Chapter 2

LITERATURE REVIEW
2.1. Oral Mucosa and Oropharyngeal Candidiasis

2.1.1. Overview of the Oral Mucosal Structure

The oral mucosa is composed of an outermost layer of stratified squamous epithelium (Figure 2.1). Below this lies a basement membrane, a lamina propria followed by the submucosa as the innermost layer. The epithelium is similar to stratified squamous epithelia found in the rest of the body in that it has a mitotically active basal cell layer, advancing through a number of differentiating intermediate layers to the superficial layers, where cells are shed from the surface of the epithelium. The epithelium of the buccal mucosa is about 40-50 cell layers thick, while that of the sublingual epithelium contains somewhat fewer. The epithelial cells increase in size and become flatter as they travel from the basal layers to the superficial layers.

Figure 2.1 Structure of the oral mucosa.
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The turnover time for the buccal epithelium has been estimated at 5-6 days\(^8\), and this is probably representative of the oral mucosa as a whole. The oral mucosal thickness varies depending on the site; the buccal mucosa measures at 500-800 µm, while the mucosal thickness of the hard and soft palates, the floor of the mouth, the ventral tongue, and the gingiva measure at about 100-200 µm. The composition of the epithelium also varies depending on the site in the oral cavity. The mucosa of areas subject to mechanical stress (the gingiva and hard palate) are keratinized similar to the epidermis. The mucosa of the soft palate, the sublingual, and the buccal regions, however, are not keratinized\(^7\). The keratinized epithelia contain neutral lipids like ceramides and acylceramides which have been associated with the barrier function. These epithelia are relatively impermeable to water. In contrast, non keratinized epithelia, such as the floor of the mouth and the buccal epithelia, do not contain acylceramides and only have small amounts of ceramide\(^9\). They also contain small amounts of neutral but polar lipids, mainly cholesterol sulfate and glucosyl ceramides. These epithelia have been found to be considerably more permeable to water than keratinized epithelia\(^10\).

2.1.2. Oropharyngeal Candidiasis

By definition oropharyngeal candidiasis is an opportunistic mucosal infection caused in a majority of incidences by *Candida albicans*. Ellepola et al\(^11\) state that there are four main types of candida infection namely pseudomembranous, erythematous, hyperblastic and denture induced stomatitis. The most common in infants are pseudomembranous (with infants presenting with white discrete plaques on an erythemous background, on the buccal mucosa, throat or tongue) and the hyperblastic (with infants presenting with white firmly adherent patches usually bilateral on the buccal mucosa). Symptoms range from none to a sore and painful mouth, leading to impaired taste and nutritional intake. Webb et al\(^12\) found that between 31 and 60% of healthy people have candida present in their mouths. In neonates, whose immunity is not compromised, spontaneous cure of oropharyngeal candidiasis usually occurs after 3–8 weeks.

Systemic factors associated with the development of upper gastrointestinal candidiasis include the use of antibiotics, diabetes, hematologic malignant neoplasms, medication induced immunosuppression, and human immunodeficiency virus (HIV) infection. Local structural or functional factors may also be associated with disease development; disorders of esophageal motility in particular are associated with
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Candida overgrowth. In the newborn, oral candidiasis may occur as a consequence of colonization or infection from the mother’s vaginal flora or other exogenous sources. In immunosuppressed patients with mucositis, oral and esophageal candidiasis must be considered potential sources of disseminated candidiasis. Most cases of oral and esophageal candidiasis are caused by *C. albicans*, but *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *S. cerevisiae* and *H. anomala* are also reported to cause this disease.\(^\text{13,14,15,16}\)

Oral candidiasis may present with different clinical manifestations. The most common is typical oral thrush, or the pseudomembranous form of oral candidiasis. In this form, white pseudomembranous plaques are seen on the surfaces of the oropharynx. Less commonly seen are erythematous candidiasis (red mucosal patches in the absence of pseudomembranes), hyperplastic candidiasis or Candida leukoplakia (rough plaques that cannot be removed by scraping) and angular cheilitis (in which the corners of the lips are involved with erythema and cracking). The diagnosis of oral candidiasis is usually made clinically by noting one of these typical appearances on physical examination. Confirmation can be obtained by direct microscopic examination. Culture is not generally helpful given the high colonization rate of the oropharynx with *Candida* species.

Esophageal candidiasis also has a range of clinical manifestations from superficial infection to deep invasive disease. This diversity is most commonly due to varying host defects, with superficial disease seen in patients with HIV infection and deep infection in neutropenic patients. It may present with or without concomitant oral infection. Symptoms are indistinguishable from those of other infectious and noninfectious causes of esophagitis and include dysphagia, odynophagia, and retrosternal chest pain. Patients may also be asymptomatic even with extensive disease. Complications of esophageal candidiasis include perforation, bleeding, a risk for dissemination and in chronic Candida esophagitis, esophageal stenosis. The diagnosis of esophageal candidiasis is frequently made on clinical grounds in high risk populations. For example, a patient with HIV infection, oral thrush and esophageal symptoms may be treated empirically for Candida esophagitis. In neutropenic patients with concomitant thrombocytopenia, the potential bleeding complications of endoscopy may preclude the use of this diagnostic procedure, and empirical treatment may again be used. In instances of esophageal symptoms when
the diagnosis is less certain or in patients who do not respond to empirical treatment, a definitive diagnosis can be made by endoscopy and biopsy.

The photographs of the Oropharyngeal candidiasis are given in the figure 2.2.

PHOTOGRAPHS OF OROPHARYNGEAL CANDIDIASIS

(A)

(B) (C)

(D) (E)
2.2. Mucoadhesion

2.2.1. Theories of Mucoadhesion

Several theories have been proposed to explain the fundamental mechanisms of adhesion\textsuperscript{7,17,18}. In a particular system, one or more theories can equally well explain or contribute to the formation of Mucoadhesive bonds.

2.2.1.1. Electronic theory

According to the electronic theory, electron transfer occurs upon contact of an adhesive polymer with biochemical contents of the mucous membrane surface because of differences in their electronic structures. This results in the formation of an electrical double layer at the interface. Adhesion occurs due to attractive forces across the double layer.
2.2.1.2. Adsorption theory
According to the adsorption theory, after an initial contact between two surfaces, the material adheres because of surface forces acting between the atoms in the two surfaces. Two types of chemical bonds resulting from these forces can be distinguished:

1. Primary chemical bonds of covalent nature, which are undesirable in bioadhesion because their high strength may result in permanent bonds.
2. Secondary chemical bonds having many different forces of attraction, including electrostatic forces, van der waals forces and hydrogen and hydrophobic bonds.

2.2.1.3. Wetting theory
Wetting theory is predominantly applicable to liquid bioadhesive systems. It analyses adhesive and contact behavior in terms of the ability of a liquid or paste to spread over a biological system.

The work of adhesion (expressed in terms of surface and interfacial tension, $Y$) is defined as the energy per square centimeter released when an interface is formed. The work of adhesion is given by:

$$W_a = Y_A + Y_B - Y_{AB}$$  \hspace{1cm} (Equation 2.1)

Where $A$ and $B$ refer to the biological membrane and the bioadhesive formulation respectively. The work of coadhesion is given by:

$$W_c = 2Y_A \hspace{1cm} \text{or} \hspace{1cm} W_c = 2Y_B$$  \hspace{1cm} (Equation 2.2)

For a Bioadhesive material $B$ spreading on a biological substrate $A$, the spreading coefficient is given by:

$$S_{B/A} = Y_A - (Y_B + Y_{AB})$$  \hspace{1cm} (Equation 2.3)

$S_{B/A}$ should be positive for a Bioadhesive material to adhere to a biological membrane.

2.2.1.4. Diffusion theory
According to diffusion theory, the polymer chains and the mucus mix to a sufficient depth to create a semipermanent adhesive bond. The exact depth to which the polymer chains penetrate the mucus depends on the diffusion coefficient and the time of contact. This diffusion coefficient, in turn, depends on the value of molecular weight between cross links and decreases significantly as the cross linking density increases.
2.2.1.5. Fracture theory
Fracture theory attempts to relate the difficulty of separation of two surfaces after adhesion. Fracture theory equivalent to adhesive strength is given by:

\[ G = (E \varepsilon / L)^{1/2} \]  
(Equation 2.4)

Where \( E \) is the Young’s modulus of elasticity, \( \varepsilon \) is the fracture energy and \( L \) is the critical crack length when two surfaces are separated.

2.2.2. Factors important to bioadhesion
The bioadhesive power of a polymer or of a series of polymers is affected by the nature of the polymer and also by the nature of the surrounding media\(^7\)\(^{,}\)\(^8\).

2.2.2.1. Polymer related factors
2.2.2.1.1. Molecular weight
Numerous studies have indicated that there is a certain molecular weight at which bioadhesion is at a maximum. The interpenetration of polymer molecules is favorable for low molecular weight polymers, whereas entanglements are favored for high molecular weight polymers. The optimum molecular weight for the maximum bioadhesion depends on the type of polymer. Their nature dictates the degree of swelling in water, which in turn determines interpenetration of polymer molecules within the biological tissues especially mucus. It seems that the bioadhesive force increases with the molecular weight of the bioadhesive polymer upto 100,000 and that beyond this level there is not much effect. To allow chain interpenetration, the polymer molecule must have an adequate length. Size and configuration of the polymer molecule are also important factors. For example, with polyethylene oxide adhesive strength increases even upto molecular weight of 4,000,000. These polymers are well known to contain molecules of highly linear configuration, which contribute to interpenetration with dextran. Molecules with molecular weights as high as 19,500,000 do not exhibit better bioadhesion than molecules with a molecular weight of 200,000\(^8\).

2.2.2.1.2. Concentration of active polymer
There is an optimum concentration of polymer corresponding to the best bioadhesion. In highly concentrated systems, the adhesive strength drops significantly. In fact, in concentrated solutions, the coiled molecules become solvent poor and the chains available for interpenetration are not numerous. This result seems to be of interest
only for more or less liquid bioadhesive form. For solid dosage forms such as tablets, showed that the higher the polymer concentration, the stronger the bioadhesion.

### 2.2.2.1.3. Flexibility of polymer chains
Flexibility is important for interpenetration and entanglement. As water soluble polymers become cross linked, the mobility of the individual polymer chain decreases. As the cross linking density increases, the effective length of the chain which can penetrate in to the biological tissue especially mucus layer decreases even further and bioadhesive strength is reduced.

### 2.2.2.1.4. Spatial Conformation
Besides molecular weight or chain length, spatial conformation of a molecule is also important. Despite a high molecular weight of 19,00,000 for dextrans, they have adhesive strength similar to that of polyethylene glycol, with a molecular weight of 200,000. The helical conformation of dextran may shield many adhesively active groups, primarily responsible for adhesion, unlike PEG polymers, which have a linear conformation.

### 2.2.2.2. Environment related factors
#### 2.2.2.2.1. pH
pH was found to have a significant effect on bioadhesion especially mucoadhesion, as observed in studies of polyacrylic polymers cross linked with -COOH groups. pH influences the charge on the surface of both mucus and the polymers. Mucus will have a different charge density depending on pH because of differences in dissociation of functional groups on the carbohydrate moiety and amino acids of the polypeptide backbone. Robinson and his group\(^\text{19}\) observed that the pH of the medium was critical for the degree of hydration of highly cross linked polyacrylic acid polymers, increasing between pH 4 and pH 5, continuing to increase slightly at pH 6 and pH 7, and decreasing at more alkaline pH levels. This behavior was attributed to differences in charge density at the different pH levels.

