3.1 HYDROGELS

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. Hydrogels swell but not dissolve when brought in contact in water. The networks are composed of homopolymers or copolymers, and are insoluble due to the presence of chemical crosslinks (tie-points, junctions), or physical crosslinks (entanglements or crystallites). The latter provide the network structure and physical integrity. These hydrogels exhibit a thermodynamic compatibility with water which allows them to swell in aqueous media. There are numerous applications of these hydrogels, in particular in the medical and pharmaceutical sectors. Hydrogels resemble natural living tissue more than any other class of synthetic biomaterials. This is due to their high water contents and soft consistency which is similar to natural tissue. Furthermore, the high water content of the materials contributes to their biocompatibility.

Hydrogels can be formulated in a variety of physical forms, including slabs, microparticles, nanoparticles, coatings, gels and films. As a result, they are commonly used in clinical practice and experimental medicine for a wide range of applications, including biosensors, tissue engineering and regenerative medicine, separation of biomolecules or cells and barrier materials to regulate biological adhesions, materials for artificial skin, and drug delivery devices. Among these applications, hydrogel-based drug delivery devices have become a major area of research interest.

3.1.1 Classification of Hydrogels

Depending on their method of preparation, ionic charge, or physical structure features, hydrogels maybe classified in several categories.

- Based on the nature of the side groups – neutral, ionic
According to their mechanical and structural characteristics – affine, phantom networks

Based on the method of preparation – homopolymer, copolymer networks, multipolymer hydrogels, interpenetrating polymeric hydrogels

Based on the physical structure of the networks – amorphous, semi-crystalline, hydrogen-bonded structures, supermolecular structures, hydrocolloidal aggregates

3.1.1.1 Environmentally responsive hydrogels (Stimuli responsive hydrogels)

Hydrogels that change their swelling behaviour upon exposure to an external stimulus, such as, e.g., change in pH, temperature, light or electric fields are known as environmentally responsive polymers. They have the ability to respond to change in their external environment. They can exhibit dramatic changes in their swelling behaviour, network structure, permeability and mechanical strength in response to change in pH \(^\text{11}\) or ionic strength of the surrounding fluid \(^\text{12}\), temperature \(^\text{13}\), nature and composition of the swelling agent, enzymatic or chemical reaction, and electrical \(^\text{14}\) or magnetic stimuli. In most responsive networks, a critical point exists at which this transition occurs. These types of environmental sensitive hydrogels are also called “Intelligent” or “Smart” hydrogels. \(^\text{9}\)

Advantages of Smart Gels (Sol-gels)

- Can be easily delivered into specific site in body
- Less amount of drug is required, so side effects can be minimized
- Prevent the emergence of drug resistance
- Better patient compliance and comfort
- Optimum concentration of drug can be achieved in the localized region
- Continuous release of drug for a prolonged period of time
Cost effective

An interesting characteristic of numerous responsive gels is that the mechanism causing the network structural changes can be entirely reversible in nature. The ability of pH- or temperature-responsive gels to exhibit rapid changes in their swelling behavior and pore structure in response to changes in environmental conditions lend these materials favorable characteristics as carriers for bioactive agents, including peptides and proteins. This type of behavior may allow these materials to serve as self-regulated, pulsatile drug delivery systems. In the long term perspective, it is hoped that sensor-activated systems could be developed from these hydrogels.

The term “in situ gelling polymers” also describes a stimulus-induced response, but is generally used more narrowly to denote formulations that gel upon contact with mucosa, that is, they gel once they are in target position. The most prominent advantage of such formulations is that they are fluid-like prior to contact with the body surface, and can thus easily be administered as a drop or by an injection or using a spray device.

There are several possible mechanisms that lead to in situ gel formation: solvent exchange, UV-irradiation, ionic cross-linkage, pH change and temperature modulation. In situ gels are injectable fluids that can be introduced into the body in a minimally invasive manner prior to solidifying or gelling within the desired tissue, organ or body cavity.
Figure 3.01: Schematic illustration showing the different types of responses of “intelligent” polymer systems to environmental stimuli.

Figure 3.02: Schematic representation of sol-to-gel transition in stimuli-sensitive polymers
Injectable gel-forming matrices offer several advantages over systems shaped into their final form before implantation. Injectable materials do not need surgical procedure for placement and removal (if not biodegradable), and various therapeutic agents can be incorporated by simple mixing. When they are used to fill a cavity or a defect, their flowing nature enables a good fit. *In situ* implant formation can occur as a result of a physical or chemical change of the system.

### 3.1.1.2 Temperature Sensitive Hydrogels\textsuperscript{10,15-17}

The environmentally sensitive hydrogels exhibit temperature-sensitive swelling behavior due to a change in the polymer/swelling agent compatibility over the temperature range of interest. Temperature-sensitive hydrogels undergo a volume phase-transition or a sol-gel phase transition at a critical temperature, known as lower critical solution temperature (LCST), or upper critical solution temperature (UCST). The LCST polymers exhibit a hydrophilic-to-hydrophobic transition with increasing temperature, whereas the UCST systems undergo the opposite transition. LCST systems have received more attention than UCST systems due to numerous advantages. The mixing of UCST systems with drugs needs to be performed at high temperature, which may be harmful to some unstable drugs or biomolecules.
Figure 3.03: Mechanism of *in situ* physical gelation driven by hydrophobic interactions

Table 3.01: LCSTs of several typical thermosensitive polymers

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Polymer</th>
<th>LCST (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Poly(N-isopropylacrylamide) (PNIPAM)</td>
<td>32</td>
</tr>
<tr>
<td>2.</td>
<td>Poly(N,N-diethylacrylamide) (PDEAM)</td>
<td>25</td>
</tr>
<tr>
<td>3.</td>
<td>Poly(N-ethylmethacrylamide) (PNEMAM)</td>
<td>58</td>
</tr>
<tr>
<td>4.</td>
<td>Poly(methyl vinyl ether) (PMVE)</td>
<td>34</td>
</tr>
<tr>
<td>5.</td>
<td>Poly(2-ethoxyethyl vinyl ether) (PEOVE)</td>
<td>20</td>
</tr>
<tr>
<td>6.</td>
<td>Poly(N-vinylcaprolactam) (PNVCa)</td>
<td>30-50</td>
</tr>
<tr>
<td>7.</td>
<td>Poly(N-vinylisobutyramide) (PNVIBAM)</td>
<td>39</td>
</tr>
<tr>
<td>8.</td>
<td>Poly(N-vinyl-n-butyramide) (PNVBAM)</td>
<td>32</td>
</tr>
</tbody>
</table>
Thermoresponsive polymers can be broadly classified basing upon their block copolymers composition, as

1. Poly (N-substituted acrylamide) based block copolymer
   Eg: Poly(N-isopropylacrylamide) - PNIPAM

2. Poly (vinyl ether) based block copolymer
   Eg: Poly (2-methoxyethyl vinyl ether) PMOVE

3. PEO/PPO based block copolymers
   Eg: Poly (ethylene oxide)-Poly (propylene oxide)- Poly (ethylene oxide) (PEO-PPO-PEO) –Poloxamer

4. Poly Ethylene Glycol (PEG)/polyester block copolymers
   Eg: PEG-poly(L-lactide)-PEG triblock copolymers (PEG-PLLA-PEG)

5. Others
   a. PEG based amphilic block copolymers, PEG-poly(trimethylene carbonate) (PEG-PTMC)
   b. Poly (organophosphazene) derivatives
   c. Poly (1,2-propylene phosphate)
   d. Polysaccharides Eg: Cellulose derivatives – Carrageenan, methyl cellulose, hydroxyl propyl methyl cellulose, xyloglucan, chitosan and glycerophosphate
3.1.1.3 pH sensitive hydrogels\textsuperscript{11,15,16,18}

pH is another important environmental parameter for drug delivery systems, because the pH change occurs at many specific or pathological body sites, such as stomach, intestine, endosome, lysosome, blood vessels, vagina and tumour extracellular sites. Hence, they are one of the most widely studied physiologically responsive \textit{in situ} gels.

\textbf{Figure 3.04}: Tissue locations applicable for \textit{in situ} gel-based drug delivery systems

For non-ionic hydrogels, the degree of swelling only depends on the chemical composition of polymers. However, for ionic hydrogels the swelling depends not only on the chemical composition but also on the pH of the surrounding medium. Therefore, the pH sensitive polymers show dramatic changes on the pH and on the compositions of the external solutions.
The pH sensitive polymers are classified as acidic weak polyelectrolytes and basic weak polyelectrolytes according to the method of ionization i.e., donating or accepting protons. Anionic hydrogels deprotonate and swell more when external pH is higher than pKa of the ionizable groups bonded on polymer chains, while cationic hydrogels protonate and swell more when external pH is lower than the pKb of the ionizable groups. Depending on the ionic monomers used to fabricate the hydrogel, the pH dependent swelling curves exhibit one or more inflection points near the pKa/pKb of the ionizable groups as shown in Figure 3.05.

![Figure 3.05: Schematic of relative ionic hydrogel swelling as a function of pH.](image)

Typical acidic pH-sensitive polymers for drug delivery are based on the polymers containing carboxylic groups, such as poly(acrylic acid), poly(methacrylic acid), poly(L-glutamic acid), and polymers containing sulphonamide groups. Typical examples of the basic polyelectrolytes include poly(tertiary amine methacrylate) such as poly(2-(dimethylamino) ethyl methacrylate) and poly(2-(diethylamino)ethyl methacrylate), poly(2-vinylpyridine) and biodegradable poly(β-amino ester).
3.1.1.4 Temperature/pH sensitive hydrogels\textsuperscript{10,17}

Intelligent polymers and gels responding to multiple stimuli, especially to both temperature and pH, have received increasing interest for their unique advantages over the systems with single stimulus-responsiveness. The pH- and temperature-sensitive block copolymer hydrogels were largely prepared by combining thermo-responsive segments with pH-responsive segments.

