Chapter 3

Plan of Work and Objectives
The aim of the present research work was to develop and evaluate Sustained release tablets and nano emulsion gel for 6-gingerol and psoralen in combination which is extensively used in the treatment of vitiligo. Towards this goal the following objectives were identified and established.

3.1. Objectives of the Study

1. To formulate sustained release tablets of 6-gingerol and psoralen for oral administration so that the system would release the therapeutic agents at different levels over the required period of time and minimize undesirable side effects.

2. To develop a nanoemulsion based topical drug delivery system of 6-gingerol with psoralen for its anti-inflammatory activity and the pharmacodynamic evaluation of the optimized formulation.

3.2. Rationale of the Study

3.2.1. Rationale for selecting drug candidate

Vitiligo or leukoderma is a chronic skin disease that causes loss of pigment resulting in irregular pale patches of skin. It occurs when the melanocytes cells responsible for skin pigmentation, die or are unable to function. The precise cause of vitiligo is not fully understood, however some evidence suggests it is caused by a combination of autoimmune, genetic and environmental factors. The effected population worldwide is considered to be between 1% and 2% and is found almost all over the world (Osman et al., 2009). Systemic PUVA and Topical PUVA are the most common and effective treatment which can be taken by all classes of patient due to its economic considerations and ease of availability. The mechanism of action of phototherapy is certainly the most complicated one. Ultraviolet (UV) radiation promotes the proliferation and differentiation of melanocytes. However, UV radiation also induces apoptosis of Langerhans and T cells, and leads to local and systemic immunosuppression and promotion of Th2 response (Norval, 2000). Psoralen is the active ingredient used along with UV radiation in the range of 320 to 400 nm. Oral PUVA therapy is used in patients with extensive vitiligo. It is important to explain the chances of repigmenting and the associated short and long-term side effects. Although 70% to 80% of patients will experience the induction of pigment with oral psoralen treatments, less than 20% of patients have total repigmentation, and 30% to 40% of patients can expect to have only a partial treatment
response. Because of these statistics, it is important to be selective when choosing patients for oral PUVA therapy. Darker pigmented patients respond better to PUVA therapy because of the increased tolerance to greater cumulative UVA dosage, and children also experience repigmentation to a greater extent than adults (Maverakis et al., 2010). The potential side effects of PUVA therapy include PUVA burn, nausea, erythema, pruritus, xerosis, fatigue, carcinogenicity (including melanoma), pigmented lesions, cataracts and aging. Among all available treatments, PUVA found to be acceptable and cheaper for vitiligo but psoralen has its own side effects like nausea, itching, sun burns, blistering and redness of the skin. Amazingly, 6-gingerol could prevent these with its own anti inflammation, anti microbial and anti emetic activity reported and minimize severe and chronic side effects caused by psoralen effectively (Soni et al., 2010).

Fig. 3.1: Rationale of the study
3.2.2. Rationale for selecting delivery systems

The following were identified as a rationale for the selection of nanoemulsion gel and sustained release tablets as delivery systems of 6-gingerol with psoralen against minimizing the undesired effects of psoralen for the treatment of vitiligo.

3.2.2.1. Sustained release tablets

Conventional drug therapy typically involves the periodic dosing of a therapeutic agent that has been formulated in a manner to ensure its stability, activity and bioavailability. For most of the drugs, conventional methods of formulation are quite effective. However, some drugs are unstable and have a narrow therapeutic range, exhibit extreme solubility problems, require localization to a particular site in the body or require strict compliance or long-term use. In such cases a method of continuous administration of drug is desirable to maintain plasma drug levels. The goal in designing sustained or sustained delivery systems is to reduce the frequency of the dosing or to increase effectiveness of the drug by localization at the site of action, reducing the dose required or providing uniform drug delivery. So, sustained release dosage form is a dosage form that release one or more drugs continuously in a predetermined pattern for a fixed period of time, either systemically or to a specified target organ. Sustained release dosage forms provide a better control of plasma drug levels, less dosage frequency, less side effect, increased efficacy and constant delivery. During the last two decades there has been remarkable increase in interest in sustained release drug delivery system. This has been due to various factor viz. the prohibitive cost of developing new drug entities, expiration of existing international patents, discovery of new polymeric materials suitable for prolonging the drug release, and the improvement in therapeutic efficiency and safety achieved by these delivery systems.

Potential advantages of sustained release dosage forms

i] Patient Compliance: Lack of compliance is generally observed with long term treatment of chronic disease, as success of drug therapy depends upon the ability of patient to comply with the regimen. Patient compliance is affected by a combination of several factors, like awareness of disease process, patient faith in therapy, his understanding of the need to adhere to a strict treatment schedule. Also the complexity of
therapeutic regimens, the cost of therapy and magnitude of local and or systemic side
effect of the dosage form (Hui et al., 1987). The problem of lack of patient compliance
can be resolved to some extent by administering sustained release drug delivery system.

ii] Reduced 'see-saw' fluctuation: Administration of a drug in a conventional dosage
form [except via intravenous infusion at a constant rate] often results in 'see-saw' pattern
of drug concentration in the systemic circulation and tissue compartments. The
magnitudes of these fluctuations depend on drug kinetics such as the rate of absorption,
distribution, elimination and dosing intervals. The 'see-saw' or 'peak and valley' pattern is
more striking in case of drugs with biological half lives of less than four hours, since
prescribed dosing intervals are rarely less than four hours. A well-designed sustained
release drug delivery system can significantly reduce the frequency of drug dosing and
also maintain a more steady drug concentration in blood circulation and target tissue
cells.

iii] Reduced total dose: Sustained release drug delivery systems have repeatedly been
shown to use less amount of total drug to treat a diseased condition. By reducing the total
amount of drug, decrease in systemic or local side effects are observed. This would also
lead to greater economy.

iv] Improved efficiency in treatment: Optimal therapy of a disease requires an efficient
delivery of active drugs to the tissues, organs that need treatment. Very often doses far in
excess to those required in the cells have to be administered in order to achieve the
necessary therapeutically effective concentration. This unfortunately may lead to
undesirable, toxicological and immunological effects in non-target tissue. A sustained
release dosage forms leads to better management of the acute or chronic disease
condition (Hui et al., 1987).