#### 2.2.2.2. Applied strength
To place a solid bioadhesive system, it is necessary to apply a defined strength. Whatever the polymer, poly (acrylic acid/divinylbenzene), poly (HEMA) or carbopol
934, the adhesion strength increases with the applied strength or with the duration of its application, up to an optimum\(^{18}\). The pressure initially applied to the bioadhesive tissue contact site can affect the depth of interpenetration. If high pressure is applied for a sufficiently long period of time, polymers become bioadhesive even though they do not have attractive interactions with biological tissue like mucin.

### 2.2.2.2.3. Initial contact time

The initial contact time between bioadhesive and the membrane determines the extent of swelling and the interpenetration of polymer chains. Along with the initial pressure, the initial contact time can dramatically affect the performance of a system. The bioadhesive strength increases as the initial contact time increases. However, longer initial contact time should be based on tissue viability. In case of bioadhesives that need to be polymerized at the application sites, the initial contact time is critical. It is easily controlled when bioadhesives are applied to exposed areas such as eye, nose or mouth. For the application of bioadhesives to the GI tract, however, the initial contact time cannot be controlled, which is one of the difficulties in applying bioadhesives to the GI tract.

### 2.2.2.2.4. Selection of the model substrate

The handling and treatment of biological substrates during the testing of bioadhesives is an important factor, since physical and biological changes may occur in the tissues under the experimental conditions. The viability of the biological substrate should be confirmed by examining properties such as permeability, electrophysiology, or histology. Such studies may be necessary before and after performing the in vitro tests using tissues.

### 2.2.2.2.5. Swelling

The swelling characteristics is related to the polymer it self, and also to its environment. Interpenetration of chains is easier as polymer chains are disentangled and free of interactions. Swelling depends both on polymer concentration and on water presence. When swelling is too great, a decrease in bioadhesion occurs; such a phenomenon must not occur too early, in order to lead to a sufficient action of the bioadhesive system. Its appearance allows easy detachment of the bioadhesive system after the discharge of the active ingredient.
2.3. Hydrogel

A hydrogel can be defined as a material that exhibits the ability to swell in water and retain a significant fraction of water within its structure. There is a wide variety of natural and synthetic hydrogels. Their ability to absorb water is due to the presence of hydrophilic groups such as - OH, - CONH+, - CONH₂, - COOH, and -SO₃H. Hydrogels can be neutral or ionic in nature. In neutral hydrogels, the driving force for swelling arises from the water-polymer thermodynamic mixing contribution to the overall free energy which is coupled with an elastic polymer contribution. In ionic hydrogels, the swelling process is due to the previous two contributions as well as the ionic interactions between charged polymer and free ions. The ionization of the pendant ionizable groups such as carboxylic acid, sulfonic acid or amine groups renders the polymer more hydrophilic and thus leads to a very high water uptake. Examples of polyelectrolytic hydrogels are proteins, polypeptides, poly(acrylic acid), poly(styrene sulfonate) and polyamines.

Molecularly designed hydrogels are used in delivery systems that should be able to maintain the blood concentration of a drug inside the therapeutic region for an extended period of time. There are situations where it is necessary to release the therapeutic agent only when needed or only in the affected area. This requires the use of an “intelligent” biomaterial. Hydrogels have a structure that can be tethered, allowing for control of drug diffusion, the sensitivity to its environment or the recognition of a specific target by incorporation of functional groups in the matrix. All of these properties make hydrogels excellent candidates for controlled release applications.

Diffusion controlled hydrogel based systems can be matrix or reservoir systems. The active agent diffuses through a hydrogel membrane and then passes to the biological fluid. In the reservoir system, the active agent is located in a core and a polymer membrane surrounds it. In matrix systems, the drug or protein is homogeneously distributed throughout the membrane and is slowly released from it. The polymer membrane can be biodegradable.

In erodible systems the polymer matrix is hydrolyzed either by bulk or surface erosion mechanisms. Bioerodible systems are attractive delivery systems, especially for implants, because they don’t need to be surgically removed and depending on the erosion kinetics, the drug can be released for an extended period of time. The most studied bioerodible polymers are poly(lactic acid), poly(glycolic acid), and their
copolymers\textsuperscript{25}. In both cases the final degradation byproducts are water and carbon dioxide. Other examples of bioerodible hydrogels include dextran and chitosan.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure2_3.png}
\caption{Diagram describing swelling controlled drug release from hydrogel.}
\end{figure}

Swelling controlled release systems\textsuperscript{24} are particularly popular for controlled release applications. The governing transport mechanism is not Fickian diffusion\textsuperscript{26,27}. As the water penetrates to the dried glassy polymer, the glass transition temperature of the local region is lowered and the polymer becomes rubbery due to the rearrangement of the macromolecular chains. Case II, anomalous or relaxation controlled transport prevails. The most common hydrogels that exhibit swelling controlled behavior are poly (2-hydroxyethyl methacrylate), poly (vinyl alcohol), poly (N-vinyl-2-pyrrolidone), poly (ethylene glycol) and their copolymers\textsuperscript{24}.

Environmentally responsive systems\textsuperscript{23} are characterized by their ability to respond to changes in pH, temperature, ionic strength, solvent composition, and magnetic fields among others. Hydrogels that respond to any of these changes in their surroundings show a dramatic change in their swelling behavior. These changes in swelling are usually characterized by a transition point where the behavior occurs. Also, these changes in the network are completely reversible. The release behavior of therapeutic
agents is more complex and usually is a combination of diffusion and anomalous transport.

The most commonly used environmentally sensitive hydrogels in controlled release are pH sensitive and temperature sensitive ones. Hydrogels that contain ionizable moieties such as carboxylic and amine are pH and ionic strength sensitive. These hydrogels swell when the ionic moieties ionize and the electrostatic repulsion causes the network to expand and water is allowed to enter. The most common pH sensitive hydrogels are poly (acrylic acid), poly (methacrylic acid), poly (diethylaminoethyl methacrylate) and poly (dimethylaminoethyl methacrylate) and their copolymers. The most studied temperature sensitive hydrogel is poly (N-isopropylacrylamide). This hydrogel has a lower critical solution temperature of 34.3 °C. Therefore, it swells at temperatures below the lower critical solution temperature. Comb type grafted poly (N-isopropylacrylamide) hydrogels were developed by Okano and associates. Such systems show major promise for rapid or oscillatory release of drugs, peptides, and proteins. Peppas and associates synthesized a terpolymer composed of N-isopropylacrylamide, acrylic acid and 2-hydroxyethyl methacrylate with combined pH and temperature sensitivity. This hydrogel exhibited pulsatile release under oscillatory pH and temperature conditions in the streptokinase controlled release studies. Hoffman and associates have also developed novel pH and temperature sensitive hydrogels by grafting temperature sensitive side chains onto a pH sensitive backbone and by copolymerizing temperature and pH sensitive units.

Novel applications of hydrogels come from the development of stimuli responsive systems using pH and temperature sensitive hydrogels, specifically in the development of polymeric carriers that could release peptides and proteins through different delivery routes. These systems are very promising not only in the area of controlled delivery of proteins and other agents, but also in the development of biosensors. The challenges in the delivery of peptides and proteins are their poor chemical stability in aqueous solutions and their low bioavailability as a consequence of degradation in the different biological barriers. Therefore, they have to be administered mainly by frequent intramuscular injections, limiting their use as therapeutic agents. Of particular interest is the release of these agents through the gastrointestinal tract.

Temperature and pH sensitive hydrogels have been the focus of extensive research in the past years, especially in the area of controlled release of peptides and proteins.
Insulin has been used extensively as the model drug for developing these new systems. In the case of insulin, Schwarte et al.\textsuperscript{31,32} developed a system able to respond to high glucose levels in blood. This system consists of cationic hydrogels containing a poly(diethylaminoethyl methacrylate) grafted with poly(ethylene glycol). The hydrogel contains glucose oxidase, catalase and insulin. The enzymes give the system specific glucose sensitivity due to the reaction of the glucose oxidase with glucose to produce gluconic acid. The local decrease in pH causes the network to swell and insulin to be released. Ramkissoon-Ganorkar et al.\textsuperscript{35} investigated pH and temperature sensitive hydrogels composed of poly(N-isopropyl acrylamide), butyl methacrylate and acrylic acid. The in vitro release of insulin showed that it is dependent on the molecular weight of the terpolymer owing to the polymer’s higher dissolution rate at lower molecular weights.

Another pH and temperature sensitive system composed of poly(N-isopropyl acrylamide) and N,N-dimethylaminopropylmethacrylamide was investigated by Park\textsuperscript{36}. In this system, the release behavior in vitro was strongly affected by the changes in temperature and to a lesser extent by changes in pH. Lowman and Peppas\textsuperscript{37,38} have investigated complexation hydrogels as possible delivery carriers for insulin. Poly (methacrylic acid) grafted poly(ethylene glycol) hydrogels were synthesized\textsuperscript{37}. The feasibility of these hydrogels as a possible oral carrier for insulin was investigated both in vitro\textsuperscript{38} and in vivo\textsuperscript{39}. These hydrogels showed an enhancement in the transport of insulin through the epithelial cell lining of the small intestine in vivo. Torres-Lugo and Peppas\textsuperscript{40} have also tested these systems in vitro as possible delivery carriers for salmon calcitonin. Salmon calcitonin was successfully released in vitro from the system at a constant rate for approximately 7 hour.

Biodegradable systems have also been extensively used for the delivery of peptides and proteins\textsuperscript{25}. These systems contain, but not exclusively, dextran, PLA, PGA, PLGA and their copolymers with PEG have been investigated for the delivery of peptide and proteins. Hydrogels composed of glycidyl methacrylate derivatized dextran were studied for the controlled release of proteins such as lysozyme and bovine serum albumin\textsuperscript{25,41}. For this system, the release behavior was affected by the amount of water in the system, the degree of GMA substitution and the protein size. Moriyama and Yui\textsuperscript{42} used dextran and dextran hydrogels containing PEG to release insulin in vitro. The release of insulin from hydrogels containing PEG was governed
by surface degradation, whereas for hydrogels containing only dextran, the release was controlled by diffusion.

2.4. Polymer Review

2.4.1. Poly (vinyl alcohol)

Poly (vinyl alcohol) (PVA) is neutral, synthetic, water soluble, hydrogel forming polymer. PVA has a relatively simple chemical structure with a pendant hydroxyl group. The monomer of PVA is vinyl alcohol, which does not exist in a stable form rearranging to its tautomer, acetaldehyde. Therefore, PVA is prepared from vinyl acetate. PVA was first prepared by Hermann and Haehnel in 1924 by hydrolyzing polyvinyl acetate in ethanol with potassium hydroxide. Polyvinyl alcohol is produced commercially from polyvinyl acetate, usually by a continuous process. Because of its mechanical properties and biocompatibility, PVA is extensively utilized in industrial, biomedical and pharmaceutical applications. PVA hydrogels have been used or are being investigated for use in many biomedical and pharmaceutical applications from drug delivery to material for contact lenses, which can be tailored by manipulating the properties by altering the process of preparation including the type and concentration of crosslinks within the material. Polyvinyl alcohol is classified into two classes namely: partially hydrolyzed and fully hydrolyzed. Partially hydrolyzed PVA is used in the foods.

2.4.1.1. Structure, Synthesis and Properties of PVA

2.4.1.1.1. Structure

The structure of PVA is given below:

\[
\text{CH}_2 \quad \text{CH} \quad \text{OH}
\]

\[n\]

PVA is also known as Ethenol, Homopolymer, Polyviol, Vinol, Alvyl, Alkotex, Covol, Gelvatol, Lemol, PVOH etc.

