FORMULATION DEVELOPMENT AND OPTIMIZATION OF CLOTRIMAZOLE MICROENCAPSULATED MUCOADHESIVE VAGINAL GEL

A THESIS SUBMITTED TO

GAUHATI UNIVERSITY

IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE DEGREE OF

MASTER OF PHARMACY

IN

PHARMACEUTICS

BY

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JUNE 2013

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CERTIFICATE

This is to certify that the thesis entitled **"Formulation Development And Optimization Of Clotrimazole Microencapsulated Mucoadhesive Vaginal Gel"** submitted to Gauhati University for the partial fulfilment of the **Master degree in Pharmacy (Pharmaceutics)** is a faithful record of bonafide and original research work carried out by **Pranabjyoti Bashistha**, with ***Registration No. 048359*** of **2007-08** and ***Roll no - MP/11/03*** during the academic session 2011-2013 at Pharmaceutics laboratory/Department of Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS) under my supervisions and guidance.

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DECLERATION

I hereby declare that the matter embodied in the dissertation entitled "***FORMULATION DEVELOPMENT AND OPTIMIZATION OF CLOTRIMAZOLE MICROENCAPSULATED MUCOADHESIVE VAGINAL GEL***" is a bonafide and genuine research work carried out by me under the joint supervision of Dr. Suvakanta Dash (Principal, GIPS) , & Mr. Pulak Deb, Department of Pharmaceutics, *Girijananda Chowdhury Institute of Pharmaceutical Scienc, Hatkhowapara, Azara, Guwahati-17.* The work embodied in this thesis is original and has not been submitted the basis for the award of degree, diploma, associate ship or fellowship of any other university or institution.

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ACKNOWLEDGEMENT

Research is a never ending process involving a team of persons striving to attain newer horizons in the field of sciences. This study would not have been completed without the encouragement and co-operation of my teachers, parents, friends, well-wishers and relatives. I take this opportunity to express my deep gratitude to one and all.

I consider myself most lucky and privileged to work under the guidance of Mr.Pulak Deb, Asst. Professor, Dept. of Pharmaceutics, GIPS, Azara, Guwahati. His discipline, principles, simplicity, caring attitude and provision of fearless work environment will be cherished in all walks of my life. I am very much grateful to him for his valuable guidance and everlasting encouragement throughout my course.

I sincerely thank Dr. Suvakanta Dash, Principal, GIPS, Azara, Guwahati for his inspiration and for being a great facilitator.

I express my sincere gratitude to my teachers Dr. Abdul Baquee Ahmed, Asst. Professor, Dept. of Pharmaceutics, GIPS, Azara, Guwahati for his meticulous guidance and encouragement provided to me for the completion of my dissertation work. Also my sincere thanks to other teaching faculty for their timely help.

I wish to express my deepest gratitude to Dr. L.K.Nath, Dept of Pharmaceutics, Dibrugarh University for his help in providing me DSC analysis facility in completing the work.

My heartfelt thanks to my friends Ranjit, Samir, Ratul, Kuldeep, Rimlee, Adiya, Somi, Pallabi, Sanjay, Shiekh Sofiur, Pranjal, Tapash, Anupam, Trailokya for their support who by their honest opinions and diligence kept me lively.

I express my sincere thanks to Bipul Sarma, GIPS, Azara, Guwahati, for his help in all analytical work in completing my work.

Last, but not at least, I wish to express my gratitude towards “god-almighty” and my father and mother who gave me the strength and courage to fulfil my dream and has showered upon me their choicest blessings.

Thankful I ever remain.....

Date: 3/06/2013

Place: Azara, Guwahati **Pranabjyoti Bashistha**

To,
Lord Almighty
And
My very loving family

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ABBREVIATIONS	FULL FORM
FTIR	Fourier Trasform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
IR	Infrared
cps	Centi poise
Mg	Miligram
Hrs	Hours
Min	Minutes
Sec	Seconds
w/v	Weight/ volume
µg/mc	Microgram
^{0}C	Degree centigrade
%	Percentage
I.P	Indian Pharmacopoeia
U.S.P	United states of Pharmacopoeia
NF	National Formulary
PhEur	European Pharmacopoeia
B.P.	British Pharmacopoeia
VC	Vaginal Candidiasis
MBVG	Microencapsulated Bioadhesive Vaginal Gel
w/w	Weight/Weight
RPM	Revolution Per Minute
DSC	Differential scanning calorimetry

CHAPTER 1 INTRODUCTION

1.1 Benefits Of Vaginal Drug Administration

1.2 Anatomy And Physiology of The Vagina

1.3 Factors Affecting the Vaginal Absorption of Drugs

1.4 Drug Absorption Mechanism From The Vagina

1.5 Mucoadhesion

1.6 Microspheres

1.7 Release kinetics of drug release

1. Introduction:

Vaginal candidiasis (VC) is a fungal infection of the female lower genital tract-the vulva and the vagina causedby *Candida species*. Vaginal candidiasis is a condition which isassociated with severe inflammation of vagina, itching and thick milky discharges. It is one of the most frequent gynecological diseases. Literature shows that 30-50% of vaginitis is due to Candida infection and two third of women experience acute episodes of vaginal candidiasis at least once during in their lifetime. [1, 2]

The vagina, as a site of drug delivery, offers certain unique features that can be exploited in order to achieve desirable effects. The vaginal route of administration is a favorable site for local and systemic delivery of drugs that can be used specifically for female related conditions. The vaginal cavity has been used for local delivery of drugs such as prostaglandins, steroids, antibiotics, antifungal, antiviral, antiprotozoal and spermicidal agents. [1, 6, 7]

For vaginal delivery systems of antifungal agents to be more effective, they need to reside at the sites of infection for a prolonged period. In addition convenience of dosing methods is an important factor in the design of vaginal application forms.Of vaginal dosage forms, patients are known to better tolerate gels than inserts or ointments .[3] However, the direct application of gels onto the infected sites of the vagina might be difficult as well as inconvenient. Moreover, conventional gels do not remain for long at the site of application, leading to frequent dosing with antifungal agents. [6] Vaginal candidiasis, treated commonly with imidazole derivative antifungal agents such as clotrimazole since these drugs are locally active with no major side effects. [8]

Current vaginal delivery systems include creams, foams, gels, tablets, pessaries and irrigations, which are limited use because of less residence time at the genitourinary tract: they are removed rather rapidly by the self-cleansing action of the vaginal tract.[4,5,6,8]

Moreover, the physiological conditions imposed by the protective mechanism of the genital tract, limiting the residence time and thus impairing the therapeutic efficacy of the drug, make multiple and frequent administration necessary for treatment.[8] Patient compliance when administering the dosage forms and following the repeated-dose therapeutic regimen is an important challenge in vaginal drug delivery. Patients are generally reported to tolerate organogels better than other dosage forms. [8]

The vagina provides a promising site for local effect as well as systemic drug delivery because of its large surface area, rich blood supply, avoidance of the first-pass effect, relatively high permeability to many drugs and self-insertion. [6]

However, this route has not been extensively exploited because of the broad inter-individual variability affecting some physiological factors like the pH and the presence of limited vaginal secretions that further vary depending on age and menstrual cycles. Although various possibilities are presently being investigated, there are only a limited number of vaginal dosage forms available.

Many different approaches have been tested to develop novel vaginal drug delivery systems that can meet both the clinical and the patient's requirements. Considerable attention has been focused on the development of controlled delivery systems providing a long-term therapeutic concentration of drugs following a single dose. The compositon of vaginal dosage forms will be the focus of interest in the future. Novel forms are

liposomes, vaginal rings, cubic gels and formulations based on polystyrene and silicone elastomers. One interesting group of auxiliary agents is the mucoadhesive polymers, which are the basis of newly designed systems.

The vaginal route appears to be highly appropriate for bioadhesive drug delivery systems in order to retain drugs for treating largely local conditions, or for use in contraception. Particularly,protection against sexually transmitted diseases. To prolong the residence time in the vaginal cavity, bioadhesive therapeutic systems have been developed in the form of semi-solid and solid dosage forms.

The main advantages of the bioadhesive systems over the existing solid and semi-solid preparations are as follows: [6]

- Low production costs,
- Avoidance of aqueous or organic solvents,
- Ease of self-administration with no need to use applicators,
- Gel-like consistency in the activated state,
- Avoidance of local irritation phenomena,
- Rapid bioadhesion, prolonged residence time in the vaginal cavity even in absence of physiological secretions associated with a controlled drug delivery, extended dosing interval,
- Improved chemical and physical stability.

1.1 **Benefits of Vaginal Drug Administration** [4, 5, 6, 9]

In the vagina, arteries and veins form a dense network which provides a rich blood supply and consequently the vagina is well suited for the rapid and steady uptake of hormones. Drugs administered via the vagina are not subject to the first-pass effect and gastrointestinal interferences with absorption of medication are avoided. This has been demonstrated by the greater bioavailability of misoprostol following vaginal as opposed to oral administration . Vaginal administration often minimizes side effects associated with the oral route. An example is the administration of bromocriptine vaginally in treatment of hyperprolactinemia in women who suffer from nausea and vomiting following oral administration.

Bioadhesive vaginal delivery systems have several advantages when compared to conventional dosage forms. Firstly, the bioadhesive vaginal formulations are readily localized in the region of application thus improving the bioavailability of drugs. Greater bioavailability of insulin , calcitonin progesterone and estrogen was observed from bioadhesive vaginal formulations. Secondly, these delivery systems provide intimate contact of the formulation with the underlying absorption surface. This allows for modification of tissue permeability for absorption of macromolecules such as proteins and peptides .Thirdly, it permits continuous and prolonged residence of the dosage form at the site of application. Lastly, it reduces side effects due to avoidance of repeated administration of the drug.

1.2 Anatomy and Physiology Of The Vagina: [4, 5, 6, 9, 10, 11, 14]

In the pharmaceutical literature, human vagina is often described as slightly S-shaped fibro muscular collapsible tubes between 6 and 10 cm long extending from cervix of the uterus. The vaginal wall consists of three layers: the epithelial layer, the muscular coat and the tunica adventia.[10,11,12]During the menstrual cycle, the thickness of the vaginal epithelial cell layer changes by approximately 200–300 μm.[13]The surface of the vagina is composed of numerous folds, which are often called rugae. The rugae provide

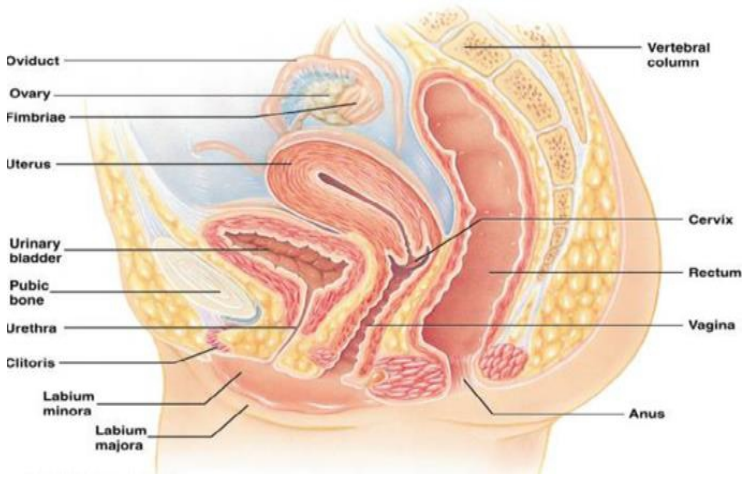


FIG1: Vaginal Anatomy

dispensability, support and an increased surface area of the vaginal wall. The vagina has an excellent elasticity because of the presence of smooth elastic fibers in the muscular coat. Loose connective tissue of tunica adventia further increases the elasticity of this organ. The network of blood vessels that supply blood to the vagina include a plexus of

arteries extending from the internal iliac artery, uterine, middle rectal and internal pudental arteries. In fact, arteries, blood vessels and lymphatic vessels are abundant in the

walls of the vagina. Drugs absorbed from the vagina does not undergo first-pass metabolism because blood leaving the vagina enters the peripheral circulation via a rich venous plexus, which empties primarily into the internal iliac veins.[14]There is some drainage to the hemorrhoidal veins as well. The lower part of the vagina receives its nerve supply from the pudental nerve and from the inferior hypogastric and uterovaginal plexuses. [11]

Although the vagina does not possess any gland, it secrets a large amount of fluid. [4]Cervical secretion and transudation from the blood vessels with desqua-mated vaginal cells and leucocytes mainly constitute the vaginal fluid. [5] Secretions from the endometrium and fallopian tubes also contribute to the vaginal fluid. [4] Like the thickness of the vaginal epithelium, the amount and composition of the vaginal fluid also changes throughout the menstrual cycle. Women of reproductive age produce fluid at a rate of 3–4 g /4 h, while the discharge produced by postmenopausal women is reduced by 50% compared to that produced by women of reproductive age. The human vaginal fluid may contain enzymes, enzyme inhibitors, proteins, carbohydrates, amino acids, alcohols, hydroxyl-ketones and aromatic compounds. [15] Sexual arousal may affect the volume and composition of vaginal fluids18 and that can alter the drug release pattern from vaginal delivery system.[16,17]Lactic acid produced from glycogen by the Lactobacillus acidophilus present in the vagina acts as a buffer to maintain the vaginal pH between 3.8 and 4.2. During menstruation, the pH of vagina l fluid increases and frequent acts of

coitus may also cause an increase in the vaginal pH because both ejaculate and vaginal transudate are alkaline. The presence of cervical mucus and the amount of vaginal transudate may also alter vaginal P^H. The vaginal epithelium has a high activity of enzymes that could potentially affect short- and long-term stability of intravaginal delivery systems and devices. [18]

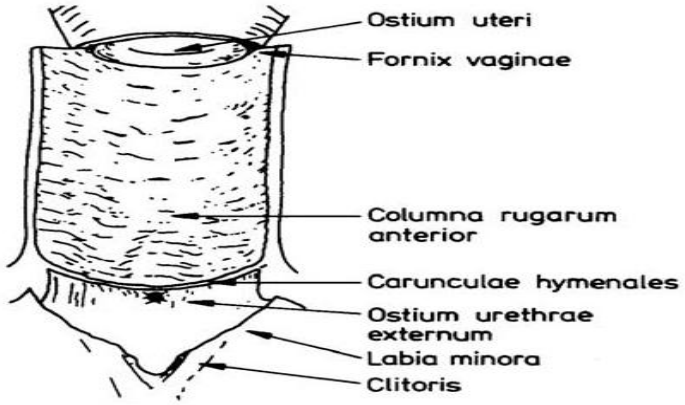
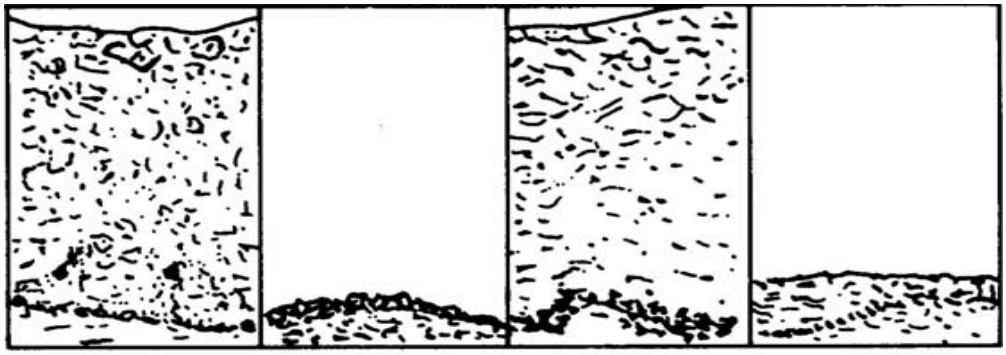


FIG 2: Inside the upper vaginal wall.



A B C D

FIG 3: Comparison of epithelial thickness of the vaginal tissue,

(A—newborn, B—child, C—adult, D—menopause)

1.3 Factors Affecting the Vaginal Absorption of Drugs [12]

Like other mucosal routes of administration, drugs administered via vaginal route are absorbed

(i) Transcellularly via concentration dependent diffusion through the cells.

(ii) Paracellularly mediated by tight junctions and

(iii) Vesicularly or receptor mediated transport as pointed out by Richard son and Illum.

Absorption of drug from vaginal delivery systems occurs in two main steps: drug dissolution in vaginal lumen and membrane penetration. Any biological or formulation factor that affects drug dissolution and membrane transport could potentially affect the absorption profile from vaginal drug delivery systems .Overall, vast and multifarious factors and processes are involved in drug absorption from the vaginal route.

1.3.1 Physiological Factors [12]

The cyclic changes in thickness of vaginal epithelium, fluid volume and composition, pH and sexual arousal could potentially affect drug release from intravaginal delivery systems. The volume, viscosity and pH of vaginal fluid may have either negative or positive impact on vaginal drug absorption. The absorption of drug that is poorly water- soluble may be increased when the fluid volume is higher. However, the presence of overly viscous cervical mucus may present a barrier to drug absorption and increased fluid volume may remove the drug from vaginal cavity and subsequently reduce absorption. Since many drugs are weak electrolytes, the pH may change their

degree of ionization and affect the absorption of drug. Any change in the vaginal pH may affect the release profiles of pH sensitive drugs from vaginal drug delivery systems.

1.3.2 **Physicochemical Properties of Drugs** [12]

Physicochemical properties such as molecular weight, lipophilicity, ionization, surface charge, chemical nature can influence vaginal drug absorption. It generally accepted that low molecular weight lipophilic drugs are likely to be absorbed more than large molecular weight lipophilic or hydrophilic drugs. Since vaginal fluid contains a large amount of water, any drug intended for vaginal delivery require a certain degree of solubility in water.

1.4 **Drug Absorption Mechanism From The Vagina** [4, 5, 6, 9, 10, 11, 12]

Like other mucosal drug delivery routes drug transport across vaginal membrane may occur by a number of different mechanisms: (a) diffusion through the cell due to a concentration gradient (transcellular route) (b) vesicular or receptor mediated transport mechanism or (c) diffusion between cells through the tight junctions (intercellular route). There are various vaginal drug delivery systems such as suppositories, gels, creams, vaginal rings, bioadhesive delivery systems etc. Vaginal absorption of a therapeutic agent from a controlled release drug delivery system, such as medicated vaginal ring, should be visualized. [14, 19, 20, 21]

For the drug dispersing vaginal ring the step consists of (a) The dissolution of the finely ground drug particles, (b) Well dispersion of drug particles into the surrounding polymer structure, (c) Diffusion through the polymer matrix to the device surface, (d) Particles into and the diffusion across vaginal secretion fluid (which is sandwiched between the ring surface and vaginal walls), (e) Uptake and then penetration of drug through the vaginal mucosa, (f) Transport and distribution of the absorbed drug molecules by circulating blood and /lymph to a target tissue. [21]

1.5 Mucoadhesion [15, 22, 23, 24]

The term bioadhesion refers to any bond formed between two biological surfaces or a bond between a biological and a synthetic surface. In case of bioadhesive drug delivery, the term bioadhesion is used to describe the adhesion between polymers, either synthetic or natural and soft tissues or the gastrointestinal mucosa. In cases where the bond is formed with the mucus the term mucoadhesion may be used synonymously with bioadhesion. [15, 22]

Mucoadhesion can be defined as a state in which two components, of which one is of biological origin, are held together for extended periods of time by the help of interfacial forces. Generally speaking, bioadhesion is an term which broadly includes adhesive interactions with any biological or biologically derived substance, and mucoadhesion is used when the bond is formed with a mucosal surface. [15, 23, 24]

Mucoadhesive drug delivery systems include the following [23, 24]

- Buccal delivery system
- Oral delivery system
- Vaginal delivery system

- Rectal delivery system
- Nasal delivery system
- Ocular delivery system

1.5.1 Mechanism of Mucoadhesion [23, 24]

Mucoadhesion is the attachment of the drug along with a suitable carrier to the mucous membrane. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains.

Mucoadhesion has the following mechanism:

- Intimate contact between a bioadhesive and a membrane (wetting or swelling phenomenon)
- Penetration of the bioadhesive into the tissue or into the surface of the mucous membrane (interpenetration)

The mucoadhesive must spread over the substrate to initiate close contact and increase surface contact, promoting the diffusion of its chains within the mucus. Attraction and repulsion forces arise and, for a mucoadhesive to be successful, the attraction forces must dominate.

Each step can be facilitated by the nature of the dosage form and how it is administered. For example, a partially hydrated polymer can be adsorbed by the substrate because of the attraction by the surface water.

Due to its relative complexity, it is likely that the process of mucoadhesion cannot be described by just one of these theories. Lee, Park, Robinson, 2000 had described the mechanism of mucoadhesion in four different approaches. These include:

• Dry or partially hydrated dosage forms contacting surfaces with substantial mucus layers (typically particulates administered into the nasal cavity).

• Fully hydrated dosage forms contacting surfaces with substantial mucus layers (typically particulates of many mucoadhesive that have hydrated in the luminal contents on delivery to the lower gastrointestinal tract).

• Dry or partially hydrated dosage forms contacting surfaces with thin/discontinuous mucus layers (typically tablets or patches in the oral cavity or vagina).

• Fully hydrated dosage forms contacting surfaces with thin/discontinuous mucus layers (typically aqueous semisolids or liquids administered into the esophagus or eye).

1.5.2 Theories of Mucoadhesion [23.24, 25]

It is reported that, although the chemical and physical basis of mucoadhesion are not yet well understood there are six classical theories adapted from studies on the performance of several materials and polymer-polymer adhesion which explain the phenomenon. Contact angle and time plays a major role in mucoadhesion.

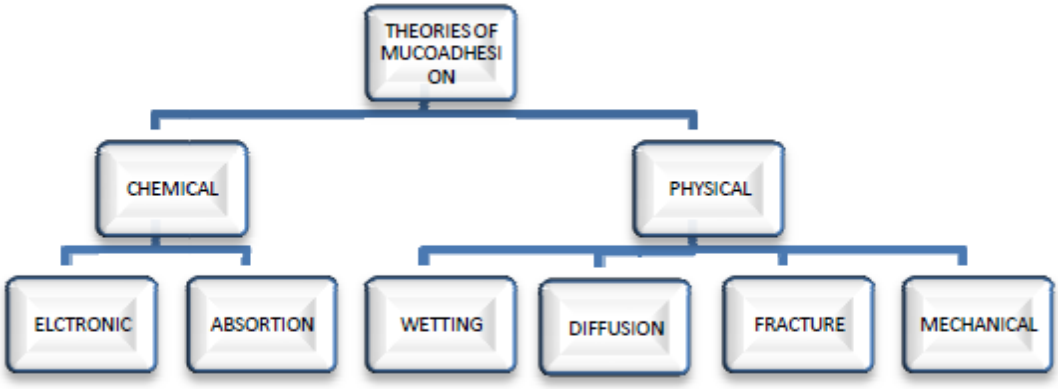


FIG 4: Theories of mucoadhesion[23.24, 25]

1.5.2.1 The electronic theory: [23.24, 25]

This theory is based on the assumption that the bioadhesive material and the glycoprotein mucin network have different electronic structures. When the two materials come in contact with each other electron transfer will occur causing the formation of a double layer of electrical charge at the interface. The bioadhesive force is due to attractive forces across this electrical double layer. The system is charged when the adhesive and the substrate are in contact and discharged when they are separated. However, this theory has caused some controversy regarding whether the electrostatic forces are an important cause or the result of the contact between the bioadhesive and the biological tissue.

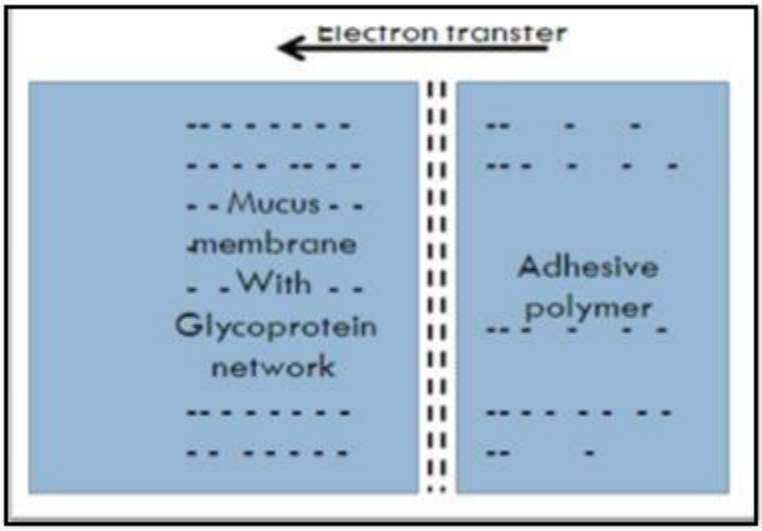


FIG 5: The electronic theory

1.5.2.2 The absorption theory: [23.24, 25]

According to this 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:

I) Primary chemical bonds of covalent nature, which areundesirable in bioadhesion because their high strength may result in permanent bonds.

II) Secondary chemical bonds having many different forces of attraction, including electrostatic forces, Vander walls forces and hydrogen and hydrophobic bonds.

1.5.2.3 The wetting theory: [23.24, 25]

The wetting theory applies to liquid systems which present affinity to the surface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angle. The general rule states that the lower the contact angle then the

greater the affinity . The contact angle should be equal or close to zero to provide adequate spreadability.

1.5.2.4 The Diffusion theory: [23.24, 25]

According to this theory, the polymer chains and the mucus mix to a sufficient depth to create a semi permanent 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 co efficient, in turn, depends on the value of molecular weight between crosslink's and decreases significantly as the crosslinking density increases. This theory suggests that interpenetration and entanglements of bio-adhesive polymer chain and mucus polymer chains produce semi permanent adhesive bonds, and bond strength is believed to increase with the depth of penetration of the polymer chains.

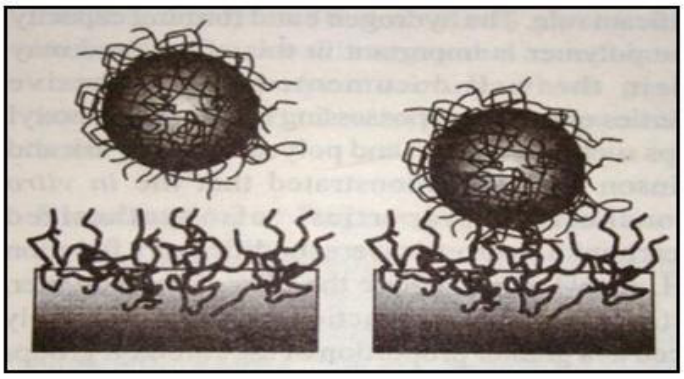


FIG 6: Diffusion theory

1.5.2.5 The Fracture theory: [23.24, 25]

This theory analyses the force that is required to separate two surfaces after adhesion. The maximum tensile stress (s_m) produced during detachment can be determined by dividing the maximum force of detachment, Fm by the total surface area (A0) involved in the adhesive interaction.

$$Sm=Fm/Ao \text{ ---------- (1)}$$

The above equation can be used for calculating fracture strengths of adhesive bonds involving hard, bioadhesive material in which the polymer chains may not penetrate the mucus layer.

1.5.2.6 The Mechanical theory: [23.24, 25]

Mechanical theory considers adhesion to be due to the filling of the irregularities on a rough surface by a mucoadhesive liquid. Moreover, such roughness increases the interfacial area available to interactions thereby aiding dissipating energy and can be considered the most important phenomenon of the process.

Lee, Park, Robinson, 2000 had described that it is unlikely that the mucoadhesion process is the same for all cases and therefore it cannot be described by a single theory. In fact, all theories are relevant to identify the important process variables. The mechanisms governing mucoadhesion are also determined by the intrinsic properties of the formulation and by the environment in which it is applied. Intrinsic factors of the polymer are related to its molecular weight, concentration and chain flexibility. For linear polymers, mucoadhesion increases with molecular weight, but the same relationship does not hold for non-linear polymers. It has been shown that more concentrated

mucoadhesive dispersions are retained on the mucous membrane for longer periods, as in the case of systems formed by *in situ* gelification. After application, such systems spread easily, since they present rheological properties of a liquid, but gelify as they come into contact the absorption site, thus preventing their rapid removal. Chain flexibility is critical to consolidate the interpenetration between formulation and mucus.

Environment-related factors include pH, initial contact time, swelling and physiological variations. The pH can influence the formation of ionizable groups in polymers as well as the formation of charges on the mucus surface. Contact time between mucoadhesive and mucus layer determines the extent of chain interpenetration. Super-hydration of the system can lead to build up of mucilage without adhesion. The thickness of the mucus layer can vary from 50 to 450 μm in the stomach to less than 1μm in the oral cavity. Other physiological variations can also occur with diseases.

1.5.3 **Factors Affecting Mucoadhesion:** [23, 25]

The mucoadhesion of a drug carrier system to the mucous membrane depends on the below mentioned factors:

- polymer based factors
- molecular weight of the polymer
- concentration of polymer used
- flexibility of polymer chains
- swelling factor
- stereochemistry of polymer

- physical factors
- pH at polymer substrate interface
- applied strength
- contact time
- physiological factors
- mucin turn over rate
- diseased state

1.5.4 Advantages Of Mucoadhesivedrug Delivery Systems: [23, 24, 25]

• Prolongs the residence time of the dosage form at the site of absorption, hence increases the bioavailability.

• Excellent accessibility, rapid onset of action.

• Rapid absorption because of enormous blood supply and good blood flow rates

• Drug is protected from degradation in the acidic environment in the GIT

• Improved patient compliance

1.5.5 Disadvantages Of Mucoadhesivedrug Delivery Systems: [23, 24, 25]

• Occurrence of local ulcerous effects due to prolonged contact of the drug possessing ulcerogenic property.

• One of the major limitations in the development of oral mucosal delivery is the lack of a good model for in vitro screening to identify drugs suitable for such administration.

• Patient acceptability in terms to taste, irritancy and mouth feel is to be checked

1.5.6 Mucoadhesive Polymers: [23, 25]

Mucoadhesive polymers are water-soluble and water insoluble polymers which are swellable networks jointed by cross-linking agents. These polymers posses optimal polarity to make sure that they permit the mutual adsorption and interpenetration of polymer and mucus to take place. Two classes of polymers are currently used for mucoadhesion which include hydrophilic polymer and hydrogels. It has been found recently that hydrophilic polymers that adhere to the mucin epithelial surface can be conveniently divided into three broad categories.

- Polymers that become sticky when placed in water and owe their mucoadhesionto stickiness.
- Polymers that adhere through nonspecific, noncovalent interactions those are primarily electrostatic in nature (although hydrogen and hydrophobic bonding may be significant).
- Polymers that bind to specific receptor site on title self surface. The promising mucoadhesive polymers include sodium alginate, hydroxypropylmethylcellulose, hydroxyethyl cellulose and cationic hydrogels such as chitosanetc. In recent years hydrophilic matrices have attracted considerable attention as sustained drug release devices. Various types of polymers can be used in the hydrophilic matrix and the hydration of these polymers results in the formation of an outer gel layer that controls drugs release. HPMC the nonionic cellulose ether is commonly used in the formulation of hydrophilic matrix systems. On the other hand acrylic acid

derivatives Carbopol have also attracted interest in their use in controlled drug delivery.

1.5.6.1 Characteristics of an ideal mucoadhesive polymer:[25]

An ideal mucoadhesive polymer has the following characteristics:

- The polymer and its degradation products should be nontoxic and should be non-absorbable from the gastrointestinal tract.
- It should be nonirritant to the mucous membrane.
- It should preferably form a strong non-covalent bond with the mucin-epithelial cell surfaces.
- It should adhere quickly to most tissue and should possess some site-specificity.
- It should allow daily incorporation to the drug and offer no hindrance to its release.
- The polymer must not decompose on storage or during the shelf life of the dosage form.
- The cost of polymer should not be high so that the prepared dosage form remains competitive.

1.5.6.1.1 Molecular characteristics[25]

The properties exhibited by a good mucoadhesive polymer may be summarized as follows:

- Strong hydrogen bonding groups (-OH, -COOH).
- Strong anionic charges.

• Sufficient flexibility to penetrate the mucus network or tissue crevices.

• Surface tension characteristics suitable for wetting mucus/mucosal tissue surface.

• High molecular weight.

Although an anionic nature is preferable for a good mucoadhesive, a range of nonionic molecules (e.g., cellulose derivatives) and some cationic (e.g., Chitosan) can be successfully used.

1.5.6.2 Classification:[23,24,25]

1.5.6.2.1 Natural polymers:

Tragacanth, Sodium alginate, Karaya gum, Guar gum, Xanthan gum, Lectin, Soluble starch, Gelatin, Pectin, Chitosan.

1.5.6.2.2 Semi-Synthetic polymers:

Cellulose derivatives (methylcellulose, ethyl cellulose, hydroxy-ethylcellulose, Hydroxyl propyl cellulose, hydroxylpropyl methylcellulose, sodium carboxy methylcellulose, Poly (acrylic acid) polymers (carbomers, polycarbophil),Poly (hydroxyethylmethylacrylate), Poly (ethylene oxide), Poly (vinyl pyrrolidone), Poly (vinyl alcohol).

1.5.6.3 New Generation Mucoadhesive Polymers:[25]

- New generation polymers are capable of forming covalent bonds with the mucus and the underlying cell layers and exhibit improved chemical interactions.
- The new generation of mucoadhesive (with the exception of thiolated polymers) can adhere directly to the cell surface,rather than to mucus. They interact with the

cell surface by means of specific receptors or covalent bonding instead of nonspecific mechanisms, which are characteristic of the previous polymers.

- Examplesof such are the incorporation of l-cysteine into thiolated polymers and the target specific, lectin mediated adhesive polymers.
- These classes of polymers hold promise for the delivery of a wide variety of new drug molecules, particularly macromolecules, and create new possibilities for more specific drug–receptor interactions and improved targeted drug delivery.

1.5.6.4 Mucoadhesive polymers as enzyme inhibitors and permeation enhancers:[25]

It has been shown that some mucoadhesive polymers can act as an enzyme inhibitor. The particular importance of this finding lies in delivering therapeutic compounds that are specifically prone to extensive enzymatic degradation such as protein and polypeptide drugs. Investigations have demonstrated that polymerssuch as poly (acrylic acid) operate through a competitive mechanism with proteolytic enzymes. This stems from their strong affinity to divalent cations(Ca^{2+}, Zn^{2+}). These cations are essential cofactors for the metalloproteinase suchas trypsin. Circular dichroism studies suggest that Ca^{2+} depletion, mediated bythe presence of some mucoadhesive polymers, causes the secondary structure of trypsin to change, and initiates a further auto degradation of the enzyme 60,61. The increased intestinal permeability of various drugs in the presence of numerous mucoadhesive polymers has also been attributed to their ability to open up the tight junctions by absorbing the water from the epithelial cells. The result of water absorption by a dry and swellable polymer is dehydration of the cells and

their subsequent shrinking. This potentially results in an expansion of the spaces between the cells

1.5.7 Mucoadhesive Vaginal Drug Delivery System: [6, 26]

Mucoadhesive vaginal drug delivery systems can be categorized as follows:[6]

1) Mucoadhesive gels
2) Mucoadhesive microparticles (microspheres or microcapsules)
3) Mucoadhesive tablets
4) Mucoadhesive films
5) Emulsion type mucoadhesive systems
6) Pessaries or suppositories
7) Vaginal niosomes and liposomes

1.5.7.1 Mucoadhesive Vaginal Gels [6, 26,]

The most widely used mucoadhesive vaginal drug delivery systems are gels [5]. In particular, for drugs designed for gynaecological use, a bioadhesive gel able to ensure prolonged contact between the active ingredient and the vaginal mucosa, and gradual release of that ingredient over time, provides the ideal solution in terms of efficacy and compliance by patients. Among vaginal formulations, gels are easy to manufacture, comfortable, and have the ability to spread onto the surface of mucous and to achieve an intimate contact with vaginal mucosa. Moreover, because of their high water content and their rheological properties, they present the further advantage of a hydrating and lubricating action, which is particularly useful in pathological situations characterized by

dryness of the vaginal mucosa. The employment of mucoadhesive polymers can improve the time of contact with the mucosa, delaying the loss of the formulation and prolonging the effect.

1.5.7.1.1 **Gels For The Moisturization Of Vagina**[6]

Vaginal dryness and pain is a problem experienced by women due to the decreasing hormone levels at the onset of menopause [11]. There are a number of patents for the moisturization of the vagina in the case of vaginal dryness. One of the first marketed products is Replens® gel which consists of 1-3 % polycarbophil. It is retained in the vaginal cavity for 3-4 days.

Robinson JR described the bioadhesive polymers in a vaginal tissue moisturizing composition . The compositions utilized in these inventions include water, a bioadhesive polymer as the moisturizing agent and a gel-like consistency-enhancing agent. The bioadhesive is water swellable, but water-insoluble, fibrous, cross-linked carboxy-functional polymer. The consistency-enhancing agent is a water-soluble or water-dispersible anionic or non-ionic polymer. It is polyacrylic acid cross-linked with polyallyl sucrose containing an average of at least three allyl groups per molecule. The composition is contacted with the vaginal mucous membrane for a time period sufficient to moisturize the contacted area.

A similar composition and method has also been described in a Russian patent by Robinson JR. A gel formulation for the local hormonotherapy of vaginal dryness was developed and disclosed in a world patent. The gel is intended for local treatment,

essentially non systemic, of vaginal dryness, particularly in menopausal women. It is characterized by the synergic combination of a natural oestrogen selected amongst 17 beta-estradiol and its salts and a biodegradable, hydrophilic lubricant and bioadhesive gel. The excipient comprises preferably carboxyvinylic acid, glycerin, a pH adjusting substance, a preservative adjuvant and an aqueous excipient.

A mucoadhesive aqueous gel formulation for vaginal use was described in the patent of Giroux. In this invention the mucoadhesive gel comprises 1-15 weight% natural polysaccharides, 0.5-15% mucoadhesion promoter and water. This invention relates to a moisturizing aqueous gel for local use, especially for the hydration of the vaginal mucosa. In this invention, the chitins (nonionic), carragenates or alginates (anionic), guar type polysaccharides (cationic) or mixtures of these polysaccharides can be used. Among the moisturizing agents physiologically acceptable, Gels or related semisolid dosage forms are also important candidates as microbicide delivery vehicles.

A vaginal gel can serve several functions:

- ✓ Delivering microbicide molecules to vaginal fluids and tissues
- ✓ Maintaining a reservoir of these molecules within a layer that can serve as a barrier to HIV migration from semen to tissue
- ✓ Providing lubrication to diminish tissue damage during sexual activity.

A patent for a prolonged-release bioadhesive vaginal gel dosage form has been described by Durrani. The dosage form comprised a synergistic formulation of carrageen, acrylic acid containing polymers (carbophil), ultra low gelling temperature agarose and

an effective amount of a therapeutic agent. The first release rate of the therapeutic agent from the composition is greater than the second rate. The therapeutic agent is released for up to about 24 hours. The polymer comprising acrylic acid is selected from the group consisting of copolymers of acrylic acid, poly-carbophil, homopolymers of acrylic acid crosslinked with divinyl glycol, polyacrylic acid homopolymers, carbomers, Carbopol 974P-NF, Carbopol 971P-NF, ETD resins, and copolymers of acrylic acid and Cl0 to C30 alkyl acrylic acid.

A method for making a bioadhesive, prolonged release drug composition consisted of the following steps: (a) dissolving appropriate amounts of soluble components comprising sodium chloride, methyl paraben, acetate buffer, and, optionally, at least one therapeutic agent, in water to provide a first mixture; (b) dispersing appropriate amounts of gelling agents comprising ultra low gelling agarose and carrageenan in the first mixture to produce a second mixture, which is stirred for about one hour; (c) dispersing an appropriate amount of at least one polymer comprising acrylic acid in the second mixture producing a third mixture, which is stirred and heated to about 90oC, and which is further cooled and stirred at about 70oC; and (d) cooling the third mixture to room temperature and stirring it until uniform, and, optionally, adding at least one therapeutic agent. The therapeutic agent was selected from the group consisting of a spermicide, an antiviral, an antibacterial, an antifungal, an antimycotic, an antipruritic, an emollient, a humectant, an anti-inflammatory, an immunomodulator, a hormonal, an antineoplastic and an analgesic.

Similary, semisolid mucoadhesive formulations for vaginal application with improved technical and organoleptic characteristics, which contain at least two bioadhesive gelling polymers and an active ingredient, useful in the prevention and/or treatment of various pathologies and disorders in human beings or animals, were described in a US patent. This semisolid mucoadhesive formulation contained: a first bioadhesive gelling polymer that is a polyacrylic acid crosslinked with divinyl glycol (Poly-carbophil AA1) (0.1- 5% by weight of the semisolid mucoadhesive formulation); a second bioadhesive gelling polymer that is a polyacrylic acid crosslinked with allyl sucrose or allylpentaerythritol (Carbopol 971P, Carbopol 940, Carbopol 941, Carbopol 980, and Carbopol 981) (0.1- 5% by weight of the semisolid mucoadhesive formulation); a neutralizing agent in sufficient quantity to position the semisolid mucoadhesive formulation's pH between 2 and 6; a pharmacologically active agent; and water. The pharmacologically active agent is selected from a group consisting of hormones, antibacterials, antimycotics, antiprotozoals, anti-STD agents, spermicides, local anaesthetics, anti-inflammatories, labour inducers, and smooth muscle relaxants. The bioadhesion of the formulation was measured in both ovariectomized and non-ovariectomized rats and bioadhesion to the vaginal mucosa of ovariectomized rats is greater than that of the nonovariectomized rats.

1.5.7.1.2 **Gels For The Treatment Of Vaginal Infections** [6]

Vaginal infections are a common problem among women. Bacterial vaginosis is the most common form of infectious vaginitis, accounting for 45% of symptomatic cases

and estimated to be present in 15% of asymptomatic sexually active women. Microbicides can be formulated as creams, films or pessaries: gel formulations appear to be preferred among researchers.

A bioadhesive aqueous composition patent assigned to Robinson and Bologna, J.R., described the use of a bioadhesive aqueous composition to control the pH of the vagina to alleviate microorganism growth and feminine odour such as presented by bacterial vaginosis. The composition comprises water and an acidic polymer, specifically one wherein 80% of the monomers contain at least one carboxyl group[-COOH] and wherein the polymer is crosslinked so as to be water-swellable, but water-insoluble (polycarbophil).

The composition of the present invention is additionally a bioadhesive agent providing for a long-lasting benefit and control of vaginal pH. The formulation further comprises an adjuvant selected from the group consisting of preservatives, lubricating oils, emulsifying agents, coloring agents, odor-providing agents, and humectants.

Another invention relates to a method for controlling the pH value in the vagina to suppress the growth of microbes and reduce female odour by means of a composition of bioadhesive and water. This composition contains water and acidic polymer, specifically the polymer whose 80% monomer contains at least one carboxyl(-COOH). This polymer is cross-linked and is swellable in water, but not dissolved in water, and this composition has bioadhesion for durable action.

Mucoadhesive antimicrobial complexes consist of a cross-linked polyacrylic acid having remarkable muco-adhesive properties, known as polycarbophil, and an imidazole or triazole derivative with antifungal or antiprotozool activity in its basic form, for use in the topical treatment of mucosal affections. Imidazole derivative is chosen from the group consisting of econazole, clotrimazole, metronidazole, tioconazole, fenticonazole, isoconazole, ketoconazole, sulconazole, bifonazole, omoconazole, azanidazole, butoconazole and oxiconazole. This formulation is produced by dissolving each of the two starting products in a common solvent or mixture of solvents, or in two different solvents compatible with each other, then joining together the two solutions in relative amounts and subsequently evaporating the solvent. Particularly preferred do formulations in gel in propylene glycol comprise an econazole-polycarbophil or omoconazole-polycarbophil complex, with an excess of polycarbophil, together with pharmaceutically acceptable carrier and excipient substances, for use as sustained-release antifungals for vaginal administration.

Another patent was for vaginal or transvaginal treatment of fungal, bacterial, viral or parasitic infections in human females. A device was developed comprising a pharmaceutical agent, a pharmaceutically acceptable non-toxic lipohilic or hydrophilic carrier, mucoadhesive agent and a penetration enhancer.

An effective amount of a pharmaceutical agent selected from the group consisting of miconazole, terconazole, isoconazole, fenticonazole, fluconazole, nystatin, ketoconazole, clotrimazole, butoconazole, econazole, metronidazole, clindamycin, 5-fluoracil, acyclovir, AZT, famovir, penicillin, tetracycline, erythromycin is formulated as

a vaginal suppository, bioadhesive tablet, bioadhesive microparticle, cream, lotion, foam, ointment, paste, solution, and gel incorporated into a vaginal device with a non-toxic pharmaceutically acceptable carrier. The composition comprises a lipophilic carrier (a semi-synthetic glyceride of saturated fatty acids of 8-18 carbon atoms) or hydrophilic carrier (polyethylene glycol of a molecular weight from 400 to 6000) and a mucoadhesive agent (alginate, pectin or hydroxypropyl methylcellulose) for intra-vaginal delivery. A penetration enhancer or sorption promoter (nonionic surfactant, bile salt or ethoxyglycol) is added to this formulation for transvaginal delivery. The device is a tampon, tampon-like device, vaginal ring, vaginal pessary, vaginal cup, vaginal tablet, vaginal suppository, vaginal sponge, vaginal bioadhesive tablet, vaginal bio-adhesive microparticle, comprising pharmaceutical agent formulated as a cream, lotion, foam, ointment, solution or gel.

An interesting patent for treating or preventing vaginal infections has been described by Bologna and Levine.In this invention the pharmaceutical vaginal composition includes a synergistic mix of a bioadhesive, extended release formulation that decreases the pH and that contains a peroxide in an amount sufficient to increase oxygen concentration without sterilising the vagina or substantially killing the normally-desired local vaginal flora. The synergistic mix releases peroxide over a period of at least 24 hours. The peroxide source is carbamide peroxide. The extended-release formulation includes a bioadhesive, water-swellable, water-insoluble, cross-linked polycarboxylic polymer (polycarbophil).

Larsen described a similar gelled vaginal pharmaceutical composition containing a water-soluble bioadhesive polymer, a peroxide source and pH buffer. The pH of the composition is between about 3.0 and 6.0. The peroxide source of the formulation is hydrogen peroxide.

The water-soluble polymer is an acrylic acid modified polymer. The composition contains a spermicide (nonoxy-nol-9) or a therapeutic agent which is selected from the group consisting of antibacterial, antiseptic, antibiotic, anti-inflammatory, antiparasitic, antiprotozoal, antiviral, antifun-gal agent and mixtures of these. The water-soluble polymer is an acrylic acid polymer and is used in amounts of about 1.0% to about 3.0% by weight of the formulation.

Another formulation for topical treatment of mixed vaginal infections was described by Bortz et al. In this invention a pharmaceutical composition comprises an antibacterial agent (clindamycin) and an antifungal agent (butoconazole). The composition is adapted for application in a unit dose amount to a vulvovaginal surface and has at least one nonlipoidal internal phase and at least one lipoidal external phase that is bioadhesive to the vulvovaginal surface. The clindamycin phosphate and butoconazole nitrate are present in substantial part in the internal phase. A phospholipid can be used as the emulsifying agent. The composition is useful for administration to a vulvovaginal surface to treat a mixed bacterial vaginosis and vulvovaginal candidiasis infection. The release period of the formulation was about 3 hours to about 10 days.

Palacin et al. developed a formulation for the treatment of vulvovaginal candidiasis. The invention relates to monodose mucoadhesive vaginal compositions of

sertaconazole or a pharmaceutically acceptable salt for the treat-ment of vulvovaginal candidiasis. The lipophilic excipients are selected from glyceryl stearates and their derivatives; ketostearyl alcohols, polyoxyethylene glycol ethers of n-alcohols, liquid paraffin, lecithin oil and glycerol are present in a total proportion of from 10 to 40%. The mucoadhesive excipients are selected from cellulose polymers, gelatin, colloidal anhydrous silica and polyacrylic acid polymers which are cross-linked with divinyl glycol and acrylic acid polymer cross-linked with sucrose or pentaerythritolallyl esters. The preservatives are selected from parabens, benzoic acid, sorbic acid, boric acid and the like.

1.5.7.1.3 Gels for Contraception [6]

A galenic form of vaginal application as a local contraceptive and/or for protection against STD and/or AIDS was described by Meignant. This formulation consists of an external envelope containing gelatine and inner non-aqueous liquid or semi-liquid phase containing a dissolved active component of spermicide, a lipophilic agent compatible with the rubber for condoms, a water-dispersible agent, a bioadhesive agent and an agent for the gelatinization of the lipophilic agent. The formulation simultaneously has spermicidic and lubrication properties. This vaginal dosage form combines the advantages associated with a soft capsule with spermicidal, antiseptic and lubricating properties. It can be safely used in conjunction with condoms without altering their rubber latex composition. The bioadhesion agent is a bio-compatible polymer selected from: carboxyvinyl acids; carboxymethylcellulose; sodium carboxymethylcellulose; methylcellulose; hydroxypropylcellulose; hydroxypro-

pylmethylcellulose; agar-agar; aluminum silicate; carrageenates; and carob gum, whereas the spermicide agent is selected from benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride, methylbenzethonium chloride, tetradecyltrimethyl ammonium bromide, benzalkonium bromide, monylphenyl ethers, lauryl ethers, and octoxynols.

1.6 Microspheres [27, 28]

Microspheres as carriers of drug become an approach of controlled release dosage form in novel drug delivery system. Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. It has a particle size of (1-1000nm).

While a variety of devices have been used for controlled release drug delivery, biodegradable polymer microspheres are one of the most common types and hold several advantages. Microspheres can encapsulate many types of drugs including small molecules, proteins, and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time.

1.6.1 Advantages Of Microspheres [27,28]

- Microspheres provide constant and prolonged therapeutic effect.
- Reduces the dosing frequency and thereby improve the patient compliance.
- They could be injected into the body due to the spherical shape and smaller size.

- Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- Microsphere morphology allows a controllable variability in degradation and drug release.

1.6.2 **Limitation** [27,28]

Some of the disadvantages were found to be as follows

- The modified release from the formulations.
- The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
- Differences in the release rate from one dose to another.
- Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
- Dosage forms of this kind should not be crushed or chewed.

1.6.3 **Applications In Drug Delivery System** [28]

- Ophthalmic drug delivery
- Gene delivery
- Intratumoral and local drug delivery
- Oral drug delivery
- Nasal drug delivery
- Buccal drug delivery

- Peroral drug delivery
- Vaginal drug delivery
- Transdermal drug delivery
- Colonic drug delivery
- Multiparticulate delivery system
- Other potential applications include

    - Conversion of oil and other liquids to solids for ease of handling
    - Taste and odor masking
    - To delay the volatilization
    - Safe handling of toxic substances

1.6.4 Drug Loading And Mechanism of Drug release [27,28]

The active components are loaded over the microspheres principally using two methods, i.e. during the preparation of the microspheres or after the formation of the microspheres by incubating them with the drug/protein. The active component can be loaded by means of the physical entrapment, chemical linkage and surface adsorption. The entrapment largely depends on the method of preparation and nature of the drug or polymer (monomer if used). Maximum loading can be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives (e.g. cross linking agent, surfactant stabilizers, etc.) heat of polymerization, agitation intensity, etc. Release of the active constituent is an important consideration in case of microspheres.The release profile from the

microspheres depends on the nature of the polymer used in the preparation as well as on the nature of the active drug. The release of drug from both biodegradable as well as non-biodegradable microspheres is influenced by structure or micro-morphology of the carrier and the properties of the polymer itself. The drugs could be released through the microspheres by any of the three methods, first is the osmotically driven burst mechanism, second by pore diffusion mechanism, and third by erosion or the degradation of the polymer. In osmotically driven burst mechanism, water diffuse into the core through biodegradable or non-biodegradable coating, creating sufficient pressure that ruptures the membrane. The burst effect is mainly controlled by three factors the macromolecule/polymer ratio, particle size of the dispersed macromolecule and the particle size of the microspheres. The pore diffusion method is named so because as penetrating water front continue to diffuse towards the core. The polymer erosion, i.e. loss of polymer is accompanied by accumulation of the monomer in the release medium. The erosion of the polymer begins with the changes in the microstructure of the carrier as water penetrates within it leading to the plasticization of the matrix. Drug release from the non-biodegradable type of polymers can be understood by considering the geometry of the carrier. The geometry of the carrier, i.e. whether it is reservoir type where the drug is present as core, or matrix type in which drug is dispersed throughout the carrier, governs overall release profile of the drug or active ingredients.

1.6.5 Method Of Preparation [27, 28]

- Spray drying
- Solvent evaporation
- Wet inversion technique
- Hot melt microencapsulation
- Single emulsion technique
- Double emulsion technique
- Polymerization techniques
- Spray congealing
- Solvent extraction

1.6.5.1 **Spray Drying** [27, 28]

In Spray Drying the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, Acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporate instantaneously leading the formation of the microspheres in a size range 1-100μm. Micro particles are separated from the hot air by means of the cyclone separator while the trace of solvent is removed by vacuum drying. One of the major advantages of process is feasibility of operation under aseptic conditions this process is rapid and this leads to the formation of porous micro particles.

1.6.5.2 **Solvent Evaporation** [27,28]

The processes are carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule. The mixture is then heated if necessary to evaporate the solvent for the polymer of the core material is disperse in the polymer solution, polymer shrinks around the core. If the core material is dissolved in the coating polymer solution, matrix – type microcapsules are formed. The core materials may be either water soluble or water insoluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous. The comparison of mucoadhesive microspheres of hyaluronicacid, Chitosan glutamate and a combination of the two prepared by solvent evaporation with microcapsules of hyaluronic acid and gelating prepared by complex coacervation were made.

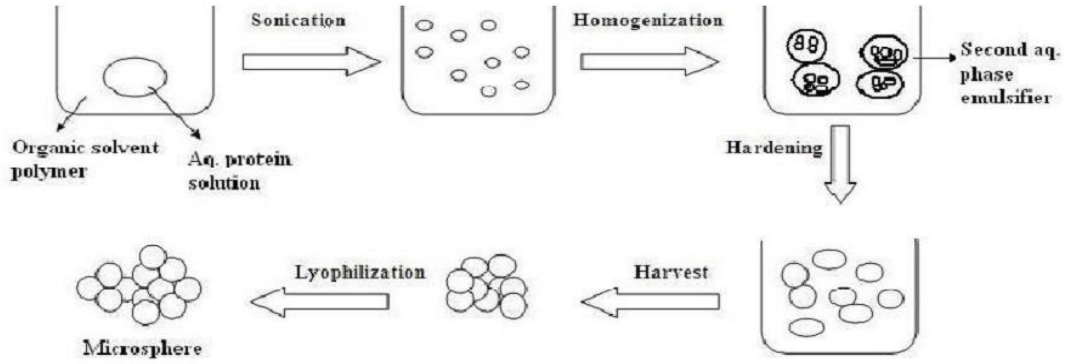


FIG 7: Solvent evaporation method for preparation of microsphere

1.6.5.3 **Wet Inversion Technique** [27, 28]

Chitosan solution in acetic acid was dropped in to an aqueous solution of counter ion sodium tripolyposphate through a nozzle. Microspheres formed were allowed to stand for 1 hr and cross linked with 5% ethylene glycol diglysidyl ether. Microspheres were then washed and freeze dried. Changing the pH of the coagulation medium could modify the pore structure of CS microspheres.

Complex Coacervation CS microparticles can also prepare by complex coacervation, Sodium alginate, sodium CMC and sodium polyacrylic acid can be used for complex coacervation with CS to form microspheres. These microparticles are formed by interionic interaction between oppositely charged polymers solutions and KCl& CaCl2 solutions. The obtained capsules were hardened in the counter ion solution before washing and drying.

1.6.5.4 **Hot Melt Microencapsulation** [27, 28]

The polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 µm. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with

petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, e.g. poly anhydrides. Microspheres with diameter of 1-1000 µm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which the drug is exposed.

1.6.5.4 **Single Emulsion Technique** [27, 28]

The micro particulate carriers of natural polymers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. Next cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, acid chloride etc. Heat denaturation is not suitable for thermolabile substances. Chemical cross linking suffers the disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing, separation The nature of the surfactants used to stabilize the emulsion phases can greatly influence the size, size distribution, surface morphology, loading, drug release, and bio performance of the final multiparticulate product.

1.6.5.5 **Double Emulsion Technique** [27,28]

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

1.6.5.6 **Polymerization Techniques** [27, 28]

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

a. Normal polymerization
b. Interfacial polymerization.

Both are carried out in liquid phase.

Normal polymerization It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be molded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerization has an advantage of formation of pure polymers.

Interfacial polymerization It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase. Phase separation coacervation technique This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important since the rate of achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed

particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment.

1.6.5.7 **Spray Drying And Spray Congealing** [27,28]

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 µm. Microparticles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying. One of the major advantages of the process is feasibility of operation under aseptic conditions. The spray drying process is used to encapsulate various penicillins. Thiamine mononitrate and sulphaethylthiadizole are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmiticacid using spray

congealing. Very rapid solvent evaporation, however leads to the formation of porous microparticles.

1.6.5.8 **Solvent Extraction** [27, 28]

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

Microencapsulation provides an effective and long lasting method for the release of antifungal drugs, and has drawn a great amount of attention due to its potential applications in the fields of medicine biomedicine and environmental engineering and as a phase change material coating and catalysis etc.[29]

1.7 Release kinetics of drug release: [30, 31]

1.7.1 Zero-order release kinetics: [30, 31]

$$Q(t)= k_0 t \text{ ----------(2)}$$

Where Q(t) is the percent of drug dissolved as a function of time t in minutes and k_0 describes the dissolution rate constant for zero-order release. A plot of the percent of drug

released against time will be linear if the release obeys zero-order release kinetics. Values of release rate constant k0 were obtained in each case from the slope of percent drug released versus time plots.

1.7.2 First-order release kinetics: [30, 31]

$$\log Q_t = \log Q_0 + (k_1 t/2.303) \text{ ----------(3)}$$

The first-order equation describes the release from systems where release rate is concentration dependent. Where Q0 is the initial amount of the drug, t is in minutes and k1 describes the dissolution rate constant for first-order release kinetics. A plot of the logarithm of the percent drug remained against time will be linear if the release obeys first-order release kinetics. Values of release rate constant kt were obtained in each case from the slope of the log percent drug remained versus time plots.

1.7.3 The simplified Higuchi model:[30,31]

$$Q(t) = k_H t^{1/2} \text{ ----------(4)}$$

Where Q(t) is the percent of drug dissolved, time t in minutes, kH is a dissolution rate constant for square root of time kinetics in percent dissolved $min^{-\frac{1}{2}}$. A plot of the fraction of drug released against square root of time will be linear if the release obeys Higuchi equation. Values of release of rate constant kH were obtained in each case from the slope of the percent drug released versus square root of time plots.

1.7.4 The Hixson-Crowell cube root model:[30,31]

$$W_0^{1/3} - W_t^{1/3} = k_{HC} t \text{ ---------- (5)}$$

Where W0 is the initial amount of drug in the dosage form, Wt is the remaining amount of drug at time t. Hixson-Crowell cube root law describes the release from systems where there is a change in surface area and diameter of the particles. kHC is the release rate constant for Hixson-Crowell rate equation. Then a graphic of the cubic root of the unreleased percent of drug versus time will be linear if the equilibrium conditions are not reached and if the geometrical shape of the dosage forms diminish proportionally over time. The release rate constant KHC corresponds to the slope. This model has been used to describe the release profile from the diminishing surface of the drug particles during the dissolution.

1.7.5 Release from spherical matrix system:[30,31]

The rate of the release of the drug from a spherical matrix controlled by diffusion, proposed by Baker-Lonsdale and its empirical form is

$$3/2[1-(1-M_t/M_\infty)^{3/2}]-M_t/M_\infty-k_{BL}t \text{ ----------(6)}$$

Where $M_\infty$is the amount of drug released at an infinite time and Mt is the amount of drug released after time t, kBL is the release rate constant. The graphic relating the left side of the equation and time will be linear if the established conditions of this model were fulfilled and the slope corresponds to the release rate constant kBL.

1.7.6 Erosion controlled release from matrix system:[30,31]

The rate of release of the drug from a dosage form controlled by erosion is constantly proportional to the actual area of the surface of the dosage form and its simplified form is

$$M_t/M_\infty= 1\ (1- k_{er}\ t)^3 \text{ ----------(7)}$$

Where M_∞ is the amount of drug released at an infinite time and Mt is the amount of drug released after time t. The model assumes that the rate limiting step of drug release is the erosion of the matrix itself and that time dependent diffusional resistance internal or external to the eroding matrix do not influence it. ker is the rate constant of erosion which takes into account the rate of erosion and obtained from the slope of the plot between $(1-Mt/M_\infty)^{1/3}$ versus time t.

1.7.7 The Fickian and non-Fickian drug release model: [30, 31]

In order to define a model, which will represent a better fit for the release from the formulations; dissolution data up to 60% can be further analyzed using Peppas and Korsemeyer equation (power law). To evaluate the contribution of the release mechanisms other than diffusion, other models of the release kinetics were employed. Since erosion of the matrix will contribute to the release, a model describing general solute release from hydrophilic polymers as employed by the Korsemeyer et al (1983) was used. Applied to the hydrophilic polymers it has the simplified empirical form (Ford et al, 1991)

$$M_t/M_\infty = kt^n \text{---------- (8)}$$

Where k is the release rate and n is the release exponent. Values of the release exponent (n) and the kinetic constant (k) obtained in each case from the slope and y-intercept of a logarithmic plot of percent released versus time respectively. Peppas (1985) used this n value in order to characterize different release mechanisms.

LIST OF TABLES

2.1 AIM:

Aim of the present work is to formulate and optimize clotrimazole microencapsulated mucoadhesive vaginal gel to ensure longer residence at the infection site, providing a favorable release profile for the antifungal drug .

2.2 OBJECTIVE:

Based on this aim the following objectives has been undertaken:

- Identification of drug as per existing standard.
- Determining the compatibility of drug-excipients
- To formulate a clotrimazole microspheres
- Characterization of the microsphere
- Incorporation of the clotrimazole microsphere to a mucoadhesive gel for local therapy of vaginal candidiasis
- To carry out the evaluation studies of clotrimazole microencapsulated mucoadhesive gel

2.3 RATIONAL BEHIND THE STUDY:

Vaginal candidiasis is a common condition and up to 75% of all women suffer atleast one episode of this infection during their lifetime. *Candidia albicans* is the most important cause of vaginal candidiasis, accounting for over 80% of the infection. Most patients with vaginal candidiasis respond to topical treatment with nystatin or imidazoles. For the treatment of vaginal candidiasis , local antimicrobial administration of clotrimazole has been favoured due to the numerous side effects, toxicity and teratogenic

potential of the systemically applied drugs. To achieve desirable therapeutic effect, vaginal delivery systems for antimicrobial agents need to reside at the sites of infection for a prolonged period. Applied by the vaginal route clotrimazole has residence time of the delivery system mostly insufficient to provide adequate therapeutic effect. Hence, there is need to develop effective drug delivery systems that should prolong the contact of the drug with a mucosal surface and enable sustained release of incorporated drug. Several novel carrier systems were suggested to be appropriate for vaginal drug delivery: bioadhesive tablets , polycarbophylic hydrogels, microspheres and liposomes.

Microencapsulation is widely used in the pharmaceutical sciences to improve bioavailability. It is therefore a rapidly expanding technology for achieving sustained-release dosage forms with improved physiologic availability of active substances. Up to now, only few studies reported the use of microspheres for the treatment of vaginal diseases.

For instance bioadhesive microparticles, PGLA microspheres and polymer lipid based mucoadhesive microspheres were assessed as vaginal delivery systems. Moreover,chitosan based microsphere loaded with an antimicrobial drug were added to different mucoadhesive excipients(cellulose derivatives, sodium alginate or carbopol® 974) to prepare mucoadhesive vaginal tablets, vaginal semisolids, particularly gels based on mucoadhesive polymers are currently receiving a great deal of interest as vaginal delivery systems.

The use of prolong-release mucoadhesive vaginal gel was thought to offer numerous benefits including prolong residence time of the dosage form at the site of absorption due

to bioadhesion to the vaginal mucosa, prolong drug release, improved bioavailability and decreased side effect of drug and ultimately improved patient compliance.

Clotrimazole will be used as a model drug in this study due to its bacteriostatic and bactericidal activity against gram negative bacteria and being used as drug of choice in various vaginal infection. Another important rationale of using clotrimazole, is its unique, low molecular weight offering the greater permeation benefit through vaginal epithelial membrane. Ethyl cellulose is assumed to offer the control release behavior of drug due to its hydrophobic coating over clotrimazole..

Bioadhesive polymer carbopol proved to provide better vaginal bioadhesion. Keeping in view of the above uniqueness, the present study is designed to develop a newer Microencapsulated Bioadhesive Vaginal Gel (MBVG) for prolong release of clotrimazole to treat vaginal infections with increased patient convenience.

2.4 PLAN OF WORK:

2.4.1 Pre-formulation studies of drug and polymer in terms of

Identification

Drug solubility

Polymer viscosity

2.4.2 Compatibility study:

IR spectroscopy,

Differential Scanning Calorimetry

2.4.3 Preparation of the drug loaded microspheres

2.4.4 Evaluation of the prepared microspheres in terms of

Particle size and shape

Production yield

Loading efficiency

In-vitro drug release study

Release kinetic study

2.4.5 Preparation of clotrimazole microcapsulated mucoadhesive vaginal gel

2.4.6 Evaluation of the prepared gel in terms of

Estimation of drug in vaginal gels

Determination of P^H

Viscosity measurement

In vitro drug diffusion studies

Release Kinetic studies of micro-encapsulated vaginal gels

Vaginal bioadhesion measurements

CHAPTER 3

REVIEW OF LITERATURE

1) U.Hani *et al*(2011) developed Metronidazole microspheres in a bioadhesive gel for local therapy of vaginal candidiasis by solvent evaporation method using Eudragit RS-100 and RL-100 polymers with different drug/polymer ratios. Microspheres were characterised by SEM, DSC, FT-IR and particle size analysis and evaluated for morphology, drug loading and *in vitro* drug release in simulated vaginal fluid.[32]
2) Patel G. et al. (2010) developed effervescent bioadhesive vaginal tablets of Ketoconazole by direct compression method. HPMC K4M, HPMC K15M, HPC, sodium CMC, Chitosan, Sodium Alginate, Methyl Cellulose, Carbopol 941 were used as bioadhesive polymers. Effervescent was incorporated in to the formulations as a disintegration agent. The swellings and in-vitro release were studied. The ex-vivo mucoadhesion was determined by self developed modified mucoadhession assembly. The ex-vivo residence test was carried out by modified USP dissolution test apparatus. Anti-fungal activity of the effervescent bioadhesive tablet of ketoconazole was determined in comparison to Candid®-V3 tablet and Candid®-V gel.[33]
3) Shivakumar Y. et al. (2010) developed Metronidazole intravaginal gel for the treatment of bacterial vaginosis. Effect of Mucoadhesive Natural Polymers on the release of Metronidazole were studied. Metronidazole (0.5 %) has been formulated in an intravaginal gel using the bioadhesive natural polymers such as Chitosan, Xanthan gum and Gelatine. To increase its aqueous solubility, Metronidazole was initially dissolved in a mixture of PVP K30 and water (5:3) and added to polymer dispersion. The intravaginal gel formulations were

evaluated for pH, Spreadability, Syringeability, Viscosity, Bioadhesion test, Antimicrobial Susceptibility Test and In vitro drug release study. V7 and V2 mucoadhesive gel formulations were selected as optimum formulations based on evaluation studies. These formulations are showing control drug release with good mucoadhesion properties due to presence of chitosan alone and with combination of Xanthan gum. All the performed experiments confirm the applicability of intravaginal gel as a novel drug carrier system for the local treatment of bacterial vaginosis. [34]

4) Ganure A. L. et al. (2011) developed Chitosan loaded Clotrimazole gel for the treatment of vaginitis. Their study describes the formulation and evaluation of gel containing chitosan (1%), various thickening agents like hydroxyl ethyl cellulose (1%, w/w), sodium carboxy methyl cellulose (1%, w/w), hydroxyl propyl cellulose (1%, w/w), methyl cellulose (1%, w/w), hydroxyl propyl methyl cellulose (1% w/w), magnesium aluminum silicate (1%, w/w) and clotrimazole (1%, w/w) as a drug. The optimized thickener (HEC 2-8% w/w) containing formulations were characterized in terms of in vitro drug release, viscosity, pH, sensitivity, content uniformity and in vitro antimycotic activity. The study revealed that drug release for all the formulations ranged from 28.29% to 70.64%, indicates that with increased concentrations of chitosan relative drug release were decreased and as the concentration of HEC increased, the rate of drug release increased to optimum and decreased, thereafter. For all the formulations, viscosity was obtained from 2166.67 cps to 144400.00 cps which showed that viscosity was increased with the increasing concentration of chitosan and HEC. Antimycotic

study showed maximum zone of inhibition for candida albicans using agar cup method. By observing all the data, the formulation C1H6 was optimized and compared with standard and marketed formulation. Same evaluation parameters were carried out for the comparison with marketed product and standard gel. Study proved that the optimized formulation (C1H6) consisting of chitosan and HEC in ratio 1:6, is best suitable for topical application. [35]

5) Francois M. et al. (2003) developed a Mucoadhesive, Cyclodextrin based vaginal cream formulation of Itraconazole for the treatment of vaginal candidiasis. They developed a novel itraconazole formulation intended for vaginal use based on hydroxypropyl-β-cyclodextrin (HPβCD), a functional excipient that increases drug solubility and generates a mucoadhesive system in the presence of other ingredients. An aqueous phase was prepared by solubilizing itraconazole with HCl in the presence of propylene glycol and then adding an aqueous solution of HPβCD. After pH adjustment, the itraconazole/HPβCD solution was added to the oil phase (paraffin oil, trihydroxystearate, and cetyl dimethicon copolyol) and the desired cream containing 1%, 2%, and 2.5% drug obtained by homogenization.Primary irritation studies and subchronic toxicity studies using a rabbit vaginal model indicated that the formulation was safe, well tolerated, and retained in the vaginal space. Clinical investigations indicated that application of 5 g of a 2% cream was very well tolerated and itraconazole was not systemically absorbed. Additional studies in women found that the itraconazole cream was highly effective in reducing or eliminating fungal cultures with few adverse effects. These studies suggested that an HPβCD-based, emulsified wax cream

formulation was a useful and effective dosage form for treating vaginal candidiasis. [36]

6) Mehta M. *et al* (2012) prepared Clotrimazole microencapsulated mucoadhesive topical gel to modify the release rate and also to reduce side effects. They prepared Clotrimazole microsphere by emulsion solvent diffusion technique by using Ethyl cellulose, HPMC K4M, Carbopol 934, Eudragit RS100, Eudragit S100, Eudragit RL100 and microspheres were evaluated for % Practical yield, % Loading efficiency and *In vitro* drug release study. Drug- excipients compatibility was performed by FTIR study. Optimized batch of microsponge was further formulated as gel formulation for topical delivery.They evaluated the prepared gel formulations for physical parameters like viscosity, pH, clarity and In vitro drug permeation study. The drug release data of optimized batch were fitted into different kinetic models which show that the drug release from gel formulations follows zero order release. They compared the optimized gel formulation with the marketed formulation and pure drug for anti fungal activity, which shows that the prepared formulation is having comparative anti fungal activity with marketed formulation. [37]

7) Bhowmik B. B. et al (2009) prepared metronidazole microencapsulated bioadhesive vaginal gel for enhanced vaginal drug delivery by optimum vaginal bioadhesion and longer retention. They prepared Metronidazole encapsulated microcapsules by thermal change method using ethyl cellulose as rate controlling polymer in different ratios. They found the microcapsules to be discrete, spherical

with free flowing properties and evaluated for particle size analysis, shape (scanning electron microscopy), flow properties, wall thickness, drug encapsulation efficiency, and in vitro release performance. They incorporated selected microcapsule formulation in gels with a variety of bioadhesive polymers. The MBVGs were evaluated for pH, spreadability, extrudability, viscosity, vaginal irritation test, in vitro drug release, drug release kinetics, bioadhesion test, accelerated stability of selected gel formulation. In vitro drug release rate for selected MBVG (F5 gel, containing 1 % w/w of drug loaded microcapsules and 0.6 % w/w of carbopol 974) was found to sustain metronidazole over 36 h obeying zero order kinetic with a good bioadhesion quality. They compared the results statistically and found with satisfactory correlation. Thus in conclusion preparation protocol of MBVG studied may be adopted for a successful development of newer drug delivery system of other drugs for administration to vagina. [38]

8) Nayak B. S. *et al* (2010) prepared Bioadhesive Vaginal Gel (BVG) of Metronidazole by variety of bioadhesive polymers (carbopol- 934, 940, 974 and 980) by mechanical stirring method in order to develop a sustained release BVG of metronidazole for prolong period of time. The BVGs were evaluated for pH, spreadability, extrudability, swelling index, viscosity, vaginal irritation test, in vitro drug release, drug release kinetics, bioadhesion test and accelerated stability of selected gel formulation. They compared the drug release data of metronidazole from BVG formulations with established marketed metronidazole vaginal gel formulation. *In vitro* drug release rate for selected BVG was found to

sustain the release of metronidazole over 8 hours obeying zero order kinetic with a good bioadhesion quality. The release profile of metronidazole from BVG was found much better than marketed metronidazole vaginal gel formulation. [39]

9) Yuen C.W.M *et al* (2012)prepared Chitosan microcapsules loaded with either miconazole nitrate or clotrimazole via emulsion technique. The mean particle size of the chi-tosan/miconazole nitrate microcapsules was 2.6 µm and that of the chitosan/clotrimazole microcapsules was 4.1 µm. They found that the encapsulation efficiency of the chitosan/miconazole nitrate microcapsules (77.58–96.81%) was relatively higher than that of the chitosan/clotrimazole microcapsules (56.66–93.82%). The in vitro drug release performance of the microcapsules shows that the chitosan/miconazole nitrate microcapsules release about 49.5% of the drug while chitosan/clotrimazole microcapsules release more than 66.1% of the drug after 12 h under a pressure of 5 kg at pH 5.5, which is similar to the pH of human skin. The pre-pared drug-loaded microcapsules could be applied onto bandages or socks, and will continuously release antifungal drugs in a controlled manner under pressure.[40]

CHAPTER 4 DRUG AND EXCIPIENT PROFILE

4.1 Drug

4.2 Polymer

4.3 Chemicals And Reagents

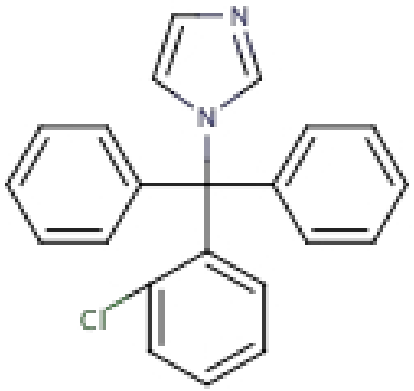
4.1 DRUG:

4.1.1 CLOTRIMAZOLE: [41]

Synonyms: Chlotrimazole

Clotrimazol

Structure:



Chemical formula: $C_{22}H_{17}ClN_2$

IUPAC name: 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole

Molecular weight: 344.84

PHYSICAL PROPERTIES:

State: solid

Melting point: 147-149 Oc

Water solubility: 1.47e-03 g/l

logP:5.48

pKa: 0

PHARMACODYNAMICS

Clotrimazole, an imidazole derivative with a broad spectrum of antimycotic activity, inhibits biosynthesis of the sterol ergostol, an important component of fungal cell membranes. Its action leads to increased membrane permeability and apparent disruption of enzyme systems bound to the membrane. Betamethasone and clotrimazole are used together to treat cutaneous tinea infections. In studies in fungal cultures, the minimum fungicidal concentration of clotrimazole caused leakage of intracellular phosphorous compounds into the ambient medium with concomitant breakdown of cellular nucleic acids, and accelerated potassium eflux. Both of these events began rapidly and extensively after addition of the drug to the cultures. The primary action of clotrimazole is against dividing and growing organisms.

Mechanism of action: Clotrimazole interacts with yeast 14-α demethylase, a cytochrome P-450 enzyme that converts lanosterol to ergosterol, an essential component of the membrane. In this way, clotrimazole inhibits ergosterol synthesis, resulting in increased cellular permeability. Clotrimazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms and the uptake of purine, impair triglyceride and/or phospholipid biosynthesis, and inhibit the movement of calcium and potassium ions across the cell membrane by blocking the ion transport pathway known as the Gardos channel.

PHARMACOKINETCS:

Absorption: Poorly and erratically absorbed orally, minimal vaginal or topical absorption.

Protein binding: 90%

Metabolism: Hepatic (metabolized to inactive metabolites)

Half life: 2 hours

PHARMACOLOGY

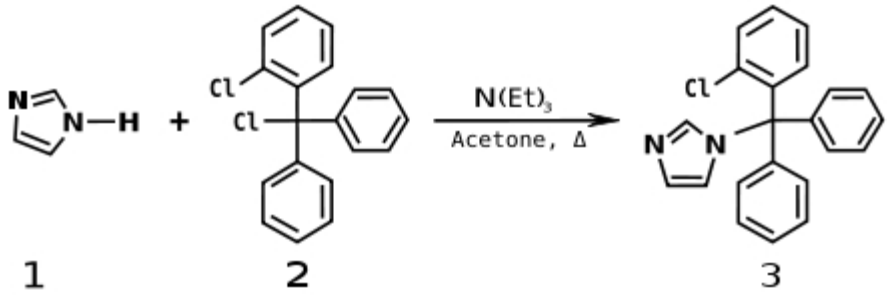
Indication: For the local treatment of oropharyngeal candidiasis and vaginal yeast infections, also used in fungal infections of the skin such as ringworm, athlete's foot, and jock itch.

DRUG INTERACTIONS

Potential for drug interactions with clotrimazole oral exists, as it is a potent, specific inhibitor of cytochrome P450 oxidase and may alter the metabolism of other drugs.

SIDE EFFECTS

Side effects include skin rash, hives, blistering, burning, itching, peeling, redness, stinging, swelling, or other sign of skin irritation



PRECAUTIONS:

GENERAL:

If irritation or sensitivity develops with the use of Clotrimazole, treatment should be discontinued and appropriate therapy instituted.

INFORMATION FOR PATIENTS:

This information is intended to aid in the safe and effective use of this medication. It is not a disclosure of all possible adverse or intended effects.

The patient should be advised to:

- Use the medication for the full treatment time even though the symptoms may have improved. Notify the physician if there is no improvement after four weeks of treatment.
- Inform the physician if the area of application shows signs of increased irritation (redness, itching, burning, blistering, swelling, oozing) indicative of possible sensitization.
- Avoid the use of occlusive wrappings or dressings.
- Avoid sources of infection or reinfection.

USAGE IN PREGNANCY

Pregnancy Category B:

The disposition of 14C Clotrimazole has been studied in humans and animals. Clotrimazole is very poorly absorbed following dermal application or intravaginal administration to humans.

In clinical trials, use of vaginally applied Clotrimazole in pregnant women in their second and third trimesters has not been associated with ill effects. There are, however, no adequate and well controlled studies in pregnant women during the first trimester of pregnancy.

Studies in pregnant rats with intravaginal doses up to 100 mg/kg have revealed no evidence of harm to the fetus due to Clotrimazole.

High oral doses of Clotrimazole in rats and mice ranging from 50 to 120 mg/kg resulted in embryotoxicity (possibly secondary to maternal toxicity), impairment of mating, decreased litter size and number of viable young and decreased pup survival to weaning. However, Clotrimazole was not teratogenic in mice, rabbits and rats at oral doses up to 200, 180 and 100 mg/kg, respectively. Oral absorption in the rat amounts to approximately 90% of the administered dose. Because animal reproduction studies are not always predictive of human response, this drug should be used only if clearly indicated during the first trimester of pregnancy.

NURSING MOTHERS:

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Clotrimazole is used by a nursing woman.

PEDIATRIC USE:

Safety and effectiveness in pediatric patients have been established for Clotrimazole when used as indicated and in the recommended dosage.

ADVERSE REACTIONS:

The following adverse reactions have been reported in connection with the use of Clotrimazole: erythema, stinging, blistering, peeling, edema, pruritus, urticaria, burning, and general irritation of the skin.

4.2 POLYMER

4.2.1 ETHYLCELLULOSE [42]

Nonproprietary names:

BP: Ethylcellulose

PhEur: Ethylcellulose

USP-NF: Ethylcellulose

Synonyms:

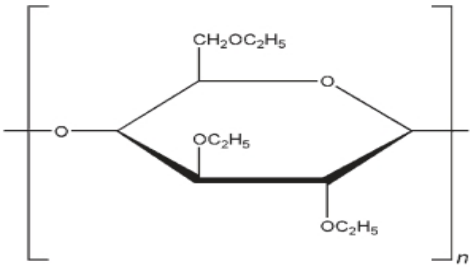
Aquacoat ECD; Aqualon; Ashacel; E462;Ethocel; ethylcellulosum;Surelease .

Chemical Name and CAS Registry Number: Cellulose ethyl ether [9004-57-3]

Empirical formula : C 12H 23O6 (C12H22O5)n C 12H 23O5

(where n can vary to provide a wide variety of molecular weights).

Structural formula



Functional category:

Coating agent; flavoring agent; tablet binder; tablet filler; viscosity-increasing agent.

Description:

Ethylcellulose is a tasteless, free-flowing, white to light tan-colored powder.

TYPICAL PROPERTIES

Density (bulk):0.4 g/cm

Glass transition temperature: 129–133^{0}C

Moisture content: Ethyl cellulose absorbs very little water from humid air or during immersion, and that small amount evaporates readily.

Solubility: Ethyl cellulose is practically insoluble in glycerin, propylene glycol, and water. Ethyl cellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%). Ethyl cellulose that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene.

Specific gravity: 1.12–1.15 g/cm

STABILITY AND STORAGE CONDITIONS

Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters.

Ethyl cellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may be prevented by the use of antioxidant and chemical additives that absorb light in the 230–340 nm range.

Ethyl cellulose should be stored at a temperature not exceeding 32^0C (90^0 F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

INCOMPATIBILITIES:

Incompatible with paraffin wax and microcrystalline wax.

APPLICATIONS IN PHARMACEUTICAL FORMULATION OR TECHNOLOGY

The main use of ethylcellulose in oral formulations is as a hydrophobic coating agent for tablets and granules.Ethylcellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation.Modified-release tablet formulations may also beproduced using ethylcellulose as a matrix former. Aqueous ethylcellulose dispersions are generally used to coat granules or pellets.Ethylcellulose-coated beads and granules have also demonstrated the ability to absorb pressure and hence protect the coating from fracture during compression.High-viscosity grades of ethylcellulose are used in drug micro-encapsulation.In tablet formulations, ethylcellulose may additionally be employed as a binder, the ethylcellulose being blended dry or wet-granulated with a solvent such as ethanol (95%). Ethylcellulose produces hard tablets with low friability, although they may demonstrate poor dissolution.

Ethylcellulose has also been used as an agent for delivering therapeutic agents from oral (e.g. dental) appliances. In topical formulations, ethylcellulose is used as a thickening

agent in creams, lotions, or gels, provided an appropriate solvent is used. Ethylcellulose has been studied as a stabilizer for emulsions. Ethylcellulose is additionally used in cosmetics and foodproducts.

REGULATORY STATUS

GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Database (oral capsules, suspensions and tablets; topical emulsions and vaginal preparations). Included in nonparenteral medicines licensed in Europe. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

4.2.2 CARBOPOL934P: [42]

Nonproprietary names:

BP: Carbomers

PhEur: Carbomers

USP-NF: Carbomer

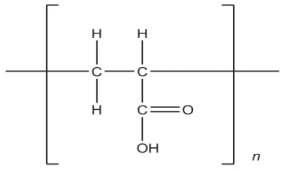
Synonyms:

Acrypol; Acritamer; acrylic acid polymer; carbomera; Carbopol; carboxy polymethylene; polyacrylic acid; carboxyvinyl polymer; Pemulen ; Tego Carbomer .

Chemical name and cas registry number: Carbomer [9003-01-4]

Alternative CAS registry numbers have been used for carbomer 934 ([9007-16-3])

Structural formula:



Functional category:

Bioadhesive material; controlled-release agent; emulsifying agent; emulsion stabilizer; rheology modifier; stabilizing agent; suspending agent; tablet binder.

APPLICATIONS IN PHARMACEUTICAL FORMULATION OR TECHNOLOGY

Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers. Formulations include creams, gels, lotions and ointments for use in ophthalmic,rectal,topical and vaginal preparations. In tablet formulations, carbomers are used as controlled release agents and/or as binders. Carbomer polymers have also been investigated in the preparation of sustained-release matrix beads,as enzyme inhibitors of intestinal proteases in peptide-containing dosage forms, as a bioadhesive for a cervical patch and for intranasally administered micro-spheres, in magnetic granules for site-specific drug delivery to the esophagus, and in oral mucoadhesive controlled drug delivery systems.Carbomers copolymers are also employedas emulsifying agents in the preparation of oil-in-water emulsionsfor external administration. Carbomers are also used in cosmetics. Therapeu-tically, carbomer formulations have proved efficacious in improving symptoms of moderate-to-severe dry eye syndrome.

DESCRIPTION:

Carbomers are white-colored, ‘fluffy’, acidic, hygroscopic powders with a characteristic slight odor. A granular carbomer is alsoavailableParticle size distribution Primary particles average about 0.2mm in diameter. The flocculated powder particles average 2–7 mm in diameter and cannot be broken down into the primary particles.A granular carbomer has a particle size in the range 150–425 mm.

Solubility: Swellable in water and glycerin and, after neutraliza-tion, in ethanol (95%). Carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally crosslinked microgels.

Specific gravity: 1.41g/cm

STORAGE CONDITIONS:

Carbomers also form pH-dependent complexes with certain polymeric excipients. Adjustment of pH and/or solubility parameter can also work in this situation.

REGULATORY ACCEPTANCE:

Included in the FDA Inactive Ingredients Database (oral suspen-sions, tablets; ophthalmic, rectal, topical, transdermal preparations; vaginal suppositories). Included in nonparenteral medicines licensed in Europe. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

4.3 CHEMICALS AND REAGENTS

4.3.1 ACETONE [42]

Nonproprietary names:

BP: Acetone

PhEur: Acetone

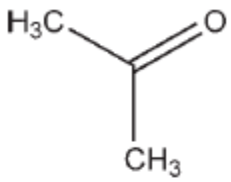
USP-NF: Acetone

Synonyms:

Acetonum; dimethylformaldehyde; dimethyl ketone; b-ketopropane; pyroacetic ether.

Chemical name and cas registry number: 2-Propanone [67-64-1]

Structural formula :



TYPICAL PROPERTIES

Boiling point: 56.2^0C

Flash point -20^0C

Melting point : 94.3^0C

Solubility : Soluble in water; freely soluble in ethanol (95%).

Vapor pressure : 185mmHg at 20^0C

APPLICATIONS IN PHARMACEUTICAL FORMULATION OR TECHNOLOGY

Acetone is used as a solvent or cosolvent in topical preparations, and as an aid in wet granulation. It has also been used when formulating tablets with water-sensitive active ingredients, or to solvate poorly water-soluble binders in a wet granulation process. Acetone has also been used in the formulation of microspheres to enhance drug release. Owing to its low boiling point, acetone has been used to extract thermolabile substances from crude drugs.

4.3.2 LIGHT LIQUID PARAFFIN [42]

PHYSICAL AND CHEMICAL PROPRTIES:

Appearance: Clear colourless liquid.

Density/specific gravity (g/ml): typical 0.845 at 15 ^{o}c

Vapour density (air=1): > 1

Vapour pressure : < 0.01 mmH g at 20 ^{0} C

SOLUBILITY DESCRIPTION:

Insoluble in water. Soluble in: petroleum solvents.

Stability and Reactivity: Stable under normal conditions of use.

CONDITIONS TO AVOID:

Avoid excess heat.

Materials to avoid: oxidising agent.

4.3.3 PETROLIUM ETHER

Petroleum ether, also known as benzine, petroleum naphtha, petroleum spirits, X4 or ligroin, is a group of various volatile, highly flammable, liquid hydrocarbon mixtures used chiefly as nonpolar solvents. Chemically, it is not an ether like diethyl ether, but a light hydrocarbon.

Petroleum ether is obtained from petrolium refineries as the portion of the distillate which is intermediate between the lighter naphtha and the heavier kerosine. It has a specific gravity of between 0.6 and 0.8 depending on its composition. The following distillation fractions of petroleum ether are commonly available: 30 to 40 °C, 40 to 60 °C, 60 to 80 °C, 80 to 100 °C, 80 to 120 °C and sometimes 100 to 120 °C. The 60 to 80 °C fraction is often used as a replacement for hexane. Petroleum ether is mostly used by pharmaceutical companies and in the manufacturing process. Petroleum ether consists mainly of pentane, and is sometimes used instead of pentane due to its lower cost. Petroleum ether is useful

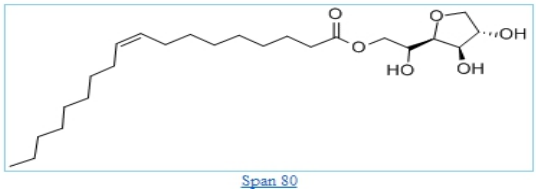
for removing the gum from self-adhesive stamps. It is the main ingredient of some 'label remover' or 'sticker remover' products.

4.3.4 SPAN 80

Molecular formula: $C_{24}H_{44}O_6$

Molecular weight: 428.6

Chemical structure:



Span 80

CHAPTER 5 MATERIALS AND METHODS

5.1 Materials

5.2 Methods

5.1 MATERIALS:

5.1.1 **Chemicals and Reagents**:

MATERIALS		SUPPLIER
DRUG	CLOTRIMAZOLE	Balaji Drugs. (30-7-37, Bhanu St.,Dabagarden)
POLYMERS	CARBOPOL 934P	Balaji Drugs. (30-7-37, Bhanu St.,Dabagarden)
	ETHYLCELLULOSE	Balaji Drugs. (30-7-37, Bhanu St.,Dabagarden)
CHEMICALS	ACETONE	Nova Biotech, Deshbondhunagar, Kolkata 700059
	LIGHT LIQUID PARAFFIN	RFCL Limited, New Delhi 110020
	PETROLIUM ETHER	Merck specialities pvt. Ltd, Worli, Mumbai 400018.
	SPAN 80	Loba chemie pvt. Ltd, Colaba, Mumbai 400 005.
	METHYL PARABENE	Loba chemie pvt. Ltd, Colaba, Mumbai 400 005.

Table 1

All the chemicals and reagents were of analytical grade.

5.1.2 Instruments:

INSTRUMENTS	COMPANY NAME
Digital Weighing Balance	Denver instrument
UV Spectrophotometer	Shimadzu (Model No.UV 1800),
FTIR	Bruker Alpha (Model no. 10059736)
Brookfield viscometer	DV-E Viscometer
Magnetic stirrer	ROLEX
Homogeniser	IKA (T25 Digital Ultra Turrax)
DSC	PERKINALMER USA
Texture analyzer	STABLE MICRO SYSTEM
Digital Melting Point apparatus	Macro Scientific Works
Digital pH Meter	335, Systronics
SEM	Zeiss, Germany

Table 2

5.2 **METHODS**:

5.2.1 **Preformulation Studies**:

5.2.1.1 **Organoleptic Properties**:

The Organoleptic properties such as colour,appearance of the drug sample were evaluated by visual observations. The taste and odour of the drug was also evaluated.

5.2.1.2 **Characterisation Of Drug**:

5.2.1.2.1 **Melting Point Determination**:

The melting point of the pure drug was determined by using the melting point apparatus (MAC, Digital Melting Point apparatus, Macro Scientific Works) and compared with the reported value from literature.

5.2.1.2.2 **Fourier Transform Infrared Spectroscopy (FTIR):**

IR spectroscopy is one of the important analytical techniques for chemical identification. The spectra of pure drug was recorded in the range of 4000cm^{-1}-400cm^{-1}(resolution 2cm^{-1}) using FTIR Bruker Alpha. The recorded spectrum was compared with recorded one in the literature.

5.2.1.3 **Analysis Of Clotrimazole**:

5.2.1.3.1 **Preparation Of Standard Curve Of Clotrimazole In Phosphate Buffer Saline (Ph 4.9): Methanol (6:4) Ratio:**

50 mg of clotrimazole was 1st dissolved in 20 ml of methanol,then required amount of pbs(4.9)(30ml)was added to the above solution and mixed well.the solution was then

filtered.the concentration of the solution was 1000µg/ml and it was taken as stock solution.

From the stock solution ,different concentration range of 10,20,30,40,50,60,70,80,100 µg/ml was prepared and absorbance was measured at 261 nm.

5.2.1.3.2 **Solubility Study Of Drug:**

50mg of clotrimazole was accurately weighed and solubility of this sample was checked in distilled water,ethanol, acetone, phosphate buffer saline pH 4.9, and methanol.

5.2.1.4 **Drug Excipient Compatibility Study:**

5.2.1.4.1 **FTIR Study**:

The excipients were mixed individually with the pure drug in a 1:1 ratio and the spectra were recorded using FTIR Bruker Alpha. The scanning range was 400-4000 cm^{-1} and the resolution was 2 cm^{-1}.

5.2.1.4.2 **DSC study:** [47]

The excipient were mixed individually with the pure drug in a 1:1 ratio and DSC study was performed. Differential scanning calorimetry (DSC) of the bulk drug clotrimazole was performed using DSC instrument (Parkin Almer, U.S.A) for measurement of the heat loss or gain resulting from physical or chemical changes within a sample as a function of temperature. About 6-7 mg of the individual components or drug-excipients combinations were weighed in aluminium DSC pans and hermetically sealed capsules were prepared with aluminium lids. An initial ramp was used to jump the

temperature to 40ºC and then a constant heating rate of 10 ºC/min was used up to 300 ºC under nitrogen atmosphere.

5.2.1.5 **Viscosity Determination:**

Viscosity of Carbopol 934P was determined by preparing a 1%w/v solution of Carbopol 934P in distilled water. Triethanolamine was used to adjust the pH. The viscosities of prepared polymeric solution were determined by using Brookfield viscometer using 25 spindle at 0.3,0.5,0.6,1 rpm.

5.2.2 Preparation Of Clotrimazole Microspheres: [43, 44, 45, 46]

Formulations	clotrimazole (mg)	Ethyl cellulose (mg)	Span80 (ml)	Acetone (ml)	Light liquid paraffin(ml)
F1	500	1000	2.5	25	100
F2	750	2250	2.5	25	100
F3	400	1600	2.5	25	100
F4	500	2500	2.5	25	100
F5	1000	1000	2.5	25	100

Table: 3 Formulation designs for the preparation of clotrimazole microspheres

The clotrmazole loaded microspheres were prepared by emulsion solvent evaporation method.

Required amount of Ethylcellulose (EC) was accurately weighed and dissolved in 25 ml of acetone. Accurately weighed required amount of clotrimazole was dispersed slowly in the polymer solution. Then the solution was slowly poured into 100ml of light liquid paraffin containing 2.5 ml of Span 80 and stirred at the rate of 2000 rpm in Homogeniser for 2 hours. The microspheres were separated by filtration. The removal of residual oil was performed by washing the microspheres with petroleum ether. Then the microspheres were dried at room temperature for 12 hours.

5.2.2.1 Evaluation test for clotrimazole microsphere: [43, 44, 45, 46]

Microsphere formulations were evaluated for particle size and shape, Production yield, Drug content and drug encapsulation efficiency (DEE) and *in- vitro* drug release.

5.2.2.1.1 Morphological and topographical characterization: [43, 44, 45, 46]

Morphological and topographicalcharacterization of microsphere formulation was performed by scanning electron Microscopy(SEM), Zeiss, Germany and optical microscopy. Their diameters were determined with a pre-calibrated graduated eyepiece of the optical microscope. One hundred measurements were averaged for each microsphere formulation prepared.

5.2.2.1.2 Production yield: [43, 44, 45, 46]

The production yield of the microsphere was determined using following equation.

$$\text{Production yield} = \frac{\text{Practical mass of microsphere}}{\text{Theoretical mass (polymer + drug)}} \times 100 \quad \text{-------- (9)}$$

5.2.2.1.3 Drug encapsulation efficiency (DEE): [43, 44, 45, 46]

Accurately weighed microspheres equivalent to 50 mg of drug, were suspended in 10ml of acetone to dissolve the polymer coat. The drug was extracted with 50 ml of simulated vaginal fluid (SVF, phosphate buffer, pH 4.9) in separating funnel and analyzed by using UV-Visible spectrophotometer after suitable dilution at 261 nm.

Drug encapsulation efficiency was calculated using the formula.

DEE (%) = (Practical drug content/Theoretical drug content) × 100 ---------- (10)

5.2.2.1.4 *In vitro* drug release studies of microsphere formulations: [43, 44, 45, 46]

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using SVF as dissolution medium (900 ml phosphate buffer pH 4.9, at 37±2°C was adjusted to 100 rpm). An aliquot sample (5 ml) was withdrawn at a definite time interval with replacement of fresh medium and analyzed for drug content by UV-Visible spectrophotometer at 261 nm. The same method was adopted for each batch of microsphere.

5.2.2.1.5 Release kinetic studies of microsphere formulations: **[48]**

In order to study the exact mechanism of drug release from the Microspheres, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsemeyer - Peppas equations. The *in vitro* release data obtained from optimised formulation in the dissolution medium (Phosphate buffer pH 4.9) was fitted to various kinetic models. Data obtained from drug release studies was analyzed according to the equations given in the Table-4

Model	Equation
Zero-order	$Q_t = Q_o - K_o t$
First-order	$\ln Q_t = \ln Q_o - K_1 t$
Higuchi matrix	$Q_t = K_H t^{1/2}$
Korsmeyer-Peppas	$\text{Log}(Q_t/Q_o) = \text{Log}K + n\text{Log}t$

Table-4

Where,

Q_t: Cumulative amount of drug released at any specific time (t).

Q_o: Amount of drug remaining in the formulation

K_o: Rate constant of Zero-order

K_1: Rate constant of First-order

K_H: Rate constant of Higuchi-matrix model

K: Release rate constant which considers structural and geometric characteristics of the microsphere.

n: The diffusion exponent; indicative of the mechanism of drug release.

The 'n' value could be used to characterize different release mechanisms as mentioned in Table-5

'n'	Mechanism
0.5	**Fickian diffusion(Higuchi matrix)**
0.5<n<1	**Anomalous transport (non-fickian diffusion)** drug release is both diffusion-controlled and swelling-controlled.
1	**Case-II transport(Zero-order)**
n>1	**Super case-II transport**

Table 5: Different release mechanism of 'n' value

5.2.2.1.6 Selection of Optimised microsphere formulation:

The optimised formulation was selected from the microsphere formulations considering the % yield, % entrapment efficiency, particle size, as well as the *in vitro* drug release study. The optimised formulation was further evaluated for the release kinetic study.

5.2.3 Preparation of microencapsulated vaginal bioadhesive gels: [38, 3940, 44]

Accurately weighed amount of carbopol 934 was taken and dissolved in water using propeller. Microsphere formulation containing Clotrimazole was added to the above solution with constant stirring. This final solution was neutralized slowly adding triethanolamine with constant stirring until the gel is formed.

Microencapsulated Bioadhesive Vaginal Gels compositions
Amount taken in percentage (w/w):

Formulation	microsphere	Carbopol934P	triethanoamine	Methyl parabene	Distilled water
MG1	1	1.0	0.5	0.2	q.s
MG2	1	0.5	0.5	0.2	q.s
MG3	1	1.0	0.5	0.2	q.s
MG4	1	0.5	0.5	0.2	q.s
MG5	1	1.0	0.5	0.2	q.s

Table-6: **Microencapsulated Bioadhesive Vaginal Gels compositions**

5.2.3.1 Evaluation of microencapsulated gel

Microencapsulated gel formulations were evaluated for the parameters like viscosity, pH, vaginal mucoadhesion measurement, drug content, in vitro drug permeation and in vitro release kinetics.

5.2.3.1.1 Viscosity measurement[49]

The viscosities of prepared gels were determined by using Brookfield viscometer using 25 spindle at 0.3, 0.5, 0.6,1 rpm.

5.2.3.1.2 P^H determination [49]

The P^Hs of the microencapsulated gels were determined by digital pH meter. 1 gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in

to gel formulation for 30 min until constant reading obtained and constant reading was noted.

5.2.3.1.3 Estimation of Clotrmazole in vaginal gels: [52]

Accurately weighed gel (0.5 g) was suspended in 25 ml of SVF. It was filtered after constant stirring and analyzed by using same UV-Visible spectrophotometer after suitable dilution at 261nm

5.2.3.1.4 In *vitro* drug diffusion studies of microencapsulated vaginal gels: [49]

Modified Franz diffusion cell was used for these studies. Cellophane membrane was used for the permeation study simulating the vaginal in vivo condition like vaginal epithelial barrier. The known quantity (1 gm gel containing 10 mg of the drug) was spread uniformly on the membrane on donor side. P^H4.9 phosphate buffer was used as the acceptor medium, from which samples were collected at regular intervals during 6hours and replaced with the same amount of buffer to maintain the receptor phase at 25ml. Samples were analyzed by UV-spectrophotometer at 261 nm.

5.2.3.1.5 Vaginal bioadhesion measurements [50, 51]

The *In vitro* bioadhesion testing for both blank as well as microsphere loaded gel was evaluated by detachment force measurements of gel by using a texture analyzer intact with mucosal membrane of goat vagina *in vitro.* The strong interaction between

both blank and drug-loaded formulation with the mucous lining of the tissue helped to increase contact time and permit localization.

5.2.3.1.6 Release Kinetic studies of microencapsulated vaginal gels: [53, 54, 55]

In order to study the exact mechanism of drug release from the microencapsulated vaginal gels, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsemeyer - Peppas equations. The *in vitro* release data obtained from optimized formulation in the dissolution medium (Phosphate buffer pH 4.9) was fitted to various kinetic models.

CHAPTER 6

RESULT AND DISCUSSION

6.1ORGANOLEPTIC PROPERTIES:

Results of the visual observation of the pure drug were found as follows:

Colour: White

Odour: Characteristic

Taste: Bitter

6.2. Characterisation of drug:

6.2.1. Melting point determination:

The melting point of the pure drug sample of clotrimazole was found to be 147^0c. As the melting point of API was within the reported ranges specified in literature (147-149^0c), it revealed that API was in its pure and pharmacologically active form.

6.2.2. Fourier Transform Infrared Spectroscopy (FTIR):

FTIR spectroscopy was performed to identify the supplied pure drug. The spectrum of the pure drug was compared with the reference spectrum of clotrimazole as shown in Fig 8 and Fig 9 respectively.

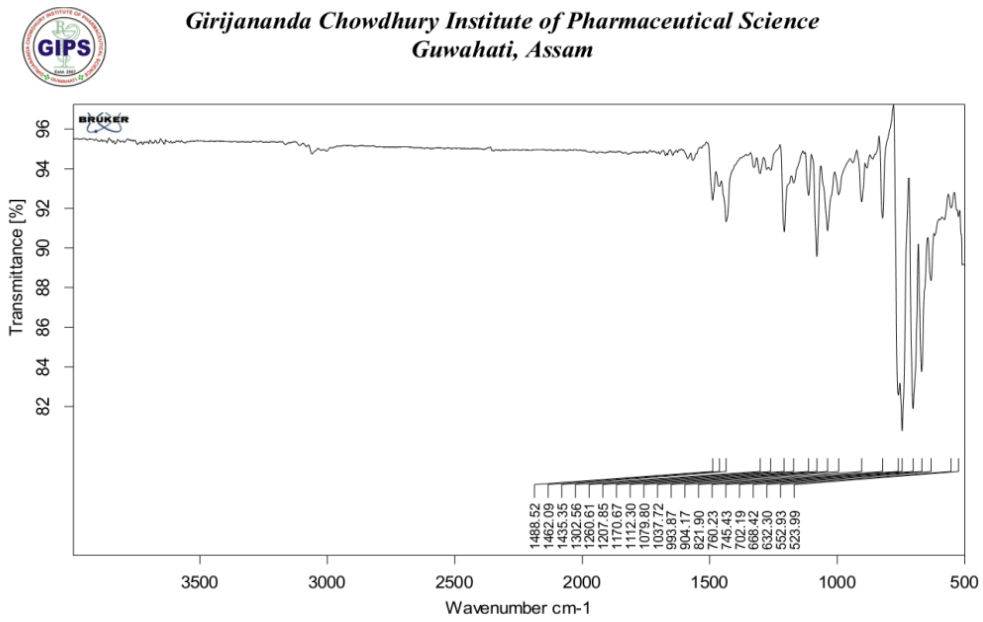


Fig 8: IR spectra of supplied clotrimazole

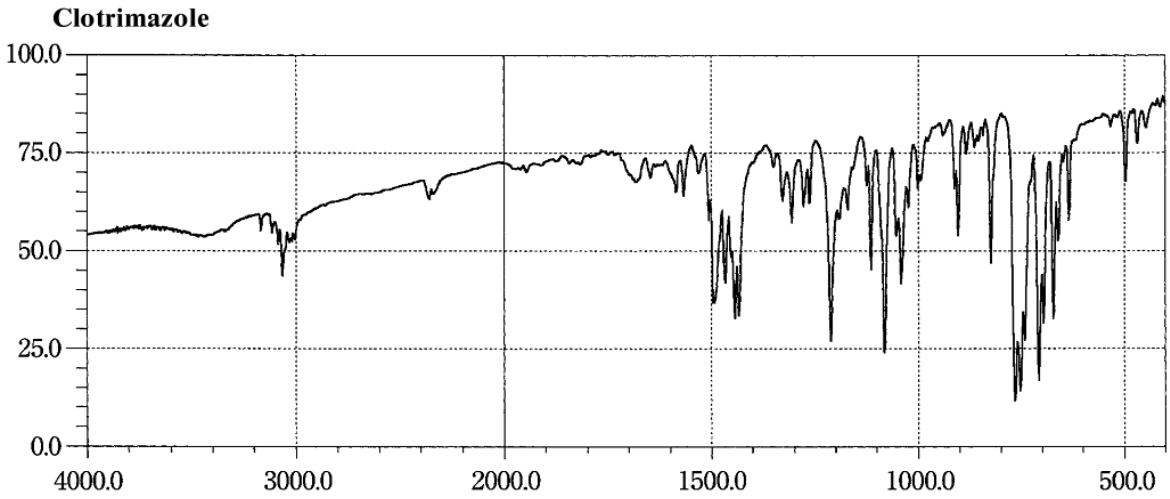


Fig 9: IR spectra of reference clotrimazole

6.2.2.1 IR interpretation of Clotrimazole

Sl no.	Functional group	IR Absorption bands (cm^{-1})	
		Reference drug	Sample drug
1.	C-H	1300	1302
2.	N-H	820	821
3.	C-Cl	900	904
4.	C-H (aromatic)	3100	3100
5.	C=N	1640	1649
6.	C-N	1550	1555
7.	C-C	1430	1435

Table 7: IR interpretation of Clotrimazole

As the IR spectrum of supplied clotrimazole matches with the IR spectrum of reference clotrimazole, it can be concluded that the supplied drug sample was pure clotrimazole.

6.3 Analysis of Clotrimazole:

6.3.1 Preparation of standard curve of clotrimazole in phosphate buffer saline (P^H4.9): methanol (6:4) ratio:

After scanning the appropriate dilute solution in the range of 200-400nm the λ_{max} was found to be 261nm.The UV spectrum and calibration curve of clotrimazole in the above mentioned solvent system were presented in Fig 10 and Fig 11 respectively. The linearity of the curve was found between concentration ranges of 10-20µg/ml.

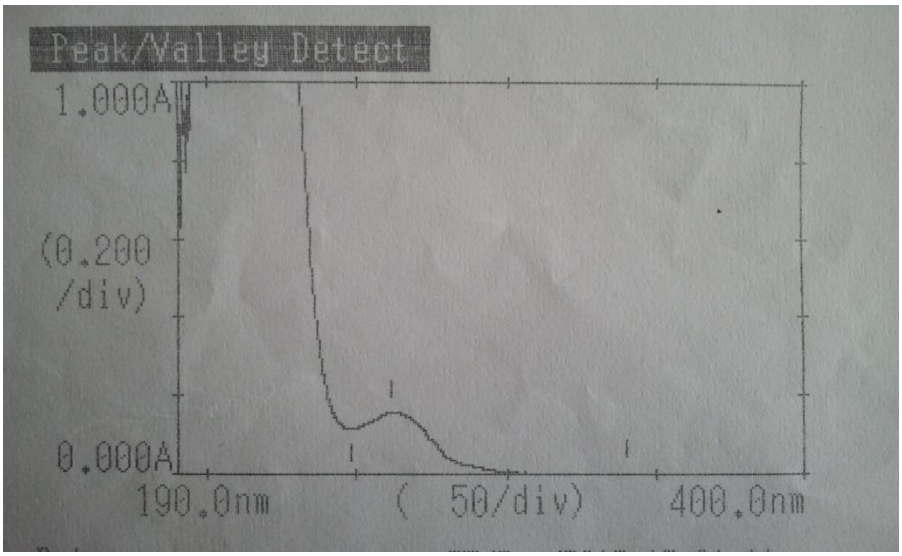


Fig10: UV spectra of clotrimazole in PBS(P^H4.9):methanol(6:4)(λ_{MAX} 261nm)

Data for standard curve of clotrimazole in pbs(p^h4.9):methanol(6:4)

Concentration(µg/ml)	Absorbance
0	0
10	0.033
20	0.0601
30	0.0892
40	0.113
50	0.1375
60	0.1642
70	0.1901
80	0.2098
100	0.2521

Table 8: Data for standard curve of clotrimazole in pbs(p^h4.9):methanol(6:4)

Standard Curve Of Clotrimazole In PBS (P^H 4.9):Methanol(6:4)

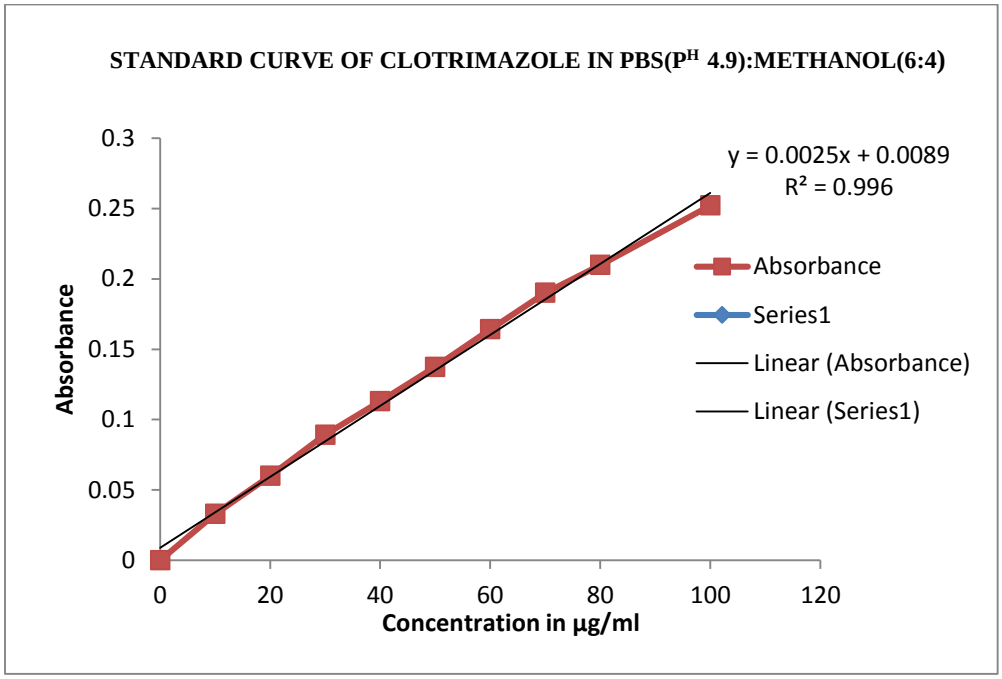


Fig 11: Standard Curve Of Clotrimazole In Pbs(P^H 4.9):Methanol(6:4)

6.4 Solubility study of drug:

Results of qualitative solubility study of the clotrimazole in different solvents were presented in Table 9.

Sample no.	Solvents	Solubility
1.	Distilled water	insoluble
2.	Phosphate buffer saline pH 4.9	insoluble
3.	Acetone	Freely soluble
4.	Ethanol	Freely soluble
5.	Methanol	Freely soluble

Table: 9

6.5 Drug excipient compatibility study:

6.5.1 FTIR study:

FTIR spectroscopy study was carried out separately to find out, the compatibility between the drug clotrimazole and the polymers ethyl cellulose and carbopol934P. The FTIR was performed for drug, polymer and the physical mixture of drug-polymer.

The spectrum obtained from FT-IR spectroscopy studies at wavelength between 4000 cm-1 to 400 cm-1 are given in Figures 12 to 15.

IR interpretation of drug, polymer and 1:1 ratio of physical mixture of drug-polymer

Sl no.	Functional group	IR Absorption bands (cm^{-1})		
		Drug	Drug + Ethyl cellulose	Drug +carbopol934p
1.	C-H	1302	1309	1259
2.	N-H	821	821	821
3.	C-Cl	904	904	902
4.	C-H (aromatic)	3100	3098	2938
5.	C=N	1649	1652	1707
6.	C-N	1555	1550	1552
7.	C-C	1435	1435	1433

Table: 10

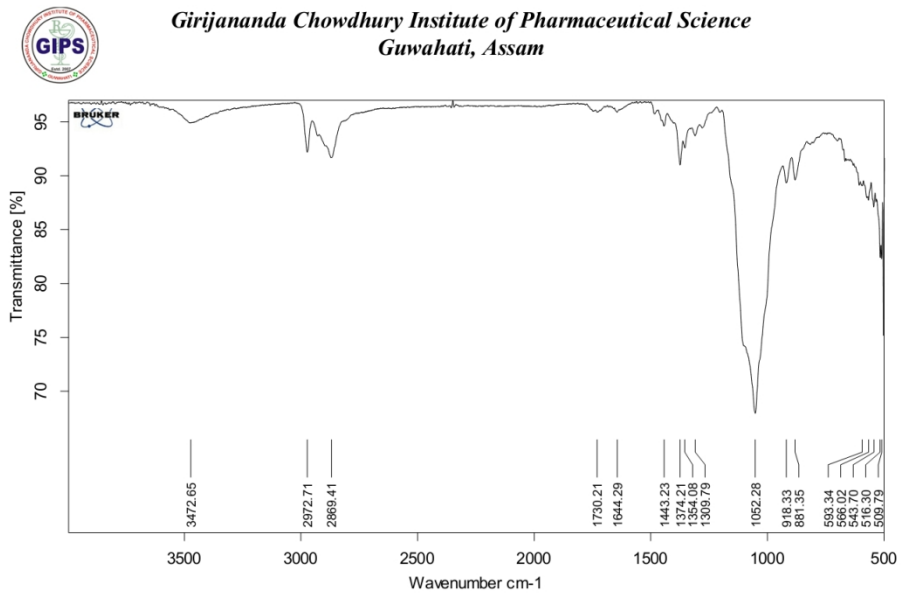


Fig 12: IR spectra of ethyl cellulose

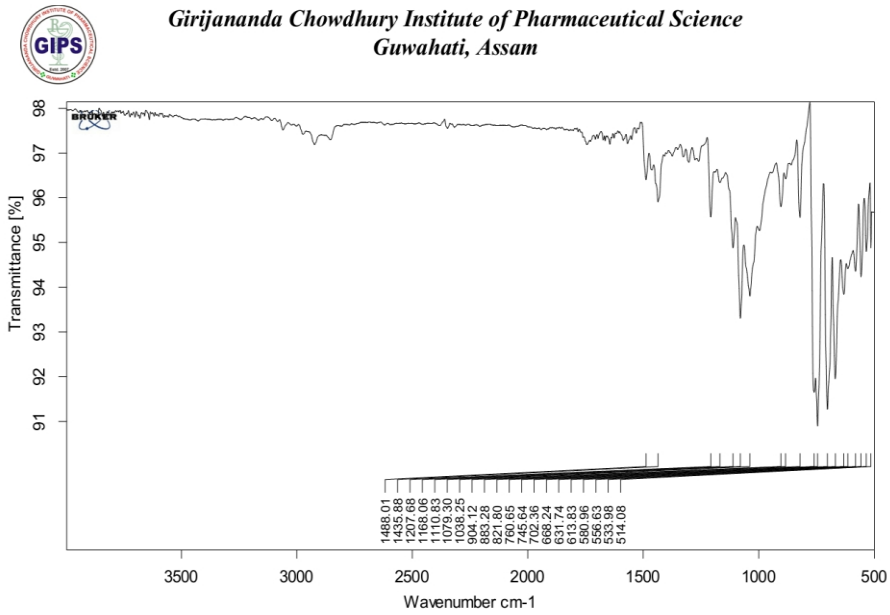


Fig13: IR spectra of ethyl cellulose:clotrimazole

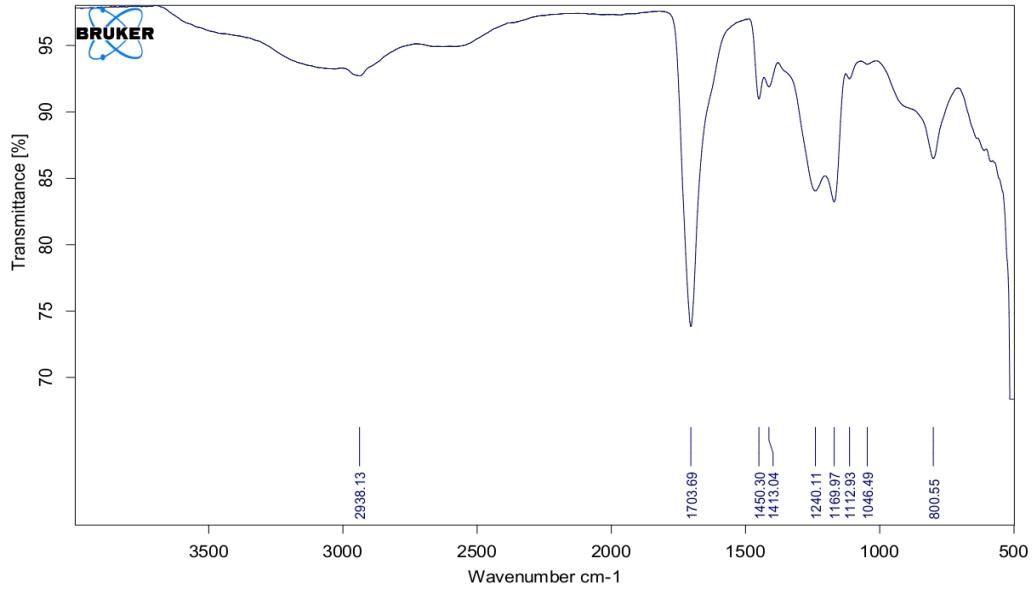


Fig14: IR spectra of carbopol 934P

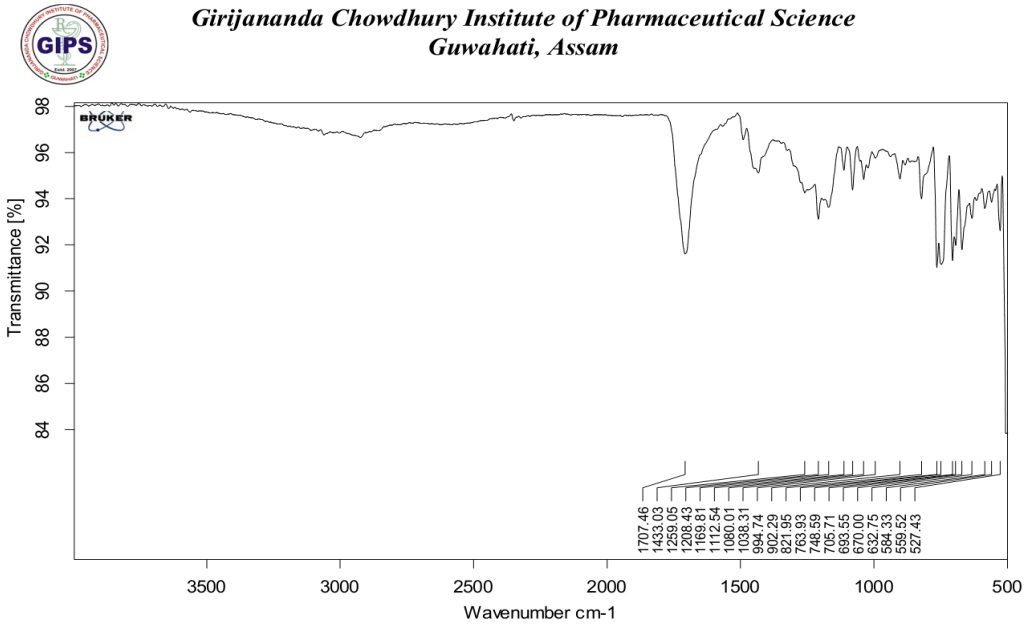


Fig15: IR spectra of carbopol 934P: clotrimazole

FTIR of clotrimazole showed the peaks at 1302,821,904,3100,1649,1555and 1435nm due to C-H, N-H, C-Cl,C-H(aromatic), C=N, C-N and C-C functional groups. The physical mixture of drug with polymer Ethyl cellulose and carbopol934P clearly shows the retention of these characteristic peaks of clotrimazole (Table 10 and Figures 12 to 15), thus revealing no interaction between the selected drug and polymers.

6.6. DSC study:

DSC study was carried out separately to find out, the compatibility between the drug clotrimazole and the polymers ethyl cellulose and carbopol934P. The DSC thermogram was performed for drug and the physical mixture of drug-polymer.The thermogram obtained from DSC studies at temperature between 20^0c to 260^0c is given in Fig 16 to 20.

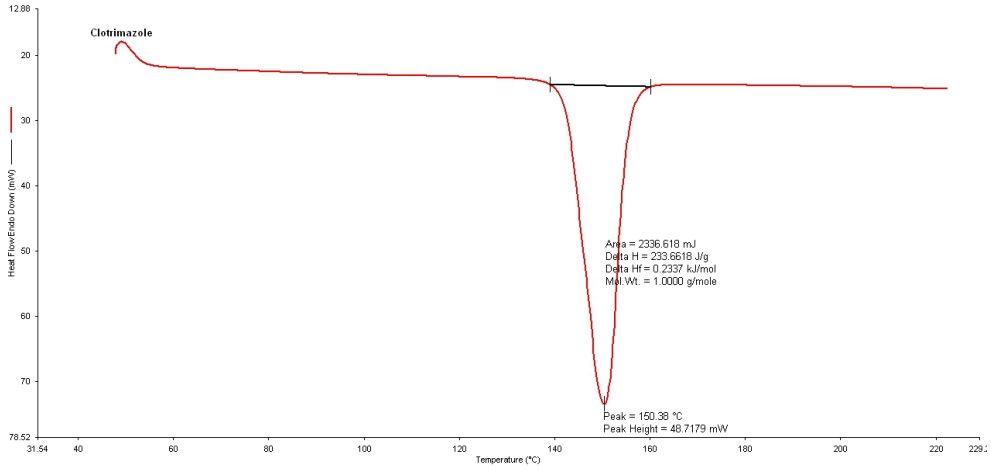


Fig16: DSC thermogram of clotrimazole

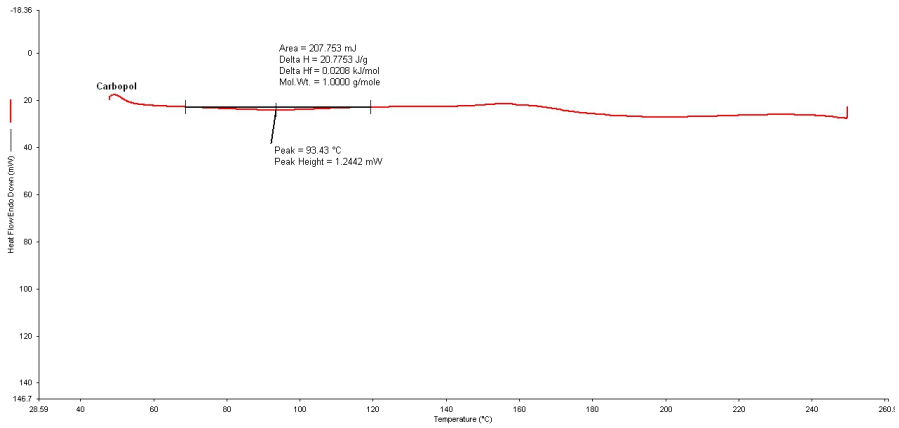


Fig17: DSC thermogram of carbopol934P

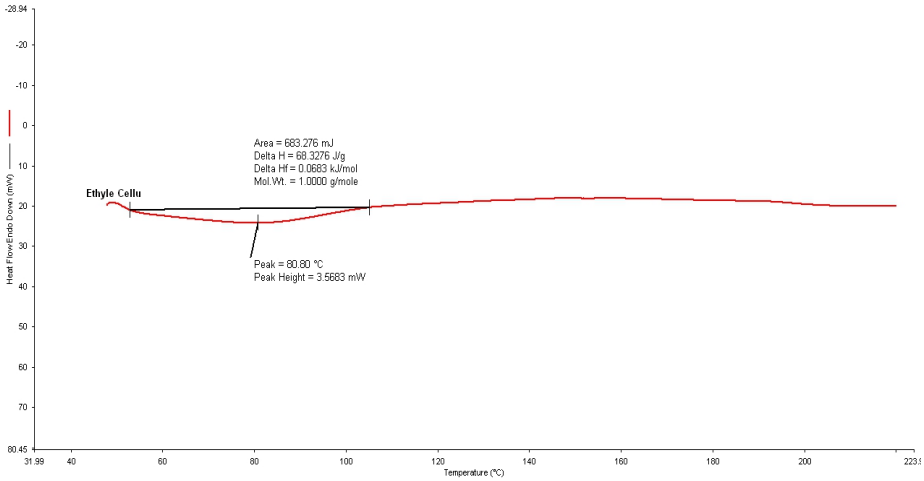


Fig18: DSC thermogram of ethyl cellulose

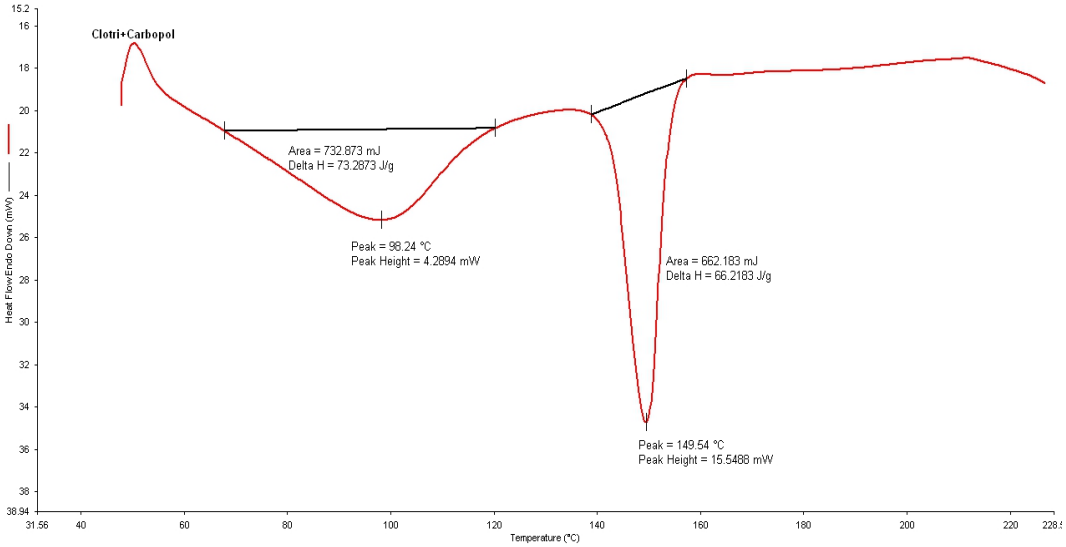


Fig19: DSC thermogram of clotrimazole:carbopol934P(1:1)

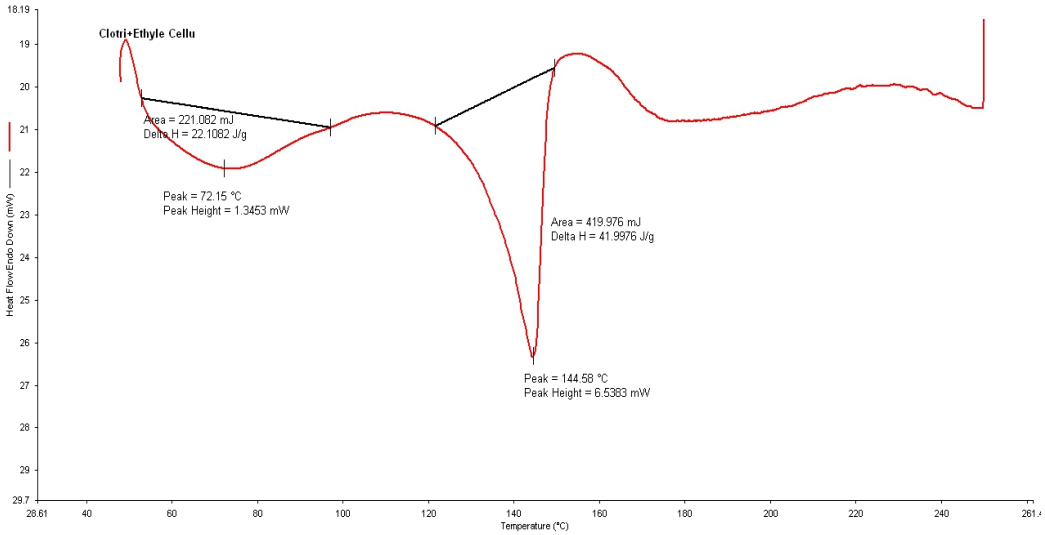


Fig20: DSC thermogram of clotrimazole:ethyl cellulose(1:1)

The thermogram of pure clotrimazole shows a sharp endothermic peak at 150.38°C which corresponds to its melting point. The thermogram of clotrimazole with excipient like ethyl cellulose and carbopol934P shows sharp endothermic peak at

144.58^{0}c and 149.54^{0}c (Fig 20&19) respectively, due to the presence of clotrimazole. Thus, the thermal data did not reveal any interaction between the drug and the excipients.

6.7 Viscosity determination:

RPM	Viscosity (1% concentration of carbopol), cps
0.3	1192000
0.5	715200
0.6	708800
1	357600

Table 11: Viscosity of carbopol 1% solution at different RPM (spindle 25)

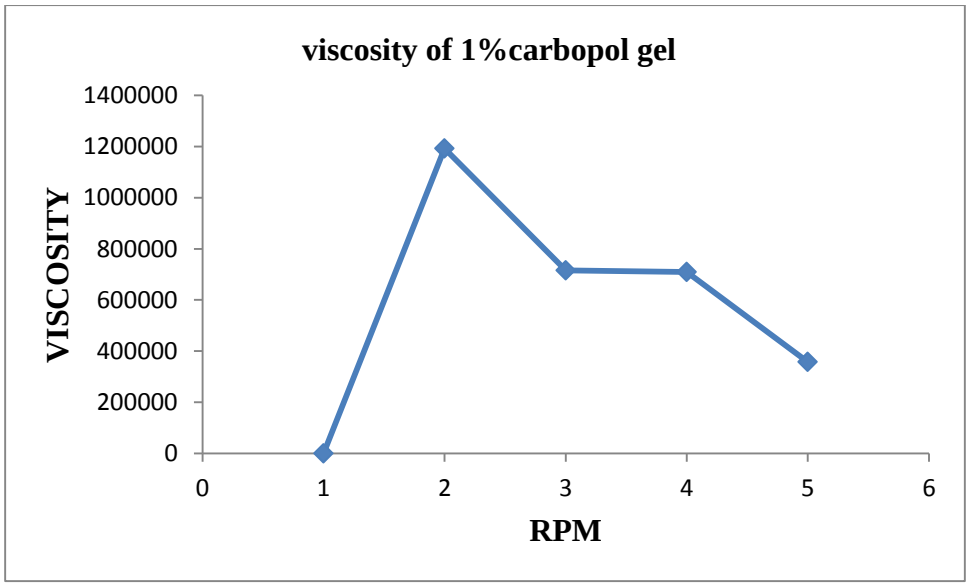


Fig 21: Viscosity of 1% carbopol gel

Viscosity of 1% carbopol gel at different rpm was determined using 25 no spindle and the viscosity was found to be decreases as the shear rate (rpm) increases.

6.8 Evaluation of Prepared clotrimazole microspheres:

6.8.1. Microspheres yield from different formulations:

Formulations	Drug to Polymer ratio (by wt)	Yield (g)	%Yield
F_1	1:2	1.8	60
F_2	1:3	1.4	70
F_3	1:4	2.2	73.33
F_4	1:5	1.52	76
F_5	1:1	0.98	49

Table12: Microspheres yield from different formulations

The microsphere yield for different formulations is shown in Table-12. The yield ranged between 49% to 73.33% w/w depending on the drug polymer ratio. The yield of microsphere is found to be increase with increase in polymer concentration.

6.8.2 Morphological and topographical characterization

Morphological and topographical analysis of microsphere formulation was performed by SEM and optical microscopy. Their diameters were determined with a pre-calibrated graduated eyepiece of the optical microscope. One hundred measurements were averaged for each microsphere formulation prepared. The mean particle size of each formulation is given in table 13.

formulation	Mean Particle size(µm)
F_1	45.950
F_2	39.933
F_3	53.633
F_4	68.830
F_5	24.016

Table13: Particle size

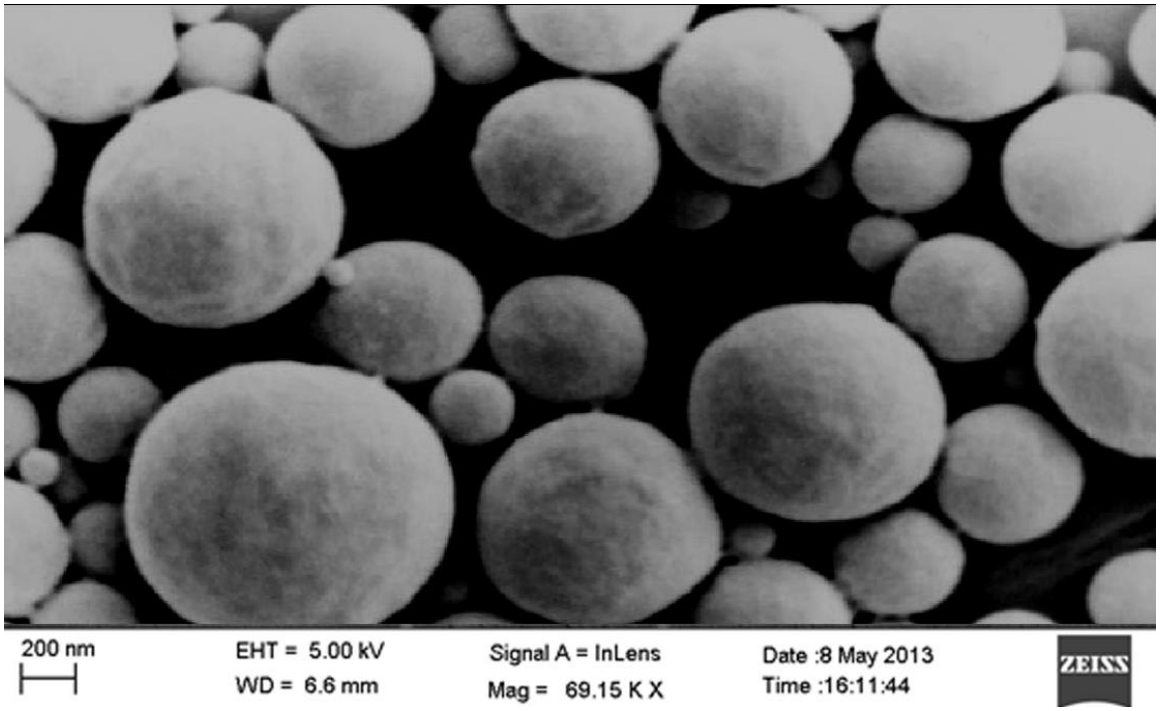


Fig 22:SEM image of optimised formulation

All particles obtained, were opaque, discrete and spherical particles with smooth surfaces further confirmed by SEM study. The results of all particle size (mean diameter) were given in Table 13. The mean diameter of the microcapsules was found to be increased with increase in proportion of coat material.

6.8.3 Drug entrapment efficiency of prepared microsphere:

Entrapment Efficiency data for different formulations

Formulations	Drug to Polymer ratio (by wt)	Amounts of drug taken in the formulation(mg)	Amounts of drug present in the formulation(mg)	% Entrapment Efficiency
F_1	1:2	51.02	30.60	59.98
F_2	1:3	35.71	35.71	80.38
F_3	1:4	22.72	19.00	83.64
F_4	1:5	30.30	29.65	97.87
F_5	1:1	55.55	30.20	54.37

Table 14: Entrapment Efficiency data for different formulations

The entrapment efficiency for different formulations is shown in Table-14. The prepared microspheres showed entrapment efficiency in the range of 54.37% to 97.87% depending on the drug polymer ratio. The entrapment efficiency of microspheres increased with increase in polymer concentration. The increased encapsulation efficiency may be attributed to the hydrophobic nature of ethyl cellulose and clotrimazole.

6.8.4 *In vitro* drug release studies of microsphere formulations:

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using SVF as dissolution medium (900 ml phosphate buffer pH 4.9, at 37±2°C was adjusted to 100 rpm) for a period of 5 hrs. An aliquot sample (5 ml) was withdrawn at a definite time interval with replacement of fresh medium and analyzed for drug content by UV-Visible spectrophotometer at 261 nm. The same method was adopted for each batch of microsphere.

Time(min)	% Cumulative Drug Release				
	F_1	**F_2**	**F_3**	**F_4**	**F_5**
0	0	0	0	0	0
30	11.25	9.04	5.89	14.94	19.84
60	19.84	15.39	15.03	19.84	28.44
90	32.04	27.54	22.59	28.44	40.86
120	40.54	32.94	28.35	33.30	45.67
180	47.25	46.80	36.94	40.86	52.47
240	54.09	54.81	45.99	45.22	61.74
300	61.65	59.85	57.10	50.58	68.44

Table 15: *In vitro* drug release studies of microsphere formulations

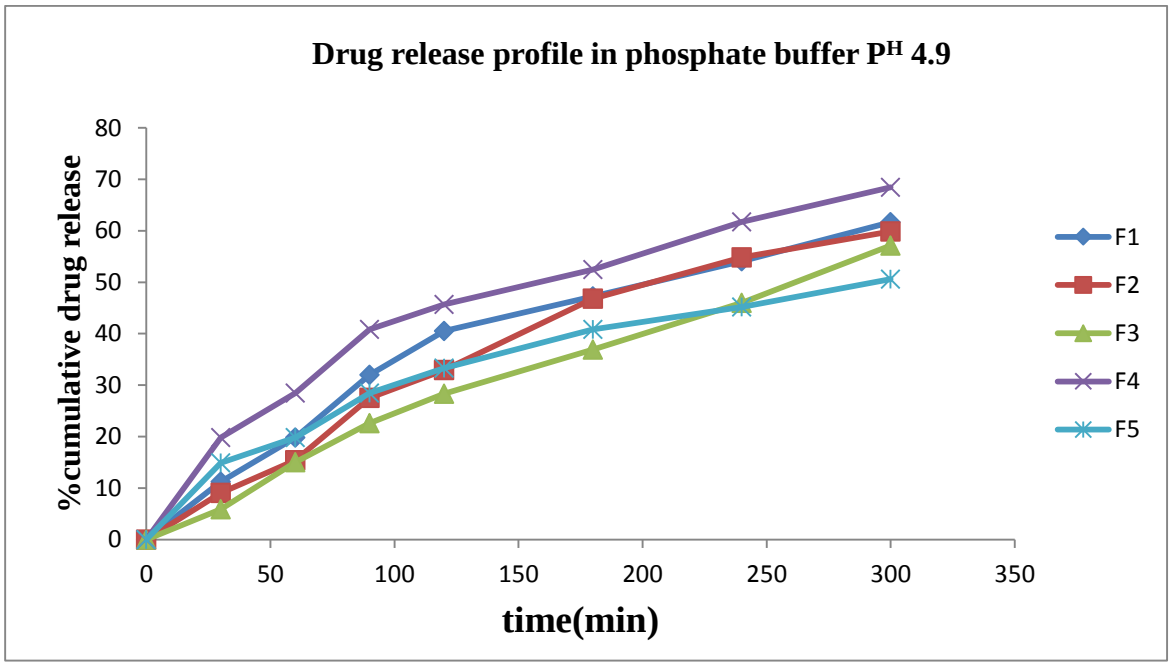


Fig 23: Drug release profile of microspheres formulation in phosphate buffer P^H 4.9

The percentage cumulative drug release of clotrimazole from prepared microsphere formulations was sustained for longer duration as the amount of polymer is increased. It is clearly shown from Fig22 that drug from the microsphere formulations

were released in controlled manner which is due to the hydration ability of ethyl cellulose, which on coming in contact with dissolution media leads to the formation of gelatinous mass which act as retardant material for the drug to diffuse out. Thus a prolonged release of drug is attained. At the end of 5hrs, the percentage cumulative drug release from F_1 was found to be 61.65%, F_2 showed 59.85%, F_3 showed 57.10%, F_4 showed 68.58% and F_5 showed 68.44% in Phosphate buffer pH 4.9. It was found that F4 formulation was able to sustain the drug release for more period of time compared to other prepared formulations.

6.8.5 Selection of Optimised formulation:

Among the different clotrimazole microsphere formulations, the formulation F4 (drug-polymer ratio 1:5) was selected as optimised formulation, after considering its optimum mean particle size, better entrapment efficiency, and drug release at sustained manner upto 5hrs. Further study was carried out on optimized formulation (F4), as release kinetics study.

6.8.6 Release Kinetics:

The *in vitro* release data obtained from optimised formulation (F4) was fitted to various kinetic models. The release rate kinetic models are shown in Fig 24 to 27.

Time (min)	Square root of time (min)	Log time (min)	% Cumulative Drug Release(Q_t)	Log Q_t	Log Q_o
30	5.47	1.47	14.94	1.17	1.92
60	7.74	1.77	19.84	1.29	1.90
90	9.48	1.95	28.44	1.45	1.85
120	10.95	2.07	33.30	1.52	1.82
180	13.41	2.25	40.86	1.61	1.77
240	15.49	2.38	45.22	1.65	1.73
300	17.32	2.47	50.58	1.70	1.69

Table16: Data for Release Kinetics of microsphere

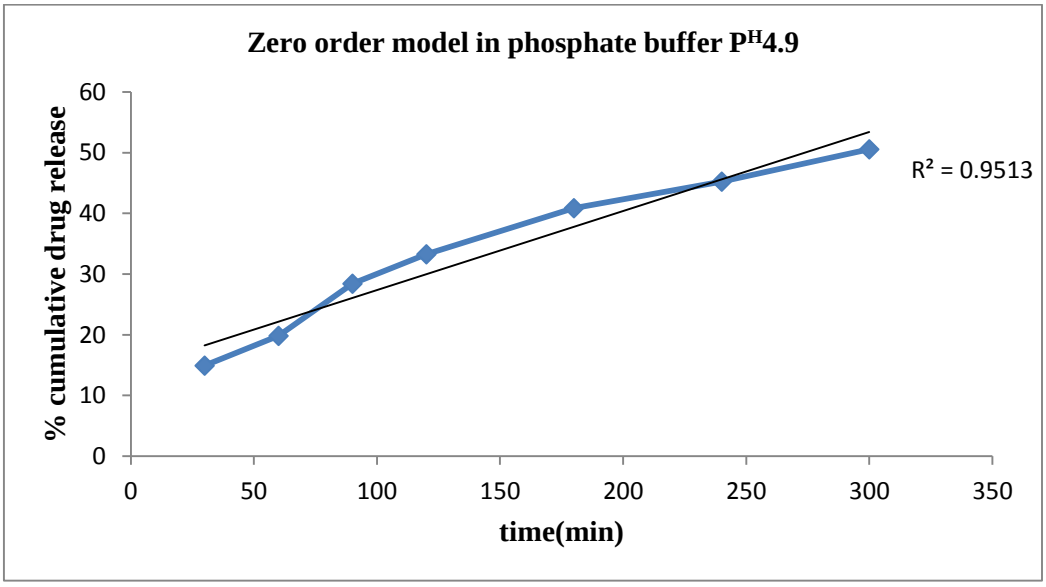


Fig 24: Zero order release kinetic study of optimized formulation in phosphate buffer P^H4.9

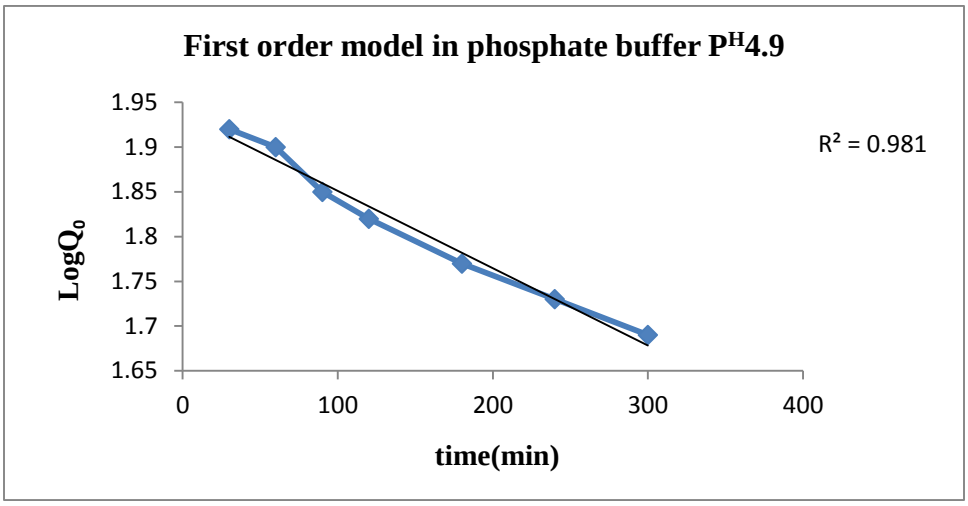


Fig 25: First order release kinetic study of optimised formulation in phosphate buffer P^H4.9

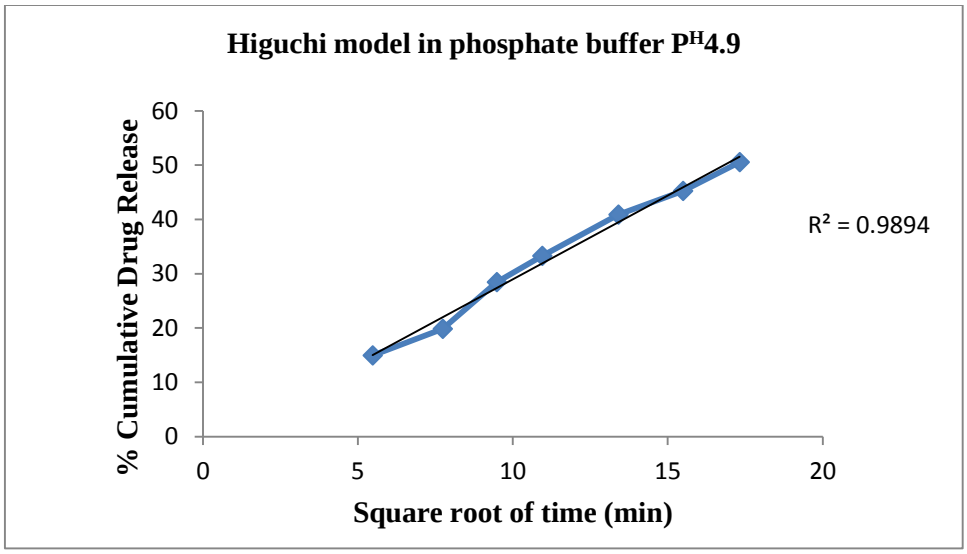


Fig 26: Higuchi release kinetic study of optimised formulation in phosphate buffer P^H4.9

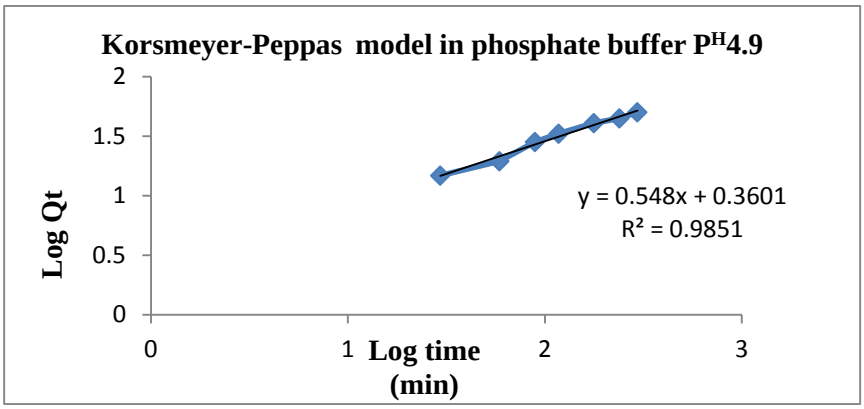


Fig 27: Korsmeyer-Peppas release kinetic study of optimised formulation in phosphate buffer P^H4.9

The value of resulting regression coefficient for each model was calculated (Fig 24 to 27). The data was also fitted into Korsemeyer-Peppas model in order to obtain the 'n' value to describe the mechanism of drug release. The 'n' value of 0.548 in Phosphate buffer pH 4.9 indicates that the drug release follows anolamous (non-fickian) diffusion mechanism which signifies that the drug release is both diffusion-controlled and swelling-controlled. From these results, it can be revealed that the release of clotrimazole from the microsphere follows first order and diffusion and swelling mechanism.

6.8.7 Evaluation of prepared mucoadhesive vaginal gel:

6.8.7.1 Viscosity

Viscosity is an important parameter for characterizing the gels as it affects the spreadability, extrudability and release of drug.. The viscosity of gels was increased with the increase in carbopol content which may be due to the increase in formation of three

dimensional cross linking structure of gel. The viscosity of the prepared gel formulation is given in table 17.

RPM	VISCOSITY				
	MG1	**MG2**	**MG3**	**MG4**	**MG5**
0.3	715200	683200	782200	684100	772340
0.5	708800	659200	751800	632400	712300
0.6	357600	326200	386500	285500	341450
1.0	304330	285690	315890	212890	259100

Table17: Viscosity of different gel formulation

6.8.7.2 P^H of the prepared mucoadhesive gel:

The P^H of the prepared gel was determined by using digital P^H meter. The P^H of gels as showed in Table18 were found to be within the range of 4.6to 5.0 which is within the limit of vaginal semisolid specifications.

FORMULATION	P^H
MG1	4.8
MG2	4.6
MG3	5.0
MG4	4.8
MG5	5.0

Table 18: P^H of the prepared mucoadhesive gel

6.8.7.3 Drug content of prepared gel:

Formulation	Drug content(%)
MG1	**68.02**
MG2	**66.32**
MG3	**66.32**
MG4	**67.66**
MG5	**50.42**

Table 19: Drug content of prepared gel

Table 19 showed the drug content and homogeneity of microencapsulated gel formulations. The drug contents of the prepared microencapsulated gels were found to be in the range of 50.42 - 68.02 % indicating the applications of the present method for the preparation of novel semi-solid MBVG system with high drug content uniformity.

6.8.7.4 *In vitro* drug diffusion studies of microencapsulated vaginal gels

Time(min)	% Cumulative Drug Release				
	MG1	**MG2**	**MG3**	**MG4**	**MG5**
0	0	0	0	0	0
30	1.98	1.17	1.26	1.98	2.97
60	5.94	5.22	5.80	9.04	9.00
90	10.53	13.54	18.04	16.47	18.49
120	18.09	21.64	24.03	25.74	27.99
180	23.49	32.94	36.99	33.16	37.39
240	37.53	41.94	46.44	42.66	47.02
300	45.04	54.04	54.72	54.45	54.99

Table20: *In vitro* drug diffusion studies of microencapsulated vaginal gels