3.2.2.2. Nanoemulsion gel as topical formulation

Nanoemulsions offer several advantages; for example, their solubilization capacity,
transparency, high stability, and simplicity of manufacture. Topical nanoemulsion can
avoid the marked disadvantages of other routes of administration. Nanoemulsion with
good stability and powerful permeation enhancing ability and suitable viscosity might be
a promising prospective carrier for topical delivery of lipophilic drugs. Some common advantages of nanoemulsions for topical delivery include:

- they have a much higher surface area and free energy that make them an effective transport system
- they avoid commonly found stability problems of emulsions like creaming, flocculation, coalescence, and sedimentation
- their versatility of application as nanoemulsions can be formulated in variety of formulations such as foams, creams, liquids, and sprays, can be easily applied to skin and mucous membranes due to non-toxic and non-irritant properties
- the surfactants employed in the preparation are Generally Recognized As Safe (GRAS) and
- nanoemulsions do not cause damage to healthy human cells

Another is the direct delivery and targetability of the drug to affected area. Both o/w and w/o nanoemulsions have been evaluated for this purpose (Devarajan and Ravichandran, 2011). Conversion of these nanoemulsions into gels (nanoemulsion gels) serves the purpose of attaining suitable viscosity and flow properties in order to achieve the optimum advantage of this novel drug delivery system on topical application. Carbopol® are the most commonly employed viscosity modifiers used to facilitate this objective.

3.3. Drug Profile:
3.3.1. Psoralen

Structural formula

The structural formula of psoralen is displayed in Fig. 3.2.

![Fig.3.2: Structural formula of psoralen](image-url)
Chemical/Physical Properties (Budavari, 1989; National, 2005)

Molecular Formula: \( \text{C}_{12}\text{H}_{6}\text{O}_{3} \)
Molecular Weight: 186.16 g/mol
Color/Form: White to off-white crystalline solid
Odor: odourless
Taste: characteristic taste
Melting Point: 158–161 °C
Octanol/Water partition coefficient: Log P value = 2
Solubilities: Soluble in ethyl acetate or acetone; insoluble in water

**Pharmacology**

The sunburn reaction in PUVA therapy is probably caused by singlet molecular oxygen generated by photoexcited psoralens. A relation has been observed between the ability to produce singlet molecular oxygen and the photosensitizing effect of the different psoralens (De Mol, 1981). The mechanism of the therapeutic efficacy of PUVA in the treatment of vitiligo is not yet fully clarified. A possible explanation is that after photoexcitation, psoralen is bound to the pyrimidine bases of DNA in the basal cell layer of the epidermis, thereby inhibiting the rapid duplication of cells in the epidermis. PUVA therapy with psoralen has been successfully used in the treatment of psoriasis and vitiligo since 1974. Irradiation usually takes place 2 h after oral administration of psoralen. The UV-A light dose is determined depending on skin type. Another possibility is determination of the minimal phototoxic dose (MPD), by testing the patients sensitivity to UV-A light after psoralen ingestion. Patients receive two to four treatments a week until clearing is obtained. During treatment the UVA dose is adjusted on the basis of MPD, skin type, presence or absence of erythema and response of the vitiligo lesions. The endpoint is complete clearing of lesions (i.e., more than 95% of the lesions have regressed). The therapeutic efficacy of PUVA has been tested in Europe and the U.S.A. in 2 multicentre trials (Henseler et al., 1981; Melski et al., 1977), which indicated that complete clearance was obtained by 65 and 88% of the patients respectively. Since PUVA therapy is only a symptomatic therapy relapses after initial therapy occur after about 5 months (Cormane et al., 1978). About the necessity and effectiveness of maintenance treatment there exists no consensus. However according to Roelandts (2002)
long-term maintenance treatment should be avoided as much as possible because of the risk of oncogenicity. For this reason PUVA therapy should be reserved for patients with moderate to severe forms of the disease that do not respond to other forms of therapy. Risk of skin cancer from PUVA therapy seems to be rather low if careful patient selection has been made and safety measures are taken. Other adverse effects of PUVA therapy like nausea, pruritus and erythema are the most commonly observed side effects.

**Dose**

Psoralen is administered orally in a dose of 0.5-0.6 mg/kg body wt. A large number of investigators in different countries have compared bioavailability of formulations containing psoralen in commercially available tablets and capsules (De Wolff and Thomas, 1986). T<sub>max</sub> is usually reached within 1-2 h after administration but large variations of T<sub>max</sub>, C<sub>max</sub>, and bioavailability have been observed. Irregular absorption is probably caused by slow dissolution of psoralen in water, because of its poor solubility in water. To overcome this problem, liquid oral formulations of psoralen have been developed. Higher, earlier (T<sub>max</sub> = 1 h) and more predictable maximum concentrations, and higher bioavailability were found after administration of psoralen dissolved in an aqueous solution ingested orally (Stolk et al., 1980). Comparable favourable results were observed with soft gelatin capsules containing psoralen dissolved in polyethylene glycol (PEG 400). A disadvantage with liquid oral preparations is that nausea is experienced more frequently. Besides oral administration, other methods of application have also been tested, e.g., topical and rectal (Siddiqui et al., 1984) using a micro-enema. Topical administration might be advantageous in that systemic toxicity might be lowered. However, after topical application of psoralen emulsion, plasma levels proved to be of the same order as those found after oral administration (Neild and Scott, 1982). Moreover it is rather difficult to achieve a uniform application on the skin; local burns due to overdosage and cosmetically unacceptable patchy pigmentation may occur. A rectal solution has been developed, using psoralen (0.75%) dissolved in glycofurol (50%), ethanol (10%) and water (40%). After rectal administration, maximum serum concentrations were reached very rapidly and predictably, 0.6 ± 0.2 h (mean ± SD, n = 31). The maximum concentration was about as high as with oral liquid preparations, although a lower dose (0.45 mg/kg compared to 0.6 mg/kg) was used. Because of rapid
and predictable absorption and subsequent quick elimination of psoralen, together with the absence of nausea, rectal administration might have certain advantages over the oral route.