- Copolymers of N-isopropylacrylamide, and acrylic acid or methacrylic acid as temperature and pH-sensitive monomers
- Copolymers containing propylacrylic acid (PAA) and NIPAAm pendant chains as pH- and thermosensitive moieties, respectively.
- poly((2-dimethyl amino) ethyl methacrylate-co-BMA)\textsuperscript{19}
- Block copolymers based on specific thermosensitive agent like pluronic, PEG-b-polyester

3.1.1.5 Complexing hydrogels\textsuperscript{20}

They exhibit environmental sensitivity due to the formation of polymer complexes. Polymer complexes are insoluble, macromolecular structures formed by the non-covalent association of polymers with affinity for one another. The complexes form as a result of the association of repeating units on different chains (interpolymer complexes) or on separate regions of the same chain (intrapolymer complexes). The stability of the associations is dependent on such factors as the nature of the swelling agent, temperature, type of dissolution medium, pH and ionic strength, network composition and structure, and length of the interacting polymer chains.

In this type of gel, complex formation results in the formation of physical cross-links in the gel. As the degree of effective cross linking is increased, the network mesh size and degree of swelling is significantly reduced. As a result, if
hydrogels are used as drug carriers, the rate of drug release will decrease dramatically upon the formation of interpolymer complexes. 

Eg: Poly(methacrylic acid) [PMAA] hydrogels in the presence of PEG. In acidic media, the PMAA chain collapsed due to the presence of linear chains of PEG and due to formation of interpolymer complexes between PMAA and PEG. The gels swelled when placed in neutral or basic media.

### 3.1.1.6 Other stimuli-sensitive hydrogels

Hydrogels respond to other stimuli including electric field, magnetic field, light, ionic strength or biomolecules (glucose, enzymes, antigens).\(^{21-32}\)

The **electro-sensitive hydrogels** were mainly based on polyelectrolytes\(^{24}\) or the polymer networks incorporating electroresponsive particles.\(^{22}\) The mechanisms of drug release from the electro-responsive hydrogels include ejection of the drug solution during deswelling, diffusion, electrophoresis of charged drugs and electro-induced gel erosion.

**Magnetic field sensitive hydrogels** were prepared by incorporating the magnetic particles into the crosslinked PNIPAM or gelatin hydrogels.\(^{23,24}\)

**Light-sensitive hydrogels** were prepared by introducing a light-sensitive molecule into the polymer network; the swelling-deswelling behavior could be controlled by UV-induced ionization-deionization\(^{26}\) or by triggering the response of a thermosensitive hydrogel via transforming light into thermal energy.\(^{27}\)

**Glucose-sensitive hydrogels** were developed as the smart insulin delivery systems responding to the blood glucose concentration. The glucose oxidase was incorporated in the polymer network and could catalyze excessive glucose into gluconic acid, which led to the swelling of a basic polyelectrolyte hydrogel\(^{29}\) or
shrinkage of an acidic polyelectrolyte hydrogel\textsuperscript{30} concomitantly with the release of insulin via different mechanisms.

**Figure 3.06:** Schematic representation of glucose-sensitive swelling changes in a poly(GEMA)–Con A hydrogel

*Enzyme-sensitive hydrogels* were designed for site-specific drug-delivery systems according to the specific enzymes in specific organs. For example, an HMDI (Hexamethylene diisocyanate) crosslinked dextran hydrogel was prepared for colon-specific drug delivery, due to the dextranases in the colon.\textsuperscript{31}

*Antigen-sensitive hydrogels* were developed based on the specific antigen-recognition function of an antibody.\textsuperscript{32} The hydrogel swelled in the presence of a free antigen but shrunk in the absence of a free antigen.
3.1.2 Advantages of *in situ* gelling systems

1. Aqueous solutions of the *in situ* gelling polymers (block copolymers) exhibit a sol–gel transition without syneresis in response to the changes in pH or/and temperature, and therefore are widely used as the injectable controlled drug-delivery systems.

2. This kind of systems has many advantages including a simple drug formulation and administration procedures, no organic solvent, site-specificity, a sustained drug release behavior, less systemic toxicity and ability to deliver both hydrophilic and hydrophobic drugs.

3.1.3 Challenges of *in situ* gelling systems

1. The main challenges for the application of a stimuli-induced *in situ* forming hydrogel include short gelation time, proper gelation temperature and/or pH, appropriate mechanical strength, biocompatibility, proper persistent time, convenient practical procedure, no significant pH decrease and desirable drug release behavior. These properties depend on copolymer composition,
hydrophilic/hydrophobic balance, hydrophilic/hydrophobic block length, molecular weight, polymer architecture, and so on.

2. In addition, drug release from the block copolymer hydrogels is based on the mechanisms of drug diffusion and gel erosion; therefore, the biodegradability or bio-eliminability of the block copolymers is important for application in vivo, and should be considered in the processes of polymeric molecular designs and choosing the synthetic methods of the non-degradable segments.

3.1.4 Applications of in situ gelling systems

Applications of in situ gels can be broadly classified into

- Biomedical applications
  - Contact lenses and ocular implants
  - Tissue prosthesis and tissue regeneration
  - Tissue engineering
  - Biosensors
  - Wound dressings

- Pharmaceutical applications
  - Drug Delivery Systems
    - conventional preparations – topical gel
    - specialized dosage forms – liposomes, niosomes, implants
  - Site specific drug delivery systems
    - Oral (Buccal), Nasal, Ocular, Periodontal (tooth), Vaginal, Rectal, Stomach floating systems
  - Injectable drug delivery systems
    - Subcutaneous, intrapericardial, intrasynovial
  - Depot formulations
Subcutaneous, Intramuscular

Protein/ gene delivery

The physical properties of hydrogels make them attractive for a variety of biomedical and pharmaceutical applications. Their biocompatibility allows them to be considered for medical applications, whereas their hydrophilicity can impart desirable release characteristics to controlled and sustained release formulations. Hydrogels exhibit properties that make them desirable candidates for biocompatible and blood-compatible biomaterials.

3.2 METHODS OF CONTROLLED RELEASE\textsuperscript{1,2,35}

All controlled release systems aim to improve the effectiveness of drug therapy. This improvement can take the form of increasing therapeutic activity compared to the intensity of side effects, reducing the number of drug administration required during treatment, or eliminating the need for specialized drug administration (e.g., repeated injections). Two types of control can be achieved over drug release viz., temporal and distribution control.

3.2.1 Temporal control

In temporal control, drug delivery systems aim to deliver the drug over an extended duration or at a specific time during treatment. Controlled release over an extended duration is highly beneficial for drugs that are rapidly metabolized and eliminated from the body after administration.
Figure 3.08: Drug concentration at site of therapeutic action after delivery as conventional injection and as a temporal controlled release system.

Figure 3.08 compares the drug concentration at therapeutic site after 4 immediate release injections and a controlled release system. With the controlled release system, the rate of drug release matches the rate of drug elimination and, therefore, the drug concentration is within the therapeutic window for the vast majority of the 24 h period but drug concentration is varying with immediate release systems. Clinically, temporal control can produce a significant improvement in drug therapy and temporally controlled release system would ensure that the maximum possible benefit is derived from the drug.
3.2.2 Distribution control

In distribution control, drug delivery systems aim to target the release of the drug to the precise site of activity within the body.

![Diagram of drug concentration at site of action and systemic drug concentration]

**Figure 3.09**: Drug delivery from an ideal distribution controlled release system.

Figure 3.09 represents the comparison of drug concentrations at the site of activity for intended action and side effect production. There are two principle situations in which distribution control can be beneficial.

1. When the natural distribution causes drug molecules to encounter tissues and cause major side effects that prohibit further treatment. Eg: Chemotherapy failure in cancer treatment due to bone marrow cell death prevents the patient from undergoing a complete drug treatment.

2. When the natural distribution of the drug does not allow drug molecules to reach their molecular site of action. For example, a drug molecule that acts on a receptor in the brain will not be active if it is distributed by the patient’s blood system but cannot cross the blood-brain barrier.
A large number of classes of drugs can benefit from temporal or distribution controlled release. These classes include chemotherapeutic drugs\textsuperscript{36,37}, immunosuppressants\textsuperscript{38}, anti-inflammatory agents\textsuperscript{39-41}, antibiotics\textsuperscript{42}, opioid antagonists\textsuperscript{43}, steroids\textsuperscript{44}, hormones\textsuperscript{45}, anesthetics\textsuperscript{46} and vaccines\textsuperscript{47}.

The need to develop new controlled release strategies has been intensified by advances in the design of peptide drugs and emergence of gene therapy. These biotechnology derived agents may dominate the next generation of drug design. However, their clinical success may be dependent on the design of controlled release devices and mechanism of drug release; which ensures that the drugs reach their target cells precisely at the required time.

3.3 DRUG RELEASE MECHANISMS IN CONTROLLED RELEASE DEVICES\textsuperscript{1,10,35}

A diverse range of mechanisms have been developed to achieve both temporal and distribution controlled release of drugs using polymers. This diversity is a necessary consequence of different drugs imposing various restrictions on the type of delivery system employed.

Controlled release devices in general, use polymers in the rate control mechanism. An important consideration in designing polymers for any controlled release mechanism is the fate of the polymer after drug release. Polymers that are naturally excreted from the body are desirable for many controlled release applications. Such polymeric devices can be classified into the following categories.

- Diffusion controlled devices
- Solvent controlled devices
- Chemically controlled devices
3.3.1 Diffusion Controlled Devices

Diffusion controlled devices work on either of two fundamental technologies; release of drug from monolithic (matrix) devices or from reservoir devices.

3.3.1.1 Monolithic (matrix) devices: The drug is intimately mixed with rate-controlling polymer, and the release occurs by diffusion of the agent from the device. It is the simplest and most convenient way to achieve prolonged release of an active agent. The drug release does not proceed by zero-order kinetics.

3.3.1.2 Reservoir devices: In a reservoir device, the drug (powder or liquid) is contained in a core surrounded by a non-biodegradable rate controlling polymeric membrane through which the drug is slowly diffused. Reservoir systems has certain drawbacks; removal of device after the drug release is complete and chances of dose dumping if membrane gets ruptured.