2.4.1.1.2. Synthesis

The primary raw material used in the synthesis of polyvinyl alcohol is vinyl acetate monomer instead of the monomer vinyl alcohol because vinyl alcohol does not exist in stable form. PVA is manufactured by the polymerization of vinyl acetate to form
Poly (vinyl acetate) (PVAc) followed by partial hydrolysis. The process of hydrolysis is based on the partial replacement of ester group in PVAc with the hydroxyl group, and is completed in the presence of aqueous sodium hydroxide. Following gradual addition of the aqueous saponification agent, PVA is precipitated, washed and dried. The degree of hydrolysis is determined by the time point at which the saponification reaction is stopped\(^4\). The hydrolysis reaction does not complete resulting in polymers with a certain degrees of hydrolysis that depends on the extent of reaction, so, the PVA is always a copolymer of PVA and PVAc. PVA of the commercial grades is available with high degree of hydrolysis (above 98.5%). The degree of hydrolysis or the content of acetate groups in the polymer has an overall effect on the properties of PVA, like chemical properties, solubility and the crystallizability\(^4\). Polyvinyl alcohol is not known to occur as a natural product.

### 2.4.1.1.3. Properties

Polyvinyl alcohol is an odourless and tasteless, translucent, white or cream coloured granular powder. It is soluble in water, slightly soluble in ethanol, but insoluble in other organic solvents. Typically a 5% solution of polyvinyl alcohol exhibits a pH in the range of 5.0 to 6.5. It has a molecular weight of between 26,300 and 30,000 and a degree of hydrolysis of 86.5 to 89%\(^4\).

Polyvinyl alcohol has excellent film forming, emulsifying and adhesive properties. It has high tensile strength, flexibility as well as high oxygen and aroma barrier. However these properties are dependent on humidity, in other words, with higher humidity more water is absorbed. The water, which acts as a plasticiser, will then reduce its tensile strength, but increase its elongation and tear strength. All PVA grades are readily soluble in water and are dependent on factors like molecular weight, particle size distribution and particle crystallinity. As a hydrophilic polymer, PVA exhibits excellent water retention or water imbibing properties\(^45\).

PVA has a melting point of 230°C and 180–190°C for the fully hydrolysed and partially hydrolysed grades respectively. It decomposes rapidly above 200°C as it can undergo pyrolysis at high temperatures. PVA is an atactic material but exhibits crystallinity as the hydroxyl groups are small enough to fit into the lattice without disrupting it.

The degree of hydrolysis during the synthesis or polymerisation affects the solubility of PVA in water\(^3\). It has been studied that PVA grades with high degree of hydrolysis have low solubility in water and more difficult to crystallize as well. Polydispersity
index of 2-2.5 is common in the most commercial grades. The molecular weight distribution is an important characteristic of PVA as it affects many of its properties like crystallizability, adhesion, mechanical strength and diffusivity.

2.4.1.2. Possible impurities (including degradation products)

Impurities resulting from the manufacturing process include sodium acetate, methanol and methyl acetate. Levels of sodium acetate, a reaction by product, are monitored using the residue on ignition test. The residual methanol and methyl acetate are monitored during the manufacturing process. No detailed information on the presence of unreacted monomer in the polymer is available.

2.4.1.3. Crosslinking methods for the preparation of PVA hydrogel

PVA hydrogel used in the pharmaceutical and biomedical purpose is the hydrophilic, crosslinked polymeric network which swells when come in contact with water or biological fluids. So, to prepare Hydrogel, PVA must be crosslinked by the any of the method mentioned below, based on its use.

2.4.1.3.1. Chemical crosslinking method

There are certain chemical agents, generally difunctional, are used for the crosslinking of PVA like: gluteraldehyde, epichlorohydrin, acetaldehyde, formaldehyde, and other monoaldehydes. Along with the crosslinking agents there must be presence of sulfuric acid, acetic acid or methanol to form the acetal bridges between the pendant hydroxyl groups of PVA chains. Hydrogels prepared by chemical crosslinking always contain residue of crosslinking agent, which is highly undesirable. So residue is removed by the extraction process which runs for very long time. This is one of the disadvantages of this method. If the residue is present in the Hydrogel, it can not be used for Pharmaceutical and Biomedical purpose because residue itself is toxic and alters the chemical stability of the active agent or drug delivered through the Hydrogel delivery. Other disadvantage of this method is that there may be presence of some other impurities like initiators, stabilisers, chain transfer agents etc. The hydrogel characteristics like swelling properties and strength can be modulated by the addition specific amount of special monomers.

2.4.1.3.2. Physical method or Freezing/Thawing Technique

Second method of producing PVA hydrogel, without utilization of chemical cross links, is the Physical crosslinking or freeze/thaw processing. The method of
producing PVA hydrogel by freeze/thaw technique was first reported by Peppas\textsuperscript{49}. This method includes the cycling of freezing at -5 to -20 °C for 2 to 20 hours and thawing at room temperature for 5 to 24 hours. PVA hydrogel prepared using freeze/thaw cycling have certain advantages over other methods and which make it excellent candidate for the use in Pharmaceutical and Biomedical field. These advantages include their non toxic, non carcinogenic and bioadhesive characteristics as well as their associated ease of processing. PVA gels exhibit a high degree of swelling in water and biological fluids, a rubbery elastic nature, higher mechanical strength and can be readily accepted in the body. The molecular weight of the polymer, concentration of aqueous PVA solution, the temperature during preparation, time of freezing and thawing and the number of freezing/thawing cycle affect the properties of hydrogel\textsuperscript{4}. In order to improve the properties of hydrogel, PVA is often combined with poly (acrylic acid)\textsuperscript{50,51}. Pure PVA hydrogels are insensitive to pH changes but the addition of PAA in the freeze/thaw process results in pH sensitive hydrogels\textsuperscript{52}, which make them suitable candidate for targeted and specialized drug delivery systems.

2.4.1.3.3. Irradiation Method
Crosslinking is also achieved by UV radiation, electron beam or the γ-irradiation. The main advantage of this method over the chemical method is the absence of residue or the toxic materials in the Hydrogel. The PVA Hydrogel prepared by γ-irradiation from \textsuperscript{60}Co source and studied for quantitative and qualitative effects of irradiation. It was found that the minimum gelation dose depend on the degree of polymerization and the concentration of polymer\textsuperscript{53}. The effects of γ-irradiation by \textsuperscript{60}Co on the physical properties of PVA fibers, hydrogels and films irradiated in water were studied by Peppas and Merill\textsuperscript{54}.

2.4.1.3.4. Co-polymerization with other polymers
Recently, PVA hydrogel prepared by copolymerization with the gelatin method with mucoadhesive, haemocompatible, biocompatible, good mechanical strength, good swelling properties and an excellent carrier for sustained drug release was reported\textsuperscript{6}. 
### 2.4.1.4. Applications of PVA

#### 2.4.1.4.1. Applications of PVA in pharmaceutical field

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<th>Crosslinking method</th>
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<td>2</td>
<td>Vaginal controlled drug delivery</td>
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## Literature Review

### 2.4.1.4.2. Applications of PVA in Biomedical field

**Table 2.2 List of applications of PVA in Biomedical field with crosslinking method and the reference.**

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The versatile properties of PVA hydrogels make it a material of choice for many biomedical applications, biotechnological applications like cell immobilization and in the pharmaceutical field like controlled release of drugs. Drug-release applications include one more property of PVA to make it polymer of choice i.e. its bioadhesive nature.

The applications in the pharmaceutical fields include drug delivery systems like vaginal, rectal, buccal, ocular, transdermal, microspheres, nanoparticles, pH and temperature sensitive drug delivery systems, swelling controlled drug delivery devices etc. and in the biomedical field include artificial organs like skin, pancreas, kidney and as material of preparation for artificial articular cartilages, embolization material, soft contact lenses etc as it is given in the table with the individual reference.

2.4.1.4.3. Other applications of PVA

- PVA is used as a cooling medium\textsuperscript{137}. 

<table>
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It is a film coating agent especially in applications where moisture barrier/protection properties are required. Polyvinyl alcohol protects the active ingredients from moisture, oxygen and other environmental components, while simultaneously masking their taste and odour.

PVA has various applications in the food industries as a binding and coating agent.

PVA may be used in high moisture foods in order to retain the overall satisfactory taste, texture and quality of the foods. Confectionery products may also contain Polyvinyl alcohol in order to preserve the integrity of the moisture sensitive constituents.

PVA may be used as adhesive and thickener material in latex paints, paper coatings, hairsprays, shampoos and glues.

PVA is used as carbon dioxide barrier in polyethylene terephthalate bottles.

PVA is used in children's play putty or slime when combined with borax.

PVA is used in feminine hygiene and adult incontinence products as a biodegradable plastic backing sheet.

PVA is used as a water soluble film useful for packaging.

PVA is used as a surfactant for the formation of polymer encapsulated nanobeads.

PVA is used with polyvinyl acetate to make Elmer's glue.

PVA is used in the manufacturing of protective chemical-resistant gloves.

PVA is used as a fixative for specimen collection, especially stool samples.

When doped with iodine, PVA can be used to polarize light.

Fishing: PVA is widely used in freshwater sport fishing. Small bags made from PVA are filled with dry or oil based bait and attached to the hook or the baited hook is placed inside the bag and cast into the water. When the bag lands on the lake or river bottom it breaks down, leaving the hook bait surrounded by ground bait, pellets etc. This method helps attract fish to the hook bait, though it does result in dissolved plastic in the water.

PVA is used as an emulsifier and protective colloid in the production of resin dispersions, in textile sizing and finishing.

PVA is used as photosensitive coatings, in specialty molded products, in water soluble, gas tight films, in paper and paperboard and as
binders for pigmented paper coatings, ceramic materials and nonwoven fabrics.

2.4.2. Gelatin

2.4.2.1. Sources for Gelatin
Gelatin (also called gelatine) is prepared by the thermal denaturation of collagen, isolated from animal skin and bones, with very dilute acid. It can also be extracted from fish skins.

2.4.2.2. Structural unit
Gelatin contains many glycine (almost 1 in 3 residues, arranged every third residue), proline and 4-hydroxyproline residues.
A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.

![Figure 2.4 Molecular structure of Gelatin.](image)

2.4.2.3. Molecular structure
Gelatin is a heterogeneous mixture of single or multi-stranded polypeptides, each with extended left handed proline helix.

conformations and containing between 50-1000 amino acids. The triple helix of type I collagen extracted from skin and bones, as a source for gelatin, is composed of two \( \alpha_1(\text{I}) \) and one \( \alpha_2(\text{I}) \) chains, each with molecular mass ~95 kD, width ~1.5 nm and length ~0.3 \( \mu \)m. Gelatin consists of mixtures of these strands together with their oligomers and breakdown (and other) polypeptides. Solutions undergo coil helix transition followed by aggregation of the helices by the formation of collagen like right handed triple helical proline/hydroxyproline rich junction zones. Higher levels of these pyrrolidines result in stronger gels. There is some dispute over whether each of the three chains in the helical structure has a 10/1 helix (the three strands forming a 10/3 helix) with a 85.8 Å axial repeat or a 7/1 helix (the three strands forming a 7/2
helix) with a 60 Å axial repeat, with tripeptides forming each unit. Although the former view seems prevalent at the present time, recent evidence indicates the latter to be correct. Each of the three strands in the triple helix require about 21 residues to complete one turn; typically there would be between one and two turns per junction zone. Gelatin films containing greater triple helix content swell less in water and are consequentially much stronger. Chemical cross links can be introduced, to alter the gel properties, using transglutaminase to link lysine to glutamine residues or by use of glutaraldehyde to link lysine to lysine.

There are two types of gelatin dependent on whether or not the preparation involves an alkaline pretreatment, which converts asparagine and glutamine residues to their respective acids and results in higher viscosity. Acid pretreatment (Type A gelatin) uses pigskin whereas alkaline treatment (Type B gelatin) makes use of cattle hides and bones.

