The preparation and characterization of poloxamer-based temperature-sensitive hydrogels for topical drug delivery

Sarath Chandra Gandra

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A Thesis

entitled

The Preparation and Characterization of Poloxamer-Based Temperature-Sensitive Hydrogels for Topical Drug Delivery.

By

Sarath Chandra Reddy Gandra

Submitted to the Graduate Faculty as partial fulfillment of the requirements for The Master of Science Degree in Pharmaceutical Sciences with Industrial Pharmacy option

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August 2013
Stimuli-sensitive hydrogels change their swelling behavior and drug release by sensing changes in the surrounding environment. One example is temperature-sensitive hydrogels which change their swelling behavior in response to a change in the environmental temperature. Poloxamers are tri-block copolymers that exhibit thermoreversible properties by transforming from a liquid-like behavior to gel-like behavior above a certain temperature called sol-gel transition temperature. By varying the concentration of poloxamer and other excipients, hydrogels with sol-gel transition point close to body temperature can be achieved. The aim of the present study was to develop poloxamer hydrogels as *in situ* gelling formulation for topical drug delivery. The anti-fungal drug fluconazole (FLZ) was used as a model drug substance. First, pre-formulation work on the solubility of FLZ in different co-solvents was performed. Then, eight different formulations containing 1% FLZ in poloxamer and a particular co-solvent (propylene
glycol or Transcutol®-P) of various concentrations were prepared. The formulations were characterized for transition temperatures, rheological, mechanical, and mucoadhesive properties. Fluconazole permeability and antifungal effect of the systems were evaluated. Except for one formulation, all hydrogels exhibited thermosensitive property, i.e. changing from Newtonian (liquid-like) behavior at 20°C to non-Newtonian (gel-like) behavior at 37°C. Transcutol®-P increased the transition temperature of the formulations, while the opposite effect was observed for propylene glycol. At body temperature, formulations with high poloxamer concentrations (17%) rendered gels with higher values in viscosity, compressibility and hardness. Formulations containing 17% poloxamer and 20% Transcutol-P and 10% propylene glycol, respectively, exhibited high values in both adhesiveness and work of adhesion. No significant differences in the permeability and antifungal activity of fluconazole were observed between the formulations. The latter suggests no influence of the gel vehicles on the biological effect of fluconazole. Based on the results, formulations containing 17% poloxamer and 20% Transcutol-P and 10% propylene glycol, respectively, seemed to be promising thermosensitive systems for topical drug delivery.
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## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge coupled device</td>
</tr>
<tr>
<td>CST</td>
<td>Critical solution temperature</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode array detector</td>
</tr>
<tr>
<td>EO</td>
<td>Ethylene oxide</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>FLZ</td>
<td>Fluconazole</td>
</tr>
<tr>
<td>G'</td>
<td>Store modulus</td>
</tr>
<tr>
<td>G”</td>
<td>Viscosity modulus</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastro intestinal tract</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally recognized as safe</td>
</tr>
<tr>
<td>HCL</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HEPT</td>
<td>Height equivalent to a theoretical plate</td>
</tr>
<tr>
<td>HOMO</td>
<td>Highest occupied molecular orbital</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HPMC</td>
<td>hydroxyl propyl methyl cellulose</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LCST</td>
<td>Lower critical solution temperature</td>
</tr>
<tr>
<td>LUMO</td>
<td>Lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>MHA-GMB</td>
<td>Mueller-Hinton agar supplemented with glucose and methylene blue</td>
</tr>
<tr>
<td>NDDS</td>
<td>Novel drug delivery system</td>
</tr>
<tr>
<td>NSAIDS</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>OH</td>
<td>Hydroxyl group</td>
</tr>
<tr>
<td>PAA</td>
<td>Poly (acrylic acid)</td>
</tr>
<tr>
<td>PAAm</td>
<td>Polyacrylamide</td>
</tr>
<tr>
<td>PDA</td>
<td>Photodiode array</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly ethylene glycol</td>
</tr>
<tr>
<td>PEO</td>
<td>Poly(ethylene oxide)</td>
</tr>
<tr>
<td>PG</td>
<td>Propylene glycol</td>
</tr>
<tr>
<td>pH</td>
<td>Measure of activity of hydronium ion</td>
</tr>
<tr>
<td>PMT</td>
<td>Photomultiplier tubes</td>
</tr>
<tr>
<td>PNIPAAm</td>
<td>poly(N-isopropylacrylamide)</td>
</tr>
<tr>
<td>PO</td>
<td>Propylene oxide</td>
</tr>
<tr>
<td>PPO</td>
<td>Poly(propylene oxide)</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>S.D</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>TCL</td>
<td>Transcutol®- P</td>
</tr>
<tr>
<td>TPA</td>
<td>Texture profile analyzer</td>
</tr>
<tr>
<td>UCST</td>
<td>Upper critical solution temperature</td>
</tr>
<tr>
<td>UV-VIS</td>
<td>Ultraviolet- visible spectrophotometer</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

1.1 What is a gel?

The gel is defined as a soft, or semi-solid or solid like material, which has both solid and liquid components, where the solid component present as a mesh/network of aggregates, which immobilizes the liquid component. This solid network prevents the liquid from flowing by increasing the surface tension (1). They have wide range of properties ranging from soft and weak to hard and tough. Gels by weight are mostly liquid, but they behave as solids due to their three dimensional cross-linked network within the liquid. In other words, gels are dispersion of liquid molecules in solid in which the solid is the continuous phase and liquid is the stationary phase.

Types of gels

Based on external solvent phase gels are mainly classified into two types:

1. Organogels

2. Hydrogels

Organogel
The organogel is a thermodynamically stable, clear, viscoelastic, biocompatible and bi-continuous system consisting of gelators and non-polar solvent, in which non-polar solvent is the external phase. The gelators undergo physical or chemical interactions, so as to form self assembled fibrous structures which get entangles with each other resulting in the formation of a three-dimensional network structure (2). Cross linking prevents flow of external polar phase, increases hydrophobicity of gels and thereby, diminishes the diffusion rate of drug.

**Hydrogels**

Hydrogel is a transparent, viscoelastic and thermo dynamically stable system consisting of a polar solvent and a polymer, where polar solvent is the external phase. The polymer which are of synthetic or natural origin assemble to form a three dimensional network which can absorb and retain significant amount of water (3).

**1.2 Hydrogels**

Hydrogels are one of the upcoming classes of polymer-based controlled release drug delivery systems(4-6). Polymeric drug delivery systems have been extensively studied in order to solve the potential problems associated with drugs or bioactive molecules including toxicity, site dependence, low effectiveness, poor solubility, short half-life, rapid degeneration and rapid clearance from the body. Considering various properties such as flexibility, structure, biocompatibility, and hydrophilicity, three dimensional matrices, hydrogels, are being extensively used as drug delivery carriers. Hydrogels are three dimensional structures of hydrophobic polymer network consisting of a single chain of monomers being cross-linked or chains of co-polymers being cross linked (4). These
are insoluble in water due to ionic interaction and hydrogen bonding between the cross-linkings, but they become swollen by imbibing large amount of biological fluids or water (6). Even then, hydrogels maintain their physical integrity and mechanical strength due to their cross-linkings. Some of the different marketed hydrogels are shown in table1.1. Hydrogels generally possess a good bio compatibility because of their hydrophilic surface, which has a low interfacial free energy. Also, the soft and rubbery nature of hydrogels minimizes irritation to surroundings tissue increasing bio compatibility (7).
Table 1.1: Marketed Hydrogel-based products (8).

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Hydrogel composition</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQZ Gel™ Oral Controlled release system</td>
<td>Macro med (UT,USA)</td>
<td>Chitosan and polyethylene glycol</td>
<td>hypertension</td>
</tr>
<tr>
<td>Hycore-V™ and Hycore R™</td>
<td>CeNeS Drug Delivery (Irvine, UK)</td>
<td>----</td>
<td>Vaginal and rectal infections respectively</td>
</tr>
<tr>
<td>Cervidil® vaginal insert</td>
<td>Controlled Therapeutics, UK; marketed by Forest Pharmaceuticals (MO,USA)</td>
<td>Poly(ethyleneoxide) and urethane</td>
<td>It relaxes and softens the cervix (cervical ripening) to allow the baby to pass through the birth canal during delivery.</td>
</tr>
<tr>
<td>Moraxen™</td>
<td>CeNeS Drug Delivery (Irvine, UK)</td>
<td>----</td>
<td>End-stage cancer pain</td>
</tr>
<tr>
<td>Smart Hydrogel™</td>
<td>MedLogic Global (Plymouth, UK)</td>
<td>Poly(acrylic acid) and poly (oxypropylene-oxyethylene) glycol</td>
<td>Used for development of ophthalmic, buccal, nasal, vaginal, transdermal, injectable, implantable, non-aerosol pulmonary drug systems</td>
</tr>
<tr>
<td>Aquamere™</td>
<td>Hydromer(NJ,USA)</td>
<td>Interpolymers of PVP and PVP-grafted copolymers with Urethane</td>
<td>Skin care, topical and oral drug delivery</td>
</tr>
<tr>
<td>Aquatrix™ II</td>
<td>Hydromer(NJ,USA)</td>
<td>Chitosan-PVP</td>
<td>Skin adhesives gels, wound and burn dressings, implants, and drug delivery matrices</td>
</tr>
<tr>
<td>Hypan ®</td>
<td>Hymedix International (NJ,USA)</td>
<td>Hydrophilic acrylate derivates with unique multiblock structure</td>
<td>Used in the manufacture of soft contact lens and moisturizing wound gels and dressings</td>
</tr>
</tbody>
</table>
Other important characteristics of hydrogels are its permeability and water holding capacity. The polar hydrophilic groups of hydrogels, upon contact with water result in the formation of primary bound water. As a result the network swells and exposes hydrophobic groups, which also have capability to interact with water. The hydrophilic groups and hydrophobic groups bound with water forming ‘primary bound water’ and ‘secondary bound water’ respectively. These hydrogels also absorb additional water called ‘free water’, assumed to fill in center of large pores or the space between network chains or voids (3). The additional swelling is mainly due to osmotic forces of network chains. This osmotic force is opposed by elasticity force of network, which balances the stretching of network and prevents hydrogel deformation. The extent of porosity and the type of porous structure determines the rate of swelling of hydrogels. The rate of swelling is one of the very important features of hydrogel swelling. Based on their extent of porosity and the type of porous structure the hydrogels can be divided into four categories (Table 1.2)
<table>
<thead>
<tr>
<th>Type</th>
<th>Pore size</th>
<th>Major swelling mechanism</th>
<th>Swelling rate</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-porous</td>
<td>1-100Å</td>
<td>Diffusion through free volumes</td>
<td>Very slow, sample size dependent</td>
<td>Various uses from contact lenses to artificial muscles</td>
</tr>
<tr>
<td>Micro-porous</td>
<td>100-1000Å</td>
<td>Combination of molecular diffusion and convection</td>
<td>Slow, sample size dependent</td>
<td>Mainly in biomedical applications and controlled release technology</td>
</tr>
<tr>
<td>Macro-porous</td>
<td>0.1-1µm</td>
<td>Diffusion in the water filled pores</td>
<td>Fast, sample size dependent</td>
<td>Mainly as super absorbents in baby diapers</td>
</tr>
<tr>
<td>Super-porous</td>
<td>Several hundred microns</td>
<td>Capillary forces</td>
<td>Very fast, sample size independent</td>
<td>Drug delivery system in GIT, Tissue engineering.</td>
</tr>
</tbody>
</table>

Hydrogels can also be classified into conventional and stimuli-sensitive hydrogels. The former conventional hydrogels will absorb water in presence of aqueous environment. These cross-linked polymer chains show no change in the swelling behavior with change in surrounding environment like temperature, pH and electric field. But the later stimuli-sensitive hydrogels or “Smart” hydrogels change their swelling behavior with the change in surrounding environment.