Polymeric membranes can be either dense or porous. The membrane properties (and drug properties) control the rate of release from such a system. In systems incorporating dense membranes, the drug particles entrapped within the reservoir system dissolve from the solid state into the medium (which the drug is suspended in) and then diffuse through the medium to the polymer membrane. Once the drug molecules have partitioned into the polymer membrane, the drug then diffuses through to the outer surface of the membrane; then partitions into the fluid surrounding the delivery device. The rate of drug release will depend on the solubility of the drug in the polymer and the thickness of the membrane. In case where the membrane is porous, the drug will diffuse through the fluid filled pores, rather than the polymer itself favoring the path of least resistance. In such a system, the release rate can be manipulated by altering the thickness of the membrane or by altering the size and number of pores in the membrane.
3.3.2 Solvent Controlled Devices

Solvent controlled release devices are a result of solvent penetration into the device. Although non-aqueous solvents can be used, only water is of importance in controlled release applications for human use. There are two types of solvent controlled systems – osmotic and swelling.

3.3.2.1 Osmotic controlled systems: They involve an external fluid moving across a semi-permeable membrane into a region within the device containing a high concentration of osmotic agent. The increased volume in the osmotic compartment forces the drug in the adjacent compartment to expel out through a small orifice.

3.3.2.2 Swelling controlled systems: These systems employ a polymer which can hold a large volume of water. Drug is mixed with polymer and the matrix is placed in system. When the device is placed in an aqueous environment, water penetrates the matrix and the polymer consequently swells. As a result of the polymer swelling, chain relaxation takes place and the drug is able to diffuse.

3.3.3 Chemically Controlled Devices

In a chemically controlled device, the rate of active agent release from the polymer is controlled by chemical reaction that can be hydrolytic or enzymatic cleavage of a labile bond, ionization or protonation. Chemically controlled devices can be divided into two classes - “pendant chain” systems and biodegradable (also known as bioerodible) systems.

3.3.3.1 Pendant chain systems: In pendant-chain systems, a drug molecule is chemically linked to a polymer backbone. The drug is either linked directly or via a spacer-group to the polymer and drug release occurs by chemical hydrolysis or enzymatic cleavage in the presence of biological fluids and enzymes in the body.
3.3.3.2 Bioerodible systems: They are designed to incorporate a drug which is dispersed throughout the polymer. As the polymer degrades, the drug diffuses out. The major advantages of such systems are that the device does not have to be removed after release and the drug does not have to be water soluble.
3.4 PERIODONTITIS

Periodontal disease is one of the most common chronic disorders of infectious origin known in humans with a prevalence of 10-60% in adults depending on the diagnostic criteria. Periodontitis is a general term for an inflammatory condition caused by specific microorganism or group of specific microorganisms affecting the physiological and structural organs supporting the teeth. In periodontal disease, inflammation of the gums around the teeth results in progressive destruction of periodontal ligaments and resorption of alveolar bone resulting in mobility and loss of tooth.\textsuperscript{36}

Chronic Periodontitis, formerly known as “adult periodontitis” or “chronic adult periodontitis” is the most prevalent form of periodontitis. It is generally considered to be a slowly progressing disease. However, in the presence of systemic or environmental factors that may modify the host response to plaque accumulation, such as diabetes, smoking or stress, disease progression may become more aggressive.\textsuperscript{36}

3.4.1 Pathophysiology

Inflammation of the periodontium may be the result of accumulation of tooth-adherent microorganisms. Bacterial flora in the periodontal pocket plays an important role in the etiology of periodontal disease, but uncertainty exists regarding the exact mechanism by which periodontal tissue is destroyed. Approximately 500 bacterial taxa inhabit periodontal pocket, which provides a moist, warm, nutritious and anaerobic environment for microbial colonization and multiplication.\textsuperscript{37} Periodontal pocket is inhabited by anaerobic pathogenic bacteria such as \textit{Actinobaccilus actinomycetemcomitans}, \textit{Bacteroides gingivalis}, \textit{Bacteroides melaninogenicus subspecies intermedius}, \textit{Porphyromonas gingivalis}, \textit{Bacteroides forsythus},...
Treponema denticola and Prevotella intermedia. Other gram-negative anaerobic rods, some gram-positive bacteria and even enterococci / pseudomonas may also play a role in etiopathogenesis of periodontitis. The abundance and diversity of periodontal pocket microorganism depends on several factors including effectiveness of oral hygiene measures, pocket depth, degree of gingivitis, flow of gingival crevicular fluid, type of interacting microbes and viruses, transmission rate of microbes from other individuals and antimicrobial efficacy of the host immune response.

3.4.2 Treatment

The primary step in periodontal treatment is to improve the oral hygiene by reduction of oral bacteria, associated calcified and non calcified deposits. The most common procedure to manage the disease involves scaling and root planing to remove bacterial deposits in the subgingival area. This may be followed by surgery to reduce the depth of the periodontal pocket. Personal plaque control procedures like flossing, frequent brushing, and use of chlorhexidine mouthwash are recommended to the patient between maintenance dental visits. The vast majority of cases (mild to moderate chronic periodontitis) can be treated by mechanical debridement of subgingival bacterial deposits alone, which does not involve surgical therapy.

3.4.3 Rationale of selecting drug

Chemotherapeutic agents may be administered systemically or delivered locally. However, use of antibiotics is sometimes recommended because of the infectious character of periodontitis. The use of antibiotic agents in conjunction with non-surgical therapy can reduce or eliminate the need for surgical intervention. Commonly prescribed antibiotics for treatment of periodontitis include erythromycin,
clarithromycin, tetracycline, minocycline, doxycycline, metronidazole, clindamycin and ciprofloxacin.

Figure 3.10: Schematic representation of normal tooth structure and periodontal pocket

Figure 3.11: The plaque-bacteria association with tooth surface and periodontal tissues
Antibiotics are suggested in both acute and chronic periodontitis. In acute infection, where therapy must not be postponed, unspecific broader spectrum antibiotics or specific antibiotics that are known to be effective in the particular disease condition should be used without delay. In chronic infections, the pathogens are first identified by microbiological examination and then, based on the results of the microbiological analysis, an antibiotic is chosen which is appropriate for the particular microorganism.\textsuperscript{39} The use of systemic antibiotics raises a number of issues, like bacterial resistance to administered antibiotic and unpleasant or toxic side effects. Large doses must be taken in order to achieve sufficient concentrations in the gingival crevicular fluid of the periodontal pockets. This leads to antibiotics related side effects and emergence of antibiotic resistance.\textsuperscript{40}

The microbial population in periodontal pocket is susceptible to antibiotics of macrolides class. Azithromycin dihydrate is a semi synthetic azalide antibiotic, a subclass of macrolide antibiotic with a broader spectrum than that of erythromycin or clarithromycin. Azithromycin dihydrate is indicated in both adjunctive and prophylactic treatment of chronic inflammatory periodontitis. Hence, it is selected for the study.

\textbf{3.4.4 Need of \textit{in situ} gels}

Because of above considerations, there is a need of specialized local delivery system which can maintain the antibiotic in gingival crevicular fluid at a concentration higher than that achieved by systemic administration. Controlled delivery of an antibiotic or antibacterial within periodontal pocket can alter the pathogenic flora (decrease total bacterial count) and improve clinical signs of periodontitis.\textsuperscript{41,42} Mechanical debridement procedures coupled with local
administration of an antibiotic susceptible to microbes in the periodontal cavity will influence the outcome of the study.

3.4.5 Advantages of in situ gels

Local drug delivery systems provide several benefits: the drug can be delivered to the site of disease activity at a bactericidal concentration; it can facilitate prolonged drug delivery, requires low dose (compared to systemic dose), reduces cost of therapy, bypasses surgical procedures and thus improves patient compliance. With respect to solid devices, semisolid (gel) formulations have some advantages, such as relatively faster release or controlled release of the incorporated drug; ease of administration, biocompatibility and rapid elimination through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site.
3.5 OCULAR DRUG DELIVERY

Eye is one of the sensitive organs in the human body. Delivering drug to eye is one of the most interesting and challenging endeavors faced by pharmaceutical scientist. As an isolated organ, the eye is very difficult to study from a drug delivery point of view. It is very difficult to obtain specimens of eye tissue containing drug from humans. Further, absorption of drug from topically applied formulations is very poor resulting in low bioavailability.\textsuperscript{44}

3.5.1 Problems encountered

The pre-corneal constraints responsible for the poor ocular bioavailability include solution drainage, lachrymation, tear dilution, tear turnover and conjunctival absorption. Drug solution drainage away from the pre-corneal area has been shown to be the most significant factor in reducing the contact time of the drug with the cornea and consequently ocular bioavailability of topical dosage forms. The instilled dose leaves the pre-corneal area within two minutes of instillation in humans.

A frequent eye drops instillation is associated with patient non-compliance. Inclusion of excess drug in the formulation in an attempt to overcome the bioavailability problems is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolachrymal duct.

3.5.2 Disadvantages of conventional dosage forms

Though, the conventional ophthalmic dosage forms [solutions (drops), ointments, lotions, suspensions] are the simplest form of the drug delivery system, but they suffer certain drawbacks such as:

1. Frequent medication.
2. Dilution by tear fluid.
3. Drainage of medication by tear fluid.
4. Rapid reduction in the drug level.
5. Reduced bioavailability.
6. Massive and unpredictable doses etc.

Despite these severe limitations, significant improvements in ocular drug delivery have been made. The improvements have been with the objective of maintaining the drug in the biophase for an extended period. It is a challenge to the formulator to circumvent the protective barriers of the eye, so that the drug reaches the biophase in required concentration. Aqueous solutions have the disadvantage of being quickly removed from the front of the eye resulting in poor ocular bioavailability. A solution or suspension form of a drug delivery system is preferred, provided retention time in the eye is extended.

3.5.3 Advances in drug delivery

An increase in drug absorption from pre-corneal surface can be attained by prolonging the contact time of the dosage form with eye surface. A considerable amount of effort has been made in ophthalmic drug delivery since 1970’s. The various approaches attempted can be divided into two main categories.