### 2.4.2.4. Functionality and concerns

Gelatin is primarily used as a gelling agent forming transparent elastic thermoreversible gels on cooling below about 35°C, which dissolve at low temperature to give 'melt in the mouth' products with useful flavor release. In addition, the amphiphilic nature of the molecules endows them with useful emulsification (for example, whipped cream) and foam stabilizing properties (for example, mallow foam). On dehydration, irreversible conformational changes take place that may be used in the formation of surface films. Such films are strongest when they contain greater triple helix content. Gelatin is also used as a fining agent to clarify wine and fruit juice.

Although gelatin is by far the major hydrocolloid used for gelling, current concerns about the possibility of such an animal derived product containing the prions that cause Creutzfeldt-Jakob Disease, plus the need generated by vegetarians and certain religions, has recently encouraged the serious search for alternatives. The combination of the melt in the mouth, elastic and emulsification characteristics of gelatin gels is, however, difficult to reproduce.

Gelatin is nutritionally lacking as a protein being deficient in isoleucine, methionine, threonine and tryptophan.
2.5. Drug Review

2.5.1. Econazole Nitrate (ECN)

2.5.1.1. General

2.5.1.1.1. Category

Antifungals (Skin & Mucous Membrane)

Econazole Nitrate, an imidazole derivative, is a synthetic azole with broad spectrum antifungal and antifungal activity. It acts as a fungistatic agent with fungicidal properties.

2.5.1.1.2. Chemical Name

1-[2-[(4-chlorophenyl) methoxy]-2-(2,4 dichlorophenyl) ethyl]-1 H-imidazole mononitrate.

1-[(2RS)-2-[(4-Chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate.

(RS)-1-[2-(4-chlorophenylmethoxy)-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate.

2.5.1.1.3. Empirical Formula

C_{18}H_{16}Cl_{3}N_{3}O_{4}

2.5.1.1.4. Structural Formula

![Molecular Structure of Econazole Nitrate](image)

Figure 2.5 Molecular structure of Econazole Nitrate.

2.5.1.1.5. Molecular weight

444.7
2.5.1.1.6. Content
99.0 per cent to 101.0 per cent (dried substance).

2.5.1.2. Physical Properties
2.5.1.2.1. Appearance, colour, odour and taste
White or almost white, crystalline powder, tasteless and odourless.

2.5.1.2.2. Melting range
Melts between 161°C and 166°C.

2.5.1.2.3. Solubility
Soluble in methanol.
Sparingly soluble in dichloromethane.
Slightly soluble in ethanol.
Very slightly soluble in water.
Practically insoluble in ether.

2.5.1.3. Identification
A: The infra red absorption spectrum is concordant with the reference spectrum of ECN or with the spectrum obtained from ECN RS.

B: The light absorption in the range 230 to 360 nm of a 0.04% w/v solution in a mixture of 1 volume of 0.1M hydrochloric acid and 9 volumes of 2-propanol exhibits maxima at about 265, 271 and 280 nm; the ratio of the absorbance at the maximum at about 271 nm to that at the maximum at about 280 nm is 1.55 to 1.70.

C: In the test for Related substances examine the chromatograms obtained under ultra-violet light (254 nm) before spraying.

2.5.1.4. Pharmacology
2.5.1.4.1. Pharmacokinetics
Oral and GIT Absorption: <10%
Metabolism: Hepatic to more than 20 metabolites
Excretion: Urine (<1%); feces (<1%)
Chapter 2

2.5.1.4.1.1. Absorption
Percutaneous absorption of ECN appears to be rapid but minimal following topical application of the drug to intact skin.

In one patient in which 70–100 mg of ECN 1% cream was applied to a 28 cm² skin area of the thigh and covered with an occlusive dressing for about 16 hours, less than 1% of the dose was recovered in urine. In another study in healthy individuals in which 1 g of ECN 2% cream was applied to intact skin and to skin stripped of the stratum corneum, peak plasma drug concentrations were less than 1 ng/mL and 20 ng/mL, respectively, and approximately 0.1 and 3.7% of the applied doses, respectively, were recovered in urine and feces.

2.5.1.4.1.2. Distribution
Following topical application of ECN 1% cream to healthy human skin in vivo in one study, about 7.6–9.6% of the applied dose was present in the stratum corneum 0.5–5 hours after application. Although the highest drug concentrations, ranging from 1070–1410 mcg/cm³, were present in the stratum corneum, concentrations present in the epidermis ranged from 0.95–20.6 mcg/cm³, which exceeded the usual MIC for most susceptible fungi; inhibitory concentrations of the drug were also attained as deep as the middle region of the dermis. Occlusive dressings appear to only slightly increase the extent of dermal penetration of the drug. It is not known if ECN is distributed into human milk following topical application; the drug and/or its metabolites are distributed into milk in rats following oral administration.

2.5.1.4.1.3. Elimination
The metabolic and excretory fate of ECN has not been fully elucidated. Following topical application of ECN cream to intact skin, less than 1% of the applied dose is excreted in urine and feces; most of the systemically absorbed fraction of the dose appears to be excreted in urine within 24 hours.

2.5.1.4.2. Clinical Pharmacology
After topical application to the skin of normal subjects, systemic absorption of ECN is extremely low. Although most of the applied drug remains on the skin surface, drug concentrations were found in the stratum corneum which, by far, exceeded the minimum inhibitory concentration for dermatophytes. Inhibitory concentrations were
achieved in the epidermis and as deep as the middle region of the dermis. Less than 1% of the applied dose was recovered in the urine and feces.

Microbiology: ECN has been shown to be active against most strains of the following microorganisms, both in vitro and in clinical infections.

2.5.1.4.2.1. Dermatophytes

*Epidermophyton floccosum*

*Trichophyton mentagrophytes*

*Microsporum audouini*

*Trichophyton rubrum*

*Microsporum canis*

*Trichophyton tonsurans*

*Microsporum gypseum*

2.5.1.4.2.2. Yeasts

*Candida albicans*

*Malassezia furfur*

2.5.1.4.3. Acute Toxicity

There has been no reports to date of overdosage with ECN. Toxicological studies have shown that the oral LD$_{50}$ of ECN in mice, rats, guinea pigs, and dogs is 462, 668, 272, and greater than 160 mg/kg, respectively.

2.5.1.4.4. Spectrum

ECN is active against many fungi, including dermatophytes and yeasts. The drug also has in vitro activity against some gram positive bacteria and *Trichomonas vaginalis*.

Results of in vitro ECN susceptibility tests are method dependent, and MIC values vary depending on the culture medium used, the presence of serum, and inoculum size. ECN is active in vitro against *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. verrucosum*, *T. violaceum*, *Epidermophyton floccosum*, *Microsporum audouini*, *M. canis*, and *M. gypseum*. Most susceptible strains of these fungi are inhibited in vitro by ECN concentrations of 1 mcg/ml or less. In vitro on a weight basis, ECN’s activity against dermatophytes appears to be similar to that of Clotrimazole, Miconazole or Tioconazole and greater than that of tolnaftate. ECN is also active in vitro against *Malassezia furfur* (*Pityrosporum orbiculare*), *Aspergillus*,...
Chapter 2  

Cladosporium and Sporothrix. In vitro on a weight basis, ECN appears to be more active than Miconazole against filamentous fungi (e.g., Aspergillus). Most susceptible strains of Candida albicans are inhibited in vitro by ECN concentrations of approximately 4 μg/mL or less. In vitro on a weight basis, ECN’s activity against C. albicans appears to be similar to that of Clotrimazole, Miconazole, NYS or Tioconazole.

ECN also is active in vitro against Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, and Corynebacterium diphtheriae. In vitro, some strains of Trichomonas vaginalis are inhibited by ECN concentrations of 62.5–125 mcg/ml.

2.5.1.4.5. Mechanism of Antifungal Action

ECN usually is fungistatic in action, but may be fungicidal in high concentrations or against very susceptible organisms.

The exact mechanism(s) of action of ECN has not been fully determined. Like other imidazole derivatives, ECN appears to exert its antifungal and antibacterial activity by altering cellular membranes and interfering with intracellular enzymes. ECN increases cell membrane permeability; the exact effect on cellular membranes has not been clearly established. Like some other imidazole derivatives, ECN blocks C-14 demethylation of sterols, which interferes with the synthesis of ergosterol and may result in alterations in membrane permeability. Since cell membrane permeability appears to be increased in non-growing cells as well as in growing cells, it has been suggested that the drug acts on formed membranes rather than causing formation of defective membranes during growth phases. An increase in cell membrane permeability may allow ECN to penetrate into the cell where it appears to interfere with RNA and protein synthesis and impair lipid metabolism. ECN inhibits biosynthesis of triglycerides and phospholipids and inhibits oxidative and peroxidative enzyme activity which may contribute to cellular necrosis and cell death. At high concentrations, the drug may cause complete lysis of cell organelles.

ECN is also active against some gram positive bacteria.

2.5.1.4.6. Resistance

Cross resistance can occur among theazole antifungals. In vitro tests using imidazoles
resistant strains of ECN indicate that resistance may result from changes in cellular membrane structure and function.

2.5.1.4.7. Drug Interactions
There are no known significant interactions.

2.5.1.4.7.1. Corticosteroids
In vitro studies indicate that corticosteroids (i.e., hydrocortisone, triamcinolone acetonide) inhibit the antifungal activity of ECN against *Saccharomyces cerevisiae* and *Candida albicans* in a concentration dependent manner, but have no effect on the antibacterial activity of ECN against *Staphylococcus*. When the concentration of the corticosteroid was equal to or greater than that of ECN on a weight basis, the antifungal activity was substantially inhibited; when the corticosteroid concentration was only one tenth that of ECN, the antifungal activity was unaffected.

Studies on healthy skin in humans showed that the presence of ECN in 10-fold higher molar concentrations did not substantially alter the blanching phenomenon elicited by topical application of triamcinolone acetonide, suggesting that the antifungal agent does not alter the activity of the corticosteroid.

2.5.1.4.8. Precautions
2.5.1.4.8.1. General: If a reaction suggesting sensitivity or chemical irritation should occur, use of the medication should be discontinued.

2.5.1.4.8.2. Carcinogenicity Studies: Long-term animal studies to determine carcinogenic potential have not been performed.

2.5.1.4.8.3. Fertility (Reproduction): Oral administration of ECN in rats has been reported to produce prolonged gestation. Intravaginal administration in humans has not shown prolonged gestation or other adverse reproductive effects attributable to ECN therapy.

2.5.1.4.8.4. Pregnancy: Pregnancy Category C. ECN has not been shown to be teratogenic when administered orally to mice, rabbits or rats. Fetotoxic or embryotoxic effects were observed in Segment I oral studies with rats receiving 10 to 40 times the human dermal dose. Similar effects were observed in Segment II or Segment III studies with mice, rabbits and/or rats receiving oral doses 40 or 80 times the human dermal dose. ECN should be used in the first trimester of pregnancy only
when the physician considers it essential to the welfare of the patient. The drug should be used during the second and third trimesters of pregnancy only if clearly needed.

2.5.1.4.8.5. **Nursing Mothers:** It is not known whether ECN is excreted in human milk. Following oral administration of ECN to lactating rats, ECN and/or metabolites were excreted in milk and were found in nursing pups. Also, in lactating rats receiving large oral doses (40 or 80 times the human dermal dose), there was a reduction in post partum viability of pups and survival to weaning; however, at these high doses, maternal toxicity was present and may have been a contributing factor. Caution should be exercised when ECN is administered to a nursing woman.

2.5.1.4.9. **Adverse Reactions**

During clinical trials, approximately 3% of patients treated with ECN 1% cream reported side effects thought possibly to be due to the drug, consisting mainly of burning, itching, stinging and erythema. One case of pruritic rash has also been reported.

2.5.1.4.10. **Contraindications and Precautions**

ECN is contraindicated in individuals who have shown hypersensitivity to any of its ingredients.

2.5.1.4.11. **Indications**

ECN is indicated for topical application in the treatment of tinea pedis, tinea cruris, and tinea corporis caused by *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans, Microsporum canis, Microsporum audouini, Microsporum gypseum, and Epidermophyton floccosum*, in the treatment of cutaneous candidiasis, vulvovaginal candidiasis, oral and oropharyngeal candidiasis and in the treatment of tinea versicolor.

2.5.1.5. **Analytical Method**

2.5.1.5.1. **HPLC (BP2004)**

**Test solution** Dissolve 0.100 g of the substance to be examined in methanol R and dilute to 10.0 ml with the same solvent.

**Reference solution (a)** Dissolve 10 mg of ECN for system suitability CRS in methanol R and dilute to 1.0 ml with the same solvent.
Reference solution (b)  Dilute 1.0 ml of the test solution to 20.0 ml with methanol R. Dilute 1.0 ml of the solution to 25.0 ml with methanol R.

Column:  Size: 1 = 0.10 m, Ø = 4.6 mm,
Stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R (3 µm),
Temperature: 35 °C.

Mobile phase:
mobile phase A: mix 20 volumes of methanol R and 80 volumes of a 0.77 g/l solution of ammonium acetate R,
mobile phase B: methanol R, acetonitrile R (40:60 V/V)

Table 2.3 HPLC parameters of ECN analysis.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 25</td>
<td>60 → 10</td>
<td>40 → 90</td>
</tr>
<tr>
<td>25 - 27</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>27 - 28</td>
<td>10 → 60</td>
<td>90 → 40</td>
</tr>
<tr>
<td>28 - 33</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

Flow rate  1.5 ml/min.
Detection  Spectrophotometer at 225 nm.
Injection  10 µl.

System suitability  Reference solution (a):
Peak to valley ratio  minimum of 1.5, where \( H_p \) = height above the baseline of the peak due to impurity C, and \( H_v \) = height above the baseline of the lowest point of the curve separating this peak from the peak due to ECN.

Limits:
Correction factor: for the calculation of content, multiply the peak area of impurity A.
Impurity A, B or C: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent),
Total: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent).
Disregard limit: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent); disregard the peak due to the nitrate ion at the beginning of the chromatogram.
2.5.1.5.2. Loss on drying
Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 100–105 °C for 4 hour.

2.5.1.5.3. Sulphated Ash
Maximum 0.1 per cent, determined on 1.0 g.

2.5.1.5.4. Assay
Dissolve 0.400 g in 50 ml of anhydrous acetic acid R. Titrate with 0.1 M perchloric acid determining the end point potentiometrically. Carry out a blank titration.
1 ml of 0.1 M perchloric acid is equivalent to 44.47 mg of C_{18}H_{16}Cl_{3}N_{3}O_{4}.

2.5.1.6. Related substances or impurities
Carry out the method for thin layer chromatography, using silica gel GF254 as the coating substance and a mixture of 60 volumes of dioxan, 40 volumes of toluene and 1 volume of strong ammonia solution as the mobile phase and allowing the solvent front to ascend 10 cm above the line of application. Apply separately to the plate 10 µl of each of four solutions in a mixture of 9 volumes of methanol and 1 volume of strong ammonia solution. Solutions (1), (2) and (3) contain 5.0% w/v, 0.50% w/v and 0.0125% w/v respectively of the substance being examined. Solution (4) contains 0.50% w/v of ECN RS. After removal of the plate, dry it in a current of air for 15 minutes and examine under ultra violet light (254 nm). Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). Spray the plate with modified potassium iodobismuthate solution and examine in daylight. Any secondary spot in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3). The test is not valid unless a spot is clearly visible in the chromatogram obtained with solution (3).

2.5.1.7. Uses
2.5.1.7.1. Dermatophytoses
ECN 1% topical cream is used for the treatment of certain dermatophytoses, including tinea corporis (body ringworm), tinea cruris (jock itch), and tinea pedis (athlete’s foot) caused by *Epidermophyton floccosum*, *Microsporum audouinii*, *M. canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum*, or *T. tonsurans*.

Clinical studies to date indicate that ECN 1% cream is effective for the topical treatment of dermatophytoses and appears to be equivalent in efficacy and safety to
topical Clotrimazole 1% cream, Miconazole nitrate 1% cream, or Tioconazole 1% or 2% cream (not commercially available in the US).

Like other imidazole derivatives (e.g., Clotrimazole, Ketoconazole, Miconazole, Oxiconazole, Sulconazole) and Ciclopirox olamine, ECN has an advantage over some other topical antifungal agents (e.g., NYS, Tolnaftate) in the treatment of mixed infections or for empiric treatment pending identification of the causative organism, since the drug is active against both dermatophytes and Candida.

2.5.1.7.2. Tinea Corporis and Tinea Cruris

Tinea corporis and tinea cruris generally can be effectively treated using a topical antifungal; however, an oral antifungal may be necessary if the disease is extensive, Dermatophyte folliculitis is present, the infection is chronic or does not respond to topical therapy, or the patient is immunocompromised because of coexisting disease or concomitant therapy.

Many clinicians consider topical imidazoles derivative azole antifungals (e.g., Clotrimazole, ECN, Ketoconazole, Miconazole, Oxiconazole, Sulconazole) or topical allylamine antifungals (e.g., Naftifine, Terbinafine) the drugs of first choice for the topical treatment of tinea corporis or tinea cruris, although other antifungals (e.g., Ciclopirox olamine, Butenafine hydrochloride, Tolnaftate, Undecylenic acid) also can be effective in the treatment of these infections.

2.5.1.7.3. Tinea Pedis

While topical antifungals usually are effective for the treatment of uncomplicated tinea pedis, an oral antifungal usually is necessary for the treatment of hyperkeratotic areas on the palms and soles, for chronic moccasin type (dry type) tinea pedis, and for the treatment of tinea unguium (fingernail or toenail dermatophyte infections, onychomycosis).

2.5.1.7.4. Pityriasis (Tinea) Versicolor

ECN 1% topical cream is used for the treatment of Pityriasis (Tinea) Versicolor, a superficial infection caused by Malassezia furfur (Pityrosporum orbiculare or P. ovale).
Pityriasis versicolor generally can be treated topically with an imidazoles derivative azole antifungal (e.g., clotrimazole, ECN, Ketoconazole, Miconazole, Oxiconazole, Sulconazole), an allylamine antifungal (e.g., Terbinafine), Ciclopirox olamine or certain other topical therapies (e.g., Selenium sulfide 2.5%). However, an oral antifungal (e.g., Itraconazole, Ketoconazole) may be indicated with or without a topical antifungal, in patients who have extensive or severe infections or who fail to respond to or have frequent relapses with topical therapy.

2.5.1.7.5. Cutaneous and Mucus Candidiasis

ECN 1% topical cream is used for the treatment of cutaneous, vulvovaginal and oropharyngeal candidiasis caused by Candida albicans.

2.5.1.7.6. Other Uses

ECN has been used effectively as a 1% vaginal cream or 150 mg vaginal suppositories for the treatment of vulvovaginal candidiasis. In a few comparative studies, these vaginal preparations produced cure rates similar to those produced by Clotrimazole or NYS vaginal tablets. ECN has also been used with some success as a 1% otic solution for the treatment of otomycoses.

2.5.1.8. Marketed products of ECN (International products)

Table 2.4 Marketed products of ECN with brand names and manufacturer.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Strength</th>
<th>Dosage form</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectazole®</td>
<td>1 %</td>
<td>Cream</td>
<td>Ortho-McNeil Pharmaceutical, Inc.</td>
</tr>
<tr>
<td>Gyno-Pevaryl®</td>
<td>150 mg</td>
<td>Vaginal Pessaries</td>
<td>Janssen Cilag Ltd</td>
</tr>
<tr>
<td>Gyno-Pevaryl®</td>
<td>1 %</td>
<td>Vaginal Cream</td>
<td>Janssen Cilag Ltd</td>
</tr>
<tr>
<td>Pevaryl®</td>
<td>1 %</td>
<td>Topical Cream</td>
<td>Janssen Cilag Ltd</td>
</tr>
<tr>
<td>Ecostatin®</td>
<td>1 %</td>
<td>cream</td>
<td>Bristol Meyers Squibb</td>
</tr>
<tr>
<td>Ecostatin®</td>
<td>150 mg</td>
<td>Vaginal ovule</td>
<td>Bristol Meyers Squibb</td>
</tr>
<tr>
<td>Ifenec®</td>
<td>1 %</td>
<td>Cream</td>
<td>Italfarmaco</td>
</tr>
<tr>
<td>Ifenec®</td>
<td>150 mg</td>
<td>pessary</td>
<td>Italfarmaco</td>
</tr>
</tbody>
</table>
2.5.1.9. Drug Delivery Research on ECN: Literature survey

Pedersen et al, 1993\textsuperscript{138}, studied the stability constants between β-cyclodextrin and miconazole and ECN. Increased ionization of the imidazole derivatives decreased the size of the stability constants. The same phenomenon was observed for miconazole and hydroxypropyl-β-cyclodextrin. In addition, the type of solubility diagram obtained was dependent on the degree of ionization of the imidazole derivatives. An ECN-β-cyclodextrin complex with a molar ratio of 1:1 was isolated. In a fluid medium the antimycotic effect of the ECN-β-cyclodextrin complex against a strain of *Candida albicans* was superior to the effect of a physical mixture of the two compounds. A small inhibitory effect of β-cyclodextrin on the growth of the test organism was noticed.

Pedersen et al, 1998\textsuperscript{139}, studied the cyclodextrin inclusion complexes of the ECN and β-cyclodextrin. They found higher antimycotic activity of inclusion complexes of the ECN and β-cyclodextrin than a physical mixture of ECN and β-cyclodextrin. Surprisingly, the ECN dissolution rate from the physical mixture was higher than the dissolution rate from the inclusion complex. A new procedure was applied to disclose the drug supersaturation. The genuine inclusion complex molar ratio ECN:β-cyclodextrin 2:3 gave rise to more hemolysis than the corresponding physical mixture. Toxicity testing on a human buccal epithelium in vitro model based on TR146 cells showed that the physical mixture was more toxic than the inclusion complex when TR146 cell mortality was evaluated. Neither measurement of the transepithelial electrical resistance of TR146 cell layers exposed to either the physical mixture or the inclusion complex nor analysis of the protein liberation from the TR146 cells during exposure revealed any differences between the two compositions.

Mura et al, 1999\textsuperscript{140}, investigated equimolar combinations of ECN with β-cyclodextrin and statistically substituted methyl-β-cyclodextrin for both solid state characterization (differential scanning calorimetry, hot stage microscopy, infrared spectroscopy, scanning electron microscopy) and dissolution properties (dispersed amount method). They evaluated the influence of the preparation method (physical mixing, ball-milling, kneading and sealed heating) on the physicochemical properties of the products. Kneading and sealed heating techniques led to amorphous products in the case of systems with methyl-β-cyclodextrin, whereas crystalline drug was still
clearly detectable in all products with β-cyclodextrin. Independently of the preparation technique, all combinations with methyl-β-cyclodextrin yielded better performance than the corresponding ones with β-cyclodextrin. In fact, the sealed heated with the β-derivative showed an increase of drug dissolution efficiency of 130% with respect to the corresponding physical mixture, in comparison to the 70% increase obtained from that with β-cyclodextrin.

Jacobsen et al, 1999\textsuperscript{141}, investigated the dissolution rate, the toxicity and the release from chewing gum of miconazole and ECN cyclodextrin products and complexes. The dissolution rate studies showed that an amorphous miconazole hydroxypropyl-β-cyclodextrin product gave drug supersaturation, whereas drug supersaturation was not present during dissolution rate testing of an ECN hydroxypropyl-β-cyclodextrin product. The miconazole hydroxypropyl-β-cyclodextrin product and genuine cyclodextrin inclusion complexes of miconazole, ECN and clotrimazole were toxic on a human TR146 buccal cell culture model. The toxicity was probably due to drug supersaturation, thereby increasing the bioavailability of the antimycotics. The ECN hydroxypropyl-β-cyclodextrin product and physical mixtures of miconazole or ECN and β-cyclodextrin did not give supersaturation and were not as toxic as the above mentioned compounds. Neat ECN and miconazole, a genuine ECN-β-cyclodextrin complex and the miconazole hydroxypropyl-β-cyclodextrin product were incorporated in chewing gum. The miconazole hydroxypropyl-β-cyclodextrin gum had a much higher drug release in vitro than the neat miconazole gum. The genuine ECN-β-cyclodextrin complex only increased the drug release moderately when compared with the release from the neat ECN gum. The release studies were performed on a mastication device.

Piemi et al, 1999\textsuperscript{142}, studied the charged submicron emulsions of ECN or miconazole nitrate using stearylamine or deoxycholic acid for the transdermal delivery. The investigation of the relationship between the physicochemical properties of the vehicles, especially the charge of the emulsion and skin permeation, was conducted ex vivo during percutaneous absorption experiments using hairless female rat skin. In addition, drug quantification was carried out using two different analytical techniques (HPLC and radioactivity measurements) in order to examine if the drug analysis approach might affect the results. The results clearly indicate that the surface modified droplets have a significant influence on the diffusion through the skin.
Furthermore, the method of preparation of the formulation and subsequently the analytical method of drug concentration measurement are able to influence the results of percutaneous experiment.

**Dellenbach et al, 2000**\(^{143}\), compared the efficacy and safety of sertaconazole and ECN sustained release suppositories in the treatment of vulvovaginal candidosis. 369 women with symptoms and signs of vulvovaginitis were enrolled in this multicenter, randomized, double blind study. After clinical examination and vaginal sampling, 183 women were treated with a 300 mg sertaconazole suppository and the other 186 with a 150 mg ECN suppository. They were evaluated 1 week after treatment and those who were clinically uncured received a second suppository and were reassessed 1 week later. All women were evaluated 1 month after the last administration. At each follow up visit, clinical efficacy was assessed and a vaginal sampling was performed for microscopic examination and culture. Of the 369 women included, only the 310 who had a positive culture for a strain of *Candida* were taken into account for efficacy analysis: 150 in the sertaconazole group and 160 in the ECN group. One hundred and five women (49 in the sertaconazole group and 56 in the ECN group) were not clinically cured after 1 week and received a second suppository. There were no differences between the two groups for the rates of clinical recovery (disappearance of signs and symptoms) and mycological recovery (negative culture), 1 week after the first application (62.1% and 67.7%, respectively), 1 week after the second application for women treated twice (72.3% and 80.6%, respectively) and for all patients 1 month after the last application (65.3% and 62.0%, respectively). Among the patients cured 1 week after the last application, the mycological recurrence rate after 1 month was significantly higher in the ECN group (32.7% vs. 19.8%, \(P=0.035\)). There was a trend towards better efficacy of sertaconazole after the first application, whereas the two treatments had similar efficacy in women treated twice. There were no serious adverse events and only local irritation was reported, without any statistically significant difference between the two groups. Single topical administration of sertaconazole and ECN had similar efficacy and safety but the former is associated with a lower rate of mycological recurrence one month after achieving a negative culture.

**Pandey et al, 2005**\(^{144}\), tried for the improvement of the bioavailability of clotrimazole and ECN. Each drug was encapsulated in nanoparticles of a synthetic polymer
polylactide-co-glycolide, or a natural polymer alginate stabilized with chitosan. The nanoparticles were prepared by the emulsion solvent evaporation technique in case of polylactide-co-glycolide and by the cation induced controlled gelification in case of alginate. Drug encapsulation efficiency was better (>90%) for the alginate formulation compared with the polylactide-co-glycolide formulation (nearly 50%).

The formulations were orally administered to mice and the drugs were analyzed in plasma by a validated HPLC technique. The biodistribution/pharmacokinetic data suggested that there was a controlled drug release for 5-6 days with each of the formulations, compared with unencapsulated drugs, which were cleared within 3-4 hour of oral/intravenous administration. There was a striking improvement in the relative and absolute bioavailability of each drug. Further, the drugs were detected in the tissues (lungs, liver and spleen) till 6-8 days in case of nanoparticles whereas free drugs were cleared by 12 hour. Overall, the alginate formulation appeared to be better than the polylactide-co-glycolide formulation.

**Moustafa et al, 2006**

Moustafa et al, 2006\(^{145}\), studied the in vitro susceptibility of fungal organisms to β-cyclodextrin complexes with the ECN and ciclopirox olamine by using laser nephelometry. The antymycotic influence of the complexes against *Candida albicans* DSM 11225 and *Candida krusei* ATCC 6258 species was determined using this method. A rapid inhibition and even killing of both fungi was observed only above certain concentrations of complex ranged between 12.5 and 100 μg/ml for β-cyclodextrin–ECN complex, while for the complex with β-cyclodextrin–ciclopirox olamine the range was between 150 and 400 g/ml. The improvement of solubility of both antymycotic agents in phosphate buffer solution was observed by complexation with β-cyclodextrin.

**Sanna et al, 2007**

Sanna et al, 2007\(^{146}\), formulated Solid lipid nanoparticles of ECN prepared by o/w high-shear homogenization method using different ratios of lipid and drug (5:1 and 10:1). After incorporation of solid lipid nanoparticles into hydrogels, rheological measurements were performed, and ex vivo drug permeation tests were carried out using porcine stratum corneum. In-vivo study of percutaneous absorption of ECN as a function of application time and composition of gels was carried out by tape stripping technique. High shear homogenization method resulted in a good technique for preparation of ECN loaded Solid lipid nanoparticles. Ex vivo tests showed that solid lipid nanoparticles were able to control the drug release through the stratum corneum;
the release rate depended upon the lipid content on the nanoparticles. In vivo studies demonstrated that solid lipid nanoparticles promoted a rapid penetration of ECN through the stratum corneum after 1 hour and improved the diffusion of the drug in the deeper skin layers after 3 hour of application compared with the reference gel.

**Furneri et al, 2008**\(^{147}\), prepared ECN bioadhesive system by addition of polycarbophil. The addition of polycarbophil increased the duration of the active drug at the site of infection, led to a greater frequency of negative culture after treatment and reduced the recurrence rate of vaginal candidiasis. 180 women with vaginal candidiasis were treated with 150 mg vaginal ovules ECN with (group A) or without (group B) polycarbophil. After 3 days of treatment the negative culture of *Candida albicans* reached 98.6% in group A and 84.8% in B group, while the overall persistence (*C. albicans, C. glabrata, C. krusei, and C. parapsilosis*) was 5.6% and 30%, respectively. During a 60 day follow up, only one case out of 85 (1.2%) in group A reported recurrence while in group B there were 6 out of 63 (9.5%) recurrences.

**Ahmad et al, 2008**\(^{148}\), demonstrated the potential of ECN and moxifloxacin individually against tuberculosis caused by multidrug resistant and latent *Mycobacterium tuberculosis*. In this study, poly-(dl-lactide-co-glycolide) nanoparticles encapsulated ECN and moxifloxacin were evaluated against murine tuberculosis (drug susceptible) in order to develop a more potent regimen for tuberculosis. Poly (dl-lactide-co-glycolide) nanoparticles were prepared by the multiple emulsion and solvent evaporation technique and were administered orally to mice. A single oral dose of PLG nanoparticles resulted in therapeutic drug concentrations in plasma for up to 5 days (ECN) or 4 days (moxifloxacin), whilst in the organs (lungs, liver and spleen) it was up to 6 days. In *M. tuberculosis* infected mice, eight oral doses of the formulation administered weekly were found to be equipotent to 56 doses (moxifloxacin administered daily) or 112 doses (ECN administered twice daily) of free drugs. Furthermore, the combination of moxifloxacin +ECN proved to be significantly efficacious compared with individual drugs. Addition of rifampicin to this combination resulted in total bacterial clearance from the organs of mice in 8 weeks. Poly (dl-lactide-co-glycolide) nanoparticles appear to have the potential for intermittent therapy of tuberculosis and combination of moxifloxacin, ECN and rifampicin is the most potent.
Passerini et al, 2009\textsuperscript{149}, investigated the suitability of the spray congealing technique to produce solid lipid microparticles for topical administration and to study the skin permeation of ECN from compared with solid lipid nanoparticles. Solid lipid microparticles had particle sizes of 18–45 micron, while solid lipid nanoparticles showed a mean diameter of 130–270 nm. The encapsulation efficiency was 80-100%. Permeation profiles of ECN were influenced by both particle size (significant difference until 9 hour) and the amount of lipid. The results confirm the usefulness of solid lipid nanoparticles as carriers for topical administration and suggest the potential of solid lipid microparticles for the ECN delivery of drugs to the skin.

Albertini et al, 2009\textsuperscript{150}, investigated mucoadhesive microparticles for innovative vaginal delivery systems of ECN able to enhance the drug antifungal activity. Seven different formulations were prepared by spray congealing: a lipid-hydrophilic matrix (Gelucire 53/10) was used as carrier and several mucoadhesive polymers such as chitosan, sodium carboxymethylcellulose and poloxamers (Lutrol F68 and F127) were added. The antifungal activity of the microparticles against a strain of \textit{Candida albicans} ATCC 10231 was investigated. Both poloxamers significantly (p<0.01) improved the solubility and in vitro bioavailability of the low solubility drug and the mucoadhesive strength. Poloxamers/Gelucire based microparticles exhibited an inhibition effect on the \textit{C. albicans} growth, suggesting their use as an effective treatment for vaginal candidiasis, with potential for reduced administration frequency. The results demonstrated that spray congealing technology can be considered a novel and solvent free approach for the production of mucoadhesive microparticles for the vaginal delivery of ECN.

2.5.2. Nystatin (NYS)
Nystatin is a polyene antifungal antibiotic obtained from \textit{Streptomyces noursei}.

2.5.2.1. General

2.5.2.1.1. Empirical formula
\[ \text{C}_{47}\text{H}_{75}\text{NO}_{17} \]

2.5.2.1.2. Molecular weight
926.13
2.5.2.1.3. Structural formula

![Figure 2.6 Molecular structure of Nystatin.](image)

2.5.2.1.4. Content

NYS has a potency of not less than 4400 Units per mg, calculated with reference to the dried substance.

2.5.2.2. Physical properties

2.5.2.2.1. Appearance, colour, odour and taste

Yellow to slightly brown powder; odour, characteristic; hygroscopic.

2.5.2.2.2. Melting range

Gradually starts melting from 160 °C with decomposition.

2.5.2.2.3. pH

Between 6.5 and 8.0, determined in a 3.0% w/v suspension in water.

2.5.2.2.4. Solubility

Freely soluble in dimethylformamide.

Slightly soluble in methanol.

Very slightly soluble in water.

Insoluble in chloroform.

Insoluble in ether.

Insoluble in ethanol.
Chapter 2  

2.5.2.3. Identification

A: The light absorption of the final solution obtained in the test for Light absorption in the range 220 to 360 nm exhibits four maxima, at about 230, 291, 305 and 319 nm. The ratios of the absorbances at the maxima at about 291 nm and about 319 nm to the absorbance at the maximum at about 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use as the blank a solution prepared exactly in the same manner without the substance being examined.

B: Shake 30 mg with 5 ml of water for 2 minutes, add 2 ml of sodium molybdotungstophosphate solution, and allow to stand for 1 hour; the green colour produced is darker than that produced by repeating the test without the substance being examined.

C: Shake 30 mg with 5 ml of water for 2 minutes, add 2 ml of a solution prepared by dissolving 0.1 g of pyrogallol in 100 ml of decolorized magenta solution, heat on a water-bath until a dark pink colour is produced, cool and allow to stand for 1 hour; the pink colour is retained.

D: To 2 mg add 0.1 ml of hydrochloric acid; a brown colour is produced.

E: To 2 mg add 0.1 ml of sulphuric acid; a brown colour is produced which becomes violet on standing.

2.5.2.4. Pharmacology

2.5.2.4.1. Pharmacokinetics

Topical Absorption: None through mucous membranes or intact skin  
Oral Absorption: Poorly absorbed  
Excretion: Feces (as unchanged drug)

Gastrointestinal absorption of NYS is insignificant. Most orally administered NYS is passed unchanged in the stool. Significant concentrations of NYS may appear occasionally in the plasma of patients with renal insufficiency during oral therapy with conventional dosage forms.

Mean NYS concentrations in excess of those required in vitro to inhibit growth of clinically significant Candida persisted in saliva for approximately two hours after the
start of oral dissolution 400,000 units NYS administered simultaneously to 12 healthy volunteers.

2.5.2.4.2. Pharmacodynamics
Onset of action: Symptomatic relief from candidiasis: 24-72 hours
NYS is both fungistatic and fungicidal in vitro against a wide variety of yeasts and yeast-like fungi. *Candida albicans* demonstrates no significant resistance to NYS in vitro on repeated subculture in increasing levels of NYS; other *Candida* species become quite resistant. Generally, resistance does not develop in vivo.
Nystatin exhibits no activity against bacteria, protozoa, trichomonads or viruses.

2.5.2.4.3. Mechanism of Action
NYS acts by binding to sterols in the cell membrane of susceptible fungi with a resultant change in membrane permeability allowing leakage of intracellular components.

2.5.2.4.4. Adverse Reactions
Frequency not defined: Dermatologic: Contact dermatitis, Stevens-Johnson syndrome
1% to 10%: Gastrointestinal: Diarrhea, nausea, stomach pain, vomiting.
<1%: Hypersensitivity reactions.
NYS topically is generally well tolerated by all age groups, even during prolonged use. Rarely, oral irritation or sensitization may occur. Nausea has been reported occasionally during therapy.

Large oral doses of NYS have occasionally produced diarrhea, gastrointestinal distress, nausea and vomiting. Rash, including urticaria has been reported rarely. Stevens-Johnson syndrome has been reported very rarely.

2.5.2.4.5. Drug Interactions
Saccharomyces boulardii: Antifungal Agents may diminish the therapeutic effect of Saccharomyces boulardii. Risk D: Consider therapy modification

2.5.2.4.6. Dosage
2.5.2.4.6.1. Oral candidiasis
Suspension (swish and swallow orally):
Premature infants: 100,000 units 4 times/day.
Infants: 200,000 units 4 times/day or 100,000 units to each side of mouth 4 times/day

Children and Adults: 400,000-600,000 units 4 times/day.

Powder for compounding: Children and Adults: 1/8 teaspoon (500,000 units) to equal approximately 1/2 cup of water; give 4 times/day.

2.5.2.4.6.2. **Mucocutaneous infections**: Children and Adults: Topical: Apply 2-3 times/day to affected areas; very moist topical lesions are treated best with powder.

2.5.2.4.6.3. **Intestinal infections**: Adults: Oral: 500,000-1,000,000 units every 8 hours.

2.5.2.4.6.4. **Vaginal infections**: Adults: Vaginal tablets: Insert 1 tablet/day at bedtime for 2 weeks.

2.5.2.5. **Storage**

Vaginal insert: Store in refrigerator. Protect from temperature extremes, moisture, and light.

Oral tablet, ointment, topical powder, and oral suspension: Store at controlled room temperature 15°C to 25°C (59°F to 77°F).

2.5.2.6. **Analysis**

2.5.2.6.1. **Sulphated ash**: Not more than 0.1%.

2.5.2.6.2. **Loss on drying**: Not more than 0.5%, determined on 1 g by drying in an oven at 105°C for 2 hours.

2.5.2.6.3. **Assay**: Weigh accurately about 0.4 g, dissolve in 50 ml of anhydrous glacial acetic acid and carry out Method A for non aqueous titration, determining the end-point potentiometrically. Perform a blank determination and make any necessary correction. Each ml of 0.1M perchloric acid is equivalent to 0.04447 g of C\textsubscript{18}H\textsubscript{15}Cl\textsubscript{3}N\textsubscript{2}O,HNO\textsubscript{3}. 
2.5.2.7. Marketed products of NYS (International products)

Table 2.5 Marketed products of NYS with brand names and manufacturer.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Strength</th>
<th>Dosage form</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycostatin®</td>
<td>100,000 units per gm</td>
<td>Topical Cream, Topical Powder</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>Mycostatin®</td>
<td>100,000 units</td>
<td>Vaginal Tablet, pastilles</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>Nilstat®</td>
<td>100,000 units per ml</td>
<td>Oral drops</td>
<td>Wyeth Ayerst</td>
</tr>
<tr>
<td>Nilstat®</td>
<td>500,000 units</td>
<td>Tablets</td>
<td>Wyeth Ayerst</td>
</tr>
<tr>
<td>Nilstat®</td>
<td>100,000 units per gm</td>
<td>Vaginal: Cream</td>
<td>Wyeth Ayerst</td>
</tr>
<tr>
<td>Bio-Statin®</td>
<td>100,000 units</td>
<td>Topical powder, Capsule</td>
<td>Bio-Tech Pharmacal Inc.</td>
</tr>
<tr>
<td>Nyamyc™</td>
<td>100,000 units per gm</td>
<td>Topical powder</td>
<td>Upsher-Smith Laboratories</td>
</tr>
<tr>
<td>Nystop®</td>
<td>100,000 units per gm</td>
<td>Topical powder</td>
<td>Paddock Laboratories</td>
</tr>
<tr>
<td>Paddock Nystatin™</td>
<td>150 million units's</td>
<td>Oral Suspension</td>
<td>Paddock Laboratories</td>
</tr>
<tr>
<td>Pedi-Dri®</td>
<td>100,000 units per gm</td>
<td>Topical powder</td>
<td>Pedinol Pharmacal Inc.</td>
</tr>
<tr>
<td>Candistatin®</td>
<td>100,000 units per gm</td>
<td>Topical powder</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>Nystan®</td>
<td>100,000 units per ml</td>
<td>Oral suspension</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>Nystan®</td>
<td>100,000 units</td>
<td>Oral tablets</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>Nystan®</td>
<td>100,000 units per gm</td>
<td>topical ointment</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>Nystan®</td>
<td>100,000 units</td>
<td>pessaries</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>Nysert®</td>
<td>100,000 units</td>
<td>vaginal suppositories</td>
<td>Procter &amp; Gamble</td>
</tr>
<tr>
<td>Korostatin®</td>
<td>100,000 units</td>
<td>vaginal tablets</td>
<td>Holland Rantos</td>
</tr>
</tbody>
</table>

2.5.2.8. Drug Delivery Research on NYS: Literature survey

Jan Knapczyk, 1992\textsuperscript{151}, prepared buccal and vaginal tablets of NYS and clotrimazole of deacetylated krill chitosan. Tablets obtained by direct compression disintegrate either slowly in water (buccal), or rapidly in a 0.1% lactic acid solution (vaginal). The stability of the tablet properties, as well as the microbiological and chemical stability
of the applied drugs, both recorded after storage, considerably extend the applicability of chitosan as an auxiliary substance for use in direct tableting.

**Encarnacion and Chin, 1994**\(^{152}\), studied mucosal oral therapeutic controlled release osmotic system of NYS. A crossover study was conducted in five healthy volunteers to evaluate the amount of NYS released (based on residual drug content) when the system is held in the mouth for 30 minute, 1 hour, and 2 hour and to compare these concentrations with those achieved with a Mycostatin\(^{®}\) pastille. An average of 37% of the NYS content was released intra orally from the system during 2 hour in the mouth, which was very similar to the percentage delivered in vitro. The study demonstrates that the osmotic system maintains high salivary NYS concentrations throughout a 2 hour dosing interval.

**Allemandi et al, 2002**\(^{153}\), designed and evaluated mucoadhesive double layered tablet of NYS. Lactose CD, caromer and hydroxypropylmethylcellulose were used as excipients. Tablets were obtained through direct compression. Properties such as in vitro mucoadhesion, water uptake, front movements and drug release were evaluated. The immediate release layer was made of lactose CD and NYS. The CB:HPMC 9:1 mixture showed the best mucoadhesion properties and was selected as excipient for the mucoadhesive polymeric layer. Nystatin showed a first order release. The mucoadhesive tablet formulated in this work releases NYS quickly from the lactose layer and then in a sustained way, during approximately 6 hours, from the polymeric layer.

**Allemandi et al, 2004**\(^{154}\), investigated the mucoadhesive matrices of NYS. The in vitro mucoadhesion, water uptake and drug release of NYS from matrices of caromer and lyophilized caromer sodium salt mixtures were evaluated. Matrices with different ratios were prepared by direct compression. In vitro mucoadhesion increased as the proportion of caromer in the matrix was raised. Matrices in which caromer was replaced by CL showed an increase of both water uptake and release rates. Besides, the release of NYS from matrices lyophilized caromer sodium salt mixtures exhibited a kinetics with Super Case II (\(n > 1\)) mechanism.

**Pelin et al, 2004**\(^{155}\), formulated occlusive bioadhesive systems of NYS for prophylaxis and treatment of oral mucositis. Gel and film formulations were prepared
using chitosans at different molecular weights and in different solvents. The in vitro release of NYS from the formulations was decreased with the increasing molecular weight of chitosan. The effect of the formulations was investigated in vivo in hamsters with chemotherapy-induced mucositis. Mucositis scores in groups treated with NYS incorporated into gel and suspension. The retention time and distribution of the gels in the oral cavity were investigated in healthy volunteers. A faster distribution of NYS in the oral cavity was obtained using the suspension compared to the gels, but the NYS saliva level decreased rapidly as well. A drug concentration above the minimum inhibitory concentration value for *Candida albicans* (0.14 μg/ml) was maintained for longer periods of time at the application site (90 min).

Allemandi et al., 2007a\(^{156}\) and 2007b\(^{157}\), investigated the preformulation and evaluation of buccoadhesive films of NYS in two separate publications. In preformulation work studies concerning the design of novel mucoadhesive films were reported. The rationality of the design was based on the utilization of mucoadhesive polymers (carbomer and carboxymethylcellulose), a plasticizer (polyethyleneglycol) and a surfactant (ascorbil palmitate, ASC16). In the gel preparation, the casting method using water as a solvent was employed. The addition of ASC16 improved the solubilization of NYS and provoked a decrease in gel viscosity.

Whereas the second paper deals with the formulation and evaluation of the mucoadhesive films containing NYS. The design and formulation of the films were based on the mucoadhesive properties of carbomer 934P and carboxymethylcellulose and also on the plasticizer properties of polyethyleneglycol 400. A surfactant (ascorbil palmitate, ASC16) was added to the system to aid in NYS dispersion. Addition of these last two components produced a significant improvement in physical and mechanical properties (flexibility and strength) as well as an increase in the NYS release rate.

Quinones and Ghaly, 2008\(^{158}\), developed and characterized a series of carbopol 934 hydroxypropyl methylcellulose and a combination of carbopol-HPMC as a gel base for topical delivery of NYS. The drug level was held constant at 1.72% w/w and the level of propylene glycol which is used as a cosolvent and penetration enhancer was also kept constant at 2% w/w. The rheological study showed that formulation containing combination of 2 carbopol and 1 HPMC ratio gave the highest viscosity, and exhibited an apparent pseudoplastic thixotropic behavior. The diffusion study
indicated that gel formulation containing carbopol-HPMC at a ratio of 2:1 gave the highest percent drug diffusion compared to formulation containing low carbopol to HPMC ratio, carbopol alone or HPMC alone. Both in-vitro release and rheological study indicated that carbopol-HPMC had the best gel strength, physical properties and ability to diffuse the drug than carbopol or HPMC alone.

Hombach et al, 2009\textsuperscript{159}, designed and evaluated a novel vaginal delivery system for NYS based on mucoadhesive polymers. L-Cysteine and cysteamine, respectively, were covalently attached to poly(acrylic acid) and the two different thiolated polymers were evaluated in vitro regarding their swelling behavior, mucoadhesive properties and release behavior. Tablets comprising these thiolated polymers and NYS demonstrated a high stability in vaginal fluid simulant pH 4.2 and an increase in weight by swelling whereas control tablets comprising unmodified poly(acrylic acid) disintegrated and dissolved. The mucoadhesion time of tablets on freshly excised bovine vaginal mucosa on a rotating cylinder and the total work of adhesion of gels and tablets increased significantly due to the formation of disulfide bonds between the thiolated polymer and cysteine rich subdomaines of the mucus layer. The drug NYS was released more slowly out of thiomer tablets and gels than out of poly(acrylic acid) control tablets and gels. The thiolated polymers were proved promising delivery systems for NYS providing a prolonged residence time and a sustained drug release in vitro under physiological relevant conditions.

Llabot et al, 2009\textsuperscript{160}, formulated mucoadhesive tablets containing NYS and evaluated in vivo. The assays were carried out with 12 healthy volunteers and the concentration of NYS in saliva was determined at different times. Tablets remained attached to the buccal mucosa during 270 min +/- 30 min. No evidence of ulceration or bleeding was observed. Typical appearance of intact human buccal mucosa was seen before and after contact with the tablet. The tablets were well accepted by the volunteers, although most of the volunteers reported a light bitter taste, probably due to NYS. Concentration of NYS in saliva was several times higher than MIC over a period of approximately 4.5 hour, which was in agreement with the behavior observed in vitro.
2.6. Experimental Design or Design of Experiment (DoE)

Statistical methods can increase the efficacy of an experiment, one such method is Design of Experiment (DoE). This technique is widely used in chemical research and pharmaceutical industry in the development of new process and in optimizing the performance of existence processes.

2.6.1. The traditional way - the COST approach

(COST: Change One Separate factor at a Time)

Most experimentation today is done by changing levels of one factor (variable) at a time in an unsystematic way in order to try and find the optimum conditions of a complex system. Is this a good (efficient, rational, economic) strategy? No!!

As shown by Fisher around 1925, changing one separate factor at a time (COST) does not give any information about the position of the optimum in the common case where there are interactions between factors. Then the COST approach gets stuck, usually far from the real optimum. However, the experimenter perceives that the optimum has been reached because changing one factor at a time does not lead to any further improvement. The COST approach is said to be pseudo convergent.

2.6.2. Problems associated with the COST approach:

1. Does not lead to real optimum.
2. Inefficient, unnecessarily many runs.
3. Provides no information about what happens when factors are varied simultaneously (ignores interactions).
4. Provides less information about the variability of the response.
5. Isolated, unconnected experiments.
6. Slow growth of knowledge, mapping of experimental space.

In 1925 Fisher started the development of methods of statistical experimental design. These methods have been further refined by Yule, Box, Stu and Bill Hunter, Scheffe, Cox, Taguchi, and others, so that today they comprise a tool box for virtually any optimization problem. The basic idea is to devise a small set of experiments, in which all pertinent factors are varied systematically. This set usually does not include more than ten to twenty experiments. The subsequent analysis of the resulting experimental data will identify the optimal conditions, the factors that most influence the results.
and those that do not, the presence of interactions and synergisms, and so on. The most important aspect of statistical experimental designs is that they provide a strict mathematical framework for changing all pertinent factors simultaneously, and achieve this in a small number of experimental runs. Most of us can only grasp the effect of one factor at a time in our minds, and that leads to the inefficient COST approach. We need the mathematics (and the computer) to keep track of the factors and their combinations.

1. All factors are varied together over a set of experimental runs
2. Noise is decreased by means of averaging
3. The functional space is efficiently mapped, interactions and synergisms are seen

Typical examples when design of experiments (DoE) is useful involve the development of new products and processes, e.g. optimizing the quality and performance of an existing product or optimizing existing manufacturing processes of chemicals, polymers, materials, drugs and pharmaceuticals, foods and beverages, cosmetics, paints and so on.

2.6.3. Design of Experiment (DoE)

In an experiment, we deliberately change one or more process variables (or factors) in order to observe the effect of changes have on one or more response variables. The (statistical) design of experiments (DoE) is an efficient procedure for planning experiments so that the data obtained can be analyzed to yield valid and objective conclusions.

2.6.4. Optimization

Defined as to make perfect, effective or functional as possible.

“Optimization may be defined as to find out the values of controllable independent variables, that gives the most desired value of dependent variables”

2.6.4.1. Advantages

1. Choosing Between Alternatives

Supplier A vs. supplier B? Which new additive is the most effective? Is catalyst `x' an improvement over the existing catalyst? These and countless other choices between alternatives can be presented to us in a never ending parade. Often we have the choice made for us by outside factors over which we have no control. But in many cases we
are also asked to make the choice. It helps if one has valid data to back up one's decision.

2. Selecting the Key Factors Affecting a Response

Often there are many possible factors, some of which may be critical and others which may have little or no effect on a response. It may be desirable, as a goal by itself, to reduce the number of factors to a relatively small set (2-5) so that attention can be focused on controlling those factors with appropriate specifications, control charts, etc.

Screening experiments are an efficient way, with a minimal number of runs, of determining the important factors. They may also be used as a first step when the ultimate goal is to model a response with a response surface.

3. Hitting a Target

This is a frequently encountered goal for an experiment. One might try out different settings until the desired target is 'hit' consistently. For example, a machine tool that has been recently overhauled may require some setup 'tweaking' before it runs on target. Such action is a small and common form of experimentation. However, rather than experimenting in an ad hoc manner until we happen to find a setup that hits the target, one can fit a model estimated from a small experiment and use this model to determine the necessary adjustments to hit the target.

4. Maximizing or Minimizing a Response

Many processes are being run at sub optimal settings, some of them for years, even though each factor has been optimized individually over time. Finding settings that increase yield or decrease the amount of scrap and rework represent opportunities for substantial financial gain.

5. Reducing Variation

A process may be performing with unacceptable consistency, meaning its internal variation is too high. Excessive variation can result from many causes. Sometimes it is due to the lack of having or following standard operating procedures. At other times, excessive variation is due to certain hard to control inputs that affect the critical output characteristics of the process.

6. Making a Process Robust

Designing an item so that it is robust calls for a special experimental effort. It is possible to stress the item in the design lab and so determine the critical components affecting its performance. A different gauge of armature wire might be a solution to
the starter motor but so might be many other alternatives. The correct combination of factors can be found only by experimentation.

2.6.4.2. Optimization parameters

Variables: These are the measurements, values, which are characteristics of the data.

Two types

1. Independent variables

Which are not dependent on any other value? They may be formulation and process variable. Directly under the control of formulator Eg: conc. Polymer-drug ratio, lubricant, compression pressure.

2. Dependent variables

They are direct result of any change in the formulation or process and they are popularly known as Response variables

Factor: Factor is an assigned variable such as concentration, temperature, lubricating agent, drug to polymer ratio, polymer to polymer ratio or polymer grade. A factor can be qualitative or quantitative.

A quantitative factor has a numerical value to it e.g., concentration. (1%, 2%…. So on), drug to polymer ratio (1:1, 1:2…etc).

Qualitative factors are the factors, which are not numerical also known as categorical. Eg: Polymer grade, Polymer type, type of equipment, etc. These are discrete in nature.

Levels: The levels of a factor are the values or designation assigned to the factor, for e.g. in concentration (factor) 1% will be one level, while 2% will be another level.

Two different plasticizer are levels for grade factor. Usually levels are indicated as low, middle or high level.

Coding: The process of transforming a natural variable into a non dimensional coded variable, so that the central value of experimental domain is zero is known as coding.

Normally for ease of calculation the numeric and discrete levels are converted to –1 (low level), +1 (high level) and 0 (middle or central level).

Response: Response is mostly interpreted as the outcome of an experiment. It is the effect, which we are going to evaluate i.e. Disintegration time, Duration of buoyancy, Thickness, $t_{1/2}$ etc.

Effect: The effect of a factor is the change in response caused by varying the levels of the factor. This describes the relationship between various factor and levels.

Interaction: It is also similar to effect, which gives the overall effect of two or more variables (factors) of a response. Depending upon whether the change in the response
is desired (positive) or undesired (negative), the phenomenon of interaction may be described as synergistic or antagonistic. For example, the combined effect of lubricant (factor) and drug conc. (factor) on dissolution (response) of a tablet. Once the DoE is conducted according to the chosen statistical design, yields a series of data as response variables. Such data can be suitably modeled to generate mathematical relationships between the dependent and independent variables. The graphical representation of the mathematical relationships is known as response surface.

A response surface plot is a 3-D graphical representation of a response plotted between two independent variables and one response variable and it helps us to understand the behavior of the system by demonstrating the contribution of the independent variable (figure 2.7).

![Response Surface Plot](image)

**Figure 2.7 Response surface plot (3-D graphical).**

*Contour plot:* A 2-D slices of corresponding 3-D graph. It is the geometric illustration of a response obtained by plotting one independent variable against another, while holding the magnitude of response and other variables as constant (figure 2.8).
2.6.5. Factorial design

- Factorial designs are used primarily for screening significant factors.
- It is used sequentially to model and refine a process.
- It uses dimensional factor space at the corner of the design space.
- It helps for simultaneous determination of the effect of several factors.
- One can investigate 2 to 15 factors using 4 to 256 runs.

It is an experimental design, which uses dimensional factor space at the corner of the design space. Factorial designs are used in experiments where the effects of different factors or conditions on experimental results are to be elucidated. These are the design of choice for simultaneous determination of the effect of several factors and their interaction.

The number of experiments is given by $2^n$, where ‘n’ is the number of factors studied at 2 levels i.e., at high (+) and low (-) levels.

2.6.5.1. $3^2$ full factorial design

It is the two factor three level factorial design where two factors are considered each at three levels leads to nine experiments given as follows
Table 2.6 Levels and factors for $3^2$ full factorial design.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Factor $X_1$</th>
<th>Factor $X_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>7</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>8</td>
<td>+1</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>

Where, -1 denotes the low level of the factor, 0 denotes the middle level of the factor, and +1 denotes the higher level of the factor.

**Mathematical model:** Normally they are referred to simply as model, is an algebraic expression defining the dependence of a response variable on the independent variables that is the variables whose optimum values we are trying to find.

For $3^2$ full factorial design the polynomial regression equation is:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$ (Equation 2.5)

where, $Y$ is the dependent variable, $\beta_0$ is the arithmetic mean response of the nine runs, and $\beta_1$ and $\beta_2$ are the estimated coefficient for the factors $X_1$ and $X_2$. The main effects ($X_1$ and $X_2$) represent the average result of changing one factor at a time from its low to high value. The interaction terms ($X_1 X_2$) show how the response changes when two factors are simultaneously changed. The polynomial terms ($X_1^2$ and $X_2^2$) are included to investigate nonlinearity.

$3^2$ full factorial design is used by many of the researchers for the development of the drug delivery systems$.161$,$162$,$163$,$164$,$165$. 