present study were to:

a. develop a stable and reproducible drug delivery system.

b. reduce side effects due to the optimization of the blood concentration time profile.

c. extend duration of activity, which allows greater patient compliance

     owing to elimination of multiple dosing schedules.

d. to obviate specific problems associated with the oral administration of drugs e.g.,

     G.I.T. irritation, low absorption, first pass effect, formation of metabolites that cause
     side effects and short half life necessitates frequent dosing.
Nanoemulsions (being a versatile technology) have the potential to increase the permeability and bioavailability of drug in many ways. They act as supersolvent for the drug. They can solubilize hydrophilic and lipophilic drugs and therefore improve the bioavailability of Class II (solubility limited poor bioavailability) and Class IV drugs (poor bioavailability due to both poor solubility and poor permeability). This is due to the existence of microdomains of different polarity within the same single-phase solution. The dispersed phase, lipophilic or hydrophilic (o/w or w/o nanoemulsions) can behave as a potential reservoir of lipophilic or hydrophilic drugs respectively. The drug partitions between dispersed and continuous phase, and when the system comes into contact with the skin, the drug can be transported through the barrier thus increasing the bioavailability and permeability of Class III drugs (permeability limited poor bioavailability). Drug release with pseudo zero order kinetics can be obtained, depending on the volume of the dispersed phase, the partition of the drug and the transport of the drug. The droplet size of nanoemulsion is less than 100 nm, thus it can be sterilized by filtration. The small size of droplets in nanoemulsion yields very large interfacial area, from which the drug can quickly be released into the external phase, when absorption takes place, maintaining the concentration in the external phase close to the initial levels. Because of the thermodynamic stability, nanoemulsions are easy to prepare and require no significant energy contribution during preparation and thus scale up of technology is easy. The use of nanoemulsions as drug delivery system can improve the efficacy of drug, allowing the total dose to be reduced thus minimizing side effects.

Therefore in the present study an attempt has been made to prepare and evaluate nanoemulsions for transdermal delivery of celecoxib (CXB) and acedofenac.

The specific objectives of the study were to:

1. enhance the permeability and solubility of the poorly soluble drugs, celecoxib and acedofenac by using nanoemulsification as the formulation technique.
2. develop pseudo ternary phase diagrams and characterize the optimized formulation.
3. evaluate the solubility and drug release in in vitro models.
4. evaluate the solubility and bioavailability enhancement of the drug in in vivo models and compare it with the conventional formulations.
5. evaluate pharmacodynamics effects of the optimized formulations.
6. predict shelf life of the optimized formulations.
Chapter 3

Objectives and work plan

3.2 SELECTION OF DRUGS

For the formulation and development of transdermal therapeutic system (TTS) the drug must possess the following characteristics:

- The drug should possess favorable oil: water partition coefficient i.e., it should be sufficiently lipophilic. The log $P$ should be in the range of 1-3.
- The molecular weight of the drug should be less than 1000; because if molecular weight is high, there is a problem of penetration of the drug through the stratum corneum as molecular weight controls diffusion process.
- Molecular size of the drug should be less than 500 daltons.
- Melting point of the drug should be less than 200° C.
- Hydrogen bonding groups should be less than 2.
- The drug should be non irritant as well as non toxic to the skin.
- The drug should not stimulate an immune reaction in the skin.
- Drug should be potent i.e. the daily systemic dose should be less than 20 mg.
- The drug should undergoes extensive hepatic first pass metabolism.
- The drug should have a low biological half life.
- Drugs used for chronic ailment like hypertension and osteoarthritis are good candidates for transdermal drug delivery.

As the drugs celecoxib (CXB) and aceclofenac possess most of the above characteristics, therefore these drugs were selected as model drugs for transdermal drug delivery system.

The important characteristics of CXB are

- Poor oral bioavailability – 20-40 %
- Molecular weight - 381.38
- Melting point - 157 - 163° C
- Hydrogen binding groups - 1
- No adverse report of skin irritation thus is quite safe for use on skin
- It undergoes extensive hepatic first pass metabolism
- Partition coefficient, log $P$ -3.683
- It is used for chronic ailment like osteoarthritis and rheumatoid arthritis, thus a good candidate for transdermal drug delivery.
The important characteristics of aceclofenac are:
- It undergoes hepatic first pass metabolism
- Molecular weight - 354.2
- Melting point - 149-153°C
- Partition coefficient, \( \log P \) (octanol/water) -4.53
- No adverse report of skin irritation
- Hydrogen binding groups - 2
- Half life - 4 h
- It is used for chronic ailment like osteoarthritis and rheumatoid arthritis, thus a good candidate for transdermal drug delivery.