1.3 Stimuli-sensitive hydrogels

Stimuli-sensitive hydrogels can change their swelling behavior by sensing changes in surrounding environment. The environmental changes or stimuli can be in the form of
temperature, pH, light, ionic strength, glucose, pressure, magnetic field or ultra sound (figure 1.1). The stimuli that induce responses can be categorized into three types

1. Physical stimuli: temperature, lights, ultrasound, electric fields, pressure and magnetic fields

2. Chemical stimuli: ionic strength and pH from biological fluids

3. Biological stimuli: enzymes, ligands or antibodies/antigens

Figure 1.1: Schematic representation of different stimuli on polymer matrix

(modified from (10))

The change in swelling behavior of stimuli-sensitive hydrogels is useful in drug delivery applications, as drug release can be triggered upon stimuli mentioned above. These “smart” or stimuli-sensitive polymers dictate not only when the drug is to be delivered,
but also potentially when and at which interval it should be released (11). Hence they can be used in controlled and targeted drug delivery systems.

Stimuli-responsive or ‘smart’ hydrogels can be used in controlled drug delivery to achieve

1. Constant concentration of therapeutically active compounds in the blood with minimum fluctuations
2. Predictable and reproducible release rates over a long period of time
3. Protection of bioactive compounds considering their very short half-life
4. Elimination of side-effects, waste of drug and frequent dosing
5. Optimized therapy and better patient compliance
6. Solution of the drug stability problem (12)

The most commonly investigated stimuli-sensitive systems are pH sensitive or ion sensitive and temperature-sensitive hydrogels. pH sensitive or ion sensitive hydrogels change their swelling behavior based on change in pH of the external environment. The pH sensitive polymers contain ionic groups, which may be acidic or basic groups. The ionic groups either accept or release protons in response to changes in environmental pH, causing changes in swelling. Some of the examples these pH sensitive polymers are poly methyl metha acrylate, polyacrylic acid and polyethylene glycol (13). The other system, temperature-sensitive hydrogels, will be described in more detail below.

1.4 Temperature-sensitive poloxamer-based hydrogels

Temperature-sensitive hydrogels are well-accepted form of hydrogels; they show variability in the swelling behavior in response to changes in the environmental
temperature (14). Temperature-sensitive hydrogels undergo phase transition (solid to liquid /liquid to solid or swelling/shrinking of polymer network) with the change in temperature above or below certain temperature called critical solution temperature (CST). Based on mechanism, these hydrogels are divided into positive and negative temperature-sensitive polymers. Most of the polymers belong to positive temperature-sensitive hydrogels, which swells in water with increase in temperature above critical point called upper critical solution temperature (UCST). In other words, polymers with UCST generally become gel with increase in temperature and by decreasing temperature below UCST the polymer network shrinks. On other hand, negative hydrogels shrinks with increase in temperature above critical temperature called lower critical solution temperature (LCST) (15). Polymers with LCST releases drug by shrinking the polymer network with increase in temperature. Depending on the type of polymers used, temperature-sensitive hydrogels can be both biodegradable and biocompatible. Various drug classes, such as hormones, anti-diabetic agents, anticancer agents and protein and peptides, have been investigated for enhanced delivery by the use of such systems (4). The polymers generally used in the preparation of these hydrogels are hydroxyl propyl methyl cellulose (HPMC), N-isopropylacrylamide copolymers (pNIPAM), poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) (poloxamers, Tetronics® or Pluronics®), poly (acrylic acid) (PAA) and polyacrylamide (PAAm) Poly(ethylene oxide)/poly (D, L-lactic acid-co-glycolic acid)(16). The focus of the present thesis will be on poloxamers in the preparation and characterization of thermosensitive hydrogels.
Poloxamer block co-polymers are introduced in 1950s are being used in large areas of liquids, pastes and solid. They are now listed in US and European pharmacopeias (17). Poloxamers consists of ethylene oxide and propylene oxide blocks arranged in a tri block structure (Figure 1.2) \( \text{EO}_a – \text{PO}_b – \text{EO}_a \). By changing \( a \) and \( b \) values the parameters such as lipophilicity, hydrophilicity and size can be easily modified. Poloxamers show reversible temperature-sensitive properties, which present great interest in optimizing drug formulation. They are registered under various commercial names Pluronic®, Synpersonic® or Tetronic® (17). Poloxamers are odorless and tasteless, waxy white granules of free-flowing nature. They are generally soluble in any solvent (organic/aqueous/polar/non-polar). The aqueous solutions of poloxamer are very stable in the presence of alkali, acid or metal ions. Due to these properties poloxamers established themselves as preferred substance in formulation techniques (18). The poloxamers are marketed in various grades, which have different physical and chemical properties. Depending upon the physical designation, the grades are assigned as F for flakes, P for paste, L for liquid(19). Some of the common poloxamers marketed are given in table 1.3

![Figure 1.2: General structure of poloxamers.](image)
Table 1.3: Different marketed poloxamers with their pluronic name (18).

<table>
<thead>
<tr>
<th>Poloxamer</th>
<th>a</th>
<th>b</th>
<th>Molecular weight (gram/mole)</th>
<th>Pluronic®</th>
</tr>
</thead>
<tbody>
<tr>
<td>124</td>
<td>12</td>
<td>20</td>
<td>2090-2360</td>
<td>L 44 NF</td>
</tr>
<tr>
<td>188</td>
<td>80</td>
<td>27</td>
<td>7680-9510</td>
<td>F 68 NF</td>
</tr>
<tr>
<td>237</td>
<td>64</td>
<td>37</td>
<td>6840-8830</td>
<td>F 87 NF</td>
</tr>
<tr>
<td>338</td>
<td>141</td>
<td>44</td>
<td>12700-17400</td>
<td>F 108 NF</td>
</tr>
<tr>
<td>407</td>
<td>101</td>
<td>56</td>
<td>9840-14600</td>
<td>F127 NF</td>
</tr>
</tbody>
</table>

Poloxamer 407 is a commonly used co-polymer in pharmaceutical formulations, also known as Pluronic F 127®. Poloxamer 407 has a HLB of 22 at 22°C and is also presented as an “inactive” ingredient by FDA in different types of formulations (20, 21). Aqueous solutions of poloxamer 407 show thermoreversible properties; i.e. they transform forth and back, from a liquid-like behavior to gel-like behavior above a certain temperature called sol-gel transition temperature or critical transition temperature. Above sol-gel transition temperature the solutions behave as solid and below, it behaves as a liquid. The gelling process of poloxamers is typically divided into two steps as shown in figure 1.3. The first step occurs when the temperature is increased reaching the critical micelle temperature and the poloxamer co-polymers aggregate to form spherical micelles. These micelles consist of an outer shell of hydrated swollen poly EO chains with dehydrated poly PO blocks as the core. The process develops into the second step when a further increase in the temperature packs the micelles in an orderly manner to form gels.
Apart from temperature, gelation is also dependent on the concentration of poloxamer molecules in solution. The formation of gels occurs when the concentration of poloxamer is above its critical micellar concentration. Poloxamer solution of 20-30% concentrations forms a clear liquid at cold temperature 4-5 °C and gel at room temperature. The gel can return to liquid by cooling. Poloxamer formulations are prepared mainly using the cold method. In the cold method, the poloxamer polymer is added to cooled water 4-5°C and stirred until a homogenous solution is formed. When poloxamers are placed in cold water, hydration layers surround the poloxamer molecule. The hydrophobic portions of poloxamer are separated due to hydrogen bonding between water and hydrophilic chains. The hydrogen bonds break by increase in the temperature, resulting in hydrophobic interactions among poly PO chains, thus forming gel. Hot water is not used in the preparation of poloxamer formulations, because of early hydrophobic interactions of poly PO blocks producing non-homogenous solution. The drug and excipients are added to the homogenous solution based on their properties. The concentrations of poloxamers up to...
30% (w/w) can be easily prepared, whereas concentrations above 35% (w/w) requires few minutes freezing to liquefy polymers and produce homogenous solution (24). To attain a suitable formulation with satisfactory tolerance and to favor formulation stability the pH and osmolarity are adjusted (25). Also, the viscosity characteristics of the poloxamer are not altered by sterilization by autoclaving at 120°C for 15 minutes at 1 bar pressure. So, the poloxamers can be used to prepare sterile formulations for injectable or ophthalmic routes (26, 27).

1.5 Evaluation of temperature-sensitive hydrogels

1.5.1 Measurement of sol-gel transition temperature

Sol-gel transition temperature is a key parameter to define a thermosensitive formulation. At sol-gel transition temperature the rheological properties of a system abruptly changes from a liquid-like state to a solid-like state. It means at the temperature above sol-gel transition the system behaves as gel and below it behaves as a liquid solution. It is important to control sol-gel transition temperature for formulations based on type of delivery. For drug delivery, the sol-gel transition points for in situ gelling formulations should be close to body temperature to exploit the temperature difference between the body, i.e. 35 ± 2°C, and the environment, i.e. ~20°C. By changing polymer and excipients concentrations the desired sol-gel transition temperature can be achieved. The sol-gel transition point of poloxamer formulations can easily be determined by the glass micro capillary tube method or the magnetic stirrer method. In glass micro capillary method, the formulation is loaded in the micro capillary tube at high temperature and then allowed to cool. The temperature at which the formulation flows as liquid is
considered as transition point (28). Although, this method is not very sensitive it is easy to carry out. As these formulations show perfect thermoreversible properties gel-sol transition is considered to be equivalent to sol-gel transition temperature. In magnetic stirrer method, the poloxamer gels are loaded in a beaker or test tube at cold temperature and allowed to heat with constant magnetic stirring. The temperature where the magnetic stirrer stops rotating is considered as sol-gel transition point (29, 30). Generally, the sol-gel transition temperature decreases with increase in the poloxamer concentration as shown in figure 1.4. Excipients in the formulation may influence the sol gel transition temperature of the poloxamers. For example, HCl, propylene glycol and ethanol increases the sol-gel transition temperature, whereas, sodium chloride, Na₂HPO₄ and sodium alginate decreases the sol-gel transition temperature (17). These substances show their action by influencing the micellization of poloxamer and alter dehydration of poly PO core.
1.5.2 Rheological properties

Rheological and viscoelastic studies determine the interactions between the poloxamer co-polymer and additives with great sensitivity. The rheological flow behavior of poloxamer solutions can be either Newtonian (Liquid-like) or non-Newtonian (Gel-like) based on the temperature and concentration of polymer. Below the sol-gel transition temperature, poloxamer solutions exhibit Newtonian properties whereas above the sol-gel transition point they exhibit non-Newtonian properties (32). Newtonian fluids (water, gas etc) are characterized by constant viscosity over a large range of stress and can be described by the equation:
\[ \tau = \eta \gamma \]  \hspace{1cm} \text{(Equation 1.1)}

Where \( \tau \) is shear stress, \( \eta \) = coefficient of viscosity and \( \gamma \) = shear strain.

Non-Newtonian fluids exhibit remarkable change in viscosity with the change in stress. This means that below a critical stress value the materials behaves like a solid but flows like a liquid in exceeded stress (\( \tau > \tau_c \)). The shear stress of these materials can be described by simple linear Bingham flow behavior

\[ \tau = \tau_0 + \mu \gamma \]  \hspace{1cm} \text{(Equation 1.2)}

Where \( \tau \) = shear stress, \( \tau_0 \) = the critical value (yield value), \( \gamma \) = shear strain and \( \mu \) = the plastic viscosity.

