2. REVIEW OF LITERATURE

Site-specific drug delivery is an important area of research that is anticipated to increase the efficacy of drugs and reduce their potential side effects. Biodegradable polymers are currently being used as drug carriers because of their inherent properties of controlled release, enhanced distribution and overall pharmacokinetic availability. The release of loaded drugs from nanoparticles may be controlled in response to changes in environmental conditions, such as temperature and pH. Biodistribution profiles and anticancer efficacy of nanonutraceuticals in vivo might differ depending upon their size, surface charge, PEGylation, and other biophysical properties. Micro and nano particulate systems formulated with these polymers have shown wide applicability for oral, subcutaneous, or intravenous delivery of lipophilic and hydrophilic drugs. They showed that $t_{1/2}$ beta and area under the curve of a paclitaxel micelle formulation was 4.0 and 2.2 fold higher than that of a Taxon injection. Their study of bio-distribution in mice showed that the paclitaxel micelle formulation not only decreased drug uptake by the liver but also prolonged drug retention in the blood and increased drug distribution in the kidney, spleen, ovaries, and uterus, suggesting that this formulation could be a useful drug carrier for intravenous administration of paclitaxel (Wang et al., 2008).

The oral delivery of drugs and vaccines is regarded as the optimal means for achieving therapeutic and prophylactic effects for a number of conditions. For both drug and vaccine delivery the enteric route has the advantages of increased patient compliance, relieves the need for injection and does not require the presence of trained personnel. In the case of vaccination, enteric delivery may result in the induction of a protective mucosal immune response against pathogens which colonize and invade the mucosa. Unfortunately, the oral delivery route is best with problems such as: gastrointestinal destruction of labile molecules; low levels of macromolecular absorption; and poor immunity usually elicited to orally apply soluble vaccine antigens. To reduce the impact of gut secretions and to ensure the absorption of bioactive agents in an unaltered form, molecules may be incorporated into
biodegradable microparticles. This oral delivery system therefore relies on the capacity of the gastrointestinal tract to absorb micro particulate materials, a function which has been demonstrated to be carried out by membranous fold (M) cells in the Peyer’s patches of the mammalian gut (Lavelle et al., 1995).

2.1 POLYMERS FOR CONTROLLED DRUG DELIVERY

Biodegradable polymers used for synthesis of microspheres can be classified into Natural polymers – which include proteins such as casein, albumin, gelatin, etc, and polysaccharides such as agarose, alginates, chitosan. Synthetic polymers include poly lactic acid, poly (lactic-glycolic acid), poly (alkyl-cyano acrylates), etc. The use of natural proteins as drug carriers proves to be advantageous because of high biodegradability, and easy clearance.

2.1.1 NATURAL HYDROPHILIC POLYMER

Among the natural macromolecules available for the manufacture of microparticles, proteins such as albumin, gelatin, legumin or vicilin, as well as polysaccharides like alginates or agarose have been extensively studied and characterized. These macromolecules have attracted wide interest as biomaterials due to their intrinsic biodegradability and biocompatibility. Natural hydrophilic polymers are conveniently classified as proteins and polysaccharides. The polymers of natural origin however, suffer some disadvantages including (a) batch to batch variation, (b) conditional biodegradability and (c) antigenicity. Alginate based delivery systems for oral and ophthalmic administrations have been approved. It is also reported as a homocompatibility and convincingly acceptable system for parenteral administration of bioactives. In contrast, dextran, albumin and gelatin, which are though acceptable materials for parenteral administration, manifest immunogenicity due to use of crosslinking agents, which are employed during their preparations. Chitosan on the other hand, is a homo non-compatible material and hence it should be restricted to extracorporeal uses only (Pangburn et al., 1984).

2.1.2 SYNTHETIC HYDROPHOBIC POLYMERS

Polymers, which are used for nanoparticles preparations are those conceivably employed in the preparation of microspheres. Most of them are typically hydrophobic
in nature. The polymers used are either pre-polymerized or synthesized before (first group) or during the (second group) process of nanoparticle preparation. The polymers from the ester class [poly (lactic acid) and poly (lactic-glycolic acid) copolymers] are representative of the first group along with poly (ε-caprolactone) and already been approved for human use (Couverur et al., 1986; Lewis, 1990; Pitt 1990). The second group is represented by poly (alkyl-cyanoacrylates), which have received the greatest attention as polymeric nanoparticulate systems but gathered number of controversies due to the toxicity of the corresponding alkylcyanoacrylate monomer (Kattan et al., 1992). With regard to bio-degradability, Couverur and Vautier (1991) reported that product of PACA (Poly(alkylcyano acrylates)) are typically non-toxic hydrophilic oligomers and are easily eliminated by the body through glomerular filtration.

2.2 PREPARATION TECHNIQUES OF MICROPARTICLES

The selection of contusive method for microparticle preparation depends on the physicochemical characteristics of the polymer and the drug to be loaded (Table 2.1). On the contrary, the preparation techniques largely determine the inner structure, in vitro release profile and the biological fate of these polymeric delivery systems (Kreuter et al., 1991). Moreover, the method of preparation and its choice are equivocally determined by some formulation and technology related factors such as particle size, adverse effects on drugs or proteins, reproducibility of the release profile and the method, stability and toxicity.
Table 2.1: Particle preparation methods & candidate drug for certain polymers.

<table>
<thead>
<tr>
<th>Polymer Used</th>
<th>Technique</th>
<th>Candidate Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydrophilic</strong></td>
<td>Heat Denaturation and crosslinking in W/O emulsion</td>
<td>Hydrophilic</td>
</tr>
<tr>
<td>Albmin, Gelatin</td>
<td>Desolvation and crosslinking in aqueous medium</td>
<td>Hydrophilic and Protein affinity</td>
</tr>
<tr>
<td>Alginate, Chitosan,</td>
<td>Crosslinking in aqueous medium</td>
<td>Hydrophilic and protein affinity</td>
</tr>
<tr>
<td>Dextran</td>
<td>Polymer precipitation in an organic solvent</td>
<td>Hydrophilic</td>
</tr>
<tr>
<td><strong>Hydrophobic</strong></td>
<td>Emulsion Polymerization</td>
<td>Hydrophilic</td>
</tr>
<tr>
<td>Poly(alkylcyanoacrylates)</td>
<td>Interfacial O/W polymerization</td>
<td>Hydrophobic</td>
</tr>
<tr>
<td><strong>Polysterers</strong></td>
<td>Solvent evaporation-extraction</td>
<td>Hydrophilic and hydrophobic</td>
</tr>
<tr>
<td>Poly(lactic acid),</td>
<td>Solvent displacement</td>
<td>Soluble in polar solvent</td>
</tr>
<tr>
<td>poly(lactide-co-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glycolide), poly (ε-caprolactone)</td>
<td>Salting out</td>
<td>Soluble in polar solvent</td>
</tr>
</tbody>
</table>

Two types of systems with different inner structures are apparently possible including a matrix type system consisting of an entanglement of oligomer or polymer units (microparticles / microspheres) and a reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell (microcapsule). The drug can either be entrapped within the reservoir or the matrix or otherwise be adsorbed on the surface of these particulate systems. The polymers are strictly structured to a micrometric size range particle(s) using appropriate methodologies. These methodologies are conveniently classified as follows.
2.2.1 EMULSIFICATION TECHNIQUES
2.2.1.1 SINGLE EMULSION TECHNIQUE:

The microparticulate carriers of natural polymers, i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium eg., oil. In the second step of preparation, crosslinking of the dispersed globule is carried out. The crosslinking can be achieved by means of heat or by using chemical crosslinking agents like glutaraldehyde, formaldehyde, terephthaloyl chloride, diacid chloride, etc.

2.2.1.2 DOUBLE EMULSION:

Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion type w/o/w and is best suited to the water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as the synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary solution subjected to homogenization or sonication before addition to aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like leutinizing hormone releasing hormone (LH-RH) agonist, vaccines, protein/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/extraction.

2.2.2 POLYMERIZATION TECHNIQUES
2.2.2.1 NORMAL POLYMERIZATION:

Normal polymerization proceeds and carried out using different techniques as bulk, suspension, precipitation, emulsion and miceller polymerization process.

In bulk polymerization, a monomer or a mixture of monomer along with the initiator is usually heated to initiate the polymerization and carry out the process. The
catalyst or the initiator is added to reaction mixture to facilitate or accelerate the rate of the reaction. The polymer so obtained may be molded or fragmented as microspheres. For loading of drug, adsorptive drug loading or adding drug during the process of polymerization may be opted.

The suspension polymerization, which is also referred to as the bead or pearl polymerization is carried out by heating the monomer or mixture of monomers with active principles (drugs) as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. The emulsion polymerization differs from the suspension polymerization as due to presence of the initiator in the aqueous phase, which later on diffuses to the surface of the micelles or the emulsion globules.