3.3 CHOICE AND SELECTION OF DRUGS
3.3.1 Celecoxib: A Drug Profile
3.3.1.1 Physicochemical properties
(Physician Desk Reference, 2005)

Structure:

\[
\text{Generic name: Celecoxib} \\
\text{Chemical name: } 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1Hpyrazol-1-yl]
\text{benzenesulfonamide} \\
\text{Trade name: } -Celecoxib \\
\quad \text{FDA Application No.: } (\text{NDA}) \ 020998 \\
\quad \text{Company: GD SEARLE} \\
\quad \text{Original Approval or Tentative Approval Date: December 31, 1998} \\
\quad \text{- Celib (India)} \\
\quad \text{-Celebrax (Pfizer)}
\]
Chapter 3

Objectives and work plan

Molecular formula: C_{17}H_{14}F_{3}N_{3}O_{2}S
Molecular weight: 381.38.
Category: NSAID (COX-2 inhibitor)
Physical form: White Powder
Melting point: 157 – 163°C (Diwan et al., 2004)
pKa: 11.1 (Diwan et al., 2004)
Partition Coefficient: 3.683 (logP) (Diwan et al., 2004)
Half life: 11h
Protein binding: 97% (Albumin & α1-acid glycoprotein)
Volume of distribution: 400L
Bioavailability: 22 – 40% (Capsule) (Susan et al., 2001)

Table IV: Solubility data of celecoxib (Seidher and Bhatia, 2003)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>Methanol</td>
<td>113.94</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>63.346</td>
</tr>
<tr>
<td>4</td>
<td>Butanol</td>
<td>29.030</td>
</tr>
<tr>
<td>5</td>
<td>Octanol</td>
<td>7.870</td>
</tr>
<tr>
<td>6</td>
<td>Ethylene glycol</td>
<td>3.856</td>
</tr>
<tr>
<td>7</td>
<td>Propylene glycol</td>
<td>30.023</td>
</tr>
<tr>
<td>8</td>
<td>Polyethylene glycol (PEG) 400</td>
<td>414.804</td>
</tr>
</tbody>
</table>

3.3.1.2 PHARMACOLOGY (Hamid et al., 2001; Srinivasu et al., 2002)

Celecoxib is a nonsteroidal anti-inflammatory agent that exhibits anti-inflammatory, analgesic, and antipyretic activities with selective cyclooxygenase-2 inhibitory activity. In animal colon tumor models, celecoxib reduced the incidence and multiplicity of tumors. The cyclooxygenase-2: cyclooxygenase-1 ratio for celecoxib (based on concentrations of the drug to produce 50% inhibition) is approximately 0.003. It does not inhibit the cyclooxygenase-1 (COX-1) isoenzyme.

It acts by suppression of production of prostaglandin E2 at inflammation sites via inhibition of the cyclooxygenase-2 isoform. Selective cyclooxygenase-2 (COX-2) inhibitors are known to inhibit the production of vascular prostacyclin (PGI2), an inhibitor of platelet aggregation and a vasodilator.
Chapter 3 Objectives and work plan

It has no effect on reduction of platelet aggregation or increase in bleeding time so is not a substitute for aspirin for cardiovascular prophylaxis. Inhibition of PEG2 synthesis may lead to sodium and water retention through increased reabsorption in the renal loop of Henle. In the collecting ducts, PGE2 appears to inhibit water reabsorption by counteracting the action of antidiuretic hormone. Unlike conventional non-steroidal anti-inflammatory drugs, COX-2 inhibitors do not reduce the endogenous production of thromboxane A2, a potent platelet activator and aggregator, thereby causing a potentially prothrombotic cascade of events that could lead to a significant increase in the risk for thrombotic cardiovascular events (myocardial infarction, occlusive stroke) in patients receiving celecoxib therapy. Selective cyclooxygenase-2 inhibition is claimed to reduce adverse effects typically observed with this class of drugs.