**Metabolism and pharmacokinetics**

Absorption of psoralen is dependent on the formulation used. After oral administration of capsules or tablets, the maximum serum concentration is usually reached within 1-4h. Considerable inter- and intra-individual variation of both the time ($T_{\text{max}}$) and the height ($C_{\text{max}}$) of the maximum serum concentrations of psoralen are observed (Herfst and De Wolff, 1983). This variation is due to the variable absorption of psoralen from the different formulations and variability of the extent of first pass elimination of psoralen. After oral administration, psoralen is subjected to extensive, saturable "first pass elimination" (Schmid et al., 1980a), which means that after administration of a low psoralen dose, none or only a small amount of unchanged psoralen reaches the general circulation. With doses higher than the "breakthrough dose", liver enzymes are saturated and serum concentrations of psoralen rise rapidly. Therefore no linearity exists between dose and the height of the serum concentrations in the dose range around the breakthrough dose, 0.23 mg kg$^{-1}$. Pharmacokinetic behaviour of psoralen is influenced by food. $T_{\text{max}}$ is delayed and $C_{\text{max}}$ is lower under postprandial in comparison with fasting conditions. It is eliminated from the serum with a half-life of about 1 h. Photosensitivity of the skin rises and declines correspondingly with psoralen serum concentrations and nearly all psoralen administered is metabolized; only traces of unchanged psoralen have been detected in urine (Schmid et al., 1980b). Concentrations of psoralen in suction blister fluid (SBF) have been determined by Herfst and De Wolff, 1983, since concentrations in this fluid may reflect the concentrations at the receptor site in the skin more accurately; blister fluid resembles interstitial fluid and can therefore serve as a model for studying pharmacokinetics of psoralen in the skin. Concentrations in SBF are about one third of $C_{\text{max}}$ in serum, because psoralen is strongly protein bound, and the concentration of serum proteins in SBF is about 30% of the serum concentrations. The psoralen level in SBF is determined by the peak concentration in serum and remains constant as long as the serum concentration exceeds the SBF concentration (Herfst and
De Wolff, 1983). Reversible binding and concentration of psoralen in the epidermis has been demonstrated *in vitro* (Artuc et al., 1979).

3.3.2. 6-Gingerol

Structural formula

The structural formula of 6-gingerol is displayed in Fig. 3.3.

![Fig. 3.3: Structural formula of 6-gingerol](image)

**Chemical/Physical Properties (National, 2005)**

- **Molecular Formula:** C_{15}H_{24}O_{6}
- **Molecular Weight:** 294.39
- **Color/Form:** Yellow oily liquid
- **Odor:** Aromatic odour of ginger
- **Taste:** Spicy pungent taste
- **Melting Point:** 32 °C
- **Octanol/Water partition coefficient:** Log P value = 2.49
- **Solubilities:** Sparingly soluble in petroleum ether, soluble in alcohol, chloroform and ether and almost insoluble in water.

**Stability/Shelf Life**

- Low volatility
- Stability is rated as fair when used as food additive

**Pharmacology**

Ginger in crude or standardized extract form has been subjected to several animal studies and cardiotonic, antiplatelet, antiemetic, anxiolytic, anti-diabetic, antidyslipidaemic, anti-inflammatory, anti-obesity, and immunomodulator, have been reported. There are many traditional uses for ginger, but more recent interest in the use of ginger centers on the
prevention and management of nausea. However, there is limited clinical information to support these uses. Limited number of clinical trials have justified efficacy of ginger in the treatment of antiplatelet, symptomatic gonoarthritis, pregnancy-induced nausea and vomiting, osteoarthritis and chronic low back pain.

**Anti-Emetic**

Ginger has demonstrated anti-emetic activity in both experimental models and human studies, the exact mechanism of which is still unknown although both shogaols and gingerols have been shown to have anti-emetic activity. It appears that several key constituents and several different mechanisms are responsible. According to both animal and human studies, 6-gingerol reduces emesis due to a peripherally acting mechanism, acting on the gastrointestinal tract alone. One constituent found in ginger, galanolactone, is a serotonin receptor antagonist, which may partly explain the anti-emetic effect. It also explains the inhibitory effect of ginger on serotonin-induced diarrhea and antispasmodic effects on visceral and vascular smooth muscle. Ginger has been shown to blunt gastric dysrhythmias and nausea evoked by acute hyperglycaemia in humans (Phillips et al., 1993). The anti-arrhythmic and anti-emetic effects are thought to be due to a blockade of prostaglandins rather than inhibition of their release. Ginger has also been shown to reduce radiation-induced gastrointestinal distress and emesis in rat models, which is thought to be due at least in part to its antioxidant properties and the ability to scavenge free radicals and inhibit lipid peroxidation.

**Gastrointestinal Activity**

Ginger exerts several effects in the gastrointestinal tract. It stimulates the flow of saliva, bile and gastric secretions and has been shown to increase gastrointestinal motility without affecting gastric emptying in several animal models and human studies. Ginger has also been observed to have prokinetic activity in mice *in vivo* and antispasmodic activity *in vitro* (Mahady et al., 2005). These findings appear to support the traditional use of ginger in the treatment of gastrointestinal discomfort, colic, diarrhea and bloating and its use as a carminative agent.