But, fluids not always exhibit a linear flow behavior. The model of Herschel-Buckley is used in such non linear cases (33). The plot of shear stress against shear rate confirms the type of behavior of a particular fluid, these plots are known as ‘rheograms’ (figure 1.5)

\[ \tau = \tau_0 + k \gamma^n \]  \hspace{1cm} \text{(Equation 1.3)}

Where \( \tau \) = shear stress, \( \tau_0 \) = the critical value (yield value), \( \gamma \) = shear strain and \( k, n \) = constants. If \( n < 1 \) the system is exhibiting shear thinning flow, \( n > 1 \) indicates shear thickening behavior.
Non-Newtonian materials exhibit elastic properties as well as viscous properties; these particular characteristics are known as viscoelasticity. Viscoelastic studies are performed using dynamic mechanical analysis, applying a small oscillatory stress and measuring resulting strain. Purely elastic materials have stress and strain in phase, but for viscous materials strain lags by 90°. Viscoelastic materials exhibit some lag in strain for applied oscillatory stress. From the dynamic mechanical analysis, two dynamic moduli, i.e. the elastic or storage modulus ($G'$) and the viscosity or loss modulus ($G''$) are obtained (32, 34).

$$G' = \frac{\tau_0}{\gamma_0} \cos \delta \quad \text{(Equation 1.4)}$$
\[ G'' = \frac{\tau_0}{\gamma_0} \sin \delta \]  

(Equation 1.5)

Where \( \tau_0 \) and \( \gamma_0 \) are the critical value of the stress and strain respectively, \( G' \) and \( G'' \) are storage and viscosity modulus respectively, \( \delta \) is the phase angle.

Storage modulus is the characteristic of stored elastic energy, whereas viscosity modulus is for viscous dissipated energy. In other words, the higher the \( G' \) the more pronounced the elastic character and higher the \( G'' \) higher the viscous properties (35).

1.5.3 Mechanical properties

Determination of in vitro mechanical properties of the formulations gives useful information about physical gel structure of semi-solid dosage forms. This is useful in predicting the product behavior in different physiological and environmental conditions (36). Texture profile analysis can be applied for mechanical characterization of semi-solid systems and pharmaceutical gels (37). To determine these mechanical properties, the analytical probe is inserted into a semi-solid system to a defined depth at defined rate and extracted out at define rate. The change in force is plotted against time gives different mechanical properties like hardness, compressibility and adhesiveness can be derived. A typical force-time is given in figure1.6.
Figure 1.6: Typical force-time plot of Texture profile analysis

- Hardness is defined as the force required to attain a given deformation, it gives information about applicability of gel at desired site.

- Compressibility is described as the work required to compress the product through a fixed distance, gives information about the removal of product from the container and its spreadability during application.

- Adhesiveness is defined as the work required to overcome the attractive forces between surface of probe and sample, in which its cohesive bonds are broken (38). Gives information about the time of residence of semi-solid dosage forms at
application site. Greater is the adhesiveness; greater will be the bio-adhesion of the formulation.

1.5.4 Mucoadhesive properties

Mucoadhesive force is defined as the force with which the product binds to the mucous membrane at physiological temperature (37°C). It is useful in assessing the potential of the formulation to interact with the mucosal surface. This helps in the development of a mucoadhesive dosage forms for nasal, ophthalmic, vaginal or rectal delivery. The mucoadhesive properties of the formulations can be determined by attaching a mucous membrane (natural origin or artificial like mucin disk) to bottom of analytical probe of texture profile analysis (TPA). The membrane containing probe is kept in contact with the sample surface. After certain amount of time the probe is retracted back to its original position. From the resulting force-time plot the maximum detachment force and work of adhesion are obtained. The higher the value of work of adhesion, the stronger the formulation binds to the mucosal surface thereby increasing the retention time and decreasing the leakage by mucosal secretions(39).

1.5.5 Drug release properties

In vitro drug release kinetics of poloxamer formulations serves as a comparative tool in process of developing the topical dosage forms. The poloxamer formulations release drug to receptor medium either by diffusion from the polymer matrix or by dissolution of poloxamer in the receptor medium. These in vitro drug releases of formulations are conducted by membrane model or non-membrane model. In the membrane model, membrane is used as a separator between the donor chamber (containing sample
formulation) and receptor chamber (containing buffer). The drug release into the receptor medium is mainly by diffusion. Whereas, in the non-membrane models the polymer matrix dissolves slowly in the receptor medium favoring controlled release. For analytical detection of drug, aliquots of samples are drawn in regular intervals and analyzed using various spectrophotometric methods such as by using UV-VIS spectrophotometer or by using high-pressure liquid chromatography (HPLC). The temperature of medium used in the experimentation is chosen based on the formulations pharmaceutical use i.e; 37±1°C for injectable and ophthalmic formulations. The experimentation is generally performed above sol-gel transition point of the formulation to avoid rapid dissolution of polymer in the receptor medium. The pH of the receptor medium is chosen based on the intended route of delivery. There are also instances where the change in pH produced change in drug release characteristics (40). An isotonic phosphate buffer or HEPES buffer is generally used as the receptor medium. These *in vitro* drug release characteristics of formulations decide the fate of a system, whether sustained or immediate release and also helps to find the kinetics of drug release. The hydrogels systems are mainly intended for the sustain release of the drug. Sustained release of drug helps to maintain therapeutic plasma concentrations over a longer period of time. Sustained release hydrogel systems help to decrease dosage intervals, thereby increasing patient compliance (8).
1.6 Pharmaceutical applications of temperature-sensitive poloxamer-based hydrogels

1.6.1 Topical drug delivery

Topical route of drug delivery can be defined as the application of drug formulation to body surfaces, such as skin or mucous membranes to prevent or treat diseases. Topical route can be further classified into extra topical and intra topical route. In extra topical the formulation is directly applied on to the cutaneous tissue or skin. Whereas, intra topical the formulation is applied into the local cavities of body for therapeutic action; example vagina, rectum and buccal cavity. Most of these topical formulations are generally used for localized effect at the site of drug delivery (41). The main advantages and disadvantages of topical drug delivery are

**Advantages:**

- Bypass first pass metabolism
- Easy to apply, suitable for self medication and increased patient compliance
- Avoidance of risks and inconveniences of intravenous drug delivery
- Ability to easily terminate the medications, when needed
- Avoids fluctuation in drug levels, inter and intrapatient variations
- Ability to deliver drug more selectively to a specific site
- Improving physiological and pharmacological response

**Disadvantages:**

- Possibility of allergic reactions.
- Skin irritation may occur due to drug or excipients.
• Poor permeability of drugs through membranes.

• Not suitable for drugs with large particle size.

Various dosage forms like powders, lotions, ointments, gels, solutions, emulsions and suspensions are used for topical delivery. Hydrogels with temperature-sensitive property are extensively studied, because of their favorable \textit{in situ} gelling ability. Poloxamers-based hydrogels are well tolerated by skin and are used in the treatment of thermal burns and in wound healing (27, 42, 43). Poloxamer407 is used in formulation of many non-steroidal anti-inflammatory drugs (NSAIDS), antifungal drugs, anti bacterial drugs for topical delivery. Most of the time, they are formulated along with the thickening agents (carbopol or cellulose derivates) to increase the mechanical strength and bioadhesiveness of these formulations. Solubilizing agents like ethanol or propylene glycol can be added to formulation to improve water solubility of the drug.

In ophthalmic delivery with the use of conventional eyedrops, most of the drug is drained away from the pre-corneal area in a few minutes. Increasing viscosity of the formulations can significantly decrease drainage rate. By using ointments the viscosity was increased but there is drastic decrease in patient compliance due to application difficulties and blurry vision (44). To overcome these problems \textit{in situ} gelling poloxamers showed promising characters, by behaving as liquids at the time of application followed by gelling in contact with eye surface. Also ideally, final products of ophthalmic preparations should be sterilized. Poloxamer formulations can withstand autoclaving without altering viscosity of the product favoring its use in ophthalmic
preparations. Also, poloxamers demonstrate good tolerance to ophthalmic mucosa, minimizing mucosal irritation and allergic reactions.

Semi-solid dosage forms like creams, ointments and solutions for rectal and vaginal administration are well accepted. However, the main disadvantage with the conventional formulations is incomplete or irregular absorption probably due to rapid clearance of drug from the site or due to incomplete administration (35). This drawback can be overcome by using temperature-sensitive formulations. The temperature-sensitive formulations of poloxamer behave as liquid at room temperature but become gel at rectal or vaginal temperature. Poloxamer is well tolerated by rectal mucosa (45). The inclusion of mucoadhesive enhancers along with the poloxamer synergistically increases drug retention at the site. Commonly used mucoadhesive enhancers are carbopol, polycarbophil, hydroxyl propyl cellulose, or sodium alginate (29). These mucoadhesive polymers containing hydrophilic OH groups increase the mucoadhesive character by binding to oligosaccharides, which were present in the rectal mucosa (46). Combination of poloxamer 407 in with poloxamer 188 is well studied by researchers; poloxamer 188 decreases the sol-gel transition temperature while increasing the viscosity of the formulations (31, 47). Thereby, increases the residence time of active ingredient at administration site.

1.6.2 Parenteral drug delivery

Parenteral drug delivery means introduction of drug into body by routes other than the gastro intestinal tract. Practically, the term is applied to injection of dosage form by subcutaneous, intra-muscular and intra-venous and intra-arterial routes. Injections can be
made into specific organs for targeted drug delivery. Some of advantages and disadvantages of parenteral administration are

**Advantages:**

- Rapid onset of action
- Predictable and almost complete bio availability
- Avoidance of gastro intestinal tract (GIT) with problems of oral drug administration
- Provides reliable route for drug administration in unco-operative, very ill and comatose patients

**Disadvantages:**

- Pain involved and decrease in patient compliance
- Difficult to reserve its physiological effect
- Not suitable for self medication.
- Invasive method with increased risk of infection

Although, parenteral injections are for fast on-set action, sustained or controlled release for some drugs drastically decrease dosage interval and increase patient compliance. *In situ* gelling hydrogels with temperature-sensitive property are extensively studied for this purpose. These hydrogels flow as liquid below sol-gel transition temperature, allowing injectability of formulation through syringe. In contact with physiological fluids, it forms semi-solid gel (“depot”) allowing sustained or controlled release of the drug.

*In situ* forming systems represent an attractive alternative to replace implantations or temporary prosthesis. It is less invasive and painful compared to implants, which require
local anesthesia and a small surgical intervention. Moreover, localized or systemic drug delivery can be achieved for prolonged periods of time, typically ranging from one day to several months (48). *In situ* forming gels of poloxamer show good bio-compatibility and no irritation when injected into body (22). Poloxamer formulations can withstand autoclaving without altering viscosity of the product favoring its use in parenteral preparations. Poloxamer formulations in combination with other polymers (other poloxamers and thickening agents) showed promising effect by decreasing the elimination rate. *In situ* gels formulated along with the thickening agents (HPMC, carbopol or cellulose derivates) or other poloxamers (poloxamer 188) to increase viscosity of product increasing residence time.

**1.6 Aim of thesis**

Based on the advantages of these temperature-sensitive systems, the aim of the present study is to develop a thermosensitive hydrogel for topical delivery. The poloxamer is used as temperature-sensitive polymer along with fluconazole as model drug is used in the preparation of thermosensitive hydrogels. Fluconazole is a synthetic bis-triazole with broad spectrum of fungistatic activity. Different thermosensitive hydrogel formulations were prepared and characterized based on their rheological, mechanical and mucoadhesive properties. *In vitro* performance of the FLZ thermosensitive hydrogels were evaluated by studying the FLZ permeability and antifungal activity of these systems.
References:


Chapter 2

Instrumentation

2.1 UV/Vis Spectrophotometer

Ultraviolet/Visible (UV/Vis) spectroscopy, using visible light (400nm to 800nm) and the adjacent near ultraviolet ranges (200nm to 400nm), can be used to characterize and quantitatively determine the amount of dissolved substances.