3.3.1.3 PHARMACOKINETICS (Saha et al, 2002)

Peak plasma levels of celecoxib occur approximately 3 h after an oral dose. Celecoxib is highly protein bound (~97%) within the clinical dose range. It binds primarily to albumin and, to a lesser extent, α1-acid glycoprotein. The apparent volume of distribution at steady state (Vss/F) is approximately 400 L, suggesting extensive distribution into the tissues. Celecoxib is not preferentially bound to red blood cells. Celecoxib is extensively metabolized in the liver via cytochrome P450 2C9 to 3 inactive metabolites, such as a primary alcohol, the corresponding carboxylic acid and its glucuronide conjugate. These metabolites are inactive as COX-1 or COX-2 inhibitors. It is eliminated via the kidney (27%) and faeces (57%). Less than 3% is eliminated as unchanged drug. The apparent plasma clearance (CL/F) is about 500 mL/min.

Coadministration of celecoxib with an aluminum and magnesium-containing antacid resulted in a reduction in plasma celecoxib concentrations with a decrease of 37% in cmax and 10% in AUC. Celecoxib, at doses up to 200 mg BID can be administered without regard to timing of meals. Higher doses (400 mg BID) should be administered with food to improve absorption. The daily recommended dose of celecoxib should be reduced by approximately 50% in patients with moderate (Child-Pugh Class B) hepatic impairment. The use of celecoxib in patients with severe hepatic impairment is not recommended.
3.3.1.4 INDICATIONS AND USAGE

Celecoxib is indicated:
1) For relief of the signs and symptoms of osteoarthritis.
2) For relief of the signs and symptoms of rheumatoid arthritis in adults.
3) For the relief of signs and symptoms of ankylosing spondylitis.
4) For the management of acute pain in adults.
5) For the treatment of primary dysmenorrhea.
6) To reduce the number of adenomatous colorectal polyps in familial adenomatous polyposis (FAP) and as an adjunct to usual care (e.g., endoscopic surveillance, surgery).

3.3.1.5 SIDE EFFECTS (Hamid et al., 2001)

The common gastrointestinal adverse effects include: dyspepsia, diarrhea, abdominal pain and flatulence. NSAIDs, including celecoxib, can cause serious gastrointestinal events including bleeding, ulceration, and perforation of the stomach, small intestine or large intestine, which can be fatal. These serious adverse events can occur at any time, with or without warning symptoms, in patients treated with NSAIDs.

3.3.1.6 Analytical Methodology

Spectrophotometric Method

Saha et al., 2002, reported a UV method for preparation of calibration curve and estimation of celecoxib from commercial capsule formulation. The $\lambda_{\text{max}}$ of celecoxib was determined by scanning a suitable dilution of stock. The $\lambda_{\text{max}}$ was found to be 251 nm in acetonitrile & sodium phosphate buffer (pH 5.6).

HPLC Method

Many chromatographic methods are available for the assay of CXB in bulk drug, pharmaceutical formulations and biological fluids. List of chromatographic methods reported for the assay of CXB are shown in Table V.

A micellar electrokinetic chromatographic method was developed, for the quantification of celecoxib by Srinivasu et al., 2002.

Capillary electrophoresis was performed using an Agilent CE system (Agilent technologies, Waldbronn Germany) with build in diode array detector able to deliver up to 30KV. An
extended light path capillary with a 50 mm inner diameter used was of 48.5 cm length. Detection wavelength was set at 252 nm. The background buffer consisted of 25 mm aqueous borate buffer (pH 9.3).

Assay of celecoxib in formulation sample was carried out. The contents of four capsules were finely grounded in an agate mortar and pestle. The ground material equivalent to 40 mg of celecoxib was weighed and transferred to a volumetric flask and extracted into acetonitrile by vortex mixing followed by ultrasonication. This solution was used as a stock solution to prepare test solutions.

**Table V: Chromatographic data of CXB**

<table>
<thead>
<tr>
<th>Sample matrix</th>
<th>Column</th>
<th>Mobile phase (v/v)</th>
<th>Detector (nm)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical dosage forms</td>
<td>Inertsil C$_{18}$ (4.6 × 250 mm)</td>
<td>Water/acetonitrile (35:65)</td>
<td>UV 230</td>
<td>Schoenberg er et al., 2002</td>
</tr>
<tr>
<td>Bulk drug</td>
<td>Nonopak C$_{18}$ (3.9 × 300 mm)</td>
<td>Potassium dihydrogen phosphate (0.01 M) / acetonitrile (45:55)</td>
<td>UV 252</td>
<td>Jayasagar et al., 2002</td>
</tr>
<tr>
<td>Bulk drug and formulations</td>
<td>Chiralpak AD (4.6 × 250 mm)</td>
<td>Cyclohexane/ethanol (94:06)</td>
<td>UV 255</td>
<td>Hamid et al., 2001</td>
</tr>
<tr>
<td>Bulk drug and formulations</td>
<td>Hichrome C$_{18}$ (4.6 × 50 mm)</td>
<td>Potassium dihydrogen phosphate (0.01 M) / acetonitrile (45:55)</td>
<td>UV 253</td>
<td>Werner et al., 2005</td>
</tr>
<tr>
<td>Formulations</td>
<td>RP C$_{18}$</td>
<td>Methanol/water (85:15)</td>
<td>MS</td>
<td>Jalalizadeh et al., 2004</td>
</tr>
<tr>
<td>Human plasma</td>
<td>Hypersil C$_{18}$ (4.6 × 250 mm)</td>
<td>Potassium dihydrogen phosphate (0.01 M) / acetonitrile/methanol (40:30:30)</td>
<td>UV 238</td>
<td>Kousy, 1999</td>
</tr>
<tr>
<td>Biological fluid</td>
<td>Novapak C$_{18}$ (3.9 × 150 mm)</td>
<td>Acetonitrile/ammonium acetate (gradient flow)</td>
<td>MS</td>
<td>Giachitti et al., 1994</td>
</tr>
</tbody>
</table>
3.3.2 Aceclofenac: A Drug Profile

3.3.2.1 Physicochemical properties

(Yamazaki et al., 1997)

Structure:

![Chemical structure of Aceclofenac](image)

Generic name: Aceclofenac

Chemical name: 2-[[2-[2-[2, 6-dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid

Molecular formula: $\text{C}_{16}\text{H}_{13}\text{Cl}_2\text{NO}_4$

Molecular weight: 354.2

Category: NSAID

Physical form: White crystalline powder

Melting point: 149 - 153°C (Reginster et al., 2001)

pKa: 9.2

Partition Coefficient: 4.53 (log P) (Reginster et al., 2001)

Half life: 4h (Gonzalez et al., 1994)

Protein binding: >99 % (Gonzalez et al., 1994)

Volume of distribution: 25 L (Gonzalez et al., 1994)

Bioavailability: 60 - 70% (Gonzalez et al., 1994)

Solubility: Aceclofenac is practically insoluble in water, freely soluble in acetone or soluble in alcohol (Yamazaki et al., 1997)
The mode of action of aceclofenac is largely based on the inhibition of prostaglandin synthesis. Aceclofenac is a potent inhibitor of the enzyme cyclooxygenase, which is involved in the production of prostaglandins. It inhibits synthesis of the inflammatory cytokines interleukin (IL)-1, tumor necrosis factor and prostaglandin E2 (PGE2) production.

Aceclofenac has shown stimulatory effects on cartilage matrix synthesis that may be linked to the ability of the drug to inhibit IL-1 activity. There is also evidence that aceclofenac stimulates the synthesis of IL-1 receptor antagonist in human articular chondrocytes when subjected to inflammatory stimuli. 4'-hydroxyaceclofenac has chondroprotective properties attributable to suppression of IL-1 mediated promatrix metalloproteinase production and proteoglycan release.

In patients with osteoarthritis of the knee, aceclofenac decrease pain reduces disease severity and improves the functional capacity of the knee. It reduces joint inflammation, pain intensity and the duration of morning stiffness in patients with rheumatoid arthritis. The duration of morning stiffness and pain intensity are reduced and spinal mobility improved, by aceclofenac in patients with ankylosing spondylitis.