2.2.2.2 INTERFACIAL POLYMERIZATION:

Interfacial polymerization essentially proceeds involving reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelopes the dispersed phase. In this technique two reacting monomers are employed; one of which is dissolved in the continuous phase while the other being dispersed in the continuous phase. The continuous phase is generally aqueous in nature throughout which the second monomer is emulsified. The monomers present in either phase diffuse rapidly and polymerize rapidly at the interface. If the polymer is soluble in the droplet it will lead to the formation of the monolithic type of the carrier on the other hand if the polymer is insoluble in the monomer droplet, the formed carrier is of capsular (reservoir) type.

The degree of polymerization can be controlled by the reactivity of the monomer chosen, their concentration and composition of the vehicle of either phase and by the temperature of the system. The polymerization reaction can be controlled by maintaining the concentration of the monomer, which can be achieved by addition of an excess of the continuous phase. The interfacial polymerization is not widely used in the preparation of the microparticles because of certain drawbacks, which are associated with the process such as toxicity associated with the unreacted monomer, high
permeability of the film, high degradation of the drug during the polymerization, fragility of microcapsules and non-biodegradability of the microparticles.

2.2.3 PHASE SEPARATION COACERVATION TECHNIQUES

Phase separation method is specially designed for preparing the reservoir type for water soluble drugs like peptides, proteins, however, some matrix type for hydrophobic drugs eg., steroids. The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called the coacervates. The Coacervation can be brought about by addition of the third component to the system which results in the formation of the two phases, one rich in the polymer, while the other one, i.e., supernatant, depleted of the polymer. There are various methods available and the choice is largely dependent upon the polymer and set of condition. The methods are based on salt addition non-solvent addition, addition of the incompatible polymer or change in pH. In this technique, the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution, if hydrophilic or dissolved in the polymer solution itself, if hydrophobic. Phase separation is then accomplished by changing the solution conditions by using any of the method mentioned above. The process is carried out under continuous stirring to control the size of the microparticles.

2.2.4 SPRAY DRYING AND SPRAY CONGEALING

Spray drying and spray congealing methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or the cooling of the solution, the two processes are named spray drying and the spray congealing respectively. The polymer was first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the microspheres in a size range 1-100 μm. Microparticles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying.
2.2.5 SOLVENT EXTRACTION

Solvent extraction method used for the preparation of microparticles, involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as isopropanol. Organic phase is removed by extraction with water. This process decreases hardening time for the microspheres. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer.

2.3 CASEIN IN DRUG DELIVERY

Recently, studies have shown that casein micelles are in fact natural Nano delivery vehicles and showed that they could be used to encapsulate, stabilize, and protect hydrophobic bioactive compounds, such as lipid-soluble vitamins (e.g., vitamin D). It is anticipated that a similar technology may be used for encapsulating hydrophobic drugs, including anticancer chemotherapeutics, for oral delivery, using either whole casein micelles or individual caseins, particularly β-casein (Semö et al., 2007).

Latha et al., (2000) prepared casein microspheres of size 150-180 μm loaded with progesterone using emulsion crosslinking method with dichloromethane as non-aqueous phase and polyurethane as stabilizer. In vitro release in phosphate buffer gives 50 to 60% of the drug in 30 days from these particles. Intramuscular injection of progesterone loaded microspheres into rabbits showed a plasma concentration of 1 to 2 ng/ml upto 5 months without any significant burst effect. In control, drug given in saline gives serum peak of 20 ng/ml, and the plasma concentration was not sustained beyond 4 days.

Latha et al., (1994) made glutaraldehyde crosslinked casein microspheres loaded with 5-Flurouracil. Drug release from the particles was about 20% in absence of protease for 5 days and its about 24 hours in presence of protease. Protein-drug conjugate via carbamoyl linkage using 6-(5-FU-1-yl) hexyl isocyanate has done to reduce the release. Implantation of these particles in the gluteal muscle of rats showed no adverse reaction for about one year. It was also suggested that the glutaraldehyde crosslinked casein
microspheres have biological half life of 6 months, which is three times more compared to albumin microspheres.

Latha et al., (1995) designed Theophylline loaded casein microspheres using emulsion crosslinked method. The particles crosslinked with glutaraldehyde saturated with toluene have size of 710-850 µm in diameter. In vitro release of drug from the particles crosslinked with different glutaraldehyde concentration didn’t show any effect on release. When the drug was administrated orally to rabbit peak serum concentration attained at 3 hr (same as when drug powder given), but maintained even after 24 hours.

Chen et al., (1987) compared the physical properties, drug loading characteristics and release rates of microspheres made of casein and albumin. Doxorubicin (Adriamycin) loaded casein and albumin microspheres, with diameters between 14 and 38 µm was prepared by glutaraldehyde stabilized oil emulsion. It was suggested that the surface charge of casein microspheres has more negative charge than albumin and also the release of drug was slower from casein microparticles. In tumour inhibition in vitro, casein microspheres containing 11µg of doxorubicin had a similar inhibitory effect on tumour growth (growth delay 20.7 days) to 85 µg of drug in albumin microspheres.

Willmott et al., (1989) analyzed the biodegradation rates of embolized protein microspheres I lung, liver, and kidney of rats. The protein microspheres of size 10-30 µm diameter were prepared from casein and albumin by w/o emulsion method. The protein microspheres radiolabelled with I^{125} were administrated to rats both intravenously (lung as a target organ, 1.4-2.2 mg/100 g) and via the hepatic artery (liver as target organ, 0.4-0.8 mg/100g). Time taken for loss of 50% of embolized microspheres from lung was: albumin 2.0 days; casein 3.5 days and from liver: albumin 3.6 days; casein 6.8 days. These data suggests that casein microspheres degraded slowly than albumin microspheres.

Desoize et al., (1986) evaluated the in vitro cytotoxic activity of crosslinked protein microcapsules. The protein microcapsules made of human serum albumin, lysozyme, haemoglobin, casein and pepsin crosslinked with glutaraldehyde and terephthaloylchloride. Cytotoxic activity was analyzed against human erythroleukaemic cell line K562, L1210 and Fibroblasts, interestingly all the microspheres showed
inhibitory effect at range of 100 μg/ml to 10 mg/ml. The effect may be non specific, reversible and to depend on contact with the cell plasma membrane.

Latha et al., (1994) prepared glutaraldehyde crosslinked casein microspheres and analyzed the release of theophylline in vitro. Casein microspheres of size 710 - 850 μm have prepared by emulsion crosslinking, containing dichloromethane/hexane oil phase and polyurethane as suspension stabilizer. Around 80% of drug incorporated in microspheres and found to stable at stimulated gastric fluid containing 0.32% pepsin at 37°C for 24 hours, also papin and α-chymotrypsin at concentration of 0.5 and 0.2% in PBS.

Knepp et al., (1993) evaluated casein microspheres loaded with mitoxantrone in lewis lung carcinoma. The microspheres of size of 1 -20 μm were prepared by steric stabilization, containing mineral oil/hexane as non aqueous phase and surfactant. The drug was loaded post-synthesis with loading of 25%. Microphage uptake of albumin and casein microspheres were compared, which shows there were only small difference in the uptake of casein and BSA microspheres at 5 and 10 min. Intratumoral administration of microspheres exhibits lower toxicity.

Willmott et al., (1992) studied incorporation of [14C] doxorubicin within glutaraldehyde crossed linked casein microspheres. The drug loaded microspheres release drug in sustained manner, the fraction of drug was slowly cleared, eliminating burst effect (growth delay 12 days).

Santinho et al., (2002) analyzed the possibility preparing casein microparticles through aqueous Coacervation technique. Particles have been prepared by Coacervation in lactate buffer containing gelatin, hydroxypropyl cellulose (HPC) and lecithin as stabilizer, glutaraldehyde as crosslinker. The data suggests that lactic acid stabilize the particles and the particles do not degrade in presence of protease.

Bayomi et al., (1994) analyzed the casein as matrix for controlled drug delivery in the form of beads. These casein microbeads can be used as a parenteral drug delivery system.

Hernandes et al., (1998) utilized casein as excipient matrix for controlled release of acetaminophen. Acylation of casein has been done using various organic acids, and it
has been utilized as matrix for formulation of sustained release dosage form. It has been suggested that acetic casein can be utilized as matrix for sustained release.

Bulgarelli et al., (1999) investigated crosslinking solution composition effect on crosslinking degree in casein/gelatin beads. Casein/gelatin beads have been evaluated for crosslinker solvent penetration, equilibrium swelling, glutaraldehyde protein reaction and biodegradation. It has been concluded that proper solvent for crosslinker has to be selected for effective crosslinking of protein beads.