**Anti-ulcer activity**

A number of *in vivo* studies have identified antiulcer activity for ginger extract and several of its isolated constituents. The orally administered acetone extract of ginger at a
dose of 1000 mg/kg and zingiberene, the main terpenoid in this extract, at 100 mg/kg significantly inhibited gastric lesions by 97.5% and 53.6%, respectively. Additionally, the pungent principle, 6-gingerol at 100 mg/kg, significantly inhibited gastric lesions by 54.5%. These results suggest that both zingiberene and 6-gingerol are important constituents responsible for ginger’s anti-ulcer activity. Other constituents demonstrating antiulcer properties in gastric ulcer models in rats include beta-sesquiphellandrene, beta-bisabolene, curcumene and shogaol (Konturek et al., 2005). In addition to direct anti-ulcer activity, ginger exerts synergistic effects with the antibiotic clarithromycin in inhibiting different *Helicobacter pylori* isolates independent of the organisms’ susceptibility to clarithromycin.

**Hypolipidaemic**

High doses of an aqueous extract of ginger (500 mg/kg) significantly reduced serum cholesterol according to an animal study that used oral doses of a raw aqueous extract of ginger administered daily for a period of 4 weeks. Effects on triglyceride levels are more difficult to determine, as one study demonstrated that 250 μg ginger extract/day reduced serum triglyceride levels by 27% in mice, whereas another study using a high dose of 500 mg/kg found no significant effects (Al-Ameen et al., 2006). An *ex vivo* study found that 250 μg/day of a standardised ginger extract significantly reduced plasma LDL-cholesterol levels, the LDL basal oxidative state, as well as LDL-cholesterol and serum cholesterol’s susceptibility to oxidation and aggregation, compared with placebo. Ginger also reduced aortic atherosclerotic lesions by 44% in atherosclerotic mouse aorta.

**Anti-Inflammatory and Analgesic**

The anti-inflammatory effects of ginger may be due to its effects on the arachidonic acid cascade, as COX-1 and -2 and lipoxygenase inhibition has been shown *in vitro* and high oral doses of an aqueous extract of ginger (500 mg/kg) significantly lowered serum PGE$_2$ and thromboxane B2 levels in rats. Ginger also suppresses leukotriene biosynthesis by inhibiting 5-lipoxygenase, thus distinguishing ginger from NSAIDs (Charlier and Michaux, 2003). Additionally, ginger extract has been shown to inhibit thromboxane synthase and inhibit the induction of several genes involved in the inflammatory response. These include genes encoding cytokines, chemokines, and the inducible enzyme COX-2, thus providing evidence that ginger modulates biochemical pathways
activated in chronic inflammation. No one single constituent seems to be responsible for the anti-inflammatory effect of ginger. An acetone extract containing gingerols, shogaols and minor compounds like gingerenone A, 6-gingerdiol, hexahydrocurcumin and zingerone have been shown synergistically to produce dose-dependent anti-inflammatory effects. Other studies have identified the gingerols and diarylheptanoids and gingerdione as the key compounds responsible. Gingerol and 8-gingerol have been found to evoke capsaicin-like intracellular Ca\textsuperscript{2+} transients and ion currents \textit{in vitro} and it has been suggested that gingerols represent a novel class of naturally occurring vanilloid receptor agonists that contribute to ginger’s medicinal properties. This is supported by the finding that topical application of ginger creams or compresses produce an analgesic capsaicin-like effect on the release of the immunoreactive substance P from primary afferent neurons. In an animal study of chemically induced inflammation, ginger extract reduced oedema that was partly caused by serotonin-receptor antagonism. Additionally, ginger oil has shown anti-inflammatory activity, significantly suppressing both paw and joint swelling in severe adjuvant arthritis in rats.

\textit{Antiplatelet}

It has been suggested that gingerols and their derivatives represent a potential new class of platelet activation inhibitors, with synthetic gingerols being found to inhibit the arachidonic acid-induced platelet release reaction \textit{in vitro} in a similar dose range as aspirin possibly due to an effect on COX activity in platelets. Powdered ginger exerted an antiplatelet activity when taken in very high doses of at least 10 g, according to one human study. A randomised double-blind study found that doses up to 2 g of dried ginger had no effect on bleeding time, platelet aggregation or platelet count. This lack of effect has been demonstrated in healthy volunteers and those with type 1 diabetes mellitus or coronary artery disease (Ali et al., 2008).

\textit{Antimicrobial and Antiparasitic}

Ginger extract and several of its main constituents exhibit antimicrobial activity \textit{in vitro} and \textit{in vivo}. Ginger extract has been shown to have an antibacterial effect against \textit{Staphylococcus aureus}, \textit{Streptococcus pyogenes}, \textit{S. pneumonia} and \textit{Haemophilus} collected from throat swaps of infected individuals. The minimum inhibitory concentration of ginger ranged from 0.0003-0.7 µg/mL, and the minimum bactericidal
concentration ranged from 0.135-2.04 μg/mL. Ginger has also shown antischistosomal activity. Gingerol (5.0 ppm) completely abolished the infectivity of Schistosoma spp. (blood flukes) in animal studies. Gingerol and shogaol exhibited potent molluscicidal activity in vivo (Ali et al., 2008). Gingerols demonstrated antibacterial activity against Bacillus subtilis and Escherichia coli in vitro, and the essential oils of ginger have been shown to have antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi in vitro. Shogaol and gingerol have demonstrated anti-nematode activities; 6.25 μg/mL. The 6-shogaol destroyed Anisakis larvae within 16 h in vitro, whereas the antinematodal medication pyranl pamoate had no lethal effect at 1 mg/mL. Ginger constituents have also been shown to be antifungal and antiviral. Shogaol and zingerone strongly inhibited Salmonella typhi, Vibrio cholerae and Tricophyon violaceum. Aqueous extracts have also been shown to be effective against Trichomonas vaginalis. Several sesquiterpenes, but especially beta-sesquiphellandrene, isolated from ginger have also been shown to have antirhino viral activity in vitro.