Aceclofenac is rapidly and completely absorbed after oral administration, peak plasma concentrations (C_max) are reached 1 to 3 hours after an oral dose. The drug is highly protein bound (7.99%). The presence of food does not alter the extent of absorption of aceclofenac but the absorption rate is reduced. The plasma concentration of aceclofenac is approximately twice that in synovial fluid after multiple doses of the drug in patients with knee pain and synovial fluid effusion. Aceclofenac is metabolized to a major metabolite, 4'-hydroxyaceclofenac and to a number of other metabolites including 5-hydroxyaceclofenac, 4'-hydroxydiclofenac, diclofenac and 5-hydroxydiclofenac. Renal excretion is the main route of elimination of aceclofenac with 70 to 80% of an administered dose found in the urine, mainly as the glucuronides of aceclofenac and its metabolites of each dose of aceclofenac, 20% is excreted in the faeces. The plasma elimination half-life of the drug is approximately 4 hours.
3.3.2.4 Drug interactions
Aceclofenac may increase plasma concentrations of lithium, digoxin and methotrexate, increase the activity of anticoagulant, inhibit the activity of diuretics, enhance cyclosporin nephrotoxicity and precipitate convulsions when co-administered with quinolone antibiotics. Furthermore, hypo or hyperglycaemia may result from the concomitant administration of aceclofenac and antidiabetic drugs, although this is rare. The coadministration of aceclofenac with other NSAIDs of corticosteroids may results in increased frequency of adverse event.

3.3.2.5 Adverse drug reaction
Aceclofenac is well tolerated, with most adverse events being minor and reversible and affecting mainly the GI system. Most common events include dyspepsia (7.5%), abdominal pain (6.2%), nausea (1.5%), diarrhea (1.5%), flatulence (0.8%), gastritis (0.6%), constipation (0.5%), vomiting (0.5%), ulcerative stomatitis (0.1%), pancreatitis (0.1%). Although the incidence of gastrointestinal adverse events with aceclofenac was similar to those of comparator NSAIDs in individual clinical trials, withdrawal rates due to these events were significantly lower for aceclofenac than with ketoprofen and tenoxicam.

Other adverse effect, which is not common includes dizziness (1%), vertigo (0.3%), and in rare cases: par aesthesia and tremor.

3.3.2.6 Dosage and administration (Movilia et al., 1989)
The usual dose of aceclofenac is 100 mg given twice daily by mouth, one tablet in the morning and one in the evening. There is no evidence that the dosage of aceclofenac needs to be modified in patients with mild renal impairment, but as with other NSAIDs caution should be exercised.

Aceclofenac balanced Cox inhibitor
It has been suggested that aceclofenac blocks PGE2 production via cyclo-oxygenase (cox)-1 and cox-2 inhibition after intracellular metabolism to 4'-hydroxy aceclofenac and diclofenac in human rheumatoid synovial and other inflammatory cells. However data from human whole blood assays show inhibition of cox-2 (with minimal effects on cox-1) by both the parent compound and 4'-hydroxy aceclofenac.

Aceclofenac may prevent the degradation of articular connective tissue in patients with rheumatoid arthritis and osteoarthritis and this should be classified as unique NSAIDs.
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Aceclofenac may prevent the degradation of articular connective tissue in patients with rheumatoid arthritis and osteoarthritis and this should be classified as unique NSAIDs.
Aceclofenac - clinical efficacy (Schattenkirchner et al., 2003)
In large trials of 2 to 6 months duration, aceclofenac significantly reduced pain and improves functional capacity and mobility relative to baseline in patients with osteoarthritis, rheumatoid arthritis or ankylosing spondylitis and reduces inflammation in patients with rheumatoid arthritis.

Aceclofenac in osteoarthritis
In patients with osteoarthritis of the knee, aceclofenac decreases pain, reduces disease severity and improves the functional capacity of the knee to a similar extent to diclofenac, piroxicam, and naproxen.

Aceclofenac in rheumatoid arthritis
The anti-inflammatory and analgesic efficacy of aceclofenac is similar to that of ketoprofen, indomethacin, tenoxicam and diclofenac in patients with rheumatoid arthritis. In randomized, double blind trials in 169 to 261 patients, aceclofenac (100 mg twice daily for 3 or 6 months) significantly reduced relative to baseline joint inflammation, pain intensity and the duration of morning stiffness and improved handgrip strength.

Aceclofenac in ankylosing spondylitis
The duration of morning stiffness and pain intensity are reduced and spinal mobility improved, by aceclofenac in patients with ankylosing spondylitis, with improvements being similar to those observed with indomethacin, naproxen or tenoxicam. These effects were observed after aceclofenac 100 mg twice daily was given for 3 months in randomized, double blind trials involving 104 to 308 patients.