Bulgarelli et al., (2000) prepared casein/gelatin beads. Effect of matrix composition, process conditions and floating properties of beads has been analyzed. beads were prepared by emulsion crosslinking method with mineral oil has non-aqueous phase, glutaraldehyde as crosslinker and fluorescein as model drug. The percentage of casein increases the drug loading in porous beads. Property of floating in gastric environment provides long lasting and drug delivery in stomach.

Santinho et al., (1999) studied the influence of formulation on physicochemical properties of casein microparticles. Microparticles prepared by aqueous coacervation using various coacervating agents like lactic acid, succinic anhydride, succinic acid and tartaric acid and glutaraldehyde as crosslinking agent. The effect of coacervating agents, ionic strength, thickener (HPMC, HPC) and plasticizing agent (gelatin) were analyzed on particle stability. The use of 0.1% HPC plus 0.25% gelatin was resulting in homogenous microparticles. Lactic acid with NaCl increasing ionic strength resulted in better stability.

Jayakrishnan et al., (1994) reported their first preparation of casein microspheres by emulsion crosslinking method using glutaraldehyde. Microspheres loaded with methotrexate at a efficiency of 15%, and 35% of drug was released within 24 hours. This was found to be similar with albumin and suggested that casein could be highly promising, low cost, biocompatible microsphere carrier system.

Wen-Ho et al., (1996) analyzed the effect of glutaraldehyde concentration on the preparation and in vitro release of drug from protein microspheres. It has been suggested that increasing the concentration of glutaraldehyde varies the release of drug from the particles; this may be due to more crosslinking of particles results in decreasing the diffusion of drug. Also microspheres prepared using the high concentration of
glutaraldehyde has a slower digestion with trypsin than those made using lower concentration.

Casein microparticles have a potential clinical use for targeting drugs. Utilizing lactic acid as the coacervating agent, there was a trend effect of adding NaCl implying that the increasing of the ionic strength resulted in better stability. Finally, the addition of 0.1% hydroxypropyl cellulose plus either 0.25 or 0.5% gelatin resulted in homogeneous formulations. The use of lactic acid plus 0.1% hydroxypropyl cellulose and 0.25% gelatin results in biodegradable and Casein microparticles homogeneous presenting a potentially useful drug delivery system.

2.4 METHOTREXATE – ANTI-CANCER DRUG

Methotrexate is an antimetabolite that is an analog of folic acid widely used in cancer chemotherapy and immunosuppression.

2.4.1 STRUCTURE

![Fig 2.1 Structure of methotrexate](image-url)
2.4.2 CHEMISTRY

The other names for the compound are Methotrexate and amethopterin. The molecular formula is C_{20}H_{22}N_{8}O_{5}. The Chemical Name is L-(d)-N-[4-((2,4-Diamino-6-pteridinyl methyl) methylamino) benzoyl glutamic acid. The Molecular weight is 454.5. The pKa (of designated functional group) is 4.8, 5.5. It is soluble in alchohol and water. It is a yellow to orange-brown crystalline powder. MTX is also prepared for use as the sodium salt (Maxtrex).

2.4.3 PHARMACOLOGY

MTX, the most widely used antimetabolite, is a folic acid antagonist. It has an essential role in the treatment of acute lymphatic leukemia, choriocarcinoma, non-Hodgkin’s lymphoma, osteosarcoma, head and neck cancer, and breast cancer. It is a therapeutic alternative in the treatment of severe psoriasis. Suppression of graft-versus-host disease after bone marrow transplantation and various rheumatic diseases.

MTX is the 4-NH_{2}, N^{10}-methyl analog of folic acid. Current concepts of the drug’s mechanism of action are illustrated in Figure 3. MTX enters cells through the active transport system used by the physiologic circulating folate N^{5}-formyl-F4 (leucovorin or folinic acid), which is used as rescue agent after high dose therapy. There is a second, albeit less efficient, drug entry mechanism that comes in to play at high concentration of MTX (in excess of 20-50µM). Because this second mechanism accounts for the major fraction of drug that enters the cell at high concentration, this carrier-independent uptake provides a rationale for the clinical use of high-dose MTX. After entering the cells, MTX quickly binds to and inactivates dihydrofolate reductase (DHFR). This enzyme plays a crucial role in maintaining intracellular reduced folate (FH_{4}) pools by reducing dihydrofolic acid (FH_{2}), which is produced during thymidylate synthesis. Since, the latter is the only reaction that converts the reduced folate to the inactive oxidized FH_{2}, the underlying rate if thymidylate synthesis is an important determinant of cytotoxicity. Thymidylate synthetase is the enzyme most sensitive to the depletion of FH_{4} or actually N^{5,10}-methylene-FH_{4}. The synthesis of thymidylate ceases at concentrations of 1X10^{-8} M MTX. 19 N 10- formyl-FH_{4}, the folate involved in both folate-dependent steps of purine synthesis, is also depleted by blockade of DHFR. The
cessation of purine synthesis occurs at MTX concentrations of $1 \times 10^{-7}$M. The lack of either thymidylate or purines blocks synthesis of DNA.

![Figure 2.2: Mechanism of action of MTX.](image)


Methotrexate has been delivered through various carrier like liposomes (Stephen and Leaf, 1989), antibody mediated liposomes (Sayed et al., 1989, Chong et al., 1994), dendrimers (Anil et al., 2005, Renu et al., 2007), implantable calcium phosphate system (Lebugle et al., 2002), hydrogels (Daniel et al., 1992, Kamal et al., 2001), Polypeptide Proteinoid (Madhankumar and Pandurangarao, 1998), Chitosan nanoparticles (Xindu et al., 2008), polymeric micelles (Ying et al., 2005), gelatin methotrexate conjugate (Narayani and Pandurangarao, 1993, 1996, Kosaaih et al., 2000, Clyde et al., 2006), Gelatin microspheres (Narayani and Pandurangarao, 1996), albumin microspheres (Chong et al., 1988, 1994, Jung-Hee et al., 1999), Erythrocytes (Mishra and Jain, 2002), Collagen Hydrogels (Narayani and Pandurangarao, 1996), Liposomes for dermal
application (Trotta et al., 2004, Vaibhav et al., 2007), polybutylcyanoacrylate nanoparticles (Kepan and Xinguo, 2006), Hydrogel transdermal (Alvarez et al., 2001), Nanohybrid system (Jae-Min et al., 2006), PLGA Microspheres (Singh and Udupa, 1997). Carrier mediated methotrexate delivery have been used for treatment of psoriasis (Alice et al., 2008), tumour (Chong et al., 1994, Narayani et al., 1993), skin permeation (Trotta et al., 1996), target to macrophages (Christophe et al., 2005), targeting arthritis (Philip, 2004).

2.5 LEVOCETIRIZINE DIHYDROCHLORIDE - ANTI-HISTAMINIC DRUG

Levocetirizine dihydrochloride, the active component of XYZAL, is an orally active H]-receptor antagonist. It is a prescription antihistamine medication that is approved to treat the following conditions: These are allergies, including seasonal allergies and allergies that occur all year long (known as perennial allergies) and Chronic hives due to unknown causes (known medically as chronic idiopathic urticaria).

2.5.1 STRUCTURE

![Figure 2.3: Structure of Levocetirizine dihydrochloride](image)

2.5.2 CHEMISTRY

The chemical name is (R)-[2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid dihydrochloride. The molecular formula is C_{21}H_{25}ClN_{2}O_{3}\cdot2HCl. The molecular weight is 461.82. The physical appearance is as white, crystalline powder. The melting point is 218–220° C. It is soluble in water and can be stored in room temperature.

2.5.3 PHARMOCOLOGY

Levocetirizine, the active enantiomer of cetirizine, is anti-histamine; its principal effects are mediated via selective inhibition of H1 receptors. The antihistaminic activity of levocetirizine has been documented in a variety of animal and human models. In vitro
binding studies revealed that levocetirizine has an affinity for the human H1-receptor 2-fold higher than that of cetirizine. The clinical relevance of this finding is unknown.

Studies in adult healthy subjects showed that levocetirizine at doses of 2.5 mg and 5 mg inhibited the skin wheal and flare caused by the intradermal injection of histamine. In contrast, dextrocetirizine exhibited no clear change in the inhibition of the wheal and flare reaction. Levocetirizine at a dose of 5 mg inhibited the wheal and flare caused by intradermal injection of histamine in 14 pediatric subjects (aged 6 to 11 years) and the activity persisted for at least 24 hours. Levocetirizine exhibited linear pharmacokinetics over the therapeutic dose range in adult healthy subjects.

Levocetirizine is rapidly and extensively absorbed following oral administration. In adults, peak plasma concentrations are achieved 0.9 hour after administration of the oral tablet. The accumulation ratio following daily oral administration is 1.12 with steady state achieved after 2 days. Peak concentrations are typically 270 ng/mL and 308 ng/mL following a single and a repeated 5 mg once daily dose, respectively. Food had no effect on the extent of exposure of the levocetirizine tablet, but Tmax was delayed by about 1.25 hours and Cmax was decreased by about 36% after administration with a high fat meal; therefore, levocetirizine can be administered with or without food.