- Bioavailability improvement drug delivery systems
  - Viscosity enhancers, penetration enhancers, prodrugs, liposomes etc

- Controlled release drug delivery systems
  - Inserts, *in situ* gels, oil-in-water emulsion, colloidal delivery systems, nanoparticles, microparticulates etc\(^\text{45,46}\)

However, these ocular drug delivery systems have not been used extensively because of drawbacks; blurred vision by ointments, low patient compliance by inserts.
3.5.4 Need of in situ gels

A significant increase in the pre-corneal residence time of drugs and consequently bioavailability can be achieved by using delivery systems based on the drug concept of in situ gel formation.\(^{47}\) These systems consist of polymers that exhibit sol-to-gel phase transitions due to a change in a specific physico-chemical parameter (pH, ion, temperature) in their environment; the cul-de-sac. Depending on the method employed to cause sol-to-gel phase transition on the eye surface, three types of systems may be utilized in devising a formulation: pH triggered systems (e.g. Cellulose acetate hydrogen phthalate latex), temperature-dependent systems (e.g. pluronics and tetronics) and ion activated systems (e.g. Gellan).\(^{48}\)

3.5.5 Advantages of in situ gels

In situ gels are one of the novel methods for drug delivery to eye. They can be conveniently dropped as a solution into the conjunctival sac of the eye. Upon contact with the eye, the polymer changes conformation and forms as transparent gel. This type of formulation has advantage of a solution being patient convenient with the favourable residence time of a gel. In situ gels can reduce the frequency of instillations, reduce the dose, increase pre-corneal residence time, and improve ocular bioavailability and patient compliance.
3.6 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a complex autoimmune disease characterized by persistent inflammation of the synovium, local destruction of bone and cartilage and a variety of systemic manifestations which may ultimately result in functional disability. RA is characterized by the inflammation and destruction of multiple joints. It not only disturbs quality of life, but also shortens the lifespan of affected patients by causing co-morbidities such as cardiovascular diseases. Joint damage occurs early in the course of RA and once present is largely irreversible. Inflammatory disease activity is an important predictor of progression of joint damage and the long-term requirement for joint replacement surgery. Inflammatory disease activity has also been shown to be the main determinant of functional capacity in RA, even in those patients with long-standing disease.

Three major aspects of RA suggest a fundamental autoimmune-mediated disease: (i) the presence of massive lymphocytic infiltrates and activated CD4+ T-lymphocytes within the inflamed synovium; (ii) production of large amounts of rheumatoid factor (RF) by B-lymphocytes and plasma cells in the synovium, and (iii) immunosuppression which further influences the course of RA.

3.6.1 Pathophysiology

RA is an autoimmune disease that causes chronic inflammation of the joints, the tissue around the joints, and other organs in the body. Although much remains uncertain, it is thought to occur when a person’s body tissues are mistakenly attacked by their own immune system. After initial injury, a continuing autoimmune reaction ensues. The cascade of immunological and inflammatory reactions involved has been determined. These reactions produce inflammatory synovitis and tissue swelling. Furthermore, fibroblasts that reside in the synovial lining significantly increase in...
number and display phenotypic transformations. This leads to irreversible destruction of adjacent cartilage and bone. Often, as part of the reparative process, RA patients develop bone spurs (osteophytes), which are formed by remaining joint cartilage. These cause further pain and inflammation, and may require surgical intervention.

In RA, fibroblast-like synoviocytes, macrophages, and other cells involved in inflammation infiltrate the synovial tissue. The complex mechanisms that lead to inflammation include signalling mediators such as nitric oxide (NO) and prostaglandins (PG). PGs are derived from fatty acids by the family of cyclooxygenase (COX) enzymes, and the COX-2 isoenzyme is considered to be a pro-inflammatory mediator. Indeed, elevated levels of PG have been found in synovial cells treated with inflammatory mediators \textit{in vitro} and in patients with RA \textit{in vivo}. Pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrosis factor-\textgreek{a} (TNF-\textgreek{a}), activate the inducible NO synthase (iNOS) pathway in bone cells. NO derived from this pathway potentiates the cytokine- and inflammation-induced bone loss. Therefore, agents that can inhibit COX-2 activity as well as the iNOS pathway have potential as anti-inflammatory drugs. Phospholipase A2 (PLA2), an upstream regulator of many inflammatory processes, also plays an important role in the pathophysiological state of RA.$^{58}$

Pathology of RA includes formation of new blood vessels, inflammatory cell infiltration and synovial proliferation. Abundant production of proteolytic enzymes such as metalloproteases, and inflammatory cytokines including tumor necrosis factor-\textgreek{a} (TNF- \textgreek{a}) and interleukin 6 (IL-6) causing cartilage breakdown and bone destruction which lead to irreversible functional disability. In addition, chemokines produced \textit{in situ} promotes recruitment of inflammatory cells in the joints.
RA affects approximately 1% of the adult population with females being two to four times more susceptible than males. Effective control of the inflammatory process in RA reduces radiographic progression of joint damage, and improves physical function and quality of life.\textsuperscript{59,60} Thus, the primary goal of therapy is to achieve rapid and effective disease control to prevent the long-term damaging effects on joint structure and function.

3.6.2 Treatment

There is no cure for RA and multiple pharmacotherapies are often required to control the disease. The drugs used to treat RA are generally divided into two categories: non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids (oral, intra-articular and parenteral routes), which provide rapid symptomatic relief but have no effect on the progression of joint damage; and disease modifying anti-rheumatic drugs (DMARDs), which reduce disease activity and slow the progression of joint damage, thereby preserving function.

1. \textit{Anti-inflammatory medications}: These include aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen.
   
   - Although NSAIDs work well, long-term use can cause problems such as ulcers and bleeding, and possible heart problems.
   
   - Celecoxib (Celebrex) is another anti-inflammatory drug, but it is labelled with strong warnings about heart disease and stroke.

2. \textit{Antimalarial medications}: This group of medicines includes hydroxychloroquine (Plaquenil) and sulfasalazine (Azulfidine), and is usually used along with methotrexate. Benefit from these medications can be seen after weeks or months after its use.
3. **Corticosteroids**: These medications work very well to reduce joint swelling and inflammation. Because of long-term side effects, corticosteroids should be taken only for a short time and in low doses, if possible.

4. **Biologic agents**: Biologic drugs are designed to affect parts of the immune system that play a role in the disease process of rheumatoid arthritis. They may be given when other medicines for rheumatoid arthritis have not worked. At times, doctor will start biologic drugs sooner, along with other rheumatoid arthritis drugs. Most of them are given either under the skin (subcutaneously) or into a vein (intravenously). There are different types of biologic agents:

   - White blood cell modulators include: Abatacept (Orencia) and Rituximab (Rituxan)
   - Tumor necrosis factor (TNF) inhibitors include: Adalimumab, Etanercept, Infliximab, Golimumab, and Certolizumab
   - Interleukin-6 (IL-6) inhibitors: Tocilizumab

   Biologic agents can be very helpful in treating rheumatoid arthritis. However, people taking these drugs must be watched very closely because of serious risk factors:

   - Infections from bacteria, viruses, and fungi
   - Leukemia
   - Possibly psoriasis

5. **Disease modifying antirheumatic drugs (DMARDs)**: These are drugs of choice for RA, in addition to rest, strengthening exercises, and anti-inflammatory drugs are advised.

   - Methotrexate is the most commonly used DMARD for RA.
• Methotrexate may have serious side effects, so frequent blood tests need to carry out while taking it.

The ultimate goal in the treatment of RA is to prevent joint damage and restore normal life. Non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids and disease-modifying anti rheumatic drugs (DMARDs) have been widely used in combination, to control disease activity without complete success to treat RA.

3.6.3 Rationale of selecting drug – Methotrexate

Methotrexate (MTX), a folic acid antagonist that inhibits DNA and RNA synthesis, is the most potent, and commonly prescribed synthetic DMARD, and can retard joint destruction. It acts by reversibly inhibiting dihydrofolate reductase which reduces dihydrofolate to tetrahydrofolate. Inhibition of this enzyme interferes with purine and pyrimidine synthesis. MTX is used in low doses for the treatment of RA and is termed as the “Gold Standard Treatment for Rheumatoid Arthritis”

Treating RA early has been recommended, however, the limitations of conventional DMARDs include insufficient capacity to prevent progression of damage and deterioration of physical function of the patients, and toxicities to the liver, the bone marrow and the lungs.

3.6.3.1 Dosage and route of administration

There are no clear clinical recommendations for optimal dosage and route of administration of MTX in patients with RA. There is lack of clinical advice/universally accepted regimen for use of MTX in treating RA, since MTX is being used in variable doses with different routes of administration for treating RA. Traditionally, the dosage regimen of MTX in RA started with initial doses of 25 mg/week or fast escalation with 5 mg/month to 25-30 mg/week orally. This regimen was associated with higher clinical effect sizes and more adverse events
Subcutaneous injection of MTX (15 mg/week) was also administered, which resulted in higher clinical efficacy but more withdrawal due to toxicity in early RA. It is proposed to start MTX 15 mg/week orally, escalating with 5 mg/month to 25-30 mg/week, or the highest tolerable dose, with a subsequent switch to subcutaneous administration in case of an insufficient response, which seems to be the optimal evidence-based dosing and routing recommendation for methotrexate in RA.\textsuperscript{66}

### 3.6.3.2 Prolonged use of MTX in RA

The prolonged usage of MTX in RA put forward many drug related side/ toxic effects which include serious manifestations like suppressing the formation of new blood cells leading to severe form of anemia, may cause liver damage, dry cough accompanied by fever and shortness of breath leading to lung damage, hair loss, sores on skin and in mouth. To keep a check on the above, complete blood tests are advised periodically. Oral folate supplements are recommended since it can decrease side effects during MTX therapy.