Aceclofenac in dental pain
The analgesic efficacy as single doses of aceclofenac was assessed in patients with moderate to severe tooth pain and in extraction of impacted third molars. The analgesic efficacy of single doses of aceclofenac 50, 100 and 150 mg was greater than that of placebo in patients with moderate to severe tooth pain or pain caused by extraction of impacted third molars.

Aceclofenac in postoperative pain
The analgesic efficacy of aceclofenac has been shown in comparisons with paracetamol in women undergoing episiotomy. Aceclofenac 100 mg was superior to paracetamol 650 mg in providing relief from postepisiotomy pain, particularly 3 to 5 hours after ingestion.
Aceclofenac in Dysmenorrhoea
In a more recent noncomparative study in 1338 women with dysmenorrhoea treated for first 3 days of 2 consecutive cycles, it was reported that aceclofenac was very effective.

Aceclofenac in acute lumbago
Aceclofenac (150 mg intramuscularly for 2 days, then 100 mg orally, both twice daily) was superior to diclofenac in alleviating functional impairment in a 7 days study in 100 patients with acute lumbago. Aceclofenac 100 mg twice daily was associated with symptomatic relief of acute low back pain in a non-comparative study in 67 patients.

Aceclofenac in musculoskeletal trauma
Aceclofenac 100 mg twice daily has also been assessed in patients with musculoskeletal trauma, although only non-comparative studies are available.

Aceclofenac in gonalgia (Knee pain)
A controlled double blind study was performed with aceclofenac comparing it with diclofenac in 40 patients with acute or chronic gonalgia. The results of the trial indicated slightly superior activity, although there was no statistically significant difference between two drugs.

3.4 PLAN OF WORK
In an attempt to develop and evaluate the effectiveness of nanoemulsion formulations for transdermal delivery of CXB and aceclofenac, following plan of work was envisaged.

1. Characterization of drugs
   1.1 Physical characterization of CXB
   a. Physical properties
   b. Identification tests
      i. Fourier transform infrared absorption (FTIR) spectra
      ii. U.V. spectral analysis
      iii. Differential scanning calorimetry (DSC) analysis
   c. Analytical methodology for CXB
      i. Preparation of working solution
      ii. Determination of absorption maxima ($\lambda_{\text{max}}$)
      iii. Development and validation of HPLC method
      iv. Preparation of calibration curve
d. Determination of solubility of CXB

e. Effect of storage on stability of drug solution

1.2 Physical characterization of aceclofenac

a. Physical properties

b. Identification tests
   i. Fourier transform infrared absorption spectrum
   ii. U.V. spectral analysis
   iii. DSC spectral analysis

c. Analytical methodology for aceclofenac
   i. Preparation of working solution
   ii. Determination of absorption spectra ($\lambda_{\text{max}}$)
   iii. Preparation of calibration curve
   iv. Determination of solubility of aceclofenac
   v. Effect of storage on stability of drug solution

2. Nanoemulsion components selection

3. Determination of solubility of CXB and aceclofenac in different components

4. Construction of pseudoternary phase diagrams for CXB and aceclofenac

5. Formulation development for CXB and aceclofenac

6. Physical stability studies for CXB and aceclofenac nanoemulsions

7. Characterization of CXB and aceclofenac nanoemulsions for
   a. Droplet size
   b. Viscosity
   c. Morphology using transmission electron microscopy (TEM)
   d. Refractive index
   e. Solubility in optimized formulations

8. In vitro skin permeation studies of CXB and aceclofenac
   a. Preparation of rat skin
   b. Stabilization of the skin
   c. In vitro skin permeation of nanoemulsion formulations
   d. Permeation data analysis

9. Skin irritation studies for optimized formulations
10. *In vivo* studies
   a. Pharmacodynamic studies (Anti-inflammatory studies)
   b. Pharmacokinetic studies (Bioavailability studies)

11. Determination of mechanism of skin permeation enhancement
   - FT-IR spectral analysis of treated and untreated rat skin
   - DSC studies of treated and untreated rat skin
   - Determination of activation of energy
   - Histopathological examination of skin specimens

12. Stability studies as per ICH guidelines