A dose of 5 mg (10 mL) of levocetirizine dihydrochloride oral solution is bioequivalent to a 5 mg dose of levocetirizine dihydrochloride tablets. Following oral administration of a 5 mg dose of levocetirizine dihydrochloride oral solution to healthy adult subjects, the mean peak plasma concentrations were achieved approximately 0.5 hour post-dose. The mean plasma protein binding of levocetirizine in vitro ranged from 91 to 92%, independent of concentration in the range of 90-5000 ng/mL, which includes the therapeutic plasma levels observed. Following oral dosing, the average apparent volume of distribution is approximately 0.4 L/kg, representative of distribution in total body water.

The extent of metabolism of levocetirizine in humans is less than 14% of the dose and therefore differences resulting from genetic polymorphism or concomitant intake of hepatic drug metabolizing enzyme inhibitors are expected to be negligible. Metabolic pathways include aromatic oxidation, N- and O-dealkylation, and taurine conjugation.
Dealkylation pathways are primarily mediated by CYP 3A4 while aromatic oxidation involves multiple and/or unidentified CYP isoforms.

The plasma half-life in adult healthy subjects was about 8 to 9 hours after administration of oral tablets and oral solution, and the mean oral total body clearance for levocetirizine was approximately 0.63 mL/kg/min. The major route of excretion of levocetirizine and its metabolites is via urine, accounting for a mean of 85.4% of the dose. Excretion via feces accounts for only 12.9% of the dose. Levocetirizine is excreted both by glomerular filtration and active tubular secretion. Renal clearance of levocetirizine correlates with that of creatinine clearance. In patients with renal impairment the clearance of levocetirizine is reduced.

Thin oral polymeric strips of levocetirizine were prepared by solvent casting method. HPMC and PVC were the polymers used and propylene glycol was the plasticizers used. Nine batches of films with drug were prepared using different polymers and plasticizers concentration. The optimized release 85% to 98% of drug within 2 minutes. Amount of drug released was varying with the concentration of polymer and plasticizer. Best amongst all was the film made with HPMC: PVA (1:2) released 96% of drug in 1 min (Patel and Darshan, 2012).

The pharmacokinetic/pharmacodynamic profile of levocetirizine was studied in 15 toddlers suffering from cough and other allergy-related symptoms, aged 12-24 months. They were treated twice a day with 0.125 mg/kg of levocetirizine for 90 days. Histamine induced wheal and flare test was performed prior treatment. Blood was sampled at 1, 2, 4, 6, 9 and 12 hours after the first dose. After one hour the peak plasma level was 286 +/- 68ng/ml. The median inhibition of the wheal was 100% at days 3-6, and day 90. That of the flare was 99.6% at days 3-6, and 98.9% at day 90. This study showed adequate pharmacokinetic/pharmacodynamic profile and the good safety profile of 0.125 mg/kg levocetirizine given twice a day. (Cranswick et al., 2005).

Absorption, distribution, metabolism and excretion of desloratadine, fexofenadine, levocetirizine, and mizolastine in humans have been compared. The time required to reach the peak plasma levels is shortest for levocetirizine (0.9 hrs) and longest for desloratadine (> or =3 h). The volume of distribution is limited for levocetirizine (0.4
L/kg) and mizolastine (1-1.2 L/kg), larger for fexofenadine (5.4-5.8 L/kg) and particularly large for desloratadine (approximately 49 l/kg). Fexofenadine and levocetirizine appear to be very poorly metabolized (approximately 5 and 14% of the total oral dose, respectively). Desloratadine and mizolastine are extensively metabolized. Urine and faeces are the preferential routes of excretion for levocetirizine with 85% and 13% respectively. In contrast, fexofenadine showed 80% in faeces and 11% in urine. The corresponding values are 41% (urine) and 47% (faeces) for 14C-desloratadine, 84-95% (faeces) and 8-15% (urine) for 14C-mizolastine. The absolute bioavailability was high for levocetirizine (Molimard et al., 2004).

2.6 DONEPEZIL HYDROCHLORIDE - ACETYLCOLINESTERASE INHIBITOR

Donepezil hydrochloride is the second drug approved by the U.S. FDA (Food and drug administration) for the treatment of mild to moderate Alzheimer’s disease (AD). Donepezil, is a centrally and selectively acting reversible acetylcholinesterase enzyme inhibitor having N-benzylpiperidine and indanone moiety in it which shows longer and more selective action. It exerts its therapeutic effect by increasing acetylcholine concentrations and enhancing cholinergic function. It is referred as E2020 in most of the pharmaceutical literatures. This is the second drug approved by U.S FDA for treatment of mild and moderate alzheimer disease. It is patented and marketed in United states of America, some European and Asian countries under the trade name Aricept by Eisai.co.,Ltd. The research on Donepezil started in 1983 (Hachiro et al., 2002). Intracerebral acetylcholine receptors are distributed throughout the brain, and donepezil influences both the pre- and the postsynaptic acetylcholinesterase positive structures in the human central nervous systems (Kasa et al., 2000). Acetylcholinesterase inhibitors improve the behavioral and attentional symptoms of Alzheimer’s disease (Kaasinen et al., 2002).
2.6.1 STRUCTURE

![Fig 2.4: Structure of Donepezil](image)

2.6.2 CHEMISTRY

It has an empirical formula of $\text{C}_{24}\text{H}_{29}\text{NO}_3\text{HCl}$ and a molecular mass of 379.492 g/mol with melting point of 218-220°C. Donepezil hydrochloride is a white crystalline powder and is freely soluble in chloroform, soluble in water and glacial acetic acid. Slightly soluble in ethanol, acetonitrile, insoluble in ethyl acetate and n-hexane. Many drugs were trialed to improve the cognitive functions in Alzheimer disease like cholinergic agonist such as oxetremorine, choline precursor phosphatidylcholine but that were ineffective. Numerous clinical trials carried out on acetylcholinesterase inhibitors such as physostigmine, this trial shown that this class of drug resulted in improvement of cognitive functions in Alzheimer disease patients. However these clinical trials also proved that physostigmine has poor brain penetration and thus less active (Muramoto et al., 1979). Later then scientist focused on development of new acetylcholinesterase inhibitors.

2.6.3 PHARMACOLOGY

Donepezil is a piperidine derivative, chemically distinct from other agents in this class. Donepezil demonstrates relatively high selectivity for central nervous system acetylcholinesterase, with minimal peripheral activity. It reversibly and noncompetitively inhibits centrally-active acetylcholinesterase, the enzyme responsible for hydrolysis of acetylcholine. This is expected to result in increased concentrations of acetylcholine that are available for synaptic transmission. Alzheimer's disease is characterized by
cholinergic deficiency in the cortex and basal forebrain, which contributes to cognitive deficits. Donepezil does not alter the course of the underlying dementing process.

**Absorption**    Donepezil hydrochloride is well absorbed from the gastrointestinal tract, maximum plasma concentrations being achieved within 3 to 4 hours after ingestion. Food did not affect the absorption of donepezil hydrochloride.

**Distribution**    It is about 95% bound to human plasma proteins, mainly albumin.

**Metabolism**    Donepezil is metabolized in the liver. Donepezil undergoes partial metabolism via the cytochrome P450 isoenzyme CYP3A4, and to a lesser extent by CYP2D6, to 4 major metabolites. About 11% of a dose is present in plasma as 6-O-desmethyldonepezil, which has similar activity to the parent compound.

**Excretion**    Over 10 days, about 57% of a dose is recovered from the urine as unchanged drug and metabolites, and about 15% from the faeces; 28% remains unrecovered suggesting accumulation. The elimination half-life is about 70 hours. Steady-state concentrations are achieved within 3 weeks of the start of therapy. Sex, race and smoking history have no clinically significant influence on plasma concentrations of donepezil hydrochloride.

It has been reported that donepezil is effective in the treatment of cognitive impairment and memory loss in patients with Alzheimer disease, and is well tolerated when 5 mg daily of the drug is prescribed (Rogers et.al., 1998). All analytical methods are used to determine donepezil in human plasma (Haginaka and Seyama, 1992), (Matsui et.al., 1999). Most of the methods for donepezil determinations involve HPLC (Nakashima et al., 2006), (Gamal et al., 2010) and cyclic voltammetry using a glassy carbon electrode as the working electrode (Golcu and Ozkan, 2006).

Donepezil hydrochloride is a reversible cholinesterase inhibitor that exhibits high specificity for centrally active cholinesterase (Yamanishi, 1990; Rho and Lipson, 1997; Rogers et al., 1998). Cholinergic deficit is one of the major pathological features of Alzheimer’s disease. This deficit has been associated with the loss of cognition and memory, the primary symptoms of this disorder (Bartus et al., 1982). The absorption, distribution, metabolism, and excretion of donepezil were investigated in male Sprague-Dawley rats after single oral administration. Orally administered 14C-labeled donepezil
was absorbed rapidly. The plasma level of unchanged donepezil declined more rapidly than that of radioactivity, and the brain level of radioactivity declined almost in parallel with the plasma level of unchanged donepezil. The ratio of donepezil to total radioactivity in brain was 86.9 to 93.0%, indicating low permeability of the metabolites through the blood-brain barrier (Matsui et al., 1999).

The efficacy and safety of donepezil as a treatment for patients with mild to moderate Alzheimer’s disease was investigated in a multicenter, double-blind study. Patients were randomly assigned to treatment with placebo (n=1621, 5 mg/d donepezil (n= 154), or 10 mg/d donepezil (n=157) for 24 weeks followed by a 6-week, single-blind placebo washout. The primary efficacy measures were the cognitive portion of the Alzheimer’s Disease Assessment Scale (ADAS-cog) and the Clinician’s Interview Based Assessment of Change-Plus (CIBIC plus), with the Mini-Mental State Examination (MMSE),Clinical Dementia Rating Scale-Sum of the Boxes (CDR-SB), and patient rated Quality of Life (QoL) used as secondary measures. Cognitive function, measured by the ADAS-cog was improved in the 5mg/d and 10-mg/d donepezil groups as compared with the placebo group at weeks 12, 18, and 24. Clinician’s global ratings on the CIBIC plus also improved in both the 5mg/d and 10mg/d donepezil groups relative to placebo At the end of the 6-week placebo washout phase, ADAS-cog scores and CIBIC plus ratings were not different for the three groups. Treatment benefits were observed consistently in both the 5mg/d and 10-mg/d groups on the MMSE and the CDR-SB, but there was no consistent effect on the patient-rated QoL. Cholinergic side effects (diarrhea, nausea, and vomiting) were reported more often in the 10mg/d group than either the 5-mgld or placebo groups. Side effects were transient and generally mild in severity. These data indicate that the donepezil is a well-tolerated drug that can improves cognition and global function in patients with mild to moderate Alzheimer’s disease (Rogers et al., 1998).

Donepezil treatment improves cognitive function of patients with Alzheimer’s disease (AD) when compared to a placebo-controlled group. The study was done to know the changes in regional cerebral blood flow (rCBF) of Alzheimer’s disease patients in short-term and long term treatment with donepezil. Regional cerebral blood flow was measured by \( N\)-isopropyl-\( p\)-123I-iodoamphetamine (IMP) autoradiography method.
Cerebral blood flow measurements were performed in 17 Alzheimer’s disease patients before treatment and after 3 months (short-term therapy) and 1 year (long-term therapy). Regional cerebral blood flow increased in short term donepezil therapy; regional cerebral blood flow was decreased after the long-term therapy (Ushijima et al., 2006).

Target validation is the investigation process by which potential drug target can be tested and given further reliability. Target validation is pharmaceutical approach in which influence of drug is observed. Acetylcholinesterase inhibition is the main target to improve cognitive functions in alzheimer disease patient. Okamura et al., (2008) studied invivo binding of donepezil in the brain of alzheimer disease patient. They radio labeled donepezil as [11C-methoxy]-donepezil for position emission tomography imaging. Evaluation of binding and distribution of donepezil performed on mild and moderate Alzheimer disease patients. PET (Position emission tomography) study was carried out after three months of neuropsychological medical examination. Region of interest analysis was carried for validation of regional distribution of donepezil. Position emission tomography images demonstrated that high volume of [11C]-donepezil distributed in all regions of brain like striatum, thalamus, striatum, and neocortex which are rich in acetylcholinesterase.

Studies were done in preparation Donepezil microparticles and evaluate its advantage in sustained release delivery system with subcutaneous injection once a month. Donepezil microparticles was prepared using poly (d,l-lactide-co-glycolide) (PLGA). Donepezil microparticles showed the loading ratio 13.2±2.1% (w/w) and yield 54.8±0.8% with mean particle size about 75 μm. In vitro release of Donepezil microparticles showed that donepezil completely released within 28 days in water, but the cumulative release percentages up to day 30 were 98.4% and 49.1% for phosphate buffer saline (PBS, pH 5.8) and PBS (pH 7.4), respectively (Zhang et al., 2007).

The effect of fatty acids on the skin permeation of donepezil base (DPB) and its hydrochloride salt (DPH) were studied in vitro using human cadaver skin and hairless mouse. Donepezil base and Hydrochloride salts were solubilized in propylene glycol (PG) containing 1% (w/v) fatty acid, after which the in vitro permeation through hairless mouse skin and human cadaver skin were evaluated using Keshary-Chien diffusion cells.
In vitro skin permeation rate of donepezil (DP) through the hairless mouse skin showed a parabolic relationship with increased carbon length of the fatty acid enhancers. Among the fatty acids tested, oleic acid for Donepezil base and palmitoleic acid for hydrochloride salts showed the highest enhancing effect, respectively. Both the permeation rates of Donepezil base and Hydrochloride salts evaluated in human cadaver skin were in good correlation with those in hairless mouse skin, regardless of the presence of fatty acids. Mouse skin model serves as vitro system that satisfactorily represents the characteristics of the human skin. In vitro results shows the optimal formulation that maintain the human plasma concentration of 50 ng/mL was determined to be 10 mg Donepezil with 1% (w/v) enhancer. When the donepezil transdermal formulations were applied to the abdominal skin of rats (2.14 cm2), the (steady-state plasma concentration ) C_{ss} was maintained for 48 h, among which the highest value of 52.21 ± 2.09 ng/mL was achieved with the Donepezil base formulation using oleic acid. These results showed that fatty acids could enhance the transdermal delivery of Donepezil and suggested the feasibility of developing a novel transdermal delivery system for clinical use (Choi et al., 2011).

2.7 PHENYTOIN SODIUM – ANTI-EPILEPTIC DRUG

Phenytoin (Dilantin) is an anti-epileptic drug, also called an anticonvulsant. It works by slowing down impulses in the brain that cause seizures. It acts to suppress the abnormal brain activity seen in seizure by reducing electrical conductance among brain cells by stabilizing the inactive state of voltage-gated sodium channels. Aside from seizures, it is an option in the treatment of trigeminal neuralgia in the event that carbamazepine or other 1st line treatment is deemed inappropriate. It is sometimes considered a class 1b anti-arrhythmic.
2.7.1 STRUCTURE

Fig 2.5 Structure of Phenytoin sodium

2.7.2 CHEMISTRY

A hydantoin-derivative, phenytoin sodium occurs as a white, hygroscopic powder which is freely soluble in water and warm propylene glycol, and soluble in alcohol. Phenytoin sodium slowly undergoes partial hydrolysis in aqueous solutions to phenytoin (base) with the resultant solution becoming turbid; the commercial injection contains 40% propylene glycol and 10% alcohol. Its empirical formula is C\textsubscript{15}H\textsubscript{11}N\textsubscript{2}NaO and chemical name is 5,5-diphenylimidazolidine-2,4-dione, with a molecular weight of 274.25.

The pH of the injectable solution is approximately 12. Phenytoin sodium is used in the commercially available capsules (both extended and prompt) and the injectable preparations. Phenytoin (base) is used in the oral tablets and suspensions. Each 100 mg of phenytoin sodium contains 92 mg of the base.

2.7.3 PHARMACOLOGY

Phenytoin is an antiepileptic drug which can be used in the treatment of epilepsy. The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited. Possibly by promoting sodium efflux from neurons, phenytoin tends to stabilize the threshold against hyper excitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient. This includes the reduction of post tetanic potentiation at synapses. Loss of post titanic potentiation prevents cortical seizure foci from detonating adjacent cortical areas. Phenytoin reduces the maximal activity of brain stem centres responsible for the tonic phase of tonic clonic
(grand mal) seizures. The plasma half-life in man after oral administration of phenytoin averages 22 hours, with a range of 7 to 42 hours. Steady-state therapeutic levels are achieved at least 7 to 10 days (5–7 half-lives) after initiation of therapy with recommended doses of 300 mg/day.

Phenytoin sodium has been in use for 60 years as an important antiepileptic drug. Its primary mechanism of action is modulation of the sustained repetitive firing of neurones by direct inhibition and blockage of voltage-gated sodium channels in the neuronal cell membrane, and by delay of cellular reactivation. The plasma protein binding of phenytoin is normally between 90% and 95%. The drug is rapidly distributed from the blood to the tissues and is almost completely metabolized in the liver. The half-life is shortened when phenytoin is given concomitantly with an enzyme-inducing drug, such as phenobarbital or carbamazepine. Phenytoin is effective for treating generalized tonic-clonic seizures, partial seizures with or without generalization, and convulsive status epilepticus (Livanainen, 1998).

Rojanasthien et al., (2007) studied the effect of doses on the bioavailability of a prompt-release and an extended release phenytoin sodium capsule after being given as single doses. They reported that the bioavailability of phenytoin sodium from both the preparations increased proportionally over the dose range of 100-300 mg. Franco et al., (2001) prepared solid dispersions of phenytoin in polyethylene glycol 6000 and polyvinylpyrrolidone K-30 with different drug: carrier ratios by the solvent method. These formulations were characterized in the solid state by FTIR spectroscopy, XRD studies and DSC methods.

Madhavi et al., (2012) published their study aimed to develop a sustained release tablet of phenytoin sodium due to narrow therapeutic window of phenytoin sodium to reduce dosing frequency. An efficient sustained release formulation of phenytoin sodium could not be designed as sustained release tablets, because up to 12 hrs it releases 60% of the drug. So it required some extent of work for desired sustained release. In this study the optimized formulation (F6) was developed by using hydroxy propyl methyl cellulose as a polymer base. Regulated drug release in Higuchi order manner was attained by using this polymer.
Sodium phenytoin, C_{13}H_{11}N_{2}NaO_{2}, in several concentrations was co-gelled with titania (TiO_{2}) by a sol–gel process. This technique is a promising method to encapsulate several drugs, in this case, phenytoin is an anticonvulsant used to control epileptic seizures. Samples were prepared by adding different concentrations of sodium phenytoin (Ph) to a solution of titanium \( n \)-butoxide. The resulting materials were characterized by transmission electron microscopy (TEM), Fourier transformed infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), and Brunauer–Emmet–Teller (BET) surface areas. The porous nanomaterials showed a wide range of particle size with a mean pore diameter of 5 nm. X-ray diffraction showed an amorphous structure of the prepared samples (Lopez et al., 2007).

Ahmed et al., (2011) reviewed the possibility of casein based formulations as promising controlled release drug delivery systems. Due to its unique physicochemical properties, casein, a natural biopolymeric surfactant has a potential use in the preparation of conventional and novel pharmaceutical drug delivery systems. A number of in vitro and in vivo studies showed that casein is a suitable material for efficient drug delivery. Researchers have noted that casein has evolved to be easily degradable by the digestive enzymes proteases.

Esher et al., (1996) conducted an investigation with the objective to formulate and prepare sustained-action microcapsules of phenytoin sodium. Using ethylcellulose and methyl acrylic acid copolymers as coating materials, microcapsules of phenytoin sodium were formulated by an organic phase separation and a granule coating method. The phase diagrams were used to study the phase separation in an ethylcellulose-petroleum ether-toluene system and the effect of temperature and amount of petroleum ether on the ethylcellulose left in the organic solvent mixture was investigated. The phase diagrams showed that increase in temperature did not significantly affect the ethylcellulose residue, and 60 ml of solvent was found adequate for microencapsulation. In vitro release of the formulated microcapsules and the commercially available preparations was performed in CO_{2}-free distilled water using the USP XXIII rotating basket method, and the profiles were evaluated by Higuchi kinetics. Geometric mean diameters of the microparticles prepared by two different methods showed differences due to different core-wall ratios.
SajidAli et al., (2010) done a study aimed to develop sustained release matrix tablets of phenytoin sodium an antiepileptic drug. Advantages of sustained release tablets are that they can often be taken less frequently than instant release formulations of the same drug, and that they keep steady levels of the drug in the blood stream. The tablets were fabricated by the wet granulation method using water as granulating agent along with matrix materials like guar gum, sodium alginate, tragacanth and xanthan gum with varying percentage. The granules were evaluated for angle of repose, bulk density, compressibility index, total porosity, and drug content. The tablets were subjected to weight variation test, drug content, hardness, friability, and in vitro release studies. The swelling behavior of matrix was also investigated. The granules showed satisfactory flow properties, compressibility, and drug content. The I.R spectral analysis studies confirmed no interaction between phenytoin with used natural gums. All the tablet formulations showed acceptable pharmacotechnical properties and complied with in-house specifications for tested parameters. In the further formulation development process, F8 (55% guar gum with 10% acacia), the most successful formulation of the study, exhibited satisfactory drug release and could extend the release up to 12 hours. The mechanism of drug release from all the formulations was diffusion coupled with erosion.

2.8 GLIMEPIRIDE – ANTIDIABETIC DRUG

Glimepiride is the first III generation sulphonyl urea it is a very potent sulphonyl urea with long duration of action. It is mainly used for concomitant use with insulin for the treatment of noninsulin-dependent (type 2) diabetes mellitus. AMARYL® (glimepiride tablets) is an oral blood-glucose-lowering drug of the sulfonylurea class. Glimepiride is a white to yellowish-white, crystalline, odorless to practically odorless powder formulated into tablets of 1-mg, 2-mg, and 4-mg strengths for oral administration. AMARYL tablets contain the active ingredient glimepiride and the following inactive ingredients: lactose (hydrous), sodium starch glycolate, povidone, microcrystalline cellulose and magnesium stearate.
2.8.1 STRUCTURE

![Figure 2.6: Structure of Glimepiride](image)

2.8.2 CHEMISTRY

The molecular weight is 490.61556 [g/mol]. The molecular formula is $C_{24}H_{34}N_{4}O_{5}S$. There are 3 H-Bond donors and 5 H-Bond acceptors. Exact mass of the compound is 490.224991. The topological polar surface area is 133.

2.8.3 PHARMACOLOGY

In Type 2 diabetes, it is considered that the lowered insulin secretion and the lowered insulin sensitivity cause hyperglycemia. Sulfonylureas have strong blood-glucose lowering effect by stimulating insulin secretion and have been widely used in the treatment of Type 2 diabetes. However, the use of sulfonylurea has several problematic issues (weight gain, hypoglycemia), which would due to stimulation of strong insulin secretion. Glimepiride, a new sulfonylurea, has a blood-glucose lowering effect as strong as those of existing sulfonylureas, but only induces mild insulin secretion. The sulfonylurea receptor has a weaker affinity for glimepiride than glibenclamide. The association and dissociation to the sulfonylurea receptor of glimepiride are faster than those of glibenclamide. Additionally, it is confirmed by basic studies that part of the glimepiride effect is attributable to improving insulin sensitivity.

The mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells, and increasing sensitivity of peripheral tissues to insulin. Glimepiride likely binds to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing
potassium conductance and causing depolarization of the membrane. Membrane depolarization stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin.

Following either an intravenous or oral dose, glimepiride is completely metabolized by oxidative biotransformation to a major metabolite, cyclohexyl hydroxymethyl derivative (M1), via the hepatic cytochrome P450 II C9 subsystem. M1 is further metabolized to the carboxyl derivative (M2) by one or several cytosolic enzymes. M1, but not M2, possessed approximately one third of the pharmacologic activity of its parent in an animal model. However, whether the glucose-lowering effect of M1 is clinically significant is not clear.

Glimepiride, like glyburide and glipizide, is a "second-generation" sulfonylurea agents. Glimepiride is used with diet to lower blood glucose by increasing the secretion of insulin from pancreas and increasing the sensitivity of peripheral tissues to insulin. With glimepiride, GI absorption is complete, with no interference of meals. Significant absorption was seen within 1 hour, and distributed throughout the body, bound to the plasma protein to an extent of 99.5%. It is metabolized by oxidative biotransformation, and 60% is excreted in the urine, the remaining being excreted in the feces.

The Glimepiride micro particles were prepared using emulsion solvent evaporation method. Micro particulates were subjected for characterization in terms of encapsulation efficiency, particle size, drug loading, FTIR, DSC, SEM analysis and drug release studies. The microparticles obtained were found to be discrete, spherical, non-sticky and free flowing. The study also proves good percentage of encapsulation efficiency and drug release profile (Sarath Chandiran et al., 2013).

Ammar et al., (2008) reported their study to develop transdermal delivery system for Glimepiride using chitosan as the polymer. Release studies showed adequate release rates from chitosan films. Permeation studies through full thickness rat abdominal skin were conducted. In vivo studies on diabetic rats for selected formulae revealed a marked therapeutic efficacy sustained for about 48 hours. The results shed light on feasibility of utilizing chitosan as an effective, safe transdermal delivery system for glimepiride characterized by increased patient compliance and better control of the disease.
Cheong et al., (2009) prepared a potential transdermal drug delivery system using an ethylene-vinyl acetate matrix containing glimepiride. Permeation studies, rate of controlled release and activation energy of drug release shows that an EVA matrix containing a permeation enhancer can be used for the transdermal controlled delivery of glimepiride.

Sandhya et al., (2013) proposed the development of controlled release formulations of Glimepiride based on osmotic technology. The effect of different formulation variable was studied to optimize release profile. The release rate increased significantly as the increase of osmogen. The release rate increased significantly with the increase of concentration of pore forming agent as noticed from the dissolution profile of the formulations. Thus drug release was inversely proportional to the concentration of osmogen in the core and the amount of pore forming agents in the coated tablets. The drug release from developed formulations was independent of pH. The manufacturing procedure was standardized and found to be reproducible.

2.9 PROPRANOLOL HCL – ANTI-HYPERTENSIVE DRUG

It is a non-selective beta blocker mainly used in the treatment of hypertension. It was the first successful beta blocker developed. It is the only drug proven effective for the prophylaxis of migraines in children. Propranolol is available in generic form as propranolol hydrochloride, as well as an AstraZeneca and Wyeth product under the trade names Inderal, Inderal LA, Avlocardyl (also available in prolonged absorption form named "Avlocardyl Retard"), Deralin, Dociton, Inderalici, InnoPran XL, Sumial, Anaprilinum (depending on marketplace and release rate), Bedranol SR (sandoz). Propranolol was derived from the early β-adrenergic antagonists dichloroisoprenaline and pronethalol.
2.9.1 STRUCTURE

Fig 2.7 Structure of Propranolol

2.9.2 CHEMISTRY

Propranolol is synthesized in two ways from the same initial substance. The first way consists of reacting 1-naphthol with epichlorohydrin. Opening of the epoxide ring gives 1-chloro-3-(1-naphthyloxy)-2-propanol, which is reacted further with isopropylamine, giving propranolol. The second method uses the same reagents in the presence of a base and consists of initially making 3-(1-naphthyloxy) propyleneoxide, the subsequent reaction with isopropylamine which results in epoxide ring opening leading to the formation of propranolol.

2.9.3 PHARMACOLOGY

Propranolol is rapidly and completely absorbed, with peak plasma levels achieved approximately 1–3 hours after ingestion. Co-administration with food appears to enhance bioavailability. Despite complete absorption, propranolol has a variable bioavailability due to extensive first-pass metabolism. Hepatic impairment will therefore increase its bioavailability. The main metabolite 4-hydroxypropranolol, with a longer half-life (5.2–7.5 hours) than the parent compound (3–4 hours), is also pharmacologically active.

Propranolol is a highly lipophilic drug achieving high concentrations in the brain. The duration of action of a single oral dose is longer than the half-life and may be up to 12 hours, if the single dose is high enough (e.g., 80 mg). Effective plasma concentrations are between 10–100 ng/mL. Toxic levels are associated with plasma concentrations above 2000 ng/ml.

Propranolol is a non-selective beta blocker, that is, it blocks the action of epinephrine and norepinephrine on both β₁- and β₂-adrenergic receptors. It has little
intrinsic sympathomimetic activity (ISA) but has strong membrane stabilizing activity (only at high blood concentrations, eg overdosage). Research has also shown that propranolol has inhibitory effects on the norepinephrine transporter and/or stimulates norepinephrine release (present experiments have shown that the concentration of norepinephrine is increased in the synapse but do not have the ability to discern which effect is taking place). Since propranolol blocks β-adrenoceptors, the increase in synaptic norepinephrine only results in α-adrenergic activation, with the α1-adrenoceptor being particularly important for effects observed in animal models. Therefore, some have suggested that it be looked upon as an indirect α1 agonist as well as a β antagonist. Probably owing to the effect at the α1-adrenoceptor, the racemate and the individual enantiomers of propranolol have been shown to substitute for cocaine in rats, with the most potent enantiomer being S-(–)-propranolol. Both enantiomers of the drug have a local anesthetic (topical) effect. In addition, some evidence suggests that propranolol may function as a partial agonist at one or more serotonin receptors (possibly 5-HT1B).

Abruzzo et al., (2012) developed and characterized chitosan/gelatin films as innovative mucoadhesive system for buccal delivery of propranolol hydrochloride. FTIR and TGA analysis confirmed the interaction between chitosan and gelatin. The presence of higher chitosan amounts in chitosan/gelatin films allowed the lowest percent water-uptake ability and the highest in vivo residence time in the buccal cavity. Chitosan/gelatin films also showed an interesting aspect of higher compatibility with buccal microflora in the absence of drug and their ability to determine growth inhibition for pathogen bacteria when loaded with drug.

Cheng et al., (2013) conducted a study to develop an in vitro–in vivo correlation (IVIVC) model for hydrophilic matrix extended-release (ER) propranolol dosage formulations. The in vitro release characteristics of the drug were determined and In vivo plasma concentrations and pharmacokinetic parameters in male beagle dogs were obtained after administering oral, ER formulations and immediate-release (IR) commercial products. The IVIVC model was developed using pooled fraction dissolved and fraction absorbed of propranolol ER formulations, ER-F and ER-S, with different release rates. An additional formulation ER-V, with a different release rate of
propranolol, was prepared for evaluating the external predictability. The results showed that the percentage prediction error for the external validation study and the observed low prediction errors demonstrated that the propranolol IVIVC model was valid.

Thacharodi and Pandurangarao, (1995) prepares membrane permeation-controlled transdermal drug delivery systems using the natural polymer, chitosan. An adhesive sealing technique was used to construct the devices. Propranolol hydrochloride was selected as the model drug for the present study. Chitosan membranes with different permeability to propranolol hydrochloride obtained by controlled cross-linking with glutaraldehyde were used to regulate the drug release in the devices. Chitosan gel was used as the drug reservoir. The drug release profiles showed that the drug delivery is completely controlled by the devices. The rate of drug release was found to be dependent on the type of membrane used.

Ramao et al., (2003) proposed a study was for in vivo evaluation of orally and transdermally administered propranolol hydrochloride in rabbits. Transdermal patches of propranolol hydrochloride (PPN) were formulated employing ethyl cellulose and polyvinylpyrrolidone as film formers. The pharmacodynamic (PD) and pharmacokinetic (PK) performance of PPN following transdermal administration was compared with that of oral administration. This study was carried out in a randomized cross-over design in male New Zealand albino rabbits. The PK parameters such as maximum plasma concentration ($C_{\text{max}}$), time for peak plasma concentration ($t_{\text{max}}$), mean residence time (MRT) and area under the curve (AUC) were significantly different following transdermal administration compared to oral administration. The terminal elimination half-life of transdermally delivered PPN was found to be similar to that following oral administration. In contrast to oral delivery, a sustained therapeutic activity was observed over a period of 24 h after transdermal administration compared to oral administration. The relative bioavailability of PPN was increased about fivefold to sixfold after transdermal administration as compared to oral delivery. This may be due to the avoidance of first pass effect of PPN. The sustained therapeutic activity was due to the controlled release of drug into systemic circulation following transdermal administration.
A novel oral controlled delivery system for propranolol hydrochloride (PPL) was developed and optimized by Gil et al., (2006). The in vitro dissolution profiles of sustained-release matrix tablets of racemic PPL were determined and compared with the United States Pharmacopeia (USP) tolerance specifications for Propranolol Hydrochloride Extended-Release Capsules. The influence of matrix forming agents (native dextran, hydroxypropyl methylcellulose (HPMC), cetyl alcohol) and binary mixtures of them on PPL release in vitro was investigated. A central composite design was applied to the optimization of a sustained-release tablet formulation. The sustained-release matrix tablets with good physical, mechanical and technological properties were obtained. The value for the similarity factor suggested that the dissolution profile of the present two sustained-release oral dosage forms are similar. Higuchi (diffusion) and Hixon–Crowell (erosion) kinetic profiles were achieved and this codependent mechanism of drug release was established.

2.10 TRANSDERMAL DRUG DELIVERY

Xiaoping et al., (2006) prepared copolymer membranes through photosynthesis three different monomer (2-hydroxy-3-phenoxypropylacrylate, 4-hydroxybutyl acrylate and sec-butyl tiglate) mixtures. The membranes reflect a linear permeation property in clonidine transdermal drug delivery system. Permeation property of the membranes with different monomer ratios and thickness were investigated. Optimized membrane was standardized by combining results from studies like FTIR, DSC and SEM.

Hemant and Shivakumar, (2010) reported that Chitosan acetate exhibits significant physicochemical properties like high drug entrapment, large water permeability and swelling, and great potency in vitro drug release. This finding proves chitosan acetate films as highly efficient for the delivery of propranolol hydrochloride via the transdermal route with several advantages.

Reinhard, (2011) reviewed many nanocarriers to understand their potential in transdermal delivery. The study shows that Micro emulsions exhibits to be most promising concerning dermal drug delivery.

Silva et al., (2012) evaluated three nonionic ether-monohydroxyl surfactants (C12E1, C12E5, and C12E8) as skin permeation enhancers in the transdermal drug
delivery of two drugs, ondansetron hydrochloride and diltiazem hydrochloride, formulated as hydrogels. The permeation studies were conducted in two conditions; one by applying chemical permeation enhancer solutions alone and other in combination with iontophoresis. Skin integrity evaluation studies, Histological studies and SEM studies, cytotoxicity studies were also conducted and realized that both drugs can be successfully delivered through the skin using a combination of chemical enhancement and iontophoresis, attaining plasma levels comparable to those obtained with oral formulations.

Wang et al., (2012) conducted their study to produce a transdermal microemulsion drug delivery system (TMDDS) for Tripterygium Wilfordii Hook f. (TWHF) to solve male reproductive toxicity problem of TWHF. The main difficulty associated with the study was the toxicity of TWHF of oral administration and later this got overcome by loading the drug in the micro emulsion and applying to the skin. Thus the novel micro emulsion based transdermal drug delivery system was developed and was proved with stronger efficacy lower toxicity.

Ochoa et al., (2012) reviewed some of the recent efforts in the design and fabrication of polymeric microdevices for transdermal and subcutaneous drug delivery. Although drug patches have been a successful platform used to deliver small lipophilic molecules across the skin, their limitations have instigated a considerable research effort in ways to enhance the skin permeability or totally bypassing it through various methods such as skin abrasion and laser microdrilling. The study also present two examples of the research towards using phase change liquids in polymeric structures to create disposable micropumps and development of MEMS based transdermal drug delivery system.

Ji et al., (2012) investigated the applicability of silver hybridized porous chitosan to the patches of Transdermal Drug Delivery System and studied mechanical strength, drug-delivery efficiency, morphology, and surface properties of the chitosan and the optimal composition (silver nitrate of 3%) for bio-patch was selected and found that can be widely used as sterilizing transdermal drug-delivery system (TDDS) in various fields.

Myung et al., (2013) prepared a drug – inorganic nanohybrid (FB - LDH) by intercalating a transdermal model drug flurbiprofen (FB), in to Layered double
hydroxides (LDHs) through co-precipitation reaction. Intercalation was confirmed by using X-ray and FTIR studies to prove how well the drug molecules are stabilized in LDHs. Gel suspension studies and diffusion cell experiments confirmed that a gel suspension of the nanohybrid containing a tailored amount of an aqueous solution of macromolecules is a novel formulation for controlled delivery of a transdermal drug.

Baek et al., (2013) prepared zaltoprofen gels using carbomer with mixture solution of polyethylene glycol (PEG) 400, Tween 80 and (2-hydroxypropyl)-β-cyclodextrin (HPCD, subsequently oleic acid as a penetration enhancer was added. Zaltoprofen gel containing T2 and oleic acid could promote the percutaneous absorption of zaltoprofen and increase AUC by 183% compared to zaltoprofen gel without oleic acid. The result reveals that zaltoprofen gel containing T2 and oleic acid did not cause dermal irritations in an experimental animal which confirms that zaltoprofen gel containing T2 and oleic acid can be used as Transdermal drug delivery systems.

Shahinaze et al., (2013) developed Microemulsion (ME) and poloxamer microemulsion-based gel (PMBG) and optimized to enhance transport of diclofenac epolamine (DE) into the skin forming in-skin drug depot for sustained transdermal delivery of drug. D-optimal mixture experimental design was applied to optimize ME that contains maximum amount of oil, minimum globule size and optimum drug solubility. The systems were assessed for drug solubility, globule size and light absorbance. The optimized ME and PMBG were assessed for pH, drug content, skin irritation, stability studies and ex vivo transport in rat skin. Contrary to PMBG and Flector gel, the optimized ME showed the highest cumulative amount of DE permeated after 8 h and the in vivo anti-inflammatory efficacy in rat paw edema was sustained to 12 h after removal of ME applied to the skin confirming the formation of in-skin drug depot. The results proposed that topical ME formulation, containing higher fraction of oil solubilized drug, could be promising for sustained transdermal delivery of drug.

Ahad et al., (2014) reviewed various attempts to develop the transdermal therapeutic system for various antihypertensive agents, including β-blockers, an important antihypertensive class. The shortcomings associated with β-blockers such as more frequent dose administration, extensive first pass metabolism and variable
bioavailability; make them an ideal candidate for transdermal therapeutic systems. The review gives a brief view of different β-blockers formulated as transdermal therapeutic system in detail to enhance the bioavailability as well as to improve patient compliance.

Chen et al., (2014) presented an review of the investigations in the field of transdermal drug delivery systems and discusses in detail about selection and design of suitable permeation enhancers.

Lam and Gambari, (2014) provides an overall discussion of microencapsulation systems for both oral and transdermal drug deliveries through their review. They reports that clinically, many drugs, especially proteins and peptides, are susceptible to the gastrointestinal tract and the first-pass metabolism after oral administration while some drugs exhibit low skin permeability through transdermal delivery route. The study concludes that medicated microcapsules as oral and transdermal drug delivery vehicles are believed to offer an extended drug effect at a relatively low dose and provide a better patient compliance.

### 2.11 TRANSDERMAL DELIVERY OF PROPRANOLOL

Singh et al., (1991) studied passive and iontophoretic transport of propranolol hydrochloride (PHCl) through human epidermis by varying the pH of the donor solution and the current density. The steady state flux during passive transport of PHCl was maximal at pH 11 and minimal at pH 7.4. The transport was enhanced during iontophoresis in comparison to the passive diffusion at all pHs. The cathodal iontophoretic flux was greater than anodal and was maximal at pH 11. The flux was also found to be proportional to the current density. One of the most important applications of this study is the possibility of enhancing and controlling the transdermal delivery of PHCl by iontophoresis.

Rajesh and Pandit, (1994) prepared three transdermal formulations containing propranolol hydrochloride in a hydrophilic polymer matrix, one without a rate controlling membrane (H-1), one with a 20µ thick Ethylene Vinyl Acetate (EVA) rate controlling membrane (H-2) and one with a 65µ thick EVA membrane. These patches were evaluated for their in-vitro performance. Cumulative % permeated across excised hair-
free rat skin was 79.2% from H-1, 65.53% from H-2 and 53.44% from H-3. Matrix diffusion profile was observed with H-1 patch.

Tacharodi et al., (1995) prepared membrane permeation-controlled transdermal drug delivery systems using the natural polymer, chitosan. An adhesive sealing technique was used to construct the devices. Propranolol hydrochloride was selected as the model drug for the present study. Chitosan membranes with different permeability to propranolol hydrochloride obtained by controlled cross-linking with glutaraldehyde were used to regulate the drug release in the devices. Chitosan gel was used as the drug reservoir. The drug release profiles showed that the drug delivery is completely controlled by the devices. The rate of drug release was found to be dependent on the type of membrane used.

Rama Rao et al., (2003) conducted a study with the purpose of in vivo evaluation of orally and transdermally administered propranolol hydrochloride in rabbits. Transdermal patches of propranolol hydrochloride (PPN) were formulated employing ethyl cellulose and polyvinylpyrrolidone as film formers. The pharmacodynamic (PD) and pharmacokinetic (PK) performance of PPN following transdermal administration was compared with that of oral administration. The terminal elimination half-life (t(1/2)) of transdermally delivered PPN was found to be similar to that following oral administration. In contrast to oral delivery, a sustained therapeutic activity was observed over a period of 24 h after transdermal administration compared to oral administration. The relative bioavailability of PPN was increased about fivefold to sixfold after transdermal administration as compared to oral delivery. The sustained therapeutic activity was due to the controlled release of drug into systemic circulation following transdermal administration.

Chomchan et al., (2005), prepared the polymeric film formulations for transdermal use by employing EC and PVP as a film former, and DBP as a plasticizer to avoid the extensive first-pass elimination and achieve the desirable penetration rate of PPL. The film successfully improved the skin penetration of PPL. They concluded that even though a remarkable synergistic effect of PG was not observed, these film
preparations would be promising ones that can safely achieve the desirable systemic absorption of PPL.

It is highly evident from the research statements cited out so far that drug delivery system using casein microparticles possess a lot of scope to signify and improve the drug delivery process. Moreover, targeted delivery of drugs is critical in improving therapeutic efficiency and minimizing the untoward effects. For the development of such delivery systems, casein possesses carious advantages such as biocompatibility, biodegradability and other unique biological properties. Unfortunately, the literature lacks data in terms of drug delivery of various novel drug molecules using casein systems and its in-vivo correlations. This has given a proper direction towards the formation of this work with the following objectives,

2.12 OBJECTIVES

i. Controlled release of MTX using biodegradable CS microparticles

ii. Designing casein microparticles based controlled delivery system for Levocetirizine dihydrochloride to improve the patient compliance in anti-histaminic medication

iii. Alzheimer’s disease management using DPZ loaded CS microparticles

iv. Formulation of Phenytoin Sodium loaded Casein microparticles for brain delivery to treat Epilepsy

v. Pharmacotherapy of diabetes using Glimepiride loaded casein microparticles

vi. Transdermal delivery of Propranolol HCl from casein films to reduce extensive hepatic metabolism