### 3.6.4 Need of \textit{in situ} gels

The toxic effects of MTX may be reduced with an optimum use of MTX during treatment with other agents in combination. Oral administration of MTX (tablets) resulted in severe gastro intestinal adverse effects and other systemic side effects due to the administration of weekly dose at once, but subcutaneous injection resulted in toxicity. To reduce the drug related toxic effects on the body, there is a need for dosage form which can ensure the availability of MTX at therapeutic concentration with less exposure to body tissues and still be effective in the treatment of RA. Conventional parenteral products failed to deliver drug at controlled rate required during management of RA.
3.6.5 Advantages of in situ gels

Injectable in situ gels offer many advantages over conventional oral dosage forms. They can be easily formulated, sterilized, could be delivered locally or at specific site in the body, can incorporate both hydrophilic and lipophilic drugs and can release drug/s at a pre-determined rate. These systems reduce the drug dose, dosing frequency and reduce systemic related side effects thereby improving patient compliance and comfort. In situ gels exhibits phase transition due to physiological change in the environment. They can be easily delivered subcutaneously or into the synovial cavity with the help of syringe equipped with the needle appropriate for the intrasynovial delivery. At physiological conditions (37°C), the polymer changes its conformation from sol to gel forming depot releasing drug in a predictable and controlled manner.\textsuperscript{18}

This delivery system has the ease of administration and has a long retention time because of the gel formation. Further, dose adjustment (total dose for a period) can be made by injecting the amount of gel corresponding to drug content. This adds to the versatility of delivery system in catering the treatment requirements of physician in response to patient’s progress/ manifestations.
3.7 GASTRO RETENTIVE DRUG DELIVERY SYSTEMS

Gastro retentive drug delivery systems prolong the drug residence time in stomach thereby increasing the absorption of drug resulting in better bioavailability. Prolonged gastric retention increases bioavailability, decreases wastage of drugs, increases solubility of drugs, which are less soluble in alkaline pH. These dosage forms prolongs the gastric residence time enabling an extended absorption phase for the local treatment of drugs and better bioavailability for the drugs that are unstable in intestinal or colonic environment. Gastric retention can be achieved by mucoadhesion or bioadhesion systems, expansion systems, high density systems, magnetic systems, superporous hydrogels, raft forming systems, low density systems and floating ion exchange resins.

In situ gels capable of floating in gastric fluid are low density systems which can be in floatation for prolonged periods releasing the drug in a controlled manner for absorption in the stomach. They are classified under Non-effervescent floating drug delivery systems.

3.7.1 Low density floating systems

These systems are also known as hydro dynamically balanced systems (HBS) or floating drug delivery systems (FDDS). They have a bulk density lower than density of gastric fluid, i.e. their bulk density is less than 1 g/cm$^3$. The specific gravity of gastric fluid is approximately 1.004-1.01g/cm$^3$, thus FDDS remains buoyant in stomach without affecting gastric emptying rate for prolonged period of time, releasing the drug slowly at desired rate. These are single-unit dosage form, containing one or more gel-forming hydrophilic polymers. Hydroxy propyl methylcellulose (HPMC), hydroxyl ethyl cellulose (HEC), hydroxyl propyl cellulose (HPC), sodium carboxy methyl cellulose (NaCMC), polycarbophil, polyacrylate,
polystyrene, agar, carrageenans or alginic acid are commonly used excipients to develop these systems.

![Intragastric floating system](image)

**Figure 3.12:** Schematic localization of an intragastric floating system in the stomach

### 3.7.2 Factors Affecting Gastric Retention

There are many factors that affect gastric emptying of an oral dosage form, viz.

- Density, Size, Shape, Single or multiple unit formulation, Fed or unfed state,
  - Nature of meal, Caloric content, Frequency of feed, Volume in stomach,
  - Biological factors can also significantly alter the gastric emptying, factors such as:
  - Gender, Age, Stress, Posture, Concomitant drug administration, Diabetes and Crohn’s disease, etc.  

- Body exercise may also influence gastric emptying.
3.7.3 Advantages of Gastric floating drug delivery systems (GRFDD)\textsuperscript{35}

- The efficacy of absorption of stomach-specific and intestine-specific drugs is equally good. e.g. Chlorpheniramine maleate
- Advantageous for drugs absorbed through the stomach e.g. ferrous salts, antacids, drugs with absorption window in stomach
- Efficacy of drugs administered from GRFDD has been found to be independent of the site of absorption of the particular medicaments.
- Complete absorption of drug can be expected from GRFDD; drug released from dosage form-present in gastric fluid, if gastric contents emptied, absorption can happen from intestine
- Drugs that are absorbed from the proximal part of the gastrointestinal tract (GIT)
Drugs that are less soluble or are degraded by the alkaline pH at the lower part of GIT.

Drugs that are absorbed due to variable gastric emptying time.

Local or sustained drug delivery to the stomach and proximal part of small intestine to treat certain conditions like duodenal ulcers.


### 3.7.4 Disadvantages of Gastric floating drug delivery systems (GRFDD)

- There are certain situations where gastric retention is not desirable. Aspirin and non-steroidal anti-inflammatory drugs are known to cause gastric lesions, and slow release of such drugs in the stomach is unwanted. Thus, drugs that may irritate the stomach lining and are unstable in its acidic environment should not be formulated in gastro retentive systems.

- Not beneficial for drugs which are well absorbed throughout the GI tract

- The floating systems in patients with achlorhydria can be questionable in case of swellable systems, faster swelling properties are required and complete swelling of the system should be achieved well before the gastric emptying time.

- In all the above systems, the physical integrity of the system is very important and the primary requirement for the success of these systems.
3.7.5 Need for gastric retention

- Drugs that are absorbed from the proximal part of the gastrointestinal tract (GIT).
- Drugs that are less soluble or are degraded by the alkaline pH they encounters at the lower part of GIT.
- Drugs that are absorbed due to variable gastric emptying time.
- Local or sustained drug delivery to the stomach and proximal small intestine to treat certain conditions.
- Particularly useful for the treatment of peptic ulcers caused by H. Pylori Infections.

3.7.6 Mechanism of drug release from GRFDD

Different mass transport processes may occur during drug release from polymer-based matrix tablets, including

1) Imbibition of water into the system
2) Polymer swelling
3) Drug dissolution
4) Drug diffusion out of the tablet and
5) Polymer dissolution

The importance of above process is dependent on the type of drug, polymer & dissolution medium and on the composition of the dosage form.
3.8 DRUG PROFILE

3.8.1 AZITHROMYCIN DIHYDRATE\textsuperscript{86-89}

**Category**: Semi synthetic azalide antibiotic, a subclass macrolide antibiotic, with a broader spectrum than that of erythromycins or clarithromycins.

**Chemical Name**: (2\text{R}, 3\text{S}, 4\text{R}, 5\text{R}, 8\text{R}, 10\text{R}, 11\text{R}, 12\text{S}, 13\text{S}, 14\text{R})-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-a-l-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[(3,4,6-trideoxy-3-(dimethylamino)-b-d-xylo-hexopyranosyl)oxy]-1-oxa-6-azacyclopentadecan-15-one dihydrate.

**Molecular Formula**: C\textsubscript{38}H\textsubscript{72}N\textsubscript{2}O\textsubscript{12}.2H\textsubscript{2}O

**Chemical Structure**:

![Chemical Structure](image)

**Molecular Weight**: 785.0

**Melting Point**: 113-115°C

**Description**: A white or almost white crystalline powder.

**Solubility**: Freely soluble in dehydrated alcohol, dichloromethane. Soluble in ethanol, methanol, DMF and acetone; practically insoluble in water.
Storage: Store in airtight containers at 15-30°C.

Mechanism of Action: Macrolides are usually bacteriostatic, although the drug may be bactericidal in high concentrations. Macrolides inhibits protein synthesis in susceptible organisms by penetrating the cell wall and binding to 50s ribosomal subunits, thereby inhibiting translocation of amino acyl transfer-RNA and inhibiting polypeptide synthesis.

The site of action of Azithromycin appears to be same as that of macrolides. The antimicrobial activity of Azithromycin is pH related i.e. only unionized Azithromycin has antimicrobial activity.

Pharmacokinetics

- Azithromycin is rapidly absorbed from GIT after oral administration.
- Absorption is incomplete, about 40% of oral dose is bioavailable.
- Peak plasma concentrations are achieved 2 to 3 h after oral administration.
- Extensively distributed to the tissues and tissue concentrations subsequently remain higher than in blood. Plasma concentrations are of little value as guide to efficacy of the drug.
- Plasma Azithromycin has a terminal half-life of 68 h and the average half-life is 1-4 days.
- Metabolism involves N-demethylation on the macrolide ring and other metabolic pathways include O-demethylation and hydrolysis.
- Excreted in feces principally as unchanged drug and biliary excretion as unchanged drug is the major route of elimination following oral
administration. Only a small portion (6%) of oral dose is excreted in urine.

**Indications/clinical use:** Indicated in the treatment of Pharyngitis and Tonsillitis, Respiratory tract infections, Otitis Media, Chlamydial infections, Chancroid, Gonorrhea, Pelvic inflammatory disease, Non-gonococcal infections, Mycobacterium avium complex (MVA) infections and in Prophylaxis of Bacterial endocarditis etc.

**Dose:**

- **For Pharyngitis, Tonsillitis and Respiratory tract infections:** 500 mg single dose on first day followed by 250 mg once daily for next 4 days.
- **For Chlamydial infections, Chancroid:** 1 g as single dose or 250 mg in 4 divided doses.
- **For Gonorrhea:** 2 g as single dose.
3.8.2 PILOCARPINE HYDROCHLORIDE\textsuperscript{86,90}

**Category** : Cholinergic Agents, Miotics, Muscarinic Agonists

**Chemical Name** : (3S, 4R)-3-Ethyldihydro-4-[(1-methyl-1H-imidazol-5-yl) methyl] - 2(3-H)-furanone- hydrochloride.

**Molecular Formula** : C\textsubscript{11}H\textsubscript{14}ClN\textsubscript{2}O\textsubscript{2}.HCl

**Chemical structure** :

![Chemical structure of Pilocarpine Hydrochloride]

**Molecular Weight** : 244.75 g/mole.

**Melting Point** : 199 to 205°C

**pH** : 3.8 to 5.3 (Conc. 2.5% w/w) [Acidic].

**Description** : A white crystalline powder, hygroscopic.

**Half life** : 0.76 hours

**Solubility** : Very soluble in water and in alcohol.

**Mechanism of action**: Pilocarpine is a cholinergic para-sympathomimetic agent. It increases secretion by the exocrine glands, and produces contraction of the iris sphincter muscle and ciliary muscle (when given topically to the eyes) by mainly stimulating muscarinic receptors.
Pharmacokinetics

- The rate of absorption decreases in presence of high fat meal
- The metabolism possibly occurs at the neuronal synapses and in the plasma.
- It is excreted in the urine

**Indications**: It is used for the treatment of radiation-induced dry mouth (xerostomia) and symptoms of dry mouth in patients with Sjogrens syndrome.