**Antioxidant**

According to in vivo research, ginger exerts significant direct and indirect antioxidant effects. Orally administered ginger significantly lowered levels of free radicals and raised the activities of endogenous antioxidants superoxide dismutase and catalase and had a sparing effect on vitamins C and E (Siddaraju and Dharmesh, 2007).

**Immunomodulation**

In vitro and in vivo research suggests ginger extract exerts some degree of immunomodulatory activity and has been shown to significantly prolong the survival of cardiac allografts in mice. Ginger oil has also been shown to have immunomodulatory activity in mice, with dose-dependent inhibition of T lymphocyte proliferation and IL-1 alpha secretion in vitro and reduced delayed type of hypersensitivity response in vivo (Ali et al., 2008).

**Hepatoprotective**

Ginger has significant hepatoprotective effects comparable to those of silymarin, according to research with experimental models of alcohol-induced liver damage (Yemitan and Izegbu, 2006).
Antihistamine

Shogaols and certain gingerols exhibit dose-dependent inhibition of drug-induced histamine release from rat peritoneal mast cells in vitro.

Anxiolytic

A combination of ginger and Ginkgo biloba has been shown to reduce anxiety in an animal model (elevated plus-maze test). The effect was similar to diazepam. A highly non-polar fraction of a ginger extract has been shown to possess anticonvulsant, anxiolytic and anti-emetic activities in animals.

Antifibrotic

Supplementation with 5 g ginger not only prevented a decrease, but also significantly increased fibrinolytic activity in 30 healthy adult volunteers who consumed 50 g fat in a meal in an open clinical study.

Apoptosis

A pungent phenolic substance found in ginger (6-paradol) effectively inhibits tumour promotion in mouse skin carcinogenesis. 6-Paradol and structurally related derivatives have also been shown to induce apoptosis through a caspase-3-dependent mechanism (caspase is a ‘suicidal’ cell protein that, when activated, induces the cell to destroy itself).

Positive Ionotrope

Gingerols and shogaols isolated from ginger have positive inotropic activity, as demonstrated on isolated heart muscle. The effect of gingerol seems to be rather specific to SR Ca\(^{2+}\)-ATPase activity.

Thermogenic

Ginger helps to maintain body temperature and inhibit serotonin-induced hypothermia in vivo. However, the addition of a ginger-based sauce to a meal did not produce any significant effect on metabolic rate in humans.

Dose

The anti-inflammatory activity of 6-gingerol has been established at a dose of 25 mg kg\(^{-1}\) in mice (Rasool et al., 2006). The anti-inflammatory effect of ginger is thought to be due to inhibition of cyclooxygenase and 5-lipoxygenase, resulting in reduced leukotriene and prostaglandin synthesis. In a study, the analgesic and anti-inflammatory effects of 6-gingerol were investigated. Intraperitoneal administration of 6-gingerol (25-50 mg/kg)
produced an inhibition of acetic acid-induced writhing response and formalin-induced licking time in the late phase. 6-gingerol (50-100 mg/kg). It also resulted in inhibition of paw edema induced by carrageenin.

6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol have been shown in different in vivo studies to be partly responsible for the ginger’s anti-emetic properties. These compounds exert their anti-emetic effect at least partly by acting on the 5-HT3 receptor ion-channel complex, probably by binding to a modulatory site distinct from the serotonin binding site. This may include indirect effects via receptors in the signal cascade behind the 5-HT3 receptor channel complex such as substance P receptors and muscarinic receptors (Heba et al., 2006).

**Metabolism and pharmacokinetics**

Nakazawa and Ohsawa investigated the metabolic fate of 6-gingerol in rats (Nakazawa and Ohsawa, 2002). The bile of rats orally administered 6-gingerol was shown to contain a major metabolite (1) by HPLC analysis. Although the metabolites derived from 6-gingerol were not detected in the urine, the ethyl acetate extract of the urine after enzymatic hydrolysis was shown to contain six minor metabolites (2-7). Their structures were determined to be (S)-6-gingerol-40-0-b-glucuronide (1), vanillic acid (2), ferulic acid (3), (S)-(++)-4-hydroxy-6-oxo-8-(4-hydroxy-3-methoxyphenyl) octanoic acid (4), 4-(4-hydroxy-3-methoxyphenyl)butanoic acid (5), 9-hydroxy 6-gingerol (6) and (S)-(++)-6-gingerol (7) based on spectroscopic and chemical data. The total cumulative amount of 1 excreted in the bile and 2–7 in the urine during 60 h after the oral administration of 6-gingerol were approximately 48% and 16% of the dose, respectively. The excretion of 2–7 in the urine decreased after gut sterilization. On the other hand, the incubations of [6]gingerol with rat liver showed the presence of 9-hydroxy 6-gingerol, gingerdiol (8), and (S)-[6]-gingerol-40-0-b-glucuronide (1). These findings suggest that the gut flora and enzymes in the liver play an important part in the metabolism of 6-gingerol.

Oral or intraperitoneal dosage (100 mg/kg) of zingerone resulted in the urinary excretion of most metabolites within 24 h, mainly as glucuronide and/or sulphate conjugates. While zingerone itself accounted for roughly 50—55% of the dose, reduction to the corresponding carbinol (11-13%) also occurred. Appreciable (40% in 12 h) biliary excretion occurred (Monge et al., 1976). A study investigated the in situ jejunal
absorption of [6]-gingerol) to determine the extent of its intestinal absorption after administering the drug solution (2 mg in 0.9% NaCl solution containing 5% Tween 80) into the closed jejunal loop in rats. Initially, the blood cell-plasma partition of 6-gingerol was investigated following intravenous administration (3 mg/kg) in rats to calculate the drug levels in whole blood from the plasma levels measured in the absorption experiments. The blood cell-plasma concentration partition ratio (K) for 6-gingerol after instantaneous equilibrium was achieved was estimated as 0.489. The cumulative percentage of 6-gingerol absorbed into the mesenteric venous blood by 60 min was estimated to be 10.86±2.61% of the initial dose. The recovery of 6-gingerol was calculated by dividing the sum of the cumulative amount of 6-gingerol absorbed in the mesenteric blood, the amount of the drug taken up by the jejunal mucosa and muscle at 60 minutes, and the remaining amount of 6-gingerol in the closed loop by the initial dose, and was estimated as 58.7±9.2%. The residual 40% of the initial dose was not recovered as 6-gingerol (Nobuhiro et al., 2003).

3.4. Rationale for selection of excipients
3.4.1. For sustained release tablets

A brief description of the various rate controlling polymers (employed chiefly as barrier layer for providing lag) and other excipients employed in the study are given. These excipients were considered and their respective concentrations were used (below the prescribed limit) in the study purely on a “trial and error” basis.

Following polymers and excipients have been used in the present research work:

1. Aerosil® (Colloidal Silicon Dioxide)
2. Avicel® PH 102 (Microcrystalline Cellulose)
3. Carbopol 934 P
4. Sodium starch glycolate
5. Magnesium Stearate

1. Aerosil®: Colloidal Silicon Dioxide is submicroscopic fumed silica with a particle size of about 15 nm. It is a light, loose, bluish-white colored, odorless, tasteless, non-gritty amorphous powder (Rowe et al., 2004). Small particle size (15 nm) and large specific surface area (200 ± 25 m²/g) gives Aerosil desirable flow characteristics that are exploited to improve the flow properties of dry powders in a number of processes such as
tabletting. Aerosil is used as a glidant in a concentration range of 0.1-0.2% to improve the flow properties of the API since it is having flow properties in the fairly passable range. Moreover, the process selected for its formulation development is direct compression (DC) for which good flow properties of the blend was desired.

2. Avicel PH 102®: Microcrystalline Cellulose (MCC) is purified, partially depolymerized cellulose that occurs as a white odorless, tasteless, crystalline powder composed of porous particles (Rowe et al., 2004). It is a unique and one of the most diverse, versatile excipient and deserves a special mention with regard to its rationale of selection. It has been used as a bulking agent, disintegrant, pore former, lubricant and even glidant (Ammon and Rina, 1999; Guo et al., 2002; Indiran et al., 2002; Saha and Sahiwala, 2009; Saigal et al., 2009). While the present research work utilizes this distinctive excipient by virtue of its special property; MCC is one of the very few excipients having the property of being hydrophilic yet being water insoluble (another excipient sharing the same property being calcium pectinate). Avicel is available in many grades PH 101, 102 and 110 to name a few, however pharmaceutical grade 102 was used here as it is a granular grade of MCC and had an excellent flow property characteristic for direct compression tabletting.

3. Carbopol® 934P

Carbopol is a commercial name for poly (acrylic acid) which is used as thickening agents to drug delivery vehicle targeting specific site in the body. Its molecular weight ranges from 2-30 x 106 with aqueous dispersion pH ranging from 2.8 – 3.2 according to the types of resin being used to prepare the solution. It is a weak acid with pKa >5. In the dry state, the carbomer chains will appear in a spiral form, but will slowly unwind when water was added which can be observed by the increase in viscosity of the solution. The unwinding of the coils can proceed through two mechanisms. The first mechanisms start when the carboxylic acid groups on the chain was neutralize with an appropriate base. This will increase the electrostatic repulsion between the chains causing the coil to come apart. The chain would then interwine with each other resulting in a 3D matrix that causes an instantaneous formation of highly viscous gel. The second mechanism occurs by the addition of hydroxyl donor structure such as polyols to the carboxyl group. The combination of the carboxyl with hydroxyl group produce a thickening affect due to
formation of hydrogen bonds in the structure. This mechanism is a time dependant mechanism. Studies conducted on the rheology of carbomer have found that the viscosity of the solution is a function of pH and the concentration (Ikhwan et al., 2012).

Carbopol was selected as the polymer to prepare sustained release tablets for the treatment of vitiligo as it possesses advantages that include:

1. The polymers and its degradation product should be non-absorbable and non-toxic.
2. Minimal protein interaction with biological surface is important for biological acceptance of foreign materials, as denaturation of proteins by surfaces may serve as a trigger for biological rejection mechanism.
3. Polymer should allow easy incorporation of drug and should offer no hindrance as to its release.
4. The polymer must not decompose on storage or during the shelf life of the dosage form.
5. It should adhere quickly to a moist surface and possess specific sites of attachment.
6. Cost of the polymer should not be high and thereby cause the device to be non-competitive.

Thus, these advantages makes carbopol suitable polymer candidate to prepare sustained release tablets for the treatment of vitiligo (Koleske, 1978; Murthy, 1997; Sinha et al., 2004).

4. Sodium starch glycolate (SSG)
Sodium starch glycolate is the sodium salt of a carboxymethyl ether of starch. Very fine, white or off white, free flowing powder; odourless or almost odourless. It is practically insoluble in water and insoluble in most organic solvents. It consists of oval or spherical granules, 30-100 μm in diameter with some less-spherical granules ranging from 10-35 μm in diameter. Sodium starch glycolate is widely used in oral pharmaceuticals as a disintegrant in capsule and tablet formulations. It is recommended to be used in tablets prepared by either direct-compression or wet-granulation processes. The recommended
concentration in a formulation is 2-8%, with the optimum concentration about 4% although in many cases 2% is sufficient. Disintegration occurs by rapid uptake of water followed by rapid and enormous swelling. The disintegrant efficiency of sodium starch glycolate is unimpaired in the presence of hydrophobic excipients, such as lubricants unlike many other disintegrants. Increasing the tablet compression pressure also appears to have no effect on disintegration time. Sodium starch glycolate has also been investigated for use as a suspending vehicle. Tablets prepared with sodium starch glycolate have good storage properties. Sodium starch glycolate are also been used as swelling agents and pore formers in many of the extended release formulations (Omidian and Park, 2008., Juergen et al., 2008). Sodium starch glycolate is stable and should be stored in a well-closed container to protect it from wide variations in humidity and temperature that may cause caking. The physical properties of sodium starch glycolate remain unchanged for up to 4 years if stored at moderate temperatures and humidity.

5. Magnesium Stearate: Magnesium stearate is a compound of magnesium with a mixture of solid organic acids that consists chiefly of variable proportions of magnesium stearate and magnesium palmitate. Magnesium stearate is hydrophobic and may retard the dissolution of a drug from a solid dosage form; the lowest possible concentration is used therefore in formulations. Tablet dissolution rate and crushing strength is decreased as the time of blending is increased; and magnesium stearate may also increase tablet friability. Blending times with magnesium stearate should therefore be controlled (Rowe et al., 2004). It has been used here as a lubricant in tablet manufacture in a concentration range of 0.25-0.5% tablet weight.

3.4.2. For nanoemulsion gel

i) Excipient Selection

Pharmaceutical acceptability of excipients and the toxicity issues of the components used, make the formulation of nanoemulsions really critical. There is great restriction in selection of the components that are orally acceptable (Ghosh and Murthy, 2006). Nanoemulsion formulation usually involves a combination of three to five components:

- An oil phase
An aqueous phase
A primary surfactant
In many cases a secondary surfactant (cosurfactant)
Sometimes an electrolyte

Hence, care was taken to select the components that are orally acceptable and fall under GRAS (Generally Regarded As Safe) category. Therefore, for the development of nanoemulsion following components were taken.

ii) Oils

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils penetrate the tail group region to a greater extent than long chain alkanes, and hence swell this region to a greater extent, resulting in an increased negative curvature (and reduced effective HLB). Various long and medium chain triglycerides have been used for the formulation of nanoemulsion (Ghosh and Murthy, 2006). Following oils were used in the present study for the development of nanoemulsion:

a) Lauroglycol FCC (Propylene glycol laurate),
b) Lauroglycol 90 (Propylene glycol monolaurate) and
c) Maisine 35 (Glyceryl monolinoleate).
d) Oleic acid
e) Olive oil
f) Jojoba oil
g) Castor oil
h) Carbitol
i) Peceol
j) Labrafil M

iii) Surfactants

The surfactant used to stabilize the nanoemulsion may be non ionic, cationic, anionic or zwitterionic. Combination of anionic or cationic surfactants of high HLB value with a cosurfactant of low HLB value, a double chained surfactant of the appropriate molecular
composition or a single chained non ionic surfactant of the polyethylene glycol alkyl ether type, at appropriate temperature are generally used for the formulation of nanoemulsion. Combination of this particularly the ionic and non-ionic surfactant can be very effective in increasing the extent of nanoemulsion region (Ghosh and Murthy, 2006). Non-ionic or zwitterionic surfactants are often considered for pharmaceutical applications and nanoemulsion formulation since these are less toxic and less affected by pH and ionic strength changes (Constantinides, 1995). Thus, for the present study following surfactants were used:

a) Tween 80 (Polyoxyethylene (20) sorbitan monooleate),
b) Tween 20 (Polyoxyethylene sorbitan monolaurate), and
c) Labrafil 1944 CS (Oleoyl macrogoglyceride).

iv) Cosurfactant

It is generally not possible to achieve the required interfacial area with the use of single surfactant. If, however, a second amphiphile is added to the system, the effects of the two surfactants can be additive provided that the adsorption of one does not adversely affect the adsorption of the other and that mixed micelle formation does not reduce the available concentration of surfactant molecule. The second amphiphile is referred to as the cosurfactant (Ghosh and Murthy, 2006). For the present study following cosurfactants were used:

a) Methanol, and
b) Polyethylene glycol (PEG) 400.

1. Tween® 80
Nonionic surfactants are the major type of surface active agents used in topical delivery systems since their advantages with respect to compatibility, stability, and toxicity are quite significant compared to the cationic, anionic, or amphoteric counterparts. They are generally less toxic and less irritating to the skin and tend to maintain near physiological pH values when in solution. The nonionic molecules are comprised of both polar and non-polar segments, possessing a broad range of interfacial activity and versatile functions as surfactants, emulsifiers and permeability enhancers (Jiao, 2008).
Polysorbates are widely used in cosmetics, food products, and oral, parenteral, and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials. Tween 80 is the most commonly employed polysorbate as emulsifying agent. Tween 80 as a surfactant forms the interfacial film in the nanoemulsions. Tween 80, is a polyoxyethylene sorbitan fatty acid ester in which the fatty acid is oleic acid. It has a CAS no. 9005-65-6. It has an empirical formula and molecular weight of $C_{64}H_{124}O_{26}$ and 1310 respectively (Rowe et al., 2004). The structural formula of Tween 80 is shown in Fig. 3.4.

![Structural formula of Tween 80](image)

**Fig. 3.4: Structural formula of Tween 80 $(w + x + y + z = 20)$**

*Description:* Polysorbates have a characteristic odor and a warm, somewhat bitter taste. Polysorbate 80 is a yellow oily liquid.

*Uses:* Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils. Tween 80 is also a wetting and dispersing/suspending agent (Rowe et al., 2004).

