"DEVELOPMENT OF POLYMERIC NANOPARTICLES BASED VAGINAL FILM OF FLUCONAZOLE"

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

BY

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May, 2018

CERTIFICATE

This is to certify that the dissertation work entitled "Development of polymeric nanoparticles based vaginal film of Fluconazole " submitted by Mr. Jagat Maniyar with Regn. No. (16MPH106) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Development of polymeric nanoparticles based vaginal film of Fluconazole", is based on the original work carried out by me under the guidance of Dr. Renuka Mishra, Assistant Professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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Development of polymeric nanoparticles based vaginal film of Fluconazole

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The aim of the present work was to develop nanoparticle based fast dissolving vaginal film containing Fluconazole. Fluconazole is a triazole derivative used in the treatment of vaginal candidiasis. Polymeric nanoparticles were made using Chitosan and sodium tripolyphosphate (TPP) as a cross linking agent. Chitosan contains positive charge due to the presence of amino groups and TPP is polyanion which acquires opposite charge and acts as cross linking agent. This cross linked structure provides entrapment to the drug molecule. Nanoparticles were made using Ionic gelation method. Blank nanoparticles were evaluated for particle size, Polydispersity Index (PI) and zeta potential. Optimized batch of blank nanoparticles had 133.5 nm particle size, 0.239 PI and +30.7 mv zeta potential. Polymeric nanoparticles were formed to provide controlled release. Fluconazole loaded chitosan nanoparticles were evaluated for particle size, PI and zeta potential. Optimized batch had 216 nm particle size, 0.117 PI and +33.4 mv zeta potential. Nanoparticles were also evaluated by transmission electron microscopy which confirmed the nano size of particles. Freeze drying was performed to obtain nanoparticles which were incorporated into the film for further evaluation. Fast dissolving film was prepared using HPMC E3 as a film forming polymer, Propylene glycol as a plasticizer and Sodium carboxymethyl cellulose as a suspending agent. Nanoparticles were loaded in fast dissolving film. Film was evaluated for % Elongation, Tensile strength, In-vitro disintegration time and Drug content. Fluconazole loaded fast dissolving film was compared with Fluconazole nanoparticles loaded fast dissolving film in terms of Tensile strength, % Elongation, In-vitro disintegration time and Drug content. Thus, nanoparticles loaded fast dissolving vaginal film will provide higher retention of the drug at the site of the application along with ease of application.

CHAPTER 1

AIM & OBJECTIVE

1. AIM

Aim of the present work is to develop nanoparticle based vaginal film for higher drug retention at the vaginal site of action along with ease of application.

Vaginal candidiasis is a fungal infection occurring in women irrespective of age groups. Candida albicans is a responsible species for this infection. The infection actually causes the imbalance of pH in vaginal area. It also inhibits the growth of useful bacteria. The symptoms include irritation, unpleasant smell and fluid discharge. Recurrent episodes of this infection are commonly observed. Around 75% of women go through vaginal candidiasis at least once in life.

Fast dissolving vaginal film can overcome drawbacks related to the conventional dosage forms like self cleansing action, continuous flow of vaginal fluid, irritation etc. Fluconazole is triazole derivative effective for the treatment of vaginal candidiasis. Various formulations of Fluconazole for topical application are available in the market. Fast dissolving film can disintegrate within seconds upon application in contact with vaginal fluid. HPMC E3 will be selected as film forming polymer, PEG 400 and Propylene glycol as plasticizer and Sodium Carboxymethyl cellulose as suspending agent. Film will be evaluated for various parameters like Tensile strength, % Elongation, In-vitro disintegration time and Drug content. Polymeric nanoparticles provide prolonged release of Fluconazole at the site of action. Nanoparticle based film provides higher retention at the site of action. Prolonged release of Fluconazole prevents the recurrent infection in women. Nanoparticles will be prepared using biodegradable, natural polymer chitosan. TPP will be used as cross linking agent by ionic gelation method. Polymeric nanoparticles will be evaluated for the particle size, PI and zeta potential. Using Freeze drying, nanoparticles collected will be evaluated for particle size, PI, zeta potential and % Entrapment Efficiency (%EE). Freeze drying technology will convert nanoparticles in solution form to the solid state leading to stabilization of the nanoparticles.

CHAPTER 2

INTRODUCTION

2. INTRODUCTION:

2.1 Anatomy of Vagina:

The human vagina is 10 cm (4 inch) long fibro muscular tube extending from the exterior to the uterine cervix. It is a passage for menstrual flow and childbirth. Vaginal wall consists of epithelial layer (stratified squamous epithelium) and muscular layer which allows the vaginal wall to increase in size and to stretch. The mucous layer forms protective layer and releases most of the lubricating secretions and it is also important for the absorption of drugs. A thin membrane called 'hymen' covers the vaginal passage. Vaginal wall is made of numerous folds called rugae provides distensibility and higher surface area helping in enlargement of the wall. Elasticity of the vaginal wall is due to the muscular layer. Thickness of the vaginal wall varies with the age. It starts increasing during puberty and decreases during menopausal phase. Vaginal wall has rich blood supply due to presence of enormous number of blood vessels in the wall. Blood vessels includes internal ilia, rectal, pudental artery. Vaginal route helps in bypassing the first pass metabolism because whatever the blood leaves the vagina enters the pheripheral circulation by rich venous plexus which initially returns to the iliac vein.

Vagina is a mucosal tissue. Though, it does not contain any gland it provides the passage for secretions. This secretion is a mixture of cervical fluid and transdute from blood vessels which forms moist film on the surface. The production of the vaginal fluid is influenced by the age factor. During reproductive age vaginal fluid is produced at the rate of 3-4g/hr. It gets reduced by almost 50% in the postmenopausal phase. Vaginal fluid production is also influenced by menstrual cycle, sexual arousal, diseased condition which affects the release of drug from the formulation.

The pH of vagina in healthy women is 3.8 to 4.2. Maintenance of pH is done by lactic acid which is produced by the *Lactobacillus acidophilus* present in vagina. Presence of these bacteria protects vagina from infection. In diseased condition like bacterial vaginitis, pH level is increased and reaches closer to neutral range. It is necessary to maintain the vaginal pH at lower side for the prevention of microbial growth and diseases.

2.2 Vaginal candidiasis:

Vagina is susceptible to the infection caused by bacteria due to the moist nature of the vagina and imbalance of pH. Maintenance of pH is required to prevent the bacterial growth and maintenance of vaginal hygiene. It is estimated that 70% of women suffer from the vaginal infection (either vaginosis or vaginitis) once in their lifetime. It causes unpleasant symptoms like vaginal irritation, fluid discharge, dysuria, redness, pain, burning and creates foul smell. It affects routine of women. Inflammation of vagina is called vaginitis and vaginosis refers to mild infection in the vagina. Vaginitis is most commonly seen infection in women.

Bacteria, fungi and protozoa are causative micro organisms for vaginal infection. Vaginitis is the overgrowth of the normally growing bacteria. Overgrowth of candida which is normally growing fungi is responsible for the vaginal infection. Normally, when vagina is healthy, Lactobacillus bacteria maintains the acidic pH of the vagina and prevent the vaginal infection by converting glycogen into the lactic acid. This acidic pH prevents the growth of other bacteria and pathogens.

About 90% of the vaginitis is caused by the yeast called candida albicans. It is usually present in healthy vagina. About 50% vaginitis caused by candida albicans is asymptomatic. Overgrowth of Candida albicans disturbs the balance of vaginal flora. Besides previously described symptoms there is appearance of “cottage cheese” like fluid discharge from the vagina. This condition is called candidiasis which is unacceptable.

Recurrence rate of vaginal candidiasis is very high. It is estimated that around 50% of women experience the recurrence of this infection. Diagnosis of vaginal candidiasis can be done by microscopic examination which can show the presence of mycelia form of candida albicans.

Treatment of candidiasis is done using azoles and antibiotics. Though antibiotics are less effective, nystatin based topical formulation is sometimes used. Most commonly azole based topical formulations are used for the treatment of vaginal candidiasis. Sometimes chemotherapeutic agents can also be used for the treatment of infection. The treatment should be done in a way that provides quick relief of signs within 24-48 hours of the administration. Topical azole treatment has success rate of around 80-90% of patients. In case of recurrence of the infection dose should be continued till 7-14 days through topical or oral treatment. Clinically this treatment is considered safe and tolerable. Several topical formulations are

available for the treatment of the infection. Single dose of 150 mg Fluconazole is found to be effective in the treatment.

2.3 Vaginal Film and Nanoparticles:

2.3.1 Vaginal Film

Film is a novel dosage form which is in the form of a thin strip intended to be used for topical application in vaginal drug delivery system. When it comes in contact with the vaginal fluid, it gets dissolved rapidly and prevents the leakage of dosage form from the vaginal tract. It consists of hydrophilic polymer, Fluconazole(Flz), permeation enhancer, suspending agent, plasticizer, disintegrant. Permeation enhancer enhances the permeation of the dosage form from the wall of the vagina. Plasticizer provides good tensile strength and elongation property.

Advantages of film

- Ease of application
- Overcomes the leakage problem of conventional dosage form
- Easy transportation
- Possibility of large scale production
- Easy storage and packaging

2.3.2 Types of film

- Fast dissolving film
- Controlled release film

Fast dissolving film

Fast dissolving film when comes in contact with liquid quickly dissolved within seconds. Due to fast dissolving property it is convenient for the pediatrics and geriatrics who have swallowing problems. Film is made up of water soluble polymer, plasticizer and suspending agent, sometimes superdisintegrant is also added. Fast dissolving film in case of vaginal drug delivery provides ease of application as it overcomes the problems like leakage of the dosage form and irritation.

Controlled release film

Controlled release film provides the release of drug at the site of action for prolonged time. When it comes in to contact with the liquid, it starts to swell and releases the drug in controlled manner.

2.3.3 Composition

Film forming Polymer

Polymer is used for the formation of backbone of the film. Various polymers like Hydroxypropyl cellulose, Hydroxymethyl cellulose, Pectin, Chitosan, Polyvinyl alcohol are used as film forming polymer. Sometimes these polymers are also used in combination for the desired characteristics of the film. The amount of polymer decides the disintegrating property of the film and robustness of the film.

Plasticizer

It is used in the formulation to reduce the brittleness of the film and provides the flexibility to the formulation. It improves the property by shifting the glass transition temperature to lower side. Glass transition temperature is a temperature at which polymer converts from glassy state to rubbery state. When heat is applied, at glass transition temperature (T_g) molecules that are locked in short chain length start to move around so amorphous rigid structure changes to the flexible structure. Various plasticizers like propylene glycol, glycerol and lower molecular weight polyethylene glycols are used for the formulation.

2.3.4 Methods of manufacturing

Various methods used for manufacturing of film include:

- Solvent casting method
- Semisolid casting method
- Hot-melt extrusion
- Rolling method
- Solid dispersion extrusion

Solvent casting method

It is the most commonly used method for the formulation of the film. In this method, first water soluble ingredients are dissolved using magnetic stirrer and viscous clear solution is

allowed to form. Separately other ingredients are also mixed in this solution. After formation of clear viscous solution, it is allowed to stand for the removal air bubbles. After removal of entrapped air, solution is poured to the petridish and allowed dry at 40^{0} C. The film is cut in to strips of the required size.

Advantages of solvent casting method over other methods

- Financially compatible method
- Uniformity of thickness
- Better drug loading can be achieved
- Thickness can be varied
- No such defects like die lines
- Industrially applicable

Semisolid Casting method

Semisolid casting method is a novel technology used for the formulation of film. This process is a complex of two processes- casting and forging to produce complex components. This process uses two types of polymers- hydrophilic and hydrophobic. Solution of water soluble film forming polymer is made and then it is added to the solution of acid insoluble polymer in ammonium or sodium hydroxide. Finally it is casted using heated drums.

Hot-melt Extrusion

Hot-melt Extrusion method is another method used for the preparation of transdermal and transmucosal drug delivery system. In this method feed is fed in feed inlet. Then the mixture is allowed to melt. Then this molten mass is passed through the orifice which produces the homogenous matrix. Extruder forces this molten mass into film die. API is introduced during the process at a higher temperature. So this method is not suitable for the thermo labile material.

Rolling Method

In this method mixture of polymer, water, mixture of water and alcohol and other excipients are introduced. This suspension is passed through the metering roller which allows specific amount to pass through the roller. Then API is mixed in the mixer for uniform distribution through the metering roller. Thickness of the film is dependent on the metering roller

application. After the film is formed, wet film is dried using dryer in the absence of external air currents.

Solid Dispersion Extrusion

Solid Dispersion Extrusion method is used for the improvement of the bioavailability and dissolution rate of the hydrophobic drug. In this method, dispersion of more than one API is dissolved in an appropriate solvent.

2.3.5 Evaluation parameters of vaginal film

1) Thickness:

Digital thickness gauge is used. Thickness is measured by placing this gauge at different places and then mean thickness is calculated.

2) % Elongation:

Digital Tensiometer (EIE Instruments, Ahmedabad) was used to measure % elongation. It is basically the increase in length of the film.

$$\% \text{ Elongation} = \frac{\text{Increase in length of strip}}{\text{Initial length of strip}} \times 100 \quad \ldots\ldots\ldots (1)$$

3) Tensile strength

It is measured using Digital Tensiometer (EIE Instruments, Ahmedabad). Here film is fit between two clamps. Then required force is applied to break the film is measured. Following Equation (2) is used to calculate the tensile strength of the film:

$$\text{Tensile strength} = \frac{\text{Load at failure}}{\text{Film thickness} \times \text{Film width}} \quad \ldots\ldots\ldots\ldots (2)$$

4) In-vitro Disintegration study

3ml SVF (Simulated Vaginal Fluid) was added in petridish and film (3cm×3cm) is placed in the center. Petridish is rotated using orbital shaker. Time for the film to disintegrate is noted. This test was performed three times and mean value is considered.

5) Drug content

A strip of film (3cm×3cm) was put in 15 ml water and 5 ml of methanol was added to this solution. Drug content was determined by UV spectrophotometer at 261 nm.

2.4 Vaginal Nanoparticles based system

Using Nano technology particles can be converted in to nano size (10^{-9} m). This provides the innovative ways to use the nano technology for novel drug delivery system. It helps in reducing side effects and increases the efficacy of the active ingredient which belongs to BCS class I and II. It provides better release profile compared to the conventional dosage form. Polymeric nanoparticles are used for better controlled release of the API through the cross linking structure.

From the stability point of view, nanoparticles show better stability in the suspension form or solution form. Currently nanoparticle technology find the vast application in the formulation of various dosage forms intended for the treatment of various disease and disorder. Using nanoparticle technology targeted drug delivery system can be achieved.

2.4.1 Methods for the preparation of the nanoparticles

- Ionic gelation method
- Emulsification and crosslinking method
- Emulsion droplet coalescence
- Emulsion solvent diffusion
- Reverse micellization
- Desolvation

Ionic Gelation Method

Generally this method is used for the preparation of chitosan based nanoparticles. In this method, specified concentration of polymer is solubilized. Then specified concentration of cross linking agent is added to the solution. Cross linking agent should have opposite charge compared to the polymer. Using magnetic stirrer, cross linking agent is added to the polymeric solution till the solution gets opalescent. Modified methods like ultra sonication method are used to obtain required particle size. Ultra sonication is carried out for the specified time as per optimization process for better particle size and better stability.

Emulsification and Cross linking Method

It was first method to be used for the formation of chitosan nanoparticles. In this method w/o emulsion is prepared and then cross linking agent is added to this emulsion. Cross linking agent hardens the formed droplets. Covalent cross linking occurred between amino group of chitosan and aldehyde group of glutaraldehyde. This bonding forms the nanoparticles.

Emulsion Droplet Coalescence

This method is derived from the emulsification and cross linking method. Formerly, this method was developed for the preparation of the microparticles but later it was modified for the preparation of chitosan nanoparticles. Chitosan is allowed to be dissolved in the solution of gadolinium and then this solution is added in to the liquid paraffin. On the other side mixture was prepared using Gadolinium solution and NaOH. Then these two solutions were simultaneously passed through the high speed homogenizer. In this way, chitosan nanoparticles were formed.

Emulsion Solvent Diffusion

This method is developed on the basis of partial miscibility of an organic solvent with water. For the preparation of chitosan nanoparticles, organic phase containing hydrophobic drug is added to the aqueous solution containing chitosan. This forms the o/w emulsion which is subjected to the high-pressure homogenization. Here acetone diffuses to the aqueous phase which decreases the solubility of chitosan and leads to the formation of nanoparticles.

Desolvation

The principle of this method is the formation of chitosan nanoparticles due to the favourable interaction between salt and water. As salt enters in to the solution of chitosan, desolvation of the surrounding water occurred. Water has higher affinity for the salt compared to its affinity for the polymer. This process leads to the insolubilization of the polymer and precipitation. This forms chitosan nano carriers

2.4.2 Evaluation of nanoparticles

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy is used for the imaging of the nano sized materials. A beam of electrons is passed through the prepared sample on copper grid and interacting with the specimen when it goes through the sample. TEM produces two-dimensional images. TEM is more preferred over Scanning Electron Microscopy (SEM) due to its higher resolution than SEM. TEM is used to image the crystalline structure, grain boundaries and core-shell-structure. Using TEM images are produced on fluorescent screen.

Particle size analysis

Particle size analysis was done using HORIBA Nano Particle Analyzer SZ-100. Principle of this instrument is scattering of light depending on the size of the particles. To analyze the size of the nanoparticles in the suspension, suspension is filled in the cuvette. Sometimes for the better measurement of the particle size dilution is carried out. It also shows polydispersity index (PI) which shows whether the particles are mono disperse or poly disperse.

Figure 2.4.2.1 HORIBA Nano Particle Analyzer SZ-100

Zeta Potential

Zeta Potential analysis was also done using HORIBA Nano Particle Analyzer SZ-100. Zeta potential gives the idea about the stability of the particles. Zeta potential measurement is

based on the charge present on the surface of the nanoparticles. For zeta potential measurement suspension is filled in zeta potential cell which has 100µl volume.

%Entrapment Efficiency (%EE)

Encapsulation of Fluconazole was determined by dispersing the weighed amount of Fluconazole in specific amount of acetic acid to hydrolyze the polymer in order to allow Fluconazole to be released. Then 1 ml methanol was added to dissolve Fluconazole. Then solution was filtered to

remove impurities. It was assayed using UV spectrophotometer at 261nm.

$$\%EE = \frac{\text{Mass of drug in nanoparticles} * 100}{\text{Mass of initial drug used}} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

2.5 Freeze Drying

Figure 2.5.1 Freeze Dryer

Freeze drying is the process of removing water directly by converting ice in to the vapor through sublimation without converting to liquid form. During freeze drying process, vacuum is applied in such amount that boiling point of the water lowers and directly ice form converts into the vapor at very low temperature (-81^0 C). In this manner here drying is carried out at very low temperature. This phenomenon is useful for drying of product which is heat sensitive. Freeze drying proceeds in two phases

- Freezing phase
- Drying phase

Freezing phase

During this phase freezing of the solution is done at -45⁰ C. Solution is converted in to solid ice form. Before freezing process cryoprotectant is added. During this stage there is possibility of rupturing of product due to the formation of crystals. Cryoprotectant protect the product from damage that occur due to the crystals.

Drying phase

Drying process occurred in further two phases- Primary drying and Secondary drying.

During **Primary drying**, vacuum is applied which lowers the boiling point of the solvent. Here drying mainly occurred at the temperature below product's critical temperature. In this temperature goes up to -81⁰ C. At this temperature and pressure ice directly converted in to vapour form due to sublimation.

During **Secondary drying**, some amount of heat is applied to remove the bound water from the product. This assures the stability of the product. Heat is applied in the controlled manner to prevent the degradation of the product.

2.6 Fluconazole

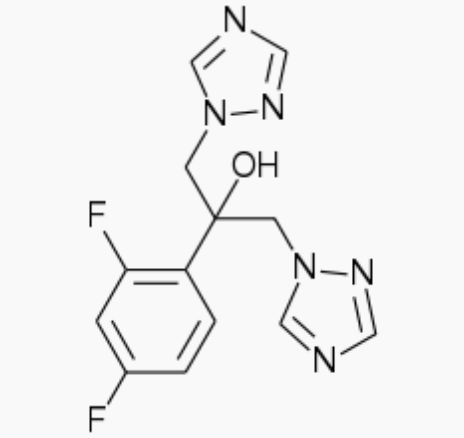

Figure 1.5 Structure of Fluconazole

Physicochemical parameters:

Chemical formula: $C_{13}H_{12}F_2N_6O$

Solubility: Slightly soluble in water

Melting point: 135-136.5ºC

Partition coefficient pKa: 2.03

Indication

To treat fungal infection

Mechanism of action:

Fluconazole converts lane sterol to ergosterol. Azoles also inhibit the transformation of candida yeast cells transformation into hyphae.

2.7 Chitosan

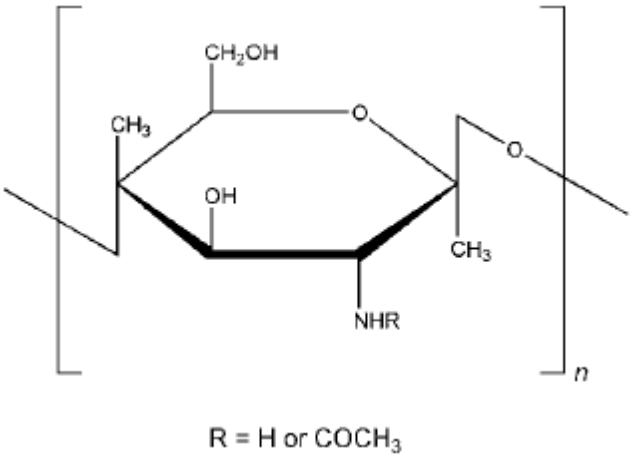

Figure 1.6 Structure of Chitosan

Chitosan is deacetylated product of chitin. Chitosan has various grades. Grades are varied depending on the molecular weights ranging from 10000-100000. Degree of deacetylation has a range of 66% to 99.8%.

Synonyms

Deacetylated chitin

Chemical Name

Poly-b-(1, 4)-2-Amino-2-deoxy-D-glucose

Category

Disintegrant, film-forming agent Coating agent, mucoadhesive, viscosity-increasing agent, tablet binder.

Solubility:

Sparingly soluble in water and practically insoluble in ethanol (95%) or other organic solvents and at pH above approximately 6.5 neutral or alkali solutions.

Stability:

Chitosan stable is a material at room temperature. It is hygroscopic after drying.

2.8 Hydroxypropyl Methyl Cellulose(HPMC)

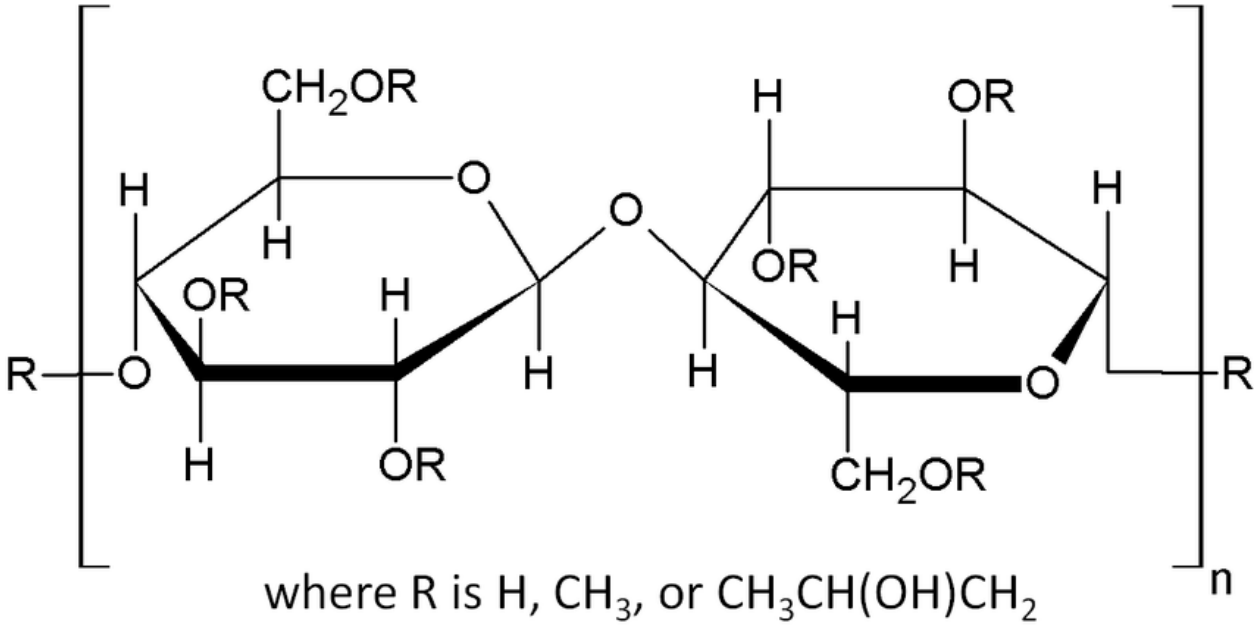

Figure 1.7 Structure of Hydroxypropyl Methyl Cellulose(HPMC)

Synonyms

hydroxypropyl methylcellulose (HPMC)

Chemical Name

Cellulose HydroxyPropyl Methyl Ether

Grade

HPMC E3 LV PREMIUM EP

Category

Bioadhesive, coating agent, controlled release agent, emulsifying agent, dispersing agent, film forming agent, solubilizing agent.

Solubility

Soluble in cold water.

Stability

In general hypromellose is stable material. Solutions are stable at 3-11 pH. It shows sol-gel transformation upon heating and cooling respectively. Aqueous solutions are more stable during long term storage. As temperature increases viscosity increases. When it is used in ophthalmic solutions as a viscosity increasing agent; preservative is used to prevent microbial spoilage. It should be stored in well closed container and in cool place.

CHAPTER 3

LITERATURE

REVIEW

3. Literature Review:

3.1 Literature Review on Vaginal Film

Zhang W et al developed film containing EFdA that could be used in case of HIV. 3^2 Full factorial design and desirability function for the comparison of two variables - ratio of polymers and concentration of plasticizer was carried out. Four responses tensile strength, elongation at break, toughness and elasticity modulus were evaluated. Optimized film consisted of PVA, HPMC E5, Propylene Glycol (7:3:3, w/w) and was compared with marketed product VCF film. Optimized film showed >95% release of drug over all media after 60 minutes. Permeability studies showed no significant differenfce in cumulative permeated amount between EFdA film and free EFdA. Epithelial study of EFdA loaded film showed much lower toxicity as compared to VCF film.

Ayman A et al developed Dapivirine containing fast dissolving film. Various charateristics water content, mechanical strength, drug release profile, permeability, compatibility with lactobacilli and bioactivity were characterized. Polymer concentration was optimized to ensure the dispersion of the Dapivirine in the film. The fast dissolving film contained PVA (38.3%) , HPMC 4000 (19.1%), PEG 8000 (25.5%), glycerin (7.9%) and propylene glycol (7.9%) In vitro and exvivo models used to confirm the anti- HIV activity of the optimized film of Dapivirine. In vitro bioactivity study was compared with NNRTI UC781. It further establishes the activity against HIV infection. Dapivirine showed fast release; 50% released in less than 10 minutes. Stability of the formulation established over the 18 months.

Ham A et al developed quick dissolving film for the delivery of Pyrimidinedione, IQP-0528 through the vaginal route. 0.1% w/w of IQP- 0528 film was made using PVA- 403 as a polymer. Film was composed of PVA- 403 (13.77w/w), glycerin (3.44%w/w), PEG (6.89%w/w), HPMC (6.89%w/w), propylene glycol (68.85%) and IQP- 0528 (0.1%w/w). Various parameters were evaluated like moisture content, pliability, thickness, color, appearance, and in-vitro disintegration. Dissolution study was performed using US apparatus-4 (flow through cell) and the drug release pattern was established. Film disintegrated within 10 minutes with over 50% release and total drug released after 30 minutes. Film did not show

toxic effect when it was established using lactobacillus strains. Stability of the formulation was established over 12 months under two environmental conditions as recommended by ICH.

Mishra R et al prepared the mucoadhesive vaginal film of Clotrimazole using Hydroxy propyl cellulose and sodium alginate which is used locally the treatment of vaginal candidiasis. Propylene glycol and Polyethylene glycol-400 were optimized as plasticizers. 3^2 full factorial design was used for the optimization of the vaginal film using two variables amount of polymers and concentration of permeation enhancer. Optimized batch showed in-vitro disintegration time 18 min, drug content 99.83%, tensile strength 502.1 g/mm^2 and 77% drug diffusion in 6 hours.

3.2 Literature review on preparation of Nanoparticles

Calvo P et al introduced a novel approach for the preparation of nanoparticles to overcome the limitations related to the previously formed nano carriers for the protein loading. This new technique called ionic gelation process involves the mixture of two aqueous phases at room temperature. Developed the nanoparticles using chitosan, propylene oxide and polyanion sodium tripolyphosphate. Nanoparticles have been evaluated for the particle size and zeta potential. Size and zeta potential can be varied by varying the concentrations. Protein loading capacity has been proved using bovine serum albumin (BSA) model. Nanoparticles showed continuous release of the entrapped protein up to 1 week.

Tang ZX et al established the use of chitosan nanoparticles as carrier for immobilized enzymes. Based on the effect of various factors such as molecular weight of chitosan, chitosan concentration, TPP concentration response surface methodology was applied. Concentration of chitosan, TPP concentration, pH of the solution showed major effect on the size of the particles. Optimized condition showed the minimum particle size of about 42 ± 5 nm. 2^{5-2} fractional factorial design was employed for designing the experimental model using factors. Morphological characterization has been done using scanning electron microscope.

Sharma K et al prepared chitosan nanoparticles using ionic gelation method. Optimized batch contained crosslinked chitosan - PEG 1000, showed greatest dispersibility and stability. Crosslinked chitosan- PEG 1000 showed aerodynamic diameter of 4.92 ± 0.3 μm which

proved to be suitable candidate for the delivery of therapeutic agents. Nanoparticles were prepared using chitosan:TPP (1:5) ratio under constant stirring. Followed by the addition of PEG in the ratio of chitosan-TPP-PEG (5:1:30) which showed no aggregation. Particle size, zeta potential and particle size dependent on the pH of the solution were evaluated.

3.3 Literature review on Fluconazole

Chopra A et al developed chitosan nanoparticles containing Fluconazole for ocular delivery using Box-Behnken design. Ionic gelation used was used. Three responses were taken into consideration namely concentration of chitosan, concentration of NaTPP, volume of NaTPP. % Encapsulation Efficiency, Particle size, in-vitro cumulative release were evaluated. Optimized batch showed 471nm particle size, 63.1% Encapsulation efficiency, 39.19% in-vitro cumulative release in 7 hours. Optimized batch was compared with the marketed product (Zocon) and showed better properties in terms of ex vivo corneal permeation and corneal hydration.

Bachhav Y et al formulated and evaluated micro emulsion based gel of Fluconazole for vaginal delivery. Various gelling agents were used to formulate micro emulsion based gel of Fluconazole. Carbopol® ETD 2020 was used to gel the micro emulsion of Fluconazole. The bioadhesive potential and anti-fungal activity was evaluated in comparison to marketed product of Clotrimazole by in-vitro methods. In-vivo irritation study was conducted in rabbits. Clinical efficacy was also evaluated of Fluconazole micro emulsion based gel and Candid V® gel in humans suffering from vaginal candidiasis. Fluconazole micro emulsion showed 24 nm globule size, 0.98 polydispersity index. The pH of fluconazole microemulsion based gel was found to be 4.53 wwhich is equivalent to the vaginal pH. Spreadibility of the formulation was also evaluated which was found good in view of patient compliance. The fluconazole microemulsion based gel showed comparatively higher bioadhesive potential and anti fungal activity in compare to Candid V® gel. It showed no irritation signs in rabbits. Clinical studies showed faster onset of action then marketed product.

CHAPTER 4

EXPERIMENTAL WORK

4. EXPERIMENTAL WORK

4.1 Material Used

Table 4.1 List of Materials used

MATERIALS	COMPANY
Chitosan	Sisco Research Laboratories Pvt. Ltd., Mumbai
Sodium Tri-polyphosphate	Central Drug House Pvt. Ltd., New Delhi
Fluconazole	Yarrow Pharma, Mumbai
HydroxyPropyl Methyl Cellulose E3,E5	Colorcon Asia Pvt. Limited, Goa
Propylene Glycol (PG)	Central Drug House Pvt. Ltd., New Delhi
Polyethylene Glycol-400 (PEG-400)	Central Drug House Pvt. Ltd. , New Delhi
Carboxy Methyl Cellulose Sodium	Central Drug House Pvt. Ltd. , New Delhi
Acetic Acid	Sisco Research Laboratories Pvt. Ltd., Mumbai
Methanol	Renkem, Gujarat

4.2 EQUIPMENT USED

Table 4.2 List of Equipments

EQUIPEMENT	COMPANY
Digital Weighing Balance	SCALE-TEC, Vadodara
Magnetic Stirrer	Remi Laboratory Instruments, Mumbai
pH meter	Analab Scientific Instruments Pvt. Ltd, Vadodara
Hot Air Oven	EIE Instruments, Ahmedabad
Freeze dryer	DELVEC Pumps Pvt. Ltd, Chennai
Particle Size Analyzer	HORIBA Scientific nano Partica SZ-100, Mumbai
UV Spectrophotometer	Shimadzu UV-1800, Mumbai
Digital Thickness Gauge	Mitutoyo, Japan
Tensiometer	EIE Instruments, Ahmedabad
Ultra Sonifier Bath	EIE Instruments, Ahmedabad
Probe Sonicator	SONICS & MATERIALS, INC., USA
Franz Diffusion cell	Orchid Scientific, Maharashtra
Fourier Transform Infrared Spectrometer	JASCO FT/IR- 6100 Type-A, Japan

4.3 Preformulation study:

4.3.1 Melting point determination:

The temperature at which solid converts to liquid indicates the melting point. For melting point determination, digital melting point apparatus was used.

Table 4.3.1 Melting Point determination

Actual Melting Point	138 - 140º C
Observed Melting Point	141.5º C

Result and Discussion:

Observed melting point of Fluconazole was found to be 141.5º C was within the range as compared to the standard.

4.3.2 Composition of Simulated Vaginal Fluid (pH 4.2)

Table 4.3.2 Composition of simulated vaginal fluid (pH 4.2)

Composition in Water	g/l
Sodium Chloride	3.51
Potassium Hydroxide	1.40
Calcium Hydroxide	0.222
Bovine serum albumin	0.018
Lactic Acid	2
Acetic Acid	1
Glycerol	0.16
Urea	0.4
Glucose	5

4.3.3 Preparation of standard curve of fluconazole using 20% methanol and 80% simulated vaginal fluid (SVF)

100 mg of Fluconazole was dissolved in 20 ml methanol and 80 ml of SVF in 100 ml volumetric flask. From above solution 1, 2, 3, 4, 5, 6 ml was pipetted out and volume was made up to 10 ml using SVF with concentration ranging from 100-600 µg/ml. Absorbance of the resulting solutions was measured using UV spectrophotometer at 261 nm using SVF as blank. The standard curve was obtained by plotting Average absorbance v/s concentration (µg/ml).

Table 4.3.3 Standard curve of Fluconazole using 20% methanol and 80% SVF at 261 nm

Sr. No.	Concentration (µg/ml)	Absorbance			Average Absorbance ± SD
		1	2	3	
1	0	0	0	0	0
2	100	0.147	0.156	0.146	0.150±0.006
3	200	0.283	0.285	0.279	0.282±0.003
4	300	0.421	0.421	0.425	0.422±0.002
5	400	0.577	0.574	0.577	0.576±0.002
6	500	0.71	0.709	0.733	0.717±0.014
7	600	0.842	0.849	0.867	0.853±0.013

Figure 4.3.3 Graphical representation of standard curve of Fluconazole using 20% Methanol and 80% SVF

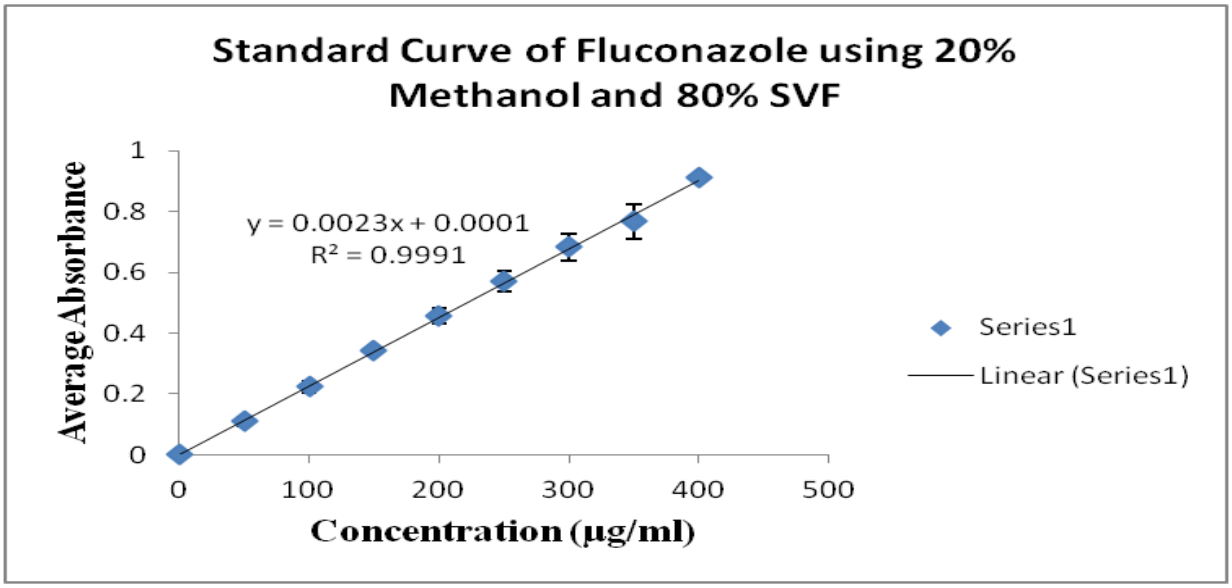

Table 4.3.4 Regression analysis for Standard curve of Fluconazole in 20% Methanolic SVF

Regression Parameter	Value
Correlation coefficient	0.999
Slope	0.0023
Intercept	0.0001

Result and Discussion

As per above observations, R^2 was 0.999 which is nearer to 1. Slope and Intercept were respectively 0.0023 and 0.0001. It gives linear graph in this concentration range of 100-600 µg/ml.

4.3.4 Fourier transform infrared spectroscopy (FTIR) of Fluconazole

FTIR of Fluconazole was performed from 4000-400 cm^{-1} wavelength using KBr pellets. Sample was initially dried using IR lamp. FTIR of test sample containing Fluconazole was compared with FTIR spectra of reference Fluconazole.

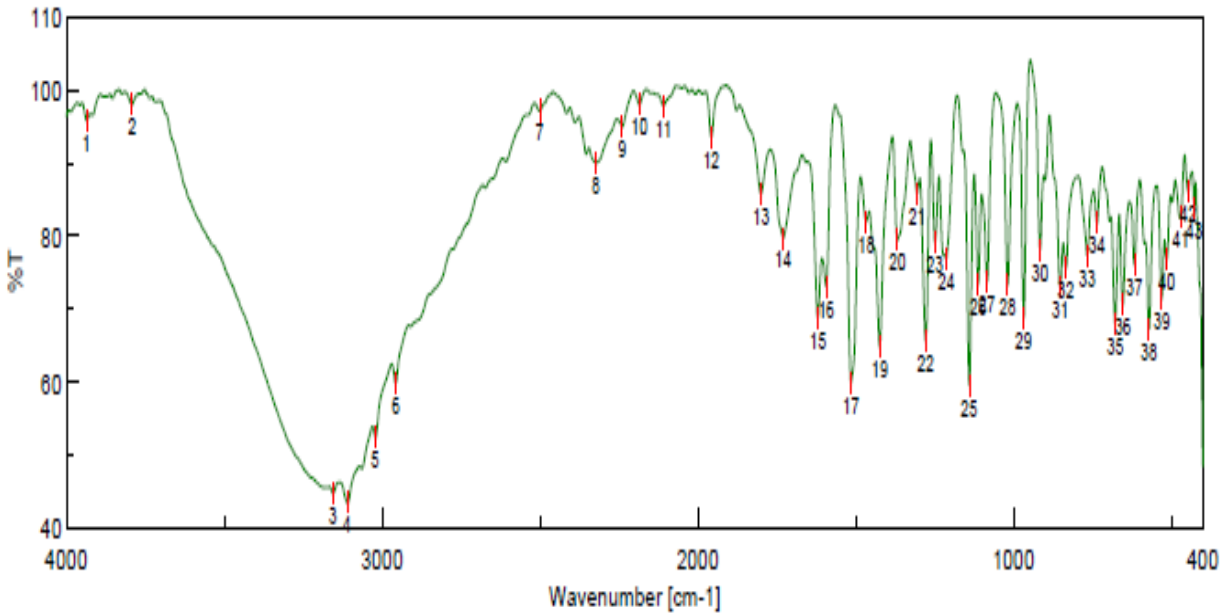

Figure 4.3.4 Test IR spectra of Fluconazole

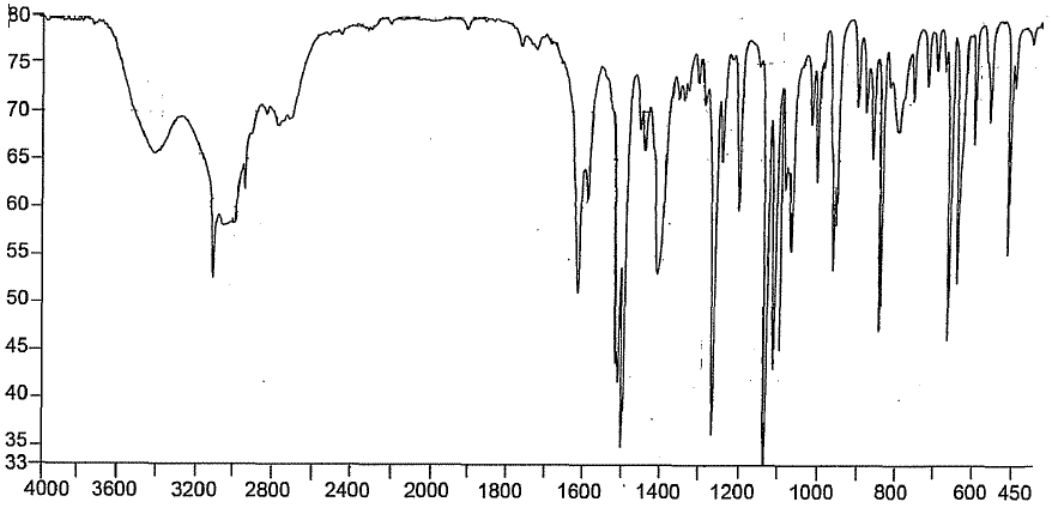

Figure 4.3.4 Reference IR spectra of Fluconazole (IP-2010)

Table 4.3.5 Interpretation of Reference and Test IR spectra of Fluconazole

Frequency of Reference sample (cm^{-1})	Vibration	Frequency of Test Sample (cm^{-1})
3550-3200	O-H Stretching	3154
2863-2843	CH_2 Stretching	2956.34
1260-1000	C=N Stretching	1619.91
860-843	C-H (Aromatic bending)	852.382

Result and Discussion

From above observations, it can be found that an IR spectra of test sample shows similar peaks as compared to IR spectra of reference. Frequency of test sample matched with each functional group of reference sample. It can be concluded that Fluconazole sample is pure.

4.4 Preliminary trials for the preparation of blank chitosan nanoparticles using Ionic gelation method

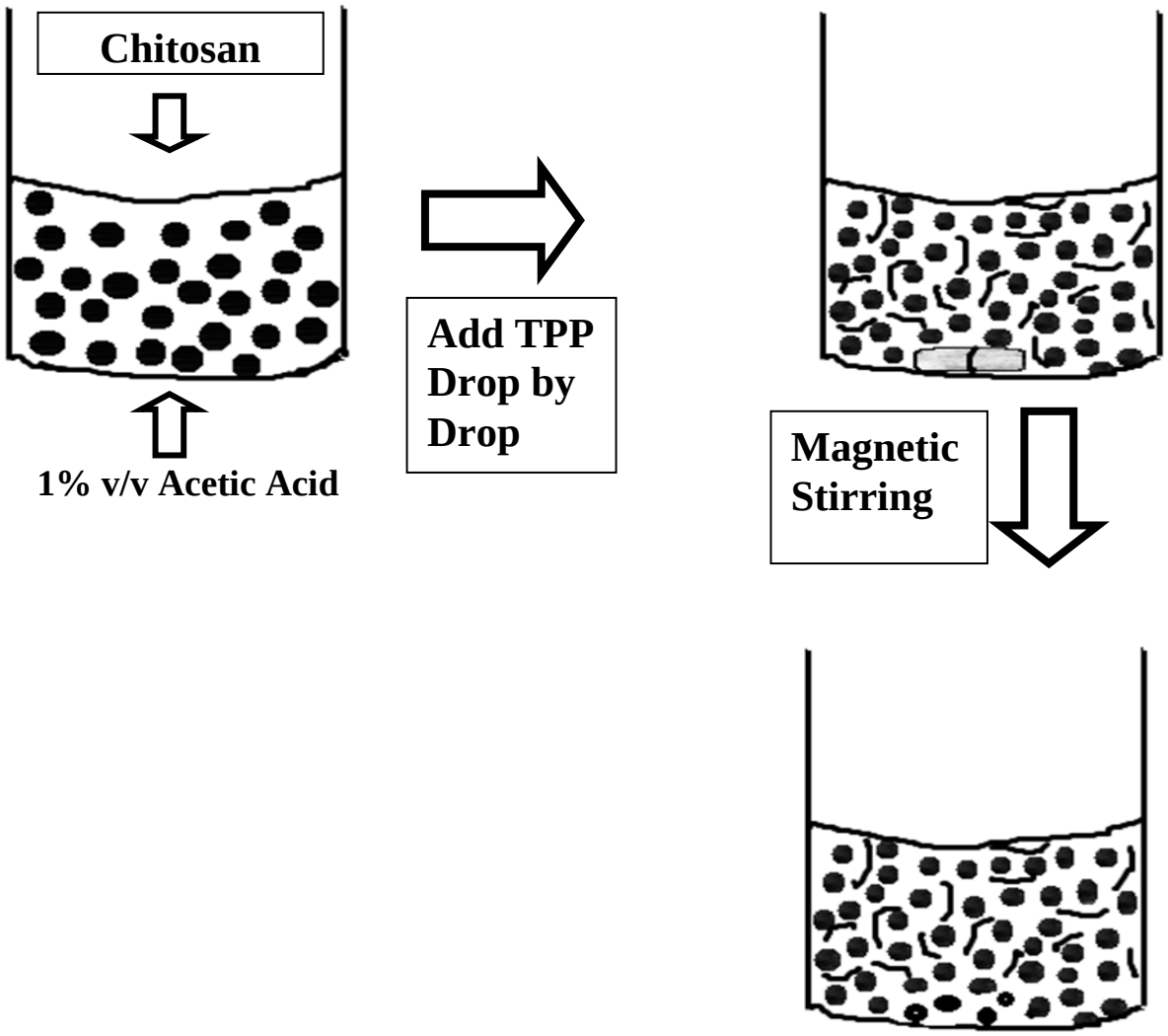

Figure 4.4 Schematic Diagram of Ionic Gelation Method

After reviewing literature, Ionic gelation method was finalized for the preparation of chitosan nanoparticles. In this method, various concentrations of chitosan with various concentration of acetic acid was prepared. This solution was then stirred overnight at 1200 RPM. pH was adjusted to 5.5 using 0.01 N NaOH. Then TPP was added to this solution as a cross linking agent in various amount. Batches containing blank chitosan nanoparticles were evaluated.

Table 4.4 Preparation and evaluation of blank Chitosan nanoparticles

Batch No.	Concentration of Chitosan (%)	Concentration of TPP (mg/ml)	Concentration of Acetic Acid (%)	Z- average	PI
CH 1	0.80	0.50	2.0	2065.6	0.731
CH 2	0.80	0.75	1.0	4864.0	3.853
CH 3	0.80	1.0	1.0	1346.0	2.465
CH 4	1.0	0.50	1.0	-	-
CH 5	2.0	0.50	1.0	2135.9	3.17

6

Result and Discussion

Table 4.4 shows that particle size and PI values were very high for batches CH 1 to CH 5. The particles were not mono disperse and impurities were also present. To remove impurities, distilled water was replaced with the double distilled water. The method was modified for improving the particle size. This did not result in any decrease in particle size and PI. Further trials were taken using probe sonication.

4.5 Modified ionic gelation method

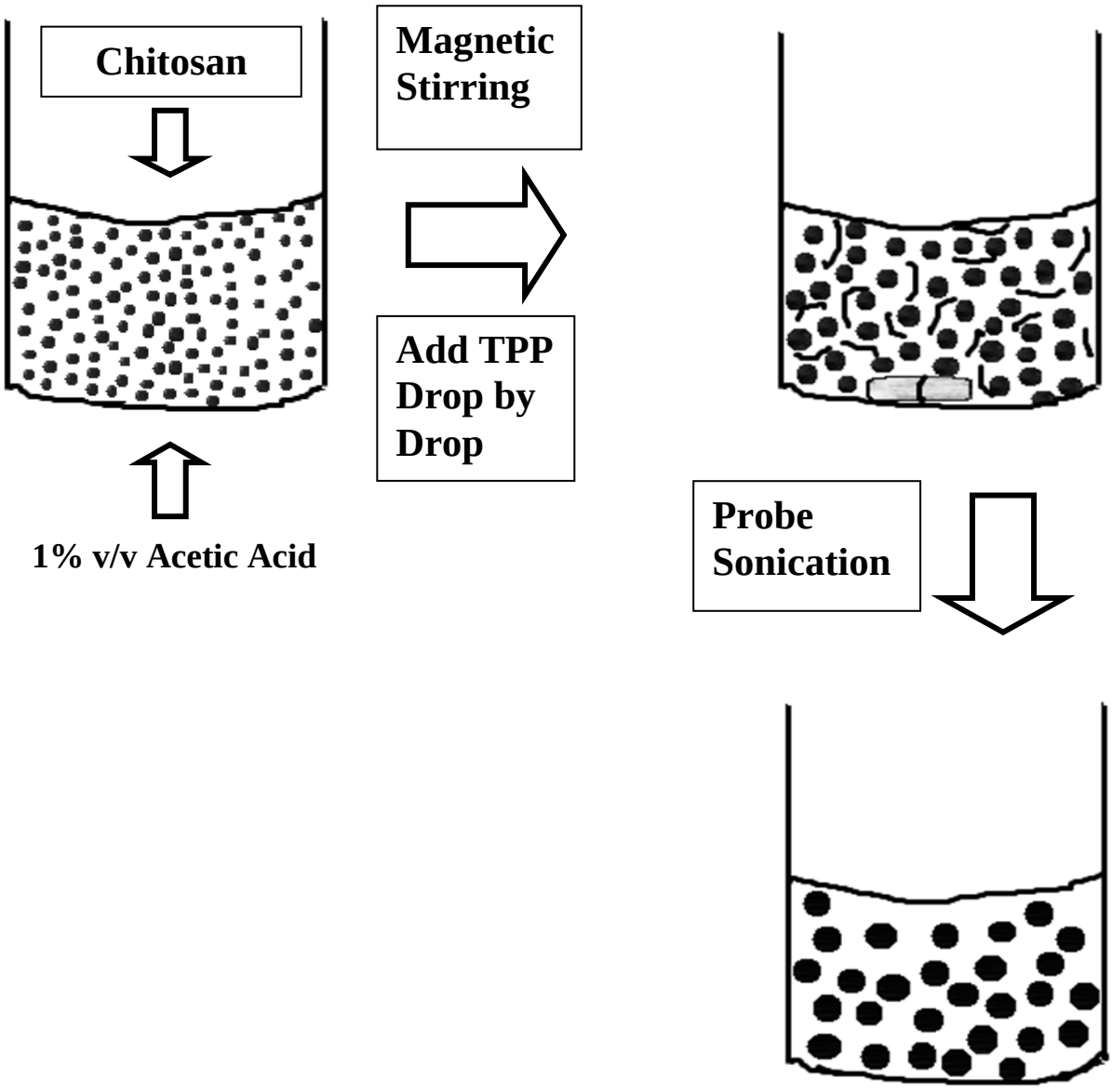

Figure 4.5 Schematic diagram of modified ionic gelation method

In this modified method Chitosan was dissolved in 1% v/v Acetic acid. The solution was passed through membrane filter to remove impurities. The solution was kept for overnight stirring at 1200 rpm. Then 2% TPP was added to the solution drop by drop under continuous stirring. Ultra Sonication in the range of (0-3 minutes) at 20% Amplitude was carried out.

Here, Chitosan and TPP concentration were kept 1% and 2% respectively throughout the formulation. After Ultra sonication, formed chitosan nanoparticles were evaluated.

Table 4.5 Preparation and evaluation of blank Chitosan nanoparticles

Batch No.	Ultra Sonication Time (Min)	Z- average (nm)	PI	Zeta Potential (mv)
CH 6	0	2611	2.677	-
CH 7	1	490.15	0.531	-
CH 8	2	693.95	0.493	-
CH 9	3	339.35	0.355	(+23.8)

Result and Discussion

Table 4.5 shows the effect of Ultra Sonication on Particle size and PI. From above observations it can be concluded that using Ultra Sonication, particle size reduction can be achieved and PI can be improved. Batch CH 9 showed lower particle size and lower PI compared to other batches. It also showed stable suspension. However, particle size and PI values were still unacceptable. Therefore, other trials were taken to optimize the concentration of chitosan and TPP keeping sonication time constant for 3 minutes.

4.6 Trials to optimize the concentration of Chitosan and TPP

Trials were taken by keeping sonication time constant for 3 minutes. Concentration of Chitosan and TPP were varied to study the effect on particle size, PI and Zeta potential.

Table 4.6 Preparation & Evaluation of blank Chitosan nanoparticles

Batch No.	Concentration of Chitosan (%)	Concentration of TPP (mg/ml)	Concentration of Acetic Acid (%)	Z-average	PI	Zeta Potential (mv)
CH 10	0.15	1.0	1.0	201.2	0.399	(+27.3)
CH 11	0.15	0.50	1.0	247.4	0.470	(+22.2)
CH 12	0.30	0.50	1.0	170.1	0.264	(+23.5)
CH 13	0.30	1.0	1.0	133.5	0.239	(+30.7)

Result and Discussion

Table 4.6 showed better results compared to the batches CH 6 to CH 9. It was observed that concentration of Chitosan and TPP had an impact on Particle size, PI and Zeta Potential of formulated nanoparticles.

4.7 Trials for the preparation of Fluconazole loaded Chitosan nanoparticles

Fluconazole was loaded into chitosan nanoparticles. As per the solubility profile, Fluconazole is freely in methanol (25% w/v). 300 mg of Fluconazole was dissolved in 1 ml methanol and then this solution was added to the solution containing chitosan in 1% Acetic acid. 1% TPP was added continuously drop by drop. Formulated nanoparticles were evaluated for particle size, PI and zeta potential.

Table 4.7 Preparation & Evaluation of Fluconazole loaded chitosan nanoparticles

Batch No.	Amount of Fluconazole (mg)	Concentration of Chitosan (%)	Concentration of TPP (mg/ml)	Z-average	PI	Zeta Potential (mv)
CHF 1	300	0.15 (100 ml)	1.0	597.2	0.449	(+23.5)
CHF 2	300	0.15 (50 ml)	1.0	417.4	0.266	(+17.6)
CHF 3	300	0.30 (50 ml)	1.0	349.6	0.267	(+27.9)
CHF 4	300	0.30 (50 ml)	1.0	216	0.117	(+33.4)

Result and Discussion

From the above Table 4.7 it can be concluded that batch CHF 4 shows lowest PI index indicating monodisperse system, 216 nm particle size and better physical stability. Further freeze drying was performed to convert the solution to the solid state for improving the stability and loading.

4.8 Freeze drying of Fluconazole loaded chitosan nanoparticles

Nanoparticles in solution form are not stable. To improve stability and conversion to solid form freeze drying was carried out. Prefreezing was done at -45^{0} C for 3 hours. Freeze drying was performed at -80^{0} C temperature and 0.290 mbar vacuum for 20 hours. Batch CH 4 was freeze dried, in which mannitol was used as cryoprotectant. Quantity of mannitol was 20% of the total solid content (chitosan, Fluconazole and TPP) present in the solution. Freeze drying was carried for the 20 hours cycle. Nanoparticles after freeze drying were evaluated for the properties.

Table 4.8 Evaluation of Freeze dried Fluconazole loaded nanoparticles

Batch No.	Amount of Fluconazole (mg)	Concentration of Chitosan (%)	Concentration of TPP (mg/ml)	Amount of Mannitol (mg)	Z-average	PI
FF 1	300	0.30 (50 ml)	1.0	90.8	-	-

Result and Discussion

Due to the higher amount of mannitol, the powder became hygroscopic and absorbed moisture. It could not be redispersed. Further evaluation could not be performed. Further trials were planned with 5% and 10% mannitol.

4.9 Freeze drying of Fluconazole nanoparticles

Quantity of mannitol was decrease to 10% (26 mg) and 5% (13 mg) of the total solid content and freeze drying was carried out for 20 hours.

Table 4.9 Evaluation of Freeze dried nanoparticles

Batch No.	Amount of Fluconazole (mg)	Concentration of Chitosan (%)	Concentration of TPP (mg/ml)	Amount of Mannitol (mg)	Z-average	PI	Zeta Potential (mv)
FF 1	300	0.30 (50 ml)	1.0	26	-	-	-
FF 2	300	0.30 (50 ml)	1.0	13	349.5	0.117	31.8

Result and Discussion

It can be concluded that when concentration of mannitol was 10%, it did not redisperse. When concentration of mannitol was decreased further to 5%, it was easily redispersed and stable.

4.10 Freeze drying of Fluconazole loaded nanoparticles using 5% mannitol

To confirm the reproducibility of nanoparticles, batch FF 2 was repeated.

Table 4.10 Evaluation of Freeze dried nanoparticles

Batch No.	Amount of Fluconazole (mg)	Concentration of Chitosan (%)	Concentration of TPP (mg/ml)	Amount of Mannitol (mg)	Mean of Z-average	PI	Zeta Potential ± SD (mv)
FF 2	300	0.30 (25 ml)	1.0	13	304.6	0.261	31.8±6.22

Result and Discussion

It can be observed that using freeze drying process nanoparticles can be converted to stable form. To formulate patient convenient dosage form and to improve its compliance, nanoparticles were required to be incorporated into film. Further trials will be taken to prepare blank film to incorporate Fluconazole nanoparticles.

4.11 Calculation of % Entrapment Efficiency (%EE) of chitosan naoparticles

Freeze dried fluconazole loaded nanoparticles were dissolved in 1% v/v acetic acid using magnetic stirrer at room temperature for 20 min to obtain solution. 1 ml of methanol was added into the solution as Fluconazole has high solubility in methanol. The solution was filtered through whatman filter paper. The filtrate was assayed spectrophotometrically at 261 nm using UV Spectrophotometer (Shimadzu UV-1800). Amount of drug solubilized was calculated from the standard curve of Fluconazole. The % yield of freeze dried nanoparticles was calculated using the formula-

$$\% \text{ Yield} = \frac{\text{Obtained nanoparticles}}{\text{Initial amount of nanoparticles in solution}} * 100$$

total amount of drug present in the polymeric nanoparticles were calculated. % Entrapment Efficiency was calculated using following equation:

%EE= Mass of drug in nanoparticles *100
Mass of initial drug used

Table 4.11 Trials for calculation of %Entrapment Efficiency

Batch No.	Amount of Fluconazole (mg)	Concentration of Chitosan (%)	Concentration of TPP (mg/ml)	Amount of Mannitol (mg)	%Yield	%EE
FF 1	300	0.30 (25 ml)	1.0	26	-	-
FF 2	300	0.30 (25 ml)	1.0	13	92.06%	94.94%
FF 3	300	0.30 (25 ml)	1.0	13	99.58%	96.66%

Result and Discussion

Batch FF 1 didn't show % EE as it could not be redispersed. Batch FF 2 and FF 3 showed 94.94% and 96.66 % EE respectively. From observations it can be concluded that chitosan nanoparticles have higher entrapment efficiency for Fluconazole.

4.12 TEM study

4.12.1 TEM study of Blank nanoparticles

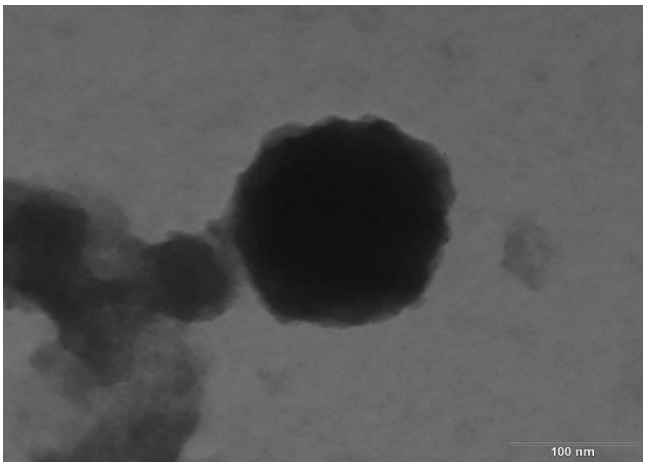

Figure 4.12.1 TEM study of blank nanoparticles

4.12.2 TEM study of Fluconazole loaded nanoparticles

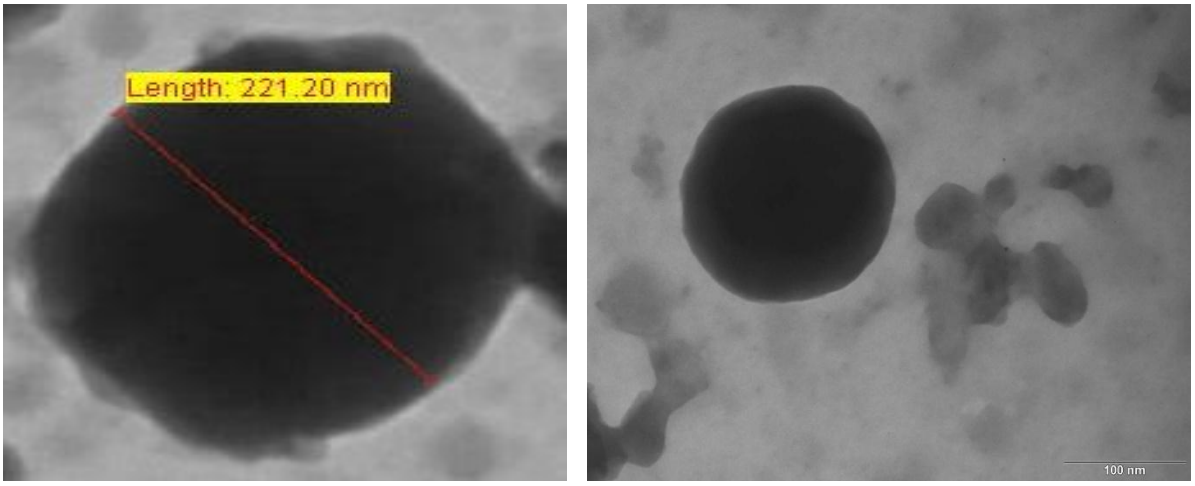

Figure 4.12.2 TEM study of Fluconazole loaded nanoparticles

Result and Discussion

Figure (4.12.1) shows blank chitosan nanoparticles with average size of 100 nm. Figure 4.12.2 (A & B) shows TEM of Fluconazole loaded chitosan nanoparticles with average size of 221.20 nm. TEM study confirmed the formation of nanoparticles in the range of 100- 250 nm. From above result it can be concluded that there is an increase in size of the

nanoparticles after loading of Fluconazole in nanoparticles. It confirmed the loading of Fluconazole in nanoparticles.

4.13 Preliminary trials for the screening of polymer

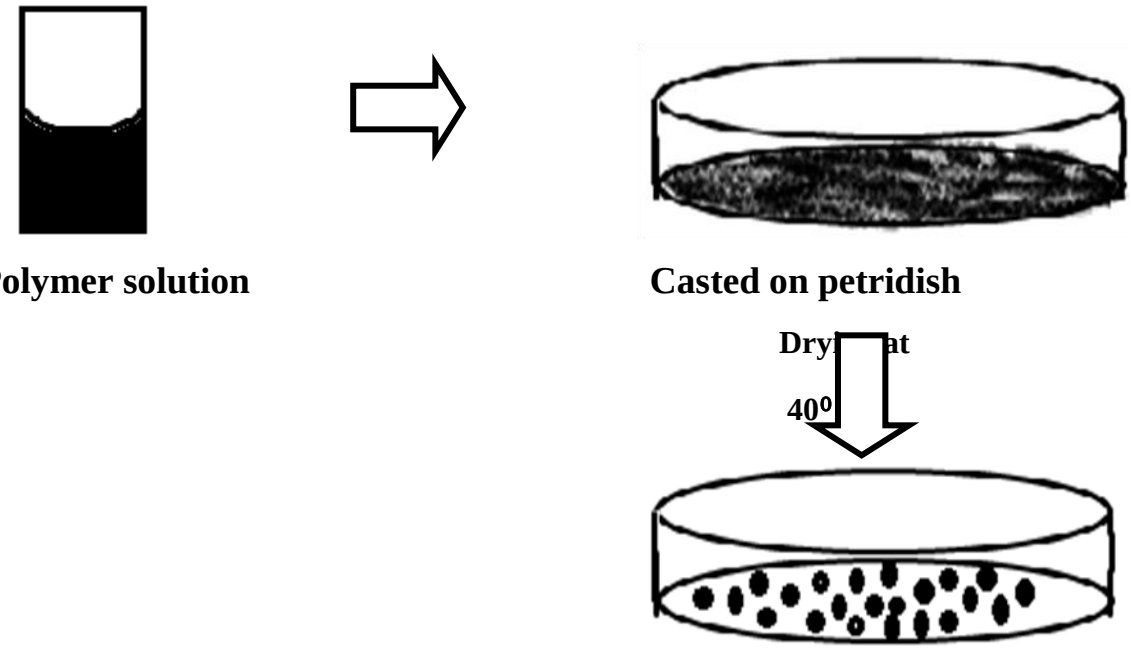

Figure 4.13 Schematic diagram of solvent casting method

Trials were taken for screening of polymers for the preparation of blank film. Hydroxypropyl Methyl Cellulose E3 (HPMC E3) , HPMC E5 and Polyvinyl Alcohol (PVA) were selected for the preparation of fast dissolving film. Films were evaluated for Tensile strength, % Elongation, In-vitro disintegration time and Drug content. Polymers were first dissolved in double distilled water using magnetic stirrer. The solution was allowed to settle to remove the air entrapment. This solution was then casted on teflon petridish and dried using hot air oven at 40^0 C for 24 h.

Table 4.13 Composition and Evaluation of fast dissolving film

Batch No.	Name of Polymer	Concentration (%)	Average Thickness (mm)	Tensile strength (N/cm^2)	% Elongation	In-vitro Disintegra-tion Time (sec)
PF 1	HPMC E5	1	0.017	0.039	-	10
PF 2	HPMC E5	2	0.020	0.026	-	42
PF 3	HPMC E3	1	0.015	0.025	-	17
PF 4	HPMC E3	2	0.018	0.022	-	3
PF 5	PVA	2	0.022	1.358	107.14	58

Result and Discussion

From Table 4.13, it can be concluded that batch PF 4 shows in-vitro disintegration time 3 seconds. Batch PF 4 had low tensile strength. Plasticizers were required to be added in further batches. % Elongation could not be obtained for batch PF 1 to PF 4. Batch PF 5 containing 2 % PVA had 107% elongation.

4.14 Effect of plasticizer on fast dissolving film

Plasticizers Propylene glycol and Polyethylene glycol were added to solution at different ratios of plasticizer: polymer to improve tensile strength. Plasticizer provides flexibility to film formulation. Batches PPF 1 to PPF 4 were prepared and evaluated.

Table 4.14 Effect of plasticizer on fast dissolving film

Batch No.	Polymer: Plasticizer ratio	Ratio	Concentration of polymer (%)	Average Thickness (mm)	Tensile strength (N/cm^2)	In-vitro Disintegration Time (sec)
PPF 1	HPMC E3: PG	1:0.2	2	0.0347	0.401	20
PPF 2	HPMC E3: PG	1:0.4	2	0.0313	0.463	18
PPF 3	HPMC E3: PEG 400	1:0.2	2	0.0617	0.235	36
PPF 4	HPMC E3:PEG 400	1:0.4	2	0.1003	2.550	27

Result and Discussion

From Table 4.14, it can be concluded that batch PPF 2 containing 0.4:1 of PG: HPMC E3 showed better tensile strength and low in-vitro disintegration time compared to other batches PPF 1, PPF 3 and PPF 4. In next trials, Fluconazole was incorporated in optimized blank film. All batches PPF 1 to PPF 4 did not show % elongation. This could be due to poor plasticization property.

4.15 Preparation of Fluconazole loaded fast dissolving film

Fluconazole was added to the solution containing 0.2:1 and 0.4:1 ratio of PG: HPMC E3. The solution was allowed to dry in hot air oven for 24 hours.

Dose calculation for Fluconazole

- ✓ Diameter of petridish : 8.5 cm
- ✓ Surface area of film: πr^2

$= 3.14*(4.25)^2$

$= 56.71$

- ✓ Area of film: $3 \times 3\ cm^2$
- ✓ Total number of films in a batch: 56.71/9

= 6 no. of films

- ✓ One film contains = 150mg

= 150mg * 6

= 945mg

- ✓ Casting surface: Teflon petridish

Table 4.15 Evaluation of Fluconazole loaded fast dissolving film

Batch No.	Polymer: Plasticizer ratio	Amount	Concentration of polymer (%)	Amount of Fluconazole	Film was not separated
PPF 5	HPMC E3: PG	1:0.2	2	945 mg	
PPF 6	HPMC E3: PG	1:0.4	2	945 mg	

Result and Discussion

Table 4.15 shows that when Fluconazole was added in the film formulation, film could not be separated. Fluconazole due to its insoluble form, settled down at the bottom of petridish. Thus film could not be obtained.

4.16 Role of ethanol as solvent for Fluconazole loaded fast dissolving film

Fluconazole was dissolved in 10 ml ethanol and added to the solution containing HPMC E3. The solution was casted on teflon petridish and allowed to dry in Hot air oven at 40^0 C for 24 hours.

Table 4.16 Evaluation of Fluconazole loaded fast dissolving film

Batch No.	Polymer: Plasticizer ratio	Amount	Concentration of polymer (%)	Amount of Fluconazole (mg)	Film was not formed
PPF 5	HPMC E3: PG	1:0.2	2	945	
PPF 6	HPMC E3: PG	1:0.4	2	945	
PPF 7	HPMC E3: PG	1:0.4	3	945	

Result and Discussion

Table 4.16 shows that when ethanol was used as a solvent for formulation of film containing Fluconazole, film was not formed.

4.17 Role of Sodium Carboxymethyl Cellulose (NaCMC) as suspending agent on film property

Trials were taken using Na CMC 0.5% (100 mg) and 1% (200 mg) as a suspending agent into solution containing 0.4:1 of PG: HPMC E3. Film was evaluated for tensile strength, % Elongation, In-vitro disintegration time and drug content.

Table 4.17 Evaluation of NaCMC based fast dissolving film

Batch No.	Polymer: Plasticizer	Concentration of polymer (%)	Amount of NaCMC	Amount of Fluconazole (mg)	Average Thickness (mm)	Tensile strength (N/cm^2)	% Elongation	In-vitro Disintegration Time (sec)
PPF 8	HPMC E3: PG	2	100mg (0.5%)	945	-	-	-	-
PPF 9	HPMC E3: PG	2	200mg (1.0%)	945	-	-	-	-
PPF 10	Total solid:PG	2	100mg (0.5%)	945	-	-	-	-
PPF 11	Total solid:PG	2	200mg (1.0%)	945	0.373	0.395	3.44	1 minute 59 seconds

Result and Discussion

Film could not be separated from batches PPF 8 to PPF 10. Batch PPF 11 could be separated and evaluated. It showed 113.71% drug content, 0.395 tensile strength, 3.44 % elongation and 1 minute and 59 seconds in-vitro disintegration time.

4.18 Incorporation of Fluconazole loaded polymeric nanoparticles in fast dissolving film

In previous batches blank fast dissolving films were prepared. In these trials, Fluconazole loaded chitosan nanoparticles were incorporated in optimized blank fast dissolving film. Freeze dried nanoparticles were dissolved in 20 ml distilled water. HPMC E3 and PG were added to the solution. The solution was kept for stirring on magnetic stirrer until clear

solution was formed. Film was evaluated for various parameters. To improve film properties, total solid: plasticizer ratio was taken. Suspending agents like NaCMC were not added.

Table 4.18 Composition and Evaluation of nanoparticles loaded fast dissolving film

Batch No.	Polymer: Plasticizer	Amount	Concentration of polymer (%)	Average Thickness (mm)	Tensile strength (N/cm^2)	% Elonga-tion	In-vitro Disintegr-ation Time (sec)
PNF 1	Total Solid: PG	1:0.4	2	0.292	0.472	33%	3 min 38 sec

Result and Discussion

Table 4.18 showed 0.472 N/cm^2 Tensile strength, 33% Elongation and 3 min 38 seconds in-vitro disintegration time. As Fluconazole loaded nanoparticles were easily soluble in distilled water. Amount of nanoparticles per film were calculated.

4.19 Calculation of Drug content

%Drug Content was calculated by dissolving film in 15 ml of water. In that 1 ml of Acetic Acid and methanol was added. This solution was assayed using UV spectrophotometer.

Calculation of amount of nanoparticles per film

- ✓ Amount of nanoparticles added in film: 300 mg
- ✓ Surface area of film: 56.71
- ✓ Area of film: 3 × 3 cm2
- ✓ Total number of films in a batch: 56.71/9

  = 6 no. of films
- ✓ Amount of nanoparticles in One film = 300/6

  =50

Table 4.19 Calculation of Drug Content

Batch No.	Polymer: Plasticizer	Amount	Concentratio of polymer (%)	Amount of nanoparticles in one film (mg)	%Drug content
PNF 1	Total Solid: PG	1:0.4	2	45.39	90.78%

Result and Discussion

Table 4.19 shows 90.78% Drug Content. Thus, the drug was uniformly distributed in the film.

4.20 Comparison of Fluconazole loaded fast dissolving film with Fluconazole nanoparticles based fast dissolving film

Table 4.20 Comparison of Fluconazole loaded fast dissolving film with Fluconazole nanoparticles based fast dissolving film

Fluconazole loaded fast dissolving film	Parameters	Fluconazole nanoparticles based fast dissolving film
0.373 mm	Average Thickness	0.292 mm
0.395 N/cm^2	Tensile strength	0.472 N/cm^2
3.44%	% Elongation	33%
1 min 59 sec	In-vitro disintegration time	3 min 38 sec
113.71%	% Drug Content	90.78%

Result and Discussion

Based on results obtained from Table 4.20, fluconazole loaded nanoparticle based film was transparent and clear in appearance compared to conventional fluconazole loaded fast

dissolving film which was translucent. Both the films had acceptable properties but mechanical properties were better for nanoparticle based film and in-vitro disintegration time was higher compared to conventional film.

CHAPTER 5

CONCLUSION

Conclusion

Nanoparticles based fast dissolving vaginal film of Fluconazole was prepared for treatment of vaginal candidiasis. Vaginal candidiasis is one of the common disease occurring in female. Fast dissolving film will avoid irritation as it will dissolve within seconds and nanoparticles will provide controlled release of Fluconazole. Film was made using solvent casting method. Ionic gelation method was used for the preparation of nanoparticles. HPMC E3 was selected as film forming polymer. Parameters like Tensile strength, % Elongation, In-vitro disintegration time and drug content were evaluated. Using PG as plasticizer, film showed better plasticity and tensile strength. Blank optimized batch contains 2% HPMC E3 with 0.4:1 ratio of plasticizer PG: polymer. Fluconazole was added to the solution to deliver 150 mg per film. Optimized batch containing 0.3% chitosan and 1% TPP showed 216 nm particle size, 0.117 PI and +33.4 Zeta potential. Fluconazole loaded nanoparticles showed 304.6 nm particle size, 0.117 PI and 96.66% EE. The drug loaded fast dissolving film was compared with Fluconazole nanoparticles based film.

CHAPTER 6

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