**Contraindications**: Miotics are contraindicated where constriction is undesirable, such as in acute iritis, and in those persons showing hypersensitivity to any of their components.

**Side effects**: Lachrymation, burning or discomfort, temporal or periorbital headache, ciliary spasm, conjunctival vascular congestion, superficial keratitis and induced myopia. Systemic reactions following topical administration are extremely rare, but occasionally patients are sensitive to develop sweating and gastrointestinal over activity following suggested dosage and administration. Ocular reactions usually occur during initiation of therapy and often will not persist with continued therapy. Reduced visual acuity in poor illumination is frequently experienced in older individuals and in those with lens opacity.
3.8.3 METHOTREXATE SODIUM

Category: Antineoplastic, anti-rheumatic drug

Chemical name: N-[4-[(2, 4-Diamino-6-pteridinyl) methyl]methyl amino) benzoyl]-L-glutamic acid.

Molecular formula: $C_{20}H_{22}N_{8}O_{5}$

Structure:

Molecular weight: 454.45

Melting point: 182°C to 189°C

Description: Yellow to orange brown crystalline powder.

Solubility: Methotrexate (base) is practically insoluble in water, alcohol, chloroform, and ether, but soluble in dilute solutions of mineral acids and alkali hydroxides and carbonates.

Mechanism of action: It acts as an antimetabolite of folic acid. Within the cell, folic acid is reduced to dihydrofolic acid and then to tetrahydrofolic acid. Methotrexate competitively inhibits the enzyme dihydrofolate reductase and prevents the formation of tetrahydrofolate which is necessary for purine and pyrimidine synthesis and consequently the formation of DNA and RNA.
Half-life: Plasma half-life is about 4 to 10 h; a longer terminal elimination phase of 10 to 70 h (mean 27 h has also been reported).

Pharmacokinetics:

- When given in low doses, methotrexate is rapidly absorbed from the gastrointestinal tract, but higher doses are less absorbed. It is rapidly and completely absorbed following intramuscular absorption.
- In plasma, about 50 to 95% is bound to plasma proteins
- It has low volume of distribution (about 0.4 to 0.8 Lt/Kg)
- It is distributed mainly in the extra cellular spaces but a proportion penetrates cell membranes and is strongly bound to dihydrofolate reductase. Small amounts of methotrexate diffuse into the cerebrospinal fluid, higher concentrations being achieved with high doses. Distribution into body spaces such as the pleural or peritoneal cavities occurs slowly.
- Methotrexate is metabolized to 7-hydroxy methotrexate (7-OH-MTX) by the liver, to diamino-methylpterioic acid (DAMPA) by intestinal bacteria during entero hepatic cycling and to methotrexate polyglutamates. The major metabolite appears to be 4-amino-10-methylpterioic acid and 7-hydroxy-4-amino-10-methyl pteric acid.
- About 40 to 50% of orally administered drug is excreted unchanged in the urine within 48 h, mostly with in first 8 h by glomerular filtration and active tubular secretion. About 30% of a dose may be excreted as metabolites as a result of the action of intestinal bacteria prior to absorption.
After I.V. administration, up to about 10% of a dose is excreted in the urine as metabolites and about 15% of a dose may be excreted in the bile.

**Indications**

It is used in acute lymphoblastic leukemia, meningeal leukemia, burkitts lymphoma, non-hodgkin’s lymphomas, osteosarcoma, tumours of the bladder, brain, breast, GIT, head and neck, lung, pancreas and prostate, retinoblastoma, mycosis fungoides, psoriasis, rheumatoid arthritis, primary biliary cirrhosis, polymycosis, wegener’s granulomatosis.

It is an effective immunosuppressive agent used for the prevention of graft versus-host reaction in bone-marrow transplantation.

**Contraindications**

Pregnancy, severe renal or hepatic dysfunction, psoriasis patients with pre-existing bone marrow depression.

**Adverse effects**

Bone marrow depression, leucopenia, thrombocytopenia, megaloblastic anemia, ulceration of mouth, gastrointestinal disturbances, stomatitis, diarrhoea, haemorrhagic enteritis, intestinal perforation, hepatic fibrosis, cirrhosis, alopecia, osteoporosis, defective oogenesis or spermatogenesis abortion and teratogenesis. Leucoencephalopathy, arachnotis and meningismus are associated particularly with intrathecal administration.

**Dose**

Methotrexate may be given orally as the base or sodium salt, or by injection as methotrexate sodium. Doses larger than 100 mg are usually given partly or wholly by intravenous infusion within 24 h.
Table 3.02: Therapeutic uses, dosage and administration of Methotrexate

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute Lymphoblastic Leukemia</strong></td>
<td>2.5 to 5 mg for children by mouth/IM</td>
</tr>
<tr>
<td></td>
<td>2.5 to 10 mg for adults by mouth/IM, once or twice weekly.</td>
</tr>
<tr>
<td><strong>Meningeal Leukemia</strong></td>
<td>12 mg/m² body-surface or 15 mg whichever is less, Once, or twice weekly, by Intrathecal injection</td>
</tr>
<tr>
<td><strong>Choriocarcinoma</strong></td>
<td>1 mg/Kg by IM, daily for four doses, at intervals of 1 to 2 weeks, for 3 to 5 courses.</td>
</tr>
<tr>
<td><strong>Lymphosarcoma</strong></td>
<td>3 to 30 mg/Kg or about 90-900 mg/m² with folinic acid.</td>
</tr>
<tr>
<td><strong>Mycosis Fungoides</strong></td>
<td>50 mg once weekly or 25 mg twice weekly.</td>
</tr>
<tr>
<td><strong>Psoriasis</strong></td>
<td>10-25 mg once weekly by IM or IV or per oral.</td>
</tr>
<tr>
<td><strong>Rheumatoid Arthritis</strong></td>
<td>7.5 mg once weekly by mouth.</td>
</tr>
<tr>
<td><strong>Breast Cancer</strong></td>
<td>(Combined cyclophosphamide and fluorouracil) 40 mg/m² on days 1 and 8, repeat monthly by IM or IV.</td>
</tr>
</tbody>
</table>

**Stability to light**: Methotrexate undergoes photo degradation when exposed to light.

**Caution**: Methotrexate is an irritant; avoid contact with skin and mucus membrane. It is extremely poisonous.
3.8.4 LOSARTAN POTASSIUM\textsuperscript{88,90,96}

**Category**: Antihypertensive \{Angiotensin II receptor (type AT1) antagonist\}

**Chemical Name**: 2-butyl-4-chloro-1-[p-(o-1Htetrazol-5ylphenyl) benzyl] imidazole-5-methanol mono potassium salt

**Molecular formula**: \(\text{C}_{22}\text{H}_{22}\text{ClKN}_6\text{O}\)

**Structure**:

\[
\begin{array}{c}
\text{N} \\
\text{N} \\
\text{Cl} \\
\text{N} \\
\text{N} \\
\text{CH}_2\text{OH} \\
\text{N} \\
\text{N} \\
\text{C}_4\text{H}_9 \\
\text{N} \\
\text{N} \\
\text{K}^+ \\
\end{array}
\]

**Molecular weight**: 461.01

**Melting Point**: 183.5-184.5\(^\circ\)C

**Description**: White to off-white free-flowing crystalline powder

**Solubility**: It is freely soluble in water, soluble in alcohols, and slightly soluble in common organic solvents, such as acetonitrile and methyl ethyl ketone

**Mechanism of action**: Losartan and its principal active metabolite block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor found in many tissues (eg. vascular smooth muscle, adrenal gland)

**Half Life**: 1.5 to 2.5 h
Pharmacokinetics:

- Losartan Potassium is absorbed from the gastrointestinal tract
- Bioavailability is about 25-35%
- Both Losartan and its active metabolite are highly bound to plasma proteins, primarily albumin, with plasma free fractions of 1.3% and 0.2% respectively
- Undergoes substantial first-pass metabolism by cytochrome P450 enzymes
- Plasma half life of Losartan Potassium is about 1.5 to 2 h.

Indications: It is used in treatment of hypertension.

Dose: It is used in the range of 25-100 mg in daily divided doses

Storage: Store in a well closed container
3.9 POLYMER PROFILE

3.9.1 PLURONIC F-127\textsuperscript{97,98}

Structure:

\[
\begin{align*}
\text{H} & \quad \text{O} \quad \text{C} \quad \text{CH}_2 \quad \text{O} \quad \text{CH}_2 \quad \text{O} \quad \text{H} \\
\text{H} & \quad \text{O} \quad \text{C} \quad \text{CH}_2 \quad \text{O} \quad \text{CH}_2 \quad \text{O} \quad \text{H} \\
\text{H} & \quad \text{O} \quad \text{C} \quad \text{CH}_2 \quad \text{O} \quad \text{CH}_2 \quad \text{O} \quad \text{H}
\end{align*}
\]

where \(a: 101\) and \(b: 56\)

Synonym: Monolan, Lutrol, Poloxamers, Superonics, Syperonics and Poloxalkol

Chemical Name: \(\alpha\)-Hydro-\(\omega\)-hydroxypoly(oxyethylene) poly(oxypropylene) poly-(oxyethylene) block copolymer

Category: Emulsifying agent, solubilising agent and wetting agent

Description: It is waxy, white granules of free flowing nature and practically odourless and tasteless

Molecular weight: 9840-14600

\(\text{pH}\): 6.0-7.4 for a 2.5% w/v aq solution

Physical form: Solid

Density: 1.06 g/cm\(^2\) at 25°C

Flowability: Free flowing

Melting Point: 56°C

Moisture Content: Generally contain less than 0.5% w/w water and hygroscopic only at greater than 80% RH

Solubility: Freely soluble in aqueous, polar and non-polar organic
solvents

**Stability and storage conditions**

Pluronics are stable materials. Aqueous solutions are stable in the presence of acids, alkalis and metal ions. However, aqueous solutions do support mold growth. The bulk material should be stored in well-closed container in a cool, dry place.

**Safety**

Pluronics® are used in a variety of oral, parenteral and Topical pharmaceutical forms and are generally regarded as nontoxic and nonirritant materials. Animal toxicity studies with dogs and rabbits have shown pluronic to be nonirritant and non-sensitizing when applied in 5%w/v and 10%w/v concentrations to the eyes, gums and skin.

**Regulatory status**

Included in FDA inactive ingredients and in non-parenteral medicines listed in United Kingdom.
3.9.2 HYDROXY ETHYL CELLULOSE \[ ^{57} \]

Structure :

\[
\text{O} \quad \text{R} \quad \text{O} \\
\text{R} \quad \text{O} \\
\text{R} \quad \text{O} \\
\text{R} \quad \text{O} \\
\text{R} = \text{H or O} \\
\text{H} \quad \text{X}
\]

Synonym : Cellosize HEC, Tylose H, Tylose PHA

Chemical Name : Cellulose, 2-hydroxyethyl ether

Category : Coating agent; suspending agent; tablet binder; thickening agent; viscosity increasing agent

Description : Hydroxyl ethyl cellulose occurs as a white yellowish-white or greyish-white, odorless and tasteless, hygroscopic powder

Solubility : It is soluble in either hot or cold water, forming clear, smooth, uniform solutions. Practically insoluble in acetone, ethanol (95%), ether, toluene, and most other organic solvents. It is non-ionic. In some polar organic solvents, such as the glycols, hydroxyethyl cellulose either swells or is partially soluble

Applications : It is primarily used as thickening agent in ophthalmic and topical formulations, although it is also used as a binder and film-coating agent for tablets. Hydroxy ethyl cellulose is also widely used in cosmetics

Safety : It is generally regarded as an essentially non-toxic and non-irritant material
3.9.3 CHITOSAN (WATER SOLUBLE)\textsuperscript{97,99}

Chitosan is a natural cationic biopolymer obtained from natural sources, namely crab and shrimp shell wastes. Commercial chitosan is derived from the shells of shrimp and other sea crustaceans, including \textit{Pandalus borealis}. Chitosan is biocompatible and biodegradable. Purified qualities of chitosan are available for biomedical applications.

\textbf{Structure}:

\begin{center}
\includegraphics[width=0.7\textwidth]{structure.png}
\end{center}

\begin{tabular}{p{8cm}p{7cm}}
\textbf{Chemical Name} & Poly-\(\beta\)-(1,4)-2-Amino-2-deoxy-D-glucose \\
\textbf{Molecular Weight} & 10000-1000000 daltons \\
\textbf{Description} & Chitosan occur as odourless, white or creamy-white powders or flakes \\
\textbf{pH} & 4 to 6 (1\% w/v aqueous solution) \\
\textbf{Density} & 1.35-1.40 g/cm\textsuperscript{3} \\
\textbf{Functional Category} & Biodegradable and biocompatible polymer, Coating agent, disintegrant, film forming agent, mucoadhesives, tablet binder, viscosity increasing agent \\
\textbf{Solubility} & Soluble in water, practically insoluble in ethanol (95\%), other organic solvents, and neutral or alkali solutions at pH above 6.5 \\
\textbf{Storage} & Store at room temperature
\end{tabular}
3.9.4 SODIUM GLYCEROPHOSPHATE

**Synonym**: Glycerol phosphate disodium salt

**Molecular Formula**: $\text{C}_3\text{H}_7\text{Na}_2\text{O}_6\text{P}$

**Molecular Weight**: 216.04

**Melting Point**: 103°C (217.4°F)

**Molecular Structure**:

![Molecular Structure of Sodium Glycerophosphate]

**Physical state and appearance**: White Solid. (Crystalline granules, solid)

**Solubility**: Soluble in water

**Stability and reactivity**: The product is stable

**Carcinogenic effects**: This material is not known to cause cancer in animals or humans

**Conditions of instability**: Excess heat, incompatible materials

**Precautions**: Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust. Keep away from incompatibles such as oxidizing agents

**Storage**: Refrigerate. Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 4°C (39.2°F)

**Toxicity of the products of biodegradation**: The product and its byproducts of degradation are not toxic
3.9.5 PLURONIC F68

Structure

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{C} & \quad \text{CH}_2 \\
\text{O} & \quad \text{CH}_2 \\
\text{O} & \quad \text{H} \\
\text{C} & \quad \text{CH}_3 \\
\text{H} & \quad \text{H} \\
\text{CH}_3 \\
\end{align*}
\]

where a: 80 and b: 27

Synonym : Lutrol, Pluronic

Chemical Name : \(\alpha\)-Hydro-\(\omega\)-hydroxypoly(oxyethylene)poly(oxypolypropylene) poly-(oxyethylene)block copolymer

Description : It is waxy, white granules of free flowing nature and practically odourless and tasteless

Molecular weight : 7680 - 9510

Solubility : Readily soluble in aqueous, polar and non-polar organic solvents.

Melting point : 52-57°C

Physical Form : Solid

pH (2.5% in water) : 5.0-7.5

Applications

- Solubilizer in concentration 0.3%
- Gelling agent in concentration 15-20%
- Spreading agent in concentration 1%
- Suppository base in concentration 4-6%
3.9.6 HYDROXY PROPYL METHYL CELLULOSE (HPMC)\textsuperscript{97}

Structure:

![Structure of HPMC]

Chemical Name : Cellulose, 2 Hydroxy Propyl methyl ether

Description : Hydroxy propyl methyl cellulose is an odorless and tasteless, white or creamy-white colored fibrous or granular powder

Functional category : Coating agent, film former, stabilizing agent, tablet binder, viscosity increasing agent

Acidity / alkalinity : pH 5.5–8.0 for 1% w/w aqueous solution

Density (Tapped) : 0.50–0.70 g/cm\textsuperscript{3}

Melting point : Browns at 190-200°C, chars at 225-230°C

Moisture content : HPMC absorbs moisture from atmosphere. The amount of water absorbed depends upon the initial moisture content, temperature and relative humidity of the surrounding air

Solubility : Soluble in cold water, forming a viscous colloidal solution. Practically insoluble in chloroform, ethanol and ether
3.9.7 POLYCARBOPHIL\textsuperscript{97}

**Brand name**: Noveon AA-1

**Description**: Polycarbophil occurs as fluffy, white to off-white,
mild acidic polymer powder with slightly acetic odor

**Structure**:

![Structural formula of Polycarbophil]

**Acidity / alkalinity**: pH 2.5-3.0

**Ash content**: 0.009 ppm

**Density (bulk)**: 0.19-0.24 g/cm\textsuperscript{3}

**Equilibrium moisture content**: 8-10%

**Dissociation constant**: pKa- 6.0 ± 0.5

**Glass transition temperature**: 100-105°C

**Moisture content**: 2.0% maximum

**Specific gravity**: 1.41

**Applications**: Thickening agent

Used in bioadhesive drug delivery system
3.9.8 CARBOPOL 934

Carbopol 934 is a high molecular weight polymer of acrylic acid cross linked with allyl ether of sucrose or penta erythritol. Carbopol 934 previously dried in vacuum at 80°C for 1 hour contains not less than 56.0% and not more than 68% of carboxylic acid group.

**Structure**: 

\[ \text{HOOC} \quad n \]

**Viscosity**: 

The viscosity of neutralized 0.5% of aqueous dispersion of carbopol 934 is between 29,400 and 39,000 centipoises

**Synonyms**: 

Acritamer, Acrylic acid polymer, Carbapol, Carboxy vinyl polymer

**Chemical Name**: 

Carboxypolymethylene

**Molecular Weight**: 

\(3 \times 10^6\) daltons

**Description**: 

White colored, fluffy acidic hygroscopic powder with a slight characteristic odor

**Functional category**: 

Emulsifying agent, suspending agent, tablet binder, viscosity increasing agent and gelling agent

**Melting point**: 

260°C

**Solubility**: 

Soluble in water and after neutralization in ethanol 95% and glycerin

**Applications**: 

Carbopol is mainly used in liquid or semi solid pharmaceutical formulation as suspending or viscosity increasing agent. Carbopol is also employed as emulsifying agent in preparation of oil in water emulsion for external use.
3.9.9 GELLAN GUM

Gellan gum is high molecular weight polysaccharide gum produced by a pure culture fermentation of carbohydrates by Pseudomonas elodea, purified by recovery with isopropyl alcohol, dried, and milled.

The high molecular mass polysaccharide is principally composed of tetracyclic repeating unit of one rhamnose, one glucuronic acid, and two glucose units and is substituted with acyl group as the O-glycosidically-linked esters.

**Structure**

```
[Diagram of Gellan gum structure]
```

**Trade Names**: Kelcogel®, Gelrite™, Phytagel, Gel-Gro

**Description**: Off white amorphous powder

**Solubility**: Soluble in water, forming viscous solution; insoluble in ethanol

**Molecular weight**: Greater than 70,000 daltons

**Legal status**: Approved by US-FDA as direct food additive, WHO and many other countries have approved the use of gellan gum in food related products for human consumption.

**Loss during drying**: Not more than 15% (105°C, 2.5 h)

**Functional category**: Thickening agent, Gelling agent, anti-settling agent, stabilizer
3.10 REVIEW OF RESEARCH PAPERS

Vemula PK et al.\textsuperscript{102} developed an injectable self-assembled nanofibrous hydrogel, which is capable of encapsulation and release the agents in response to specific enzymes that are significantly present in a diseased state. The self-assembled nanofibrous gels withstood shear forces that may be experienced in dynamic environments such as joints, and remained stable following injection into healthy joints of mice. The prepared hydrogels were capable of disassembling \textit{in vitro} to release encapsulated agents in response to synovial fluid from arthritic patients. This novel approach represented a next-generation therapeutic strategy for localized treatment of proteolytic diseases. The hydrogels remained stable within normal joints for at least 8 weeks, yet disassembled and released encapsulated agents in response to enzymes that are known to be overexpressed during flares of RA, and in the presence of synovial fluid from arthritic human joints.

Giuseppe Perale et al.\textsuperscript{103} developed a hydrogel which had shown the promising results in the spinal cord injury, which when injected through 40 IM needle in the solution phase, converted into gel inside the target tissue. Formulation was prepared by poly-condensation, using two polymers viz. Polyacrylic acid (Carbomer 947P) and Agarose, a common polysaccharide. Solution was injected in spinal cord of mouse and \textit{in situ} gel formation was confirmed by magnetic resonance imaging that showed the presence of polymeric network at injection site. Hydrogel, so produced, had provided enough data to be considered as a new biocompatible tool that can be used as a local reservoir for \textit{in situ} delivery of drugs.

Miyazaki et al.\textsuperscript{104} evaluated the gelling property for oral delivery of the cimetidine. Formulations prepared were dilute solution of the enzyme treated xyloglucan which form thermo sensitive gel on body temperature along with Gellan
gum and sodium alginate. Complexed calcium ions were also added which on release in the acidic environment form gel on contact with the polymers, gellan gum and sodium alginate. In vitro study for cimetidine release was conducted over a period of 6 h. Plasma levels of cimetidine after oral administration to rabbits were compared with commercially available cimetidine/alginate suspension and in vivo release characteristics were found to be identical with commercial preparation.

C. Roques et al.\textsuperscript{105} investigated the gene delivery into the heart to prevent inherited cardiopathies. They delivered plasmid DNA by interpericardial injection using thermosensitive gel of poloxamer 407 to improve the retention time at the site of injection. Protection and condensation of plasmid DNA was initially performed through complexation with polyethyleneimine (PEI). Characterization of the size and zeta potential of the complexes suggested interactions between the polyplexes and the Poloxamer gel through significant increase in the size of the polyplexes and shielding of the surface charges. In vivo evaluation had highlighted the toxicity of PEI/DNA polyplexes toward the myocardium.

Qin Wang et al.\textsuperscript{106} prepared in situ gellable thermosensitive poly(N-isopropylacrylamide-co-acrylamide) (designated as PNIP/AAm) nanogel aqueous dispersions and their thermosensitive volume phase transition and in situ gel-forming behavior were investigated. 5-Fluorouracil (5-Fu) and bovine serum albumin (BSA) were used as model drugs. The drug-loading properties of PNIP/AAm nanogel particles and release behavior from in situ gelatinized PNIP/AAm nanogel aqueous dispersions were investigated. The prepared PNIP/AAm nanogel particles and aqueous dispersions showed good thermosensitivity. The presence of the drugs in the systems had no significant influence upon the thermosensitivity of the systems. In addition, the amount of cross-linker used in the preparation of the PNIP/AAm nanogel
had little influence upon the drug-loading capability of PNIP/AAm nanogel particles and also on the release behavior from gelatinized dispersions. They found that the drug-loading efficacy and entrapment efficiency of PNIP/AAm nanogel particles for low molecular weight 5-Fu was higher than that for biomacromolecular BSA. Furthermore, the cumulative release ratios of 5Fu from in situ gelatinized PNIP/AAm nanogel aqueous dispersions were distinctly higher than that of BSA. The results proved the potential application of prepared thermosensitive nanogel dispersions as embolizing and tissue engineering materials.

Kwang-Mi Jin et al.\textsuperscript{107} prepared injectable and thermo-reversible physical combination gels in aqueous solution. The complex coacervate consisted two oppositely charged biomacromolecules that composed of negatively charged chondroitin 6-sulfate and positively charged high molecular weight gelatin type A and co-formulating with a negative, thermo-sensitive polysaccharide, methylcellulose containing a salting-out salt, ammonium sulfate. The combination of complex coacervation and a thermo-reversible gel demonstrated synergistic effects on the complex coacervate formation, the release rates of model proteins and in situ gel depot formation. Gels indicated sustained release patterns of the protein over 25 days with minimal initial bursts. Optimized novel in situ gel depot systems containing dual advantages of complex coacervation and temperature responsiveness demonstrated a potential for efficient protein drug delivery in terms of high protein loading, sustained protein release, ease of administration, an aqueous environment without toxic organic solvents, and a simple fabrication method.

Francois Plourde et al.\textsuperscript{108} proposed an approach based on the spontaneous self-assembly of low-molecular weight amphiphilic amino acid derivatives in a hydrophobic pharmaceutical vehicle. The injectable, in situ-forming organogels were
obtained by mixing N-stearoyl l-alanine (m)ethyl esters with a vegetable oil and a biocompatible hydrophilic solvent. The gels in vivo-delivering properties were evaluated in rats with leuprolide, a luteinizing hormone-releasing hormone agonist used in prostate cancer, endometriosis and precocious puberty treatment. Following subcutaneous injection, the gels degraded and gradually released leuprolide for 14 to 25 days. Drug release was accompanied by sustained castration lasting up to 50 days, as assessed by testosterone levels. The study demonstrated that in situ-forming implants based on l-alanine derivatives represent a novel injectable platform for the controlled delivery of hydrophilic compounds, which is simpler than currently available implant and microsphere technologies.

Rajnikanth et al.\textsuperscript{109} prepared gellan gum based floating beads containing clarithromycin (FBC) by ionotropically gelation method for stomach-specific drug delivery against Helicobacter pylori. Kinetic treatment of the in vitro drug release data with different equations revealed matrix diffusion mechanism. Prepared beads showed good anti-microbial activity against isolated H. pylori strain. The prepared beads have shown good in vivo floating efficiency in rabbit stomach. The preliminary results from this study suggest that floating beads of Gellan can be used to incorporate antibiotics like clarithromycin and may be effective when administered locally in the stomach against H. pylori.

Erem Bilensoy et al.\textsuperscript{110} formulated anticancer agent 5-fluorouracil (5-Fu) in a vaginal gel using the thermosensitive polymer Pluronic F-127 together with mucoadhesive polymers to achieve a better therapeutic efficacy and patient compliance in the treatment for Human Papillomavirus induced cervical cancers. To increase its aqueous solubility and to achieve the complete release of 5-Fu from the gel, the drug was incorporated as its inclusion complex with 1:1 molar ratio with
either β-cyclodextrin or hydroxypropyl-β-cyclodextrin. The drug: cyclodextrin complexes were characterized in vitro by determining the gelation temperature, rheological behaviour and in vitro release profiles in pH 5.5 citrate buffer. They demonstrated that complexation with cyclodextrin accelerated the release of 5-Fu from thermosensitive gels which could effective therapeutically and has better patient compliance.

Young Cho et al.\textsuperscript{111} have developed chemically conjugated doxorubicin to acrylated chitosan in order to obtain sustained-release from thermo-responsive and photo-crosslinkable hydrogels. Chitooligosaccharide was acrylated with glycidyl methacrylate and subsequently conjugated to doxorubicin via an amide linkage. A mixture of doxorubicin–chitosan conjugates, acrylated Pluronic, and doxorubicin formed physical gels at 37°C. Chitooligosaccharide–doxorubicin conjugates in the doxorubicin hydrogels significantly reduced burst release of free doxorubicin from doxorubicin hydrogels compared to hydrogels without the conjugates. In vitro cytotoxicity assay using released media from doxorubicin hydrogels showed that degraded chitosan–doxorubicin had cytotoxicity comparable to free doxorubicin. They proved that the new system can release doxorubicin in sustained manner for prolonged period.

Sultana Y et al.\textsuperscript{112} developed and characterized a series of carbopol and methyl cellulose based solutions as the gelling vehicles for ophthalmic drug delivery of pefloxacin mesylate. They found that mixture of 0.3% carbopol and 1.5% methyl cellulose solution showed enhanced gel strength in the physiological condition. The rheological behaviours of carbopol/methyl cellulose solution were not affected by the incorporation of drug. They compared 0.18% of pefloxacin mesylate with 0.3% marketed eye drops. The prepared formulation showed a similar effect at half the
concentration of marketed preparation. They concluded that the carbopol/methyl cellulose mixture can be used as a gelling vehicle to enhance the ocular bioavailability of pefloxacin mesylate.

Sh. Abashzadeh et al.\textsuperscript{113} prepared and evaluated novel physical hydrogels composed of chitosan or its water soluble derivatives as a controlled delivery system for triptorelin acetate, a luteinizing-releasing hormone agonist. The chitosan blends were prepared at different ratios and suspended in sesame oil as non-aqueous vehicle at different solid content (10–30%). The \textit{in vitro} characteristics and \textit{in vivo} performance of \textit{in situ} gel was evaluated and compared with Diphereline SR 3.75 mg, a commercially available controlled delivery system of triptorelin. \textit{In vitro} release studies showed a sustained release profile for about 192 h with first order kinetics. Results of \textit{in vivo} studies conducted for a period of 35 days on male rats exhibited acceptable performance of \textit{in situ} gels in comparison with commercial product. They demonstrated the potential of the novel \textit{in situ} gel system for controlled delivery of peptides.

Debasish Mishra et al.\textsuperscript{114} synthesised and characterized an enzymatically crosslinked injectable gel (iGel) suitable for cell based bone tissue engineering application. The gel comprised of carboxymethyl–chitosan (CMC)/gelatin/nano-hydroxyapatite (nHAp) susceptible to tyrosinase/p-cresol mediated \textit{in situ} gelling at physiological temperature. Study revealed that a combination of tyrosinase (60U) and p-cresol (2 mM) as crosslinking agents yield rigid gels at physiological temperature when applied to CMC/gelatin within 35 min in presence or absence of nHAp. Application of iGels in mice revealed that stability of the \textit{in situ} formed gels depends on the degree of crosslinking and CMC concentration. In conclusion, the iGels may
be used in treating irregular small bone defects with minimal clinical invasion as well as for bone cell delivery.

Techawanitchai et al.\textsuperscript{115} prepared a light responsive hydrogel system composed of poly(N-isopropylacrylamide-o-2-carboxyisopropylacrylamide) (P(NIPAAm-co-IPAAm)) and o-nitrobenzaldehyde (NBA) with light controlled shrinking and drug release. The NBA-integrated gel was shown to shrink rapidly upon UV irradiation without polymer “skin layer” formation due to a uniform decrease of pH inside the gel. The NBA-integrated gel was successfully employed for the controlled release of entrapped dextran, where dextran was successfully entrapped into the gel and then released into water in a controlled manner under 365 nm UV illumination. This system showed significant promise as a smart platform for triggered and programmed delivery of drugs.

\textbf{Table 3.03:} Patents related to \textit{in situ} gels

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