2. Ethanol

However, surfactants in general cannot reduce the interfacial free energy sufficiently to increase spontaneously the interfacial area. Therefore, in many cases the addition of a cosurfactant is necessary to produce thermodynamically stable systems. The length of cosurfactant alkyl chain, the composition of the oil phase, and the ratio of the surfactant in the formulation directly influence the formation and the properties of nanoemulsions.
Short chain alcohols like ethanol act as co-surfactant system in nanoemulsions. Ethanol as co-surfactant confers flexibility to the interfacial layer of the nanoemulsions. Ethanol is a straight-chain alcohol, and its molecular formula is C$_2$H$_5$OH. Its empirical formula is C$_2$H$_6$O and the structural formula is shown in Fig. 3.5.

![Structural formula of ethanol](image)

**Description:** Ethanol is a volatile, colorless liquid that has a slight odor.

**Boiling point:** 78.3 °C

**Uses:** Ethanol is used in medical wipes and in most common antibacterial hand sanitizer gels at a concentration of about 62% v/v as an antiseptic. Ethanol kills organisms by denaturing their proteins and dissolving their lipids and is effective against most bacteria and fungi, and many viruses, but is ineffective against bacterial spores. Ethanol is miscible with water and is a good general purpose solvent. It is found in paints, tinctures, markers, and personal care products such as perfumes and deodorants.

3. **Carbopol® 934P**

Carbopols are very well suited for aqueous based topical dosage forms. Many commercial topical products available today have been formulated with these carbopols, as they provide the following numerous benefits to topical formulations:

- Safe & effective — Carbopols have a long history of safe and effective use in topical gels, creams, lotions, and ointments. They are also supported by extensive toxicology studies.
- Non-sensitizing — Carbopols have been shown to have extremely low irritancy properties and are non-sensitizing with repeat usage.
- No effect on the biological activity of the drug — Carbopols provide an excellent vehicle for drug delivery. Due to their extremely high molecular weight, they cannot penetrate the skin or affect the activity of the drug.
Excellent thickening, suspending, & emulsification properties for topical formulations

Products with a wide range of viscosities and flow properties have been successfully formulated and commercialized using carbopols. Carbopol is chemically a carbomer with a CAS registry number 9003-01-4. Carbopol is chemically a carbomer with a CAS registry number 9003-01-4. Carbopols are synthetic high-molecular-weight polymers of acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 56% and 68% of carboxylic acid (COOH) groups calculated on the dry basis (Rowe et al., 2004).

**Melting point:** 100–105°C

**Glass transition temperature:** Decomposition occurs within 30 min at 260°C.

**Structural formula:** Carbomers are formed from repeating units of acrylic acid. The monomer unit is shown in Fig. 3.6. The figure displays acrylic acid monomer unit in carbomer resins. The polymer chains are crosslinked with allyl sucrose or allyl pentaerythritol (Rowe et al., 2004).

![Structural formula of Carbopol](image)

**Description:** Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a slight characteristic odor.

**Uses:** Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents. Formulations include creams, gels, and ointments for use in ophthalmic, rectal, and topical preparations. In tablet formulations, carbomers are used as dry or wet binders and as a rate controlling excipient. In wet
granulation processes, water or an alcohol–water blend is used as the granulating fluid. Anhydrous organic solvents have also been used, with the inclusion of a polymeric binder. The tackiness of the wet mass can be reduced with the addition of certain cationic species to the granulating fluid or, in the case of water, with talc in the formulation. Carbomer resins have also been investigated in the preparation of sustained-release matrix beads, as enzyme inhibitors of intestinal proteases in peptide-containing dosage forms, as a bioadhesive for a cervical patch and for intranasally administered microspheres, in magnetic granules for site-specific drug delivery to the esophagus and in oral mucoadhesive controlled drug delivery systems. Carbomers are also employed as emulsifying agents in the preparation of oil-in-water emulsions for external use. For this purpose, the carbomer is neutralized partly with sodium hydroxide and partly with a long-chain amine such as stearylamine. Carbomers are also used in cosmetics. Therapeutically, carbomer gel formulations have proved efficacious in improving symptoms of moderate-to-severe dry eye syndrome. Carbomers are used extensively in nonparenteral products, particularly topical liquid and semisolid preparations. Carbomers are generally regarded as essentially nontoxic and nonirritant materials; there is no evidence in humans of hypersensitivity reactions to carbomers used topically (Rowe et al., 2004).

3.5. Plan of Work
In order to accomplish the study objectives, the following plan of work was envisaged.

- **Characterization of psoralen and 6-gingerol**
  - Organoleptic properties
    - Appearance
    - Colour
    - Odour
    - Taste
  - Identification of drugs
    - Ultraviolet spectroscopy
    - Differential scanning calorimetry
    - Thin layer chromatography

- **Establishment of analytical methodology**
High pressure liquid chromatography (HPLC)
Calibration curve in various media (pH: 1.2, 4.5, 6.8 & 7.2)
Linearity
Accuracy as recovery
Precision
Reproducibility
Detection (LOD) and quantification (LOQ) limits
Robustness & Force degradation studies

- **Formulation and evaluation of SR Tablets**
  Drug-Excipient Compatibility studies
  Formulation of SR Tablets
  Evaluation of Sustained release tablets
  *In vitro* release studies
  *In vivo* studies
  IVIVC

  Accelerated stability studies
  - As per ICH Q1A (R2) guidelines
  - Photostability as per ICH Q1B guidelines
  - By conventional method using Arrhenius equation

- **Formulation and evaluation of Nanoemulsion gel**
  Formulation and evaluation of nanoemulsion
  - Selection of S_mix ratio
  - Stress testing of nanoemulsions

  Characterization of the nanoemulsion formulations
  - Refractive index
  - Droplet size and polydispersity index
  - Zeta potential
  - Transmission electron microscopy

  Preparation of nanoemulsion gels
  Evaluation of nanoemulsion gels
  Viscosity determination
In-vitro & Ex-vivo skin-permeation studies

Accelerated stability studies

As per ICH Q1A (R2) guidelines

Photostability as per ICH Q1B guidelines