Principle: UV/Vis spectroscopy is also called electronic spectroscopy. Because, when energy irradiated on the molecules the electronic nature of molecule changes, i.e. the electrons in the ground level (bonding orbitals) excite to higher energy levels (anti-bonding orbitals) within the molecule. Based on type of bond between atoms, the electrons in a molecule can be of one of three types. They are, \( \sigma \) (single bond), \( \pi \) (multiple-bond), or non-bonding (\( n \)- caused by lone pairs). These electrons when imparted with energy in the form of light radiation get excited from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) and the resulting species is known as the excited state or anti-bonding state (1). Most of the absorption in ultraviolet-visible spectroscopy occurs due to \( \pi \)-electron transitions or \( n \)-electron transitions (Figure 2.1). These transitions generally occur in compounds with
unsaturated bonds termed as chromophores (e.g; alkenes, aromatics, dienes and trienes).

Generally the peaks in UV/Vis spectrum are broad, due to the electronic transitions not only from vibrational energy levels but also from the rotational energy levels.

![Energy diagram](image)

Figure 2.1: Electronic excitation of electrons between molecular orbitals.

According to Beer-Lambert law (Equation 2.1), the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorbing species in a solution by using the equation given below.

$$A = - \log \left( \frac{I}{I_0} \right) = \varepsilon \cdot c \cdot L \quad \text{(Equation 2.1)}$$

Where

- $A =$ the measured absorbance
- $I_0 =$ the intensity of incident light at a given wave length
- $I =$ the transmitted intensity
\[ L = \text{the path length through the sample} \]

\[ c = \text{the concentration of the absorbing species.} \]

\[ \varepsilon = \text{the molar absorptivity or extinction coefficient} \]

**Instrumentation:** A UV/VIS spectrophotometer generally consists of a light source (halogen, deuterium or tungsten lamps), sample compartment and sample cells (cuvette for single sample or 96-well plate for analyzing multi sample), dispersion element (prism, interferometer, grating, monochromator), detector (photomultiplier tubes (PMT), photodiode array (PDA) and charge coupled device (CCD) cameras) and a data acquisition system. Spectrophotometers are generally classified into two types: a) single beam and b) dual beam.

a) **Single beam:** In a single beam UV/VIS spectrophotometer (Figure 2.2), there is only single beam of light which passes through the sample. The energy of light beam striking the detector is first measured without sample, followed by taking the measurement with the sample in the holder (2). The absorbance is calculated by taking the ratio of negative logarithm of \( I \) to \( I_0 \) where \( I \) and \( I_0 \) refers to energies of transmitted and incident light beams respectively. This type of spectrophotometer is employed in the present study.
b) Dual beam: In a dual beam UV/VIS spectrophotometer (Figure 2.3), a beam splitter is used to split single beam of light into two equal beams of equal energy with equivalent optical paths. One of the beams passes through the sample and the other through reference (may be air, solvent or any sample background). The absorbance is calculated by taking the ratio of negative logarithm of $I$ to $I_0$, where $I$ and $I_0$ refer to energies of transmitted and incident light beams respectively (2).

**Sample Preparation:** Samples for UV-Visible spectroscopy are generally solutions. Blank or reference solutions are prepared with all ingredients except the active drug. If
needed, samples are prepared by diluting them prior to analysis. Sample or/and blank are placed in a cuvette, which is made of UV transparent material such as fused silica, quartz glass, or plastic. Commonly used solvents with their wavelength cutoff for UV/Visible measurements are acetonitrile (190 nm), water (191 nm), cyclohexane (195 nm), methanol (201 nm), ethanol (204 nm), ether (215 nm), and methylene chloride (220 nm).

**Applications:** The UV/VIS spectrophotometer is routinely used in the quantitative determination of wide range of analytes such as transition metal ions, metal ions, highly conjugated organic compounds, inorganic complexes and biological molecules such as proteins and nucleic acids. Results obtained from UV/Vis spectroscopy are often used in conjugation with other techniques such as nuclear magnetic resonance (NMR), infra red (IR), and mass spectroscopy (MS) for complete chemical structure elucidation (5). UV/VIS can also be used to detect impurities and to calculate molecular weights. By using Woodward-Fieser rule the $\lambda$ max of conjugated dienes and polyenes can be calculated (6). UV-Visible derivative spectroscopy is another analytical tool to study the resolution enhancement of overlapping peaks and elimination or reduction of background matrix absorption.

### 2.2 Rheometer

Rheology is defined as the science of the deformation and flow of matter (7). Rheometer is a device that can measure both viscosity and yield stress of a material. Whereas, viscometer can measure only the viscosity of the material (8).

**Principle:**
The rheometers are classified into shear or rotational rheometers and extensional rheometers. The principle of only rotational rheometers is discussed here, as extensional rheometers are not generally used for low viscous substances like gels, solutions or suspensions. The rotational rheometers are used to measure complex flow properties as well as oscillatory properties of a substance. The flow properties of a substance can be determined by two methods. In controlled stress mode a known torque (stress) is applied to the sample and the resultant displacement (strain or strain rate) is measured. In controlled rate mode a defined speed (shear rate) is applied and the resultant torque (stress) is measured. The measured shear plotted against strain gives flow curves, which are used to determine the flow character of the substance.

Based on type of flow curve the behavior of substance can be either

- Newtonian: Constant viscosity over a large range of stress
- Non-Newtonian or Bingham bodies: Remarkable changes in viscosity with change in stress. Non-Newtonian further classified to
  - Shear thinning: Viscosity decreases with increase in stress
  - Shear thickening: Viscosity increases with increase in stress

In oscillatory measurements of a substance, a sinusoidal oscillating stress is applied to a material and the resulting strain is measured. Measuring the resultant stress and strain, at a point of time reveals differences between substances. A typical schematic stress response to oscillatory strain deformation for different substances is shown in figure 2.5.
Figure 2.5: Schematic stress response to oscillatory strain deformation for an elastic solid, a viscous fluid and a viscoelastic material.

Based on results, an Ideal elastic (solid) material will not deform on application of deformation energy. Ideal elastic solids retain all of the deformation energy applied to it, so phase difference angle ($\delta$) is $0^\circ$. And, an ideal viscous fluid will deform completely on
application of deformation energy. Ideal viscous fluid dissipates all the applied energy, so the phase angle is 90°. But, viscoelastic material will neither retain nor dissipates all the deformation energy applied on it. So the viscoelastic material have the phase angle between 0° and 90°. The viscoelastic behavior of the system characterized by the storage modulus, $G'$ and the loss modulus, $G''$, which respectively characterize the solid-like and fluid-like contributions to the measured stress response. The higher the $G'$ the more pronounced the elastic character and higher the $G''$ higher the viscous properties (9)

$$G' = \frac{\tau_0}{\gamma_0} \cos \delta \quad \text{(Equation 2.3)}$$

$$G'' = \frac{\tau_0}{\gamma_0} \sin \delta \quad \text{(Equation 2.4)}$$

Where $\tau_0$ and $\gamma_0$ are the critical value of the stress and strain respectively, $G'$ and $G''$ are storage and viscosity modulus respectively, $\delta$ is the phase angle.

**Instrumentation:** The schematic representation of rheometer is showed in figure 2.6. The sample is loaded into the gap of a measuring system specifically designed to impose simple shear flow when rotated. The measuring system can be either cone and plate or parallel plate or cylinder system based on type substance analyzed. The measuring system is supported by a virtually frictionless air-bearing and driven by an ultra-low inertia motor coupled to an ultra-high precision position encoder. The sample and measuring system can be temperature controlled (peltier plate or upper heated plate (UHP)). Various rheological characteristics of the sample can be determined by rotating, oscillating or applying a step function to the measuring system. It can be either by
controlling motor torque (stress controlled rheometry) or position change (strain controlled rheometry).

Figure 2.6: Schematic representation of a rheometer(10).

Common test modes are (a) rotational (or flow) to measure shear viscosity and (b) oscillation to measure dynamic material properties such as viscoelastic modulus and phase angle.

- **Rotational mode**: In rotational mode the rheometer is a constant speed motor with a torque detection system. The torsion bar is suspended on an air bearing to give a virtually frictionless bearing. When the drive system turns, the sample resistance (viscosity) tries to twist the torsion bar. By measuring the resultant twist and knowing the stiffness of the bar, the torque is obtained (11). The geometries generally used are illustrated in figure 2.7.
b) **Oscillatory mode:** In oscillatory mode as shown in figure 2.8, the motor oscillates rotating shaft and plate at varying frequencies keeping strain constant. In response to the applied strain, the material will exert a torque on the rheometer. If the sample is viscoelastic, the torque will be dependent on the applied frequency, as will the phase angle between the torque and the strain. Based on torque and phase angle storage (G’) and loss modulus (G’”) of the sample are calculated.
**Sample preparation:** A wide variety of samples can be analyzed ranging from low viscous substances (Newtonian) to high viscous substances (Non-Newtonian). Sample size required for the cylinder geometry is large compared to cone and plate or parallel plate geometries. The sample is introduced directly on or into the sample plate or cylinder respectively. The excess of sample flowing out of measuring system is removed carefully by gentle blotting with paper.

**Applications:** The rheometer has a wide range of applications. It is used to measure the rheology of semi-solids, suspensions, emulsions, and polymers in industries such as pharmaceuticals, foods, cosmetics, and consumer products. These measurements can help predict shelf life of products under various stress conditions. In medical field, the rheometer is used to measure viscosity of biological fluids. Rheometer in combination temperature control system is used to find the approximate glass transition temperature of polymers.

**2.3 Texture Profile Analyzer (TPA)**

A texture profile analyzer (TPA) measures mechanical properties such as hardness, springiness, adhesiveness, cohesiveness, firmness, gumminess and chewiness of a product.

**Principle:**

Texture analysis is performed by applying controlled forces to the product and record the responses in the form of deformation and time (12). The force responses of the sample are recorded and presented in the form of graph (force Vs. time) called texture profile (figure2.9). Based on the texture profile, the mechanical textural characteristics of a
sample can be divided into primary parameters (hardness, cohesiveness, viscosity, elasticity and adhesiveness) and secondary parameters (brittleness, chewiness, and gumminess) (13).

Figure 2.9: A typical force-time plot obtained from texture profile analysis.

Hardness is peak force during compression of sample. Cohesiveness also known as compressibility is the work required to deform a product during the first compression of the probe. Adhesiveness is the work necessary to overcome the attractive forces between surface of the probe and surface of the sample (14).
Texture profile analyzers primarily consist of five elements. The drive mechanism exerts pressure on the sample by simple weight or variable electric motor or hydraulic system. The probe element will be in contact with sample. There are many types of probes like shearing jaws, piercing rod, spindle and cutting blade, which can be used for various analytical purposes. Based on type of analysis different types of force are applied on system like pulling, puncturing, compressing and shearing. The deformation produced by sample is measured using sensing element and is reported out through read-out system. The read out system can be an oscilloscope, maximum-force dial or a recorder tracing force-distance relationship.

**Instrumentation:** A texture profile analyzer (figure 2.10) consists of a calibration platform on which reference weights can be positioned for simple calibration of the installed load cell. And, transducer fitted to the arm of the texture analyzer, which measures force in compression or tension and sends results to analyzer. The analyzer is connected to a computer for software control and analysis. It also consists of sample testing area, where the different probes or fixtures were attached for the sample location and testing.
Sample preparation: A wide variety samples ranging from soft viscous substances to hard brittle substances can be analyzed using texture profile analyzer. Based on type of study the probe is selected and the sample is carefully placed in sample testing area. In the present study, the sample gel is placed in a clean glass beaker with uniform upper surface and absence of air bubbles in the sample. The beaker is placed in sample testing area for testing.

Applications: Texture profile analyzer has wide range of application in food, pharmaceutical, cosmetics, packaging and medical device industries. Texture analyzers are commonly used in food industry to check the texture of food, ranging from extremely
soft gels (pectin, gums, and protein gels) to the extremely hard products (almonds, nuts, hard candy). In pharmaceutical industry it is used to analyze different properties of bandages, creams, pastes, lotions and ointments. In quality control and quality assurance it is used to ensure consistency between different batches and shifts to establish reproducibility. In medical industry it can be used to measure extrusion force and sharpness of syringes.

2.4 High performance liquid chromatography (HPLC)

HPLC is a chromatographic technique often used to separate mixtures of compounds in pharmaceuticals. It can be used to identify and quantify the individual components of mixture, and determine the purity of a sample. 

**Principle:** Chromatography is a separation technique. The components of a mixture are distributed between two phases, the stationary phase and the mobile phase. The mobile phase moves through or over the surface of the fixed (stationary) phase. The components of the mixture have different affinities for each phase, hence some are retained longer on the stationary phase than others causing separation (16).

High-performance liquid chromatography (HPLC) is a chromatographic technique in which, a small volume of liquid sample is injected into a column packed with tiny particles generally 3-5µm in diameter (stationary phase). The sample move down the column by means of pressurized flow of a liquid mobile phase, where components migrate at different rates due to different relative affinities. Components of sample that have a higher affinity for the mobile phase migrate more rapidly towards end of column,
whereas the components that have a higher affinity for the stationary phase are eluted from the column later (figure 2.11). These separated components are detected at the end of column and identified by an external measurement technique, such as a spectrophotometer that measures the intensity of the color, or by another device that can measure their amount. The detector quantify amount of sample and gives output as chromatogram (17).

Figure 2.11: Principle of chromatography (18).

There are several types of HPLC. In adsorption HPLC, the components of sample adsorb on to the surface of stationary phase or mobile phase causing separation and are most common type of HPLC. In Normal phase HPLC, the stationary phase is polar and mobile phase is non-polar (hexane or tetrahydrofuran). Whereas, the reverse phase HPLC the stationary phase is non-polar and mobile phase is polar (water, methanol). In ion-
exchange chromatography surface of the stationary phase charged opposite to that of sample ions. The separation of sample components is due to the ionic interactions between stationary surface and sample component. Size exclusion chromatography is used for separating the samples based on size. The stationary phase consists of precise pores, which allow smaller molecules to elute first followed by larger molecules. Selecting the correct stationary phase and mobile phase is necessary for the effective separation of compounds. To achieve this, a mixture of solvents can be used as mobile phase. The solvents pressurized in to chromatographic column with the help of high pressure pumps. If the composition of mobile phase remains constant throughout the procedure, is termed as isocratic elution. With isocratic elution the peak width of the later eluting component increases in chromatogram, which is a disadvantage. To overcome this gradient elution can be used, in which the composition of mobile phase changes. This decreases the time required for later eluting compound, thereby increasing efficiency of system.

The principle of HPLC is based on Van Demeter equation which relates the efficiency of the chromatographic column to the particle size of the column, molecular diffusion and thickness of the stationary phase:

$$H \text{ or } HEPT = A + \frac{B}{v} + C.v$$  \hspace{1cm} (Equation 2.8)

where $H$ or $HEPT$ is a measure of the resolving power of the column, $A$ is the Eddy-diffusion parameter which is related to channeling through a non-ideal packing, $B$ is the diffusion coefficient of the eluting particles in the longitudinal direction, $C$ is the
resistance to mass transfer coefficient of the analyte between mobile and stationary phase and $\nu$ is linear velocity.

**Instrumentation:** The HPLC equipment consists of a high pressure solvent delivery system to pump the mobile phase, a sample auto injector to inject the sample into the stream of mobile phase, a column (the stationary phase) that separates the sample components of interest. A detector (UV/Visible spectrophotometer or photo diode array (PDA) or diode array detector (DAD)) at the end of column connected to a computer to analyze the data. Some of the modern HPLC systems consist of an oven for temperature control of the column and a pre-column or guard column that protects the analytical column for impurities.

Figure 2.11:Schematic representation of HPLC instrumentation (19).
**Sample preparation:** The samples are either injected manually or automatically by using an auto sampler which injects samples from vials placed in the sampling area. Many types of vials are commercially available ranging from a dead volume as low as 10µL to as high as 10L in scale-up processes. The sample should be filtered through 0.45µm membrane filter to remove impurities. This helps to increase the lifetime of the column.

**Application:** HPLC has over the past decade become the method of choice for analysis of wide variety of compounds in pharmaceutical, food, environmental and chemical industry. In pharmaceutical qualitative analysis HPLC is used for identification and checking purity of compound. It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate product stability and monitor degradation. Its applications also include the monitoring the environmental samples and also in measuring hazardous compounds such as pesticides and insecticides. Its qualitative analysis is limited unless it is interfaced along with mass spectrometry.
References:


Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Poloxamer 407 NF

Source: PCCA (Houston, USA), Lot#C144872

Synonym: Pluronic F127; Polyethylene-polypropylene glycol;
Polyoxyethylene-polyoxypropylene Block Copolymer; Poly(Ethylene oxide-co-Polypropylene oxide), Block; Block Copolymer of Ethylene Oxide and Propylen Oxide

Chemical name: α-Hydro-w-hydroxy-poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) block copolymer

Molecular formula: HO (C₂H₄O)ₐ (C₃H₆O)ₐ (C₂H₄O)ₐ H ; where a = 101 units and b = 56

Molecular weight: 9840-14600 gram/mole

Physical state: Solid (Granular solid)

Solubility: Very soluble in water and in alcohol(1)
Poloxamer 407 is a triblock copolymer that exhibits thermoreversible behavior in aqueous solutions. Poloxamer 407 consists of ~70% polyoxyethylene units and ~30% polyoxypropylene blocks. This polymer has low toxicity, excellent compatibility with other chemicals, and a high solubilizing capacity for different drugs (2). It is widely used in pharmaceutical systems as solubilizing, suspending and emulsifying agents, adhesives; packaging and coating materials and increasingly, components of controlled and site specific drug delivery systems.

3.1.2 Propylene glycol

Source: MP biochemicals (Solon, USA), Lot#9742k

Chemical Name: Propylene glycol

Synonym: 1, 2-propanediol, 1, 2-dihydroxypropane

Chemical Formula: C₃H₈O₂

Molecular weight: 76.1 gram/mole

Solubility: soluble in water, acetone, chloroform and ethanol

Physical state: oily liquid
Figure 3.2: Chemical structure of propylene glycol

Propylene glycol is mainly used as a solvent for a wide range of active ingredients in oral, injectable and topical drug formulations. It is mainly used to solubilize drugs which are unstable or insoluble in water. In topical formulations it is used as emollient for softening and smoothing, and a humectant for promoting moisture retention in skin care medications.

3.1.3 Transcutol®-P

Source: TCI Co. Ltd. (Portland, USA), Lot#NP6EFDF

Chemical Name: 2-(2-Ethoxyethoxy) ethanol

Synonym: Diethylene glycol monoethyl ether, Carbitol,

Chemical Formula: C₆H₁₄O₃

Molecular weight: 134.1 gram/mole

Solubility: soluble in water, ethanol, and chloroform

Physical state: liquid

Figure 3.3: Chemical structure of Transcutol-P

Transcutol®-P is a hygroscopic liquid mainly used as a solvent to dissolve poorly water soluble active pharmaceutical ingredients. Moreover, enhances the per-cutaneous
absorption through the skin and mucosal barriers (3). It is also used in the pharmaceutical dosage forms such as topical ointments, emulsions, aqueous gels (Hydrogels) and also as co-surfactant in preparation of self micro emulsifying drug delivery systems (SMEDDS) (4).

3.1.4 Polyethylene glycol (PEG) 400

Source: Fisher Scientific (Fair Lawn, USA), Lot # YW0048

Chemical name: α-Hydroxy-ω-hydroxy-poly(oxy-1, 2-ethanediyl)

Chemical formula: H(OCH₂CH₂)ₙ OH

Molecular weight: 380-420 (average) gram/mole

Solubility: Miscible with water, very soluble in alcohol, acetone and dichloromethane.

Physical state: colorless viscous liquid

Figure 3.4: Chemical structure of PEG 400.

Polyethylene glycols are used combination with numeric value, which indicates its mean molecular weight. Whereas, the number n in the structure refers to number of EO-units in the molecule (5). Polyethylene glycol in liquid state is used to solubilize poorly water
soluble drugs. It is soluble in both polar and non-polar liquids. They are widely used in pharmaceutical manufacturing as water soluble bases for topical preparations and suppositories, as solvents and vehicles. They have also been reported to have antibacterial properties (1).

3.1.5 Lauryl glycol™ 90

Source: Gattefosse Pharmaceuticals (Saint-Priest Cedex, France), Batch # 118148

Chemical Name: propylene glycol monolaurate

Synonym: 1, 2-Dihydroxydodecane,

Chemical Formula: C_{15}H_{30}O_{3}

Molecular weight: 258.3 gram/mole

Solubility: very soluble in alcohol, methyl alcohol and dichloromethane

Physical state: colorless oily liquid

![Figure 3.5: Chemical structure of lauryl glycol™ 90](image)

Lauryl glycol™ 90 is type II of propylene glycol ethers consists of 90% of mono-esters and 10% of di-esters (1). It is used as solubilizing agent in pharmaceutical formulations. It is also used as emulsifying agent in the preparation of self micro emulsifying systems.

3.1.6 Capryol™ 90

Source: Gattefosse Pharmaceuticals (Saint-Priest Cedex, France), Batch # 118148.

Chemical name: Propylene glycol monocaprylate

Chemical formula: C_{11}H_{22}O_{3}
Molecular weight: 202.29 gram/mole

Physical state: Colorless oily liquid

Solubility: Soluble in ethanol, chloroform, methylene chloride, and vegetable oils, insoluble in water.

![Chemical structure of Capryol™ 90](image)

Figure 3.6: Chemical structure of Capryol™ 90

Capryol™ 90 is type II of propylene glycol moncaprylate consists of 90% of monoesters and 10% of di-esters of caprylic acid(6). It is used as an emulsifier in the preparation of oil-in-water emulsions and self-emulsifying drug delivery systems.

### 3.1.7 Fluconazole

Source: PCCA (Houston, USA), Lot#86386

Chemical Name: 2-(2, 4-Difluorophenyl)-1, 3-bis (1H-1, 2, 4-triazol-1-yl) propan-2-ol

Chemical Formula: C_{13}H_{12}F_{2}N_{6}O

Molecular weight: 306.3 gram/mole

Solubility: poorly water soluble.

Physical state: solid or crystalline solid
Figure 3.7: Chemical structure of Fluconazole

Fluconazole is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections (7). It is available as tablets for oral administration, as a powder for oral suspension and as a sterile solution for intravenous use (8). Fluconazole inhibits the fungal cytochrome P450 enzyme 14α-demethylase. This inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols (9). FLZ has emerged as the primary treatment option for virtually all forms of susceptible Candida infections in both immunocompetent and immunocompromised hosts (10). It is thus widely used in the treatment of vaginal candidiasis, oropharyngeal and esopharyngeal candidiasis, candida urinary tract infections, peritonitis and systemic candida infections including candidemia, disseminated candidiasis and pneumonia (8).

Marketed products: Diflucan®, Trican®, Alfumet, Afungil, Dofil
3.2 Methods

3.2.1 Solubility Studies

The solubility of FLZ was determined by adding excess amount of FLZ to microcentrifuge tubes containing 4ml of selected vehicle. Sealed vials were shaken at room temperature (25°C) on a nutating mixer for 72 hours and centrifuged at 5000 rpm for 15min to remove undissolved drug. The supernatants were separated, filtered through a 0.45 µm membrane filter and diluted with methanol. The amount of dissolved drug in each vehicle was quantified using the Agilent 8453 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, USA) at 261nm and also using Reversed-Phase HPLC (Waters, Milford, MA, USA) equipped with a separation module (Model 2695) was carried out at 35±5°C using a Waters Symmetry® C18 analytical column, 3.5µm, 75mm×4.6mm inner diameter. The Waters 2998 Photo diode array (PDA) detector was set to monitor at 261nm. The mobile phase consists of methanol:water (35:65v/v) with a flow rate of 0.6ml/min. The samples were diluted in methanol to an appropriate concentration prior to analysis and the amount of fluconazole was quantified using a calibration curve.

3.2.2 Preparation of temperature-sensitive hydrogels

Temperature-sensitive hydrogels containing fluconazole and also blank solution without FLZ were prepared by using the cold method (11, 12). Distilled water was cooled to 4°C and poloxamer was added slowly under continuous agitation. The formulations were kept refrigerated 4°C until clear solutions were obtained. Fluconazole was dissolved in
selected vehicles based on the solubility studies. Dissolved fluconazole was added to the clear poloxamer solutions and mixed with glass rod until clear solutions were formed. Also, the blank formulations were prepared without FLZ in vehicle. The hydrogel formulations and blank formulations were stored in the refrigerator (4°C) until further investigation.

3.2.3 Measurement of sol-gel transition temperatures

3.2.3.1 Method A

A 10ml transparent vial containing 5gram of each FLZ hydrogel formulation with a magnetic bar placed in the bottom of vial. The vial was kept in low temperature (4-5°C) water bath. A digital thermo sensor was placed in the formulation. The formulation was heated at a rate of 1°C/min till 45°C with continuous stirring at 60rpm. The temperature on the thermosensor was recorded, when the magnetic bar stopped rotating. This temperature point was considered as the sol-gel transition point (13). If magnetic stirrer did not stop rotating even at 45°C, it was considered as no-gelation in formulation.

3.2.3.2 Method B

The sol-gel transition temperatures of FLZ hydrogel formulations were also measured using AR 2000 controlled stress/controlled rate rheometer (T.A Instruments, New Castle, Delaware, U.S.A.) using stainless steel parallel plate (diameter 40mm) geometry. It was operated in temperature ramp oscillatory mode with the frequency fixed at 1Hz. The samples were subjected to a strain of 15 Pa where the stress was directly proportional to strain (linear viscoelastic region). The samples were carefully applied to the lower plate of the rheometer and allowed to equilibrate for at least 5min prior to analysis. A linear
temperature sweep was performed over the temperature range of 15°C - 45°C. The samples were heated at a rate of 2°C min\(^{-1}\). The sol-gel transition temperatures were determined graphically by plotting temperature sweeps against G´ using Rheology Advantage® software provided by T.A Instruments. The mid-point between the values of solution and gel was considered as the sol-gel transition point. The experiment was performed at least in triplicates.

3.2.4 Rheological and viscoelastic studies

The rheological flow properties of the FLZ hydrogel formulations were performed at both 20±0.1°C and 37±0.1°C by using the rheometer described above. The samples were carefully applied to the lower plate of the rheometer and allowed to equilibrate for at least 5 min prior to analysis. The rheometer was set to operate in continuous shear analysis mode. The upward and downward curves for each formulation were measured over a range of shear rates (10 s\(^{-1}\) - 1000 s\(^{-1}\)), increased over a period of 120 s, held at upper limit for 10 s then decreased over a period of 120 s. The rheological flow curves were determined by plotting viscosity (Pa.s) against shear rate (s\(^{-1}\)) using the rheology advantage software provided by T.A instruments.

The viscoelastic properties of the formulations were performed at both 20±0.1°C and 37±0.1°C. The rheometer was set to operate in oscillatory analysis mode. Frequency sweep analysis was performed over the frequency range of 0.1 Hz – 10 Hz following application of a constant strain of 15 Pa which is within the linear viscoelastic range. The standard gap between plates was 0.1 mm for each sample. The storage or elastic modulus (G’), loss or viscosity modulus (G”), and the loss tangent (tan δ) were calculated using the
Rheology Advantage® software. In both continuous shear mode and in oscillatory mode, the experiment was performed in triplicates for each formulation.

3.2.5 Texture profile analysis (TPA)

A texture analyzer TA-XT plus (Texture Technologies, Scarsdale, NY, USA) was used to determine texture properties of the temperature-sensitive hydrogel formulations. Approximately, 40 gram of each of the FLZ hydrogel formulation at 37°C was filled in a 100ml beaker ensuring no air bubbles were formed and the formulations had a smooth upper surface. An analytical probe (25mm in diameter cylinder lap perplex) was inserted to each formulation at a rate of 1mm/s to a depth of 10mm and redrawn at same rate. From the resultant force-distance curve, gel parameters like hardness, compressibility and adhesiveness were derived using Macro Software (Texture technologies, Scarsdale, NY, USA). The experiments were performed in triplicates for each formulation.

3.2.6 Mucoadhesive studies

The mucoadhesive strength of the different formulations was evaluated by measuring the force required to detach the hydrated mucin disks from the formulations using the texture analyzer previously. The mucin disks were prepared by compressing 250mg of porcine gastricmucin (Fisher scientific, Fair Lawn, USA) using a carver ring press with a 12.5mm die at the defined pressure of 10 tones for 10 seconds. The mucin disks were attached to the base of the analytical probe (7mm aluminum probe) horizontally with the aid of double sided adhesion tape. The disks were hydrated by immersing it for 30 seconds in 5% mucin solution (prepared by dissolving 5gram mucin in 100ml water). Excess mucin was removed by gently blotting the disks. Formulations at 37°C were transferred to a
50ml standard beaker ensuring that no air bubbles were formed and the formulations had a smooth upper surface before inserting the analytical probe. The probe was lowered slowly until it reaches the surface. A downward force of 0.1N was applied for 30 seconds. The probe was then moved vertically upward at a speed of 1.00mm/s. From the resultant force-distance curves, the maximum detachment force was obtained. Work of adhesion was calculated as the area under the curve. The experiment was performed in triplicates for each formulation.

3.2.7 In-vitro drug permeability

The in-vitro permeation of the drug was performed on Franz Diffusion cells using 0.45µm artificial cellulose acetate membranes (Sartorius StedimNorth America inc., Bohemia, USA). The membranes, having effective diffusion area of 0.785 cm² (10mm orifice diameter), were hydrated with phosphate buffer, pH 7.4, for 30 min before placing them between the donor and the receptor compartments. The receptor compartment contained 15ml phosphate buffer at pH 7.4, under magnetic stirring. The temperature in the outer jacket of the Franz diffusion cells was maintained at 37±0.5°C. Accurately weighed 3gram of each formulation was placed in the donor compartment. Samples of 0.6ml were drawn from the receptor compartment hourly for 8 hours and the same amount was replaced by fresh phosphate buffer to maintain sink conditions. The samples were stored in refrigerator at 4°C until analyzed by HPLC. FLZ was quantitatively determined by using reversed-phase high performance liquid chromatography (HPLC). Reversed-Phase HPLC (Waters, Milford, MA, USA) equipped with a separation module (Model 2695) was carried out at 35±5°C using a Waters Symmetry® C18 analytical
column, 3.5µm, 75mm×4.6mm inner diameter. The Waters 2998 Photo diode array (PDA) detector was set to monitor at 261nm. The mobile phase consists of methanol:water (35:65v/v) with a flow rate of 0.6ml/min. The samples were diluted in methanol to an appropriate concentration prior to analysis and the amount of fluconazole was quantified using a calibration curve. The experiments were performed in triplicates for each formulation. The average cumulative amount of drug permeated per unit surface area of the membrane was plotted versus time. The linear slope of the plot is the flux J (µg/cm²/h). The permeability was calculated by using the formula:

$$K = \frac{J}{C_0}$$

(Equation 3.1)

where K is the permeability coefficient and C₀ is the total initial amount of drug.

### 3.2.9 In-vitro antifungal activity test

#### 3.2.9.1 Preparation of Mueller-Hinton agar supplemented with glucose and methylene blue (MHA-GMB) medium

The MHA was prepared according to the manufacturer’s instructions. 2% glucose and 0.5µg/ml of methylene blue was added. After autoclaving at 20psi for 15mins, the agar was cooled down to 40-45°C in a water bath and poured in flat bottomed Petri dishes to give uniform depths of about 4mm. The agar was allowed to cool to room temperature and, unless used on the same day, stored in refrigerator (2°C - 8°C).

#### 3.2.9.2 Preparation of drug impregnated disks

The hydrogel formulations were prepared as previously described containing 25µg FLZ/20µl formulation. Blank disks were impregnated with the formulations
and were placed in the freezer (-20°C) for about 1 min for complete absorption onto the disks. The sample disks were then dried under laminar airflow and used immediately. The sample drug impregnated disks are compared against the standard Neo-Sensi® tabs FLZ 25µg disks (Sparks, USA) to check antifungal activity of FLZ on *Candida albicans*.

3.2.9.3 Disk diffusion test

*Candida albicans* *ATCC90028* (MicroBiologics, St. Cloud, USA) was used according to protocol M44-A2 by Clinical and Laboratory Standards Institute (CLSI). *Candida albicans* present in the KwikStik® were subcultured (Figure 3.8) onto Sabouraud dextrose agar and incubated at 35°C±2°C for 24 hours.

![Figure 3.8: The streaking procedure during sub-culturing on Sabourand agar.](image)

Inoculum was prepared by selecting five distinct colonies of approximately 1mm in diameter from 24 hour old culture of *Candida albicans*. Colonies were suspended in 5ml sterile 0.145mol/L saline solutions and vortexed for 15 seconds. The turbidity of the suspensions was measured at 530 nm using a spectrophotometer (Thermo Scientific, Fair Lawn, USA). The turbidity of the
suspension was adjusted by adding sterile saline or Candida colonies to obtain the same transmittance of that produced by a 0.5 McFarland standard (Becton Dickenson, Franklin lakes, USA).

To inoculate onto the MHA-GMB test plates, a sterile cotton swab is dipped into adjusted suspension and streaked over the surface of the agar plates. The procedure was repeated for two additional times; each time the plate was rotated approximately 60˚ to ensure even distribution of the inoculum. The plates were left for 5mins to allow excess moisture to be absorbed before applying the drug impregnated disks. The standard and the sample disks were applied on the surface of the agar plates manually using a sterile forceps in such a way that each disk is at least 24mm away from other disks and from the edge of the Petri plate to avoid contamination. The disks were pressed down to ensure complete contact with the agar surface. The plates were covered with lids and inverted, then placed in an incubator at 35°C±2°C for 24 hours before examining the zones of inhibition.

3.10 Statistical analysis

Statistical analysis of the data was performed using one-way ANOVA followed by Tukey’s post hoc test. When two groups were compared, an unpaired t-test was used. A difference was considered statistically significant when p< 0.05.
References:


5. Henning T. Polyethylene glycols (PEGs) and the pharmaceutical industry2002 [cited 2013 1/12]. Available from: http://www.clariant.com/C125720D002B963C/picklist/C0EB1376B40AC1C9C125726500432C94/$file/Polyethylene_glycols_(PEGs_and_the_pharmaceutical_industry.pdf.


Chapter 4

Results and Discussion

4.1 Solubility analysis

FLZ is poorly water soluble drug about 5.5mg/ml(1). Since, the aim of our study was to develop hydrogels; the solvent selection was mainly based on drug solubility and also its miscibility with water without precipitating the drug. Based on these characteristics the solubility of fluconazole in different co-solvents was performed and reported using calibration curve (figure 4.1) in table 4.1. The fluconazole exhibited greatest solubility in Transcutol®-P (TCL), propylene glycol (PG) and in polyethyleneglycol400 (PEG 400). So these co-solvents were therefore chosen to be used in the hydrogel formulation. Moreover, they are well accepted excipients in pharmaceutical formulations listed under GRAS (generally recognized as safe by F.D.A)(2-4).
Figure 4.1: Calibration curve of Fluconazole in methanol using UV/VIS spectrophotometer (n=3) $r^2=0.999$

Figure 4.2: Calibration curve of fluconazole in methanol $r^2=0.999$ using HPLC
Table 4.1: Solubility of fluconazole in different co-solvents (n=3).

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Solubility using UV/VIS (mg/ml)±S.D</th>
<th>Solubility using HPLC (mg/ml)±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>6.6±0.3</td>
<td>8.8±0.2</td>
</tr>
<tr>
<td>Lauryl glycol</td>
<td>9.8±0.5</td>
<td>10.8±0.1</td>
</tr>
<tr>
<td>Capryol</td>
<td>20.4±1.2</td>
<td>23.3±0.8</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>73.5±0.7</td>
<td>69.5±0.8</td>
</tr>
<tr>
<td>Methanol</td>
<td>92.5±2.2</td>
<td>90±1.7</td>
</tr>
<tr>
<td>PEG 400</td>
<td>121.4±1.5</td>
<td>120.4±1.2</td>
</tr>
<tr>
<td>Transcutol</td>
<td>157±2.1</td>
<td>157.6±1.5</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>163.5±1.9</td>
<td>160.3±1.8</td>
</tr>
</tbody>
</table>

4.2 Preparation of temperature sensitive hydrogels

In all preliminary studies, method A was used to measure the sol-gel transition temperature. Based on the solubility of fluconazole, three different vehicles (PEG 400, TCL and PG) were selected as co-solvents. To find the formulation showing optimal sol-gel transition temperature different concentrations of poloxamer and co-solvents were investigated. In all the formulations drug concentration of 1% fluconazole was fixed. First to determine the optimal concentration of poloxamer, different concentrations of poloxamer 10%, 15%, 20%, 25%, 30% (w/w) in water were investigated with no co-solvents and FLZ (table 4.2).

Table 4.2: Sol-geltransition temperatures of the aqueous hydrogels with poloxamer alone.
The aqueous solutions with poloxamer concentration ranging between 15% to 20% (w/w) poloxamer showed Newtonian or liquid-like behavior at room temperature (25°C) and Non-Newtonian or gel-like behavior at physiological temperature (37°C). In contrast, the formulations with 25% and 30% (w/w) poloxamer concentration showed non-Newtonian or gel-like behavior at both the temperatures. On further investigation the concentration 16% and 17% of poloxamer was fixed which showed optical sol-gel transition temperature (~35°C). The co-solvents along with 1% fluconazole of different concentrations 10%, 20% and 30% were added to the poloxamer solutions to see the effect of co-solvents on the sol-gel transition temperature (Table 4.3).

Table 4.3: Effect of co-solvent on sol-gel transition temperature.

<table>
<thead>
<tr>
<th>Poloxamer407NF (%w/w)</th>
<th>Co-solvent</th>
<th>Concentration (%w/w)</th>
<th>Sol-gel transition temperature (°C)±S.D (Method-A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Transcutol</td>
<td>10%</td>
<td>32 ± 0.2</td>
</tr>
<tr>
<td>16</td>
<td>Transcutol</td>
<td>20%</td>
<td>43 ±1.5</td>
</tr>
<tr>
<td>16</td>
<td>Transcutol</td>
<td>30%</td>
<td>No gelation</td>
</tr>
<tr>
<td>17</td>
<td>Transcutol</td>
<td>10%</td>
<td>30 ± 0.5</td>
</tr>
<tr>
<td>17</td>
<td>Transcutol</td>
<td>20%</td>
<td>34 ± 0.6</td>
</tr>
<tr>
<td>17</td>
<td>Transcutol</td>
<td>30%</td>
<td>No gelation</td>
</tr>
<tr>
<td>16</td>
<td>Propylene Glycol</td>
<td>10%</td>
<td>31 ± 0.5</td>
</tr>
<tr>
<td>16</td>
<td>Propylene Glycol</td>
<td>20%</td>
<td>30 ± 0.5</td>
</tr>
<tr>
<td>16</td>
<td>Propylene Glycol</td>
<td>30%</td>
<td>25 ±1.2</td>
</tr>
<tr>
<td>17</td>
<td>Propylene Glycol</td>
<td>10%</td>
<td>28 ±0.1</td>
</tr>
<tr>
<td>17</td>
<td>Propylene Glycol</td>
<td>20%</td>
<td>25 ±0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>-----</td>
<td>---------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>17</td>
<td>Propylene Glycol</td>
<td>30%</td>
<td>22 ± 0.1</td>
</tr>
<tr>
<td>16</td>
<td>PEG400</td>
<td>10%</td>
<td>No gelation</td>
</tr>
<tr>
<td>16</td>
<td>PEG400</td>
<td>20%</td>
<td>No gelation</td>
</tr>
<tr>
<td>16</td>
<td>PEG400</td>
<td>30%</td>
<td>No gelation</td>
</tr>
<tr>
<td>17</td>
<td>PEG400</td>
<td>10%</td>
<td>34 ±0.5</td>
</tr>
<tr>
<td>17</td>
<td>PEG400</td>
<td>20%</td>
<td>No gelation</td>
</tr>
<tr>
<td>17</td>
<td>PEG400</td>
<td>30%</td>
<td>No gelation</td>
</tr>
</tbody>
</table>

The formulation with vehicle PEG400 showed negative effect by not forming gels, so the PEG 400 was discarded for further investigation. The formulations with vehicles TCL and PG of concentration 30% did not produce gels or produced weak gels with low viscosity. Finally, the formulations with 16% and 17% (w/w) poloxamer along with either co-solvents TCL or PG of concentrations 10% or 20% showed optimal sol-gel transition temperature. The final formulations were free flowing liquids at 20°C storage temperature and formed gels or semi-solids at a temperature of 37°C reported. Final formulations were reported in table 4.4.

### 4.3 Measurement of sol-gel transition temperature

Sol-gel transition temperature is defined as the temperature at which the liquid or sol phase makes transition to the gel phase. Generally, sol-gel transition for formulations should be in the range of 25-35°C for effective drug delivery(5, 6). Transition temperatures above 37°C result in a liquid formulation at physiological temperature, which in turn can results in rapid clearance of drug. On the other hand, transition temperatures below 25°C, a gel may be formed at room temperature creating application difficulties. Aqueous solutions of poloxamer exhibit thermoreversible properties, by carefully monitoring the concentration of poloxamer the sol-gel transition can occur at
any temperature. At the sol-gel transition temperature the rheological properties of the system change from Newtonian to Non-Newtonian behavior (Figure 4.3). In the present study, the transition temperatures of eight formulations were evaluated using rheometer and the results given in table 4.4.

![Room temperature](image1)
![Physiological temperature](image2)

Figure 4.3: FLZ hydrogel behavior at room temperature (~25°C) and physiological temperature (~37°C)

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Poloxamer407 NF (%w/w)</th>
<th>Transcutol(%w/w)</th>
<th>Propylene Glycol</th>
<th>Sol-gel transition</th>
</tr>
</thead>
</table>

Table 4.4: The composition and transition temperatures of fluconazole hydrogel formulations (n=3).
The formulations containing high poloxamer concentration (17%) exhibited lower
transition temperatures than formulations with lower poloxamer concentration (16%).

This result is in agreement with current literature that describes a decrease in the
transition temperature with the increase of poloxamer concentration (7, 8). The type of co-
solvent in the formulation also influenced the transition temperature. Formulations
containing the co-solvent TCL (F1 to F4) exhibited significantly higher transition
temperatures than formulations containing PG (F5 to F8) (Table 2). The addition of co-
solvents in the formulation can interfere with the poloxamermicellization and alter the
dehydration of hydrophobic PPO blocks. The presence of different co-solvents can
therefore modify the transition temperature either by disturbing the micellar packing
(increasing the transition temperature as observed with TCL) or by favoring micelle
formation (decreasing the transition temperature as observed with PG) (8, 9). This may
explain the difference in the transition temperatures observed between the TCL- and PG-
containing hydrogels. Based on the transition temperatures, formulations F1 and F3 to F7
exhibited transition temperatures within the range appropriate for drug delivery.
Figure 4.4: Change in viscosity with respect to temperature (°C) of hydrogels formulations containing co-solvent (A) TCI and (B) PG.

4.4 Rheological and viscoelastic studies
Rheological studies can be used to predict the *in situ* behavior of semi-solid dosage forms in the body. The flow characteristics of the formulations affect the spreadability and the residence time of the formulation at the application site. The measure of changes in shear stress with shear rates has been used to determine whether the rheological behavior of the samples is Newtonian or Non-Newtonian (10, 11). Poloxamers exhibit pseudoplastic flow when in gel form and upon increasing the shear rate the gel viscosity decreases. As shown in figure 4.5, the formulations at $37^\circ$C exhibited a decrease in viscosity with an increase in shear ($10 \text{ s}^{-1} - 1000\text{s}^{-1}$), whereas at $20^\circ$C no significant change in viscosity was observed (except from formulation F8). This confirms the formulations change in behavior from liquid-like (Newtonian) to gel-like (non-Newtonian) when the temperature increases. Formulation F8 showed shear thinning characteristics at both high ($37^\circ$C) and low ($20^\circ$C) temperatures. This may be due to the high concentration of PG and poloxamer in formulation F8 which decreased the sol-gel transition temperature (table 4.4).
Figure 4.5: Flow curves of FLZ formulations at (A) 20°C and (B) 37°C.

In oscillatory rheometry the effect of sinusoidal shear stress on the viscoelastic properties are measured and two dynamic moduli, i.e. the elastic or storage modulus (G’) and the viscosity or loss modulus (G’”), are obtained as a function of oscillatory frequency. In this
study, the gel structure was examined over a frequency range of 0.1-10Hz, which is a common tested region in rheological studies (12-14). The elastic modulus G’ is a measure of the energy stored and recovered per cycle of deformation, and reflects the solid-like component of elastic behavior (15). A higher G’ than G” value means that under a shearing force, the material is able to store energy and not deform or flow. In contrast, a higher G” value than G’ means that under a shearing force, the material is not able to store energy and deforms to flow as liquid(16). It is preferred that the formulation is free flowing at room temperature (25°C) allowing for an easy application. Thus, at room temperature (25°C) the viscosity modulus G” should be higher than the storage modulus G’ (G”>G’). After administration, the formulation is expected to form gel due to rise in temperature, where G’ should be higher than the G” (G’>G”). Figure 4.6(A) (B) and figure 4.7(A) (B) shows the frequency dependence at 20°C and 37°C of the elastic modulus (G’) and viscosity modulus (G”) of all formulations. All the formulations showed gel-like characteristics (G’>G”) at 37°C and sol-like character at 20°C (G”>G’). However, the formulations with co-solvent PG (F5-F8) showed higher G’ values than the corresponding formulations with TCL (F1-F4).This indicates that the formulations with PG are able to form gels with strong cross-linkings and with greater elasticity than formulations with TCL. The greater elasticity of these formulations can be expected to enhance their retention at the application site (10, 17).
Figure 4.6: Frequency dependent changes of viscoelastic properties of the FLZ formulations containing co-solvent TCL; (A) at 37°C and (B) at 20°C
Figure 4.7: Frequency dependent changes of viscoelastic properties of the FLZ formulations containing co-solvent PG; (A) at 37°C and (B) at 20°C
The value of loss tangent ($\tan \delta = G''/G'$), used as a measure of the relative contribution of viscoelastic properties of the materials. The phase angle ($\delta$) was found higher at 20°C than at 37°C for all formulations (Fig. 4.8). This lead to the values of loss tangent greater than 1 for all formulations at 20°C indicating the dominance of the viscous component ($G''$), and was less than 1 at 37°C indicating the prevalence of the elastic component ($G'$).
Figure 4.8: The frequency dependent changes of delta values of FLZ formulations containing co-solvents (A) TCL (B) PG

4.5 Texture profile analysis

A dosage form for topical delivery should possess properties such as good skin spreadability, easy removal of product or dosage form from the package, acceptable viscosity, good bioadhesion and predictable release of active ingredient(18, 19). TPA is used to gather information about the physical gel structure of semi-solid dosage forms, which is useful in predicting the product behavior in different physiological and environmental conditions. In this study, information about the mechanical properties of the gels such as compressibility, hardness and adhesiveness were obtained by the force-time plots.
The applicability of the gel at the desired site is expressed by hardness, whereas compressibility gives information about the removal of product from the package and its spreadability at the targeted site. The values of hardness and compressibility should be low to easily remove the product from the package and administer with good spreadability. These attributes may contribute to increased patient compliance. All the formulations exhibited low hardness and compressibility at room temperature at which administration is performed. The formulations were tested at physiological temperature (37°C) and results shown in table 4.5. At 37°C, both the hardness and compressibility values increased as a function of increased poloxamer concentration in the formulation (F3, F4, F7 and F8; Table 4.5). The difference between formulations containing high and low poloxamer concentrations in hardness and compressibility was found significant ($p > 0.05$). This relationship has also been reported for other types of polymers (19) and is also consistent with the present viscoelastic measurements of the formulations. Above the transition temperature, increased poloxamer concentration in the formulation increases micelle packing and entanglements leading to stronger polymer network.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Hardness (N) ±S.D</th>
<th>Compressibility(N. mm)±S.D</th>
<th>Adhesiveness(N.mm)±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.42 ± 0.24</td>
<td>9.7 ± 1.3</td>
<td>10.1 ± 0.9</td>
</tr>
<tr>
<td>F2</td>
<td>1.45 ± 0.14</td>
<td>9.4 ± 0.4</td>
<td>11.1 ± 0.9</td>
</tr>
<tr>
<td>F3</td>
<td>1.66 ± 0.14</td>
<td>11.1 ± 0.6</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>F4</td>
<td>1.83 ± 0.12</td>
<td>12.1 ± 0.3</td>
<td>14.3 ± 0.2</td>
</tr>
<tr>
<td>F5</td>
<td>1.52 ± 0.01</td>
<td>10.5 ± 0.5</td>
<td>10.1 ± 0.5</td>
</tr>
<tr>
<td>F6</td>
<td>1.46 ± 0.19</td>
<td>9.9 ± 0.7</td>
<td>10.7 ± 0.7</td>
</tr>
<tr>
<td>F7</td>
<td>1.69 ± 0.16</td>
<td>12.2 ± 0.5</td>
<td>13.1 ± 0.9</td>
</tr>
<tr>
<td>F8</td>
<td>1.71 ± 0.16</td>
<td>11.8 ± 0.7</td>
<td>11.6 ± 0.5</td>
</tr>
</tbody>
</table>
Adhesiveness has been defined as the work required to overcome the attractive forces between the surface of the sample and the surface of the probe in which its cohesive bonds are broken (20). High adhesiveness values therefore indicate greater adhesion at the tissue surface with subsequent increase in retention time (21). At 37°C, formulations F4 and F7 with high content of poloxamer demonstrated the significant higher adhesiveness values among the formulations tested with enhanced adhesion to a tissue surface.

4.6 Mucoadhesive studies

Mucoadhesive force is defined as the force with which the formulation binds to mucosa or mucous membranes (22); the higher the value the stronger the formulation binds to the mucosal surface. Mucoadhesive properties are important when prolonged residence time is desirable at mucosal sites to improve absorption and/or reduce rapid drainage from the application site. In this study compressed mucin disks in combination with tensile analysis were used to investigate the in vitro mucoadhesive properties of the hydrogels at 37°C. Maximum detachment force and work of adhesion are common indicators to assess the mucoadhesive strength of semi-solid dosage forms.

The results are reported in Table 4.6. No significant difference ($p<0.05$) in the maximum detachment force was observed between the tested formulations. However, the difference in the work of adhesion values of formulations F2, F4, and F7 were statistically significantly ($p>0.05$). F2 expressed the lowest work of adhesion, whereas F4 and F7 expressed the highest values. In surface science, work of adhesion is described as the work required to separate the liquid from the solid. It has been proposed that work of
adhesion provides a more reliable and reproducible indicator of the mucoadhesion phenomenon because it represents the total sum of established adhesive joints (23, 24). Mucoadhesive character generally increases with gel strength (25, 26). Due to high polymer concentrations, the swelling and formation of strong and viscous gels of F4 and F7 at 37°C strengthened the interactions between the formulations and the mucin disks. This observation was consistent with the adhesiveness results. Having transition temperature ~40°C, the low mucoadhesion values of F2 may be due to its proximity to liquid nature in vitro at body temperature.

Table 4.6: Mucoadhesive properties of fluconazole hydrogel formulations at 37°C (n=3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Maximum detachment force (N) ± S.D</th>
<th>Mucoadhesion (mJ) ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.22 ± 0.030</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>F2</td>
<td>0.20 ± 0.005</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>F3</td>
<td>0.22 ± 0.001</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>F4</td>
<td>0.23 ± 0.012</td>
<td>0.73 ± 0.05</td>
</tr>
<tr>
<td>F5</td>
<td>0.21 ± 0.017</td>
<td>0.48 ± 0.04</td>
</tr>
<tr>
<td>F6</td>
<td>0.19 ± 0.004</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td>F7</td>
<td>0.22 ± 0.004</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td>F8</td>
<td>0.20 ± 0.006</td>
<td>0.50 ± 0.03</td>
</tr>
</tbody>
</table>

4.7 In vitro permeation studies

The absorption process of a drug is affected by the nature of the vehicle by influencing the partitioning behavior of the drug between the vehicle and the tissue surface (27). The in vitro permeation studies were performed on cellulose acetate membranes using Franz diffusion apparatus, to investigate how the different gel formulations influence the permeation of the drug from the vehicle. The amount of drug permeated through the membrane was calculated using the calibration curve plotted using HPLC figure 4.2.
The permeation of drug through membrane for all formulations reported in figure 4.11. Viscosity is inversely related to the release of active substances from formulation and its penetration through diffusion barriers (14). Hence, drugs permeate more slowly through viscous vehicles, allowing for prolonged drug release. It was thus expected that formulations containing high poloxamer concentrations (F3, F4, F7 and F8) express low permeability coefficients (K). However, no significant difference in the permeability values was found among the formulations (figure 4.11). The flux (J) and permeation (K) of all the formulations were calculated from graphs reported in table 4.7.

Table 4.7: Results from the in vitro permeation studies of the fluconazole hydrogel formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Flux J (µg/cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>18667±1609</td>
</tr>
<tr>
<td>F2</td>
<td>20517±1565</td>
</tr>
<tr>
<td>F3</td>
<td>16544±1107</td>
</tr>
<tr>
<td>F4</td>
<td>16512±2253</td>
</tr>
<tr>
<td>F5</td>
<td>18116±1231</td>
</tr>
<tr>
<td>F6</td>
<td>17770±1137</td>
</tr>
<tr>
<td>F7</td>
<td>15900±1753</td>
</tr>
<tr>
<td>F8</td>
<td>14637±1553</td>
</tr>
</tbody>
</table>
4.8 *In vitro* antifungal activity

The antifungal activity of the hydrogel formulations was assessed using the disk diffusion method. The results are presented in table 4.8. The test for zone of inhibition measures the ability of an antimicrobial product to prevent growth of microorganisms in a rapid manner. The zone of inhibition is defined as the clear area around the paper disk containing an antimicrobial agent on an agar surface (28). The clear region is an indication of the absence, or the effective inhibition, of microbial growth by the antimicrobial product. The size of the region is related to the intensity of antimicrobial activity. The zone of inhibition measured for the standard disks showed that the selected *Candida* species ATCC 90028 is susceptible to the dose of FLZ administered (Table 4.8). No significance difference (*p*<0.05) in the zone of inhibition was observed between
the sample disks and the standard disks (Figure 4.12). This indicates that all FLZ containing hydrogel formulations expressed antifungal effect comparable to FLZ standard and that the gel vehicle did not influence the antifungal activity of fluconazole.

Table 4.8: Antifungal activity of 25µg fluconazole standard and different fluconazole hydrogel formulations containing 25µg FLZ/20µl formulation (n=3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zone of Inhibition (mm) ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>33.0±0.4</td>
</tr>
<tr>
<td>F2</td>
<td>33.4±0.5</td>
</tr>
<tr>
<td>F3</td>
<td>33.3±1.3</td>
</tr>
<tr>
<td>F4</td>
<td>33.9±0.8</td>
</tr>
<tr>
<td>F5</td>
<td>32.7±0.5</td>
</tr>
<tr>
<td>F6</td>
<td>32.4±0.6</td>
</tr>
<tr>
<td>F7</td>
<td>33.6±0.1</td>
</tr>
<tr>
<td>F8</td>
<td>33.6±0.4</td>
</tr>
<tr>
<td>Standard FLZ 25µg</td>
<td>33.6±0.3</td>
</tr>
</tbody>
</table>

Figure 4.10: A representative plate showing comparable zones of inhibition between the sample disks and the standard disk. Standard disk contains 25µg fluconazole and sample disks contain 25µg FLZ/20µl formulation, i.e. 17% poloxamer and 10% and 20% TCL, respectively (F3 and F4).
Conclusions:
In this study, different poloxamer concentrations 10 to 30 % (w/w) were evaluated to produce a hydrogel. The concentrations between 15 to 20% (w/w) poloxamer showed Newtonian or liquid-like behavior room temperature (25°C) and non-Newtonian or gel-like behavior at physiological temperature (37°C). 1% Fluconazole the active ingredient of the formulation is dissolved in co-solvents Transcutol-P, propylene glycol and PEG400 and poloxamer as polymer. PEG 400 did not show desired effect so discarded from study. Eight different formulations (F1-F8) were selected for further investigation. All formulations except F8 exhibited Newtonian (liquid-like) behavior at 20°C and non-Newtonian (gel-like) behavior at 37°C. F8, containing high poloxamer and PG concentrations, exhibited shear thinning behavior at both 20°C and 37°C. Formulations containing high poloxamer concentrations (F3, F4, F7 and F8) formed stronger and more viscous gels with subsequent higher values in hardness and compressibility at physiological temperature. Moreover, formulations F4 and F7 rendered gels with highest adhesiveness and the most mucoadhesive character. These properties are advantageous to prolong residence time and reduce leakage of the dosage form, when applied on mucosal layers. In-vitro performance of the hydrogels, No significant differences in the permeability and antifungal activity of fluconazole were observed between the formulations. The type and amount of co-solvent included in the formulation influenced the transition point of the thermosensitive systems; TCL increased the transition temperature, while the opposite is true for PG. Based on the transition temperature, and
the rheological, mechanical and mucoadhesive properties, formulations F4 and F7, containing 17% poloxamer and 20% TCL and 10% PG, respectively, seemed to be the most promising thermosensitive systems for efficient delivery of fluconazole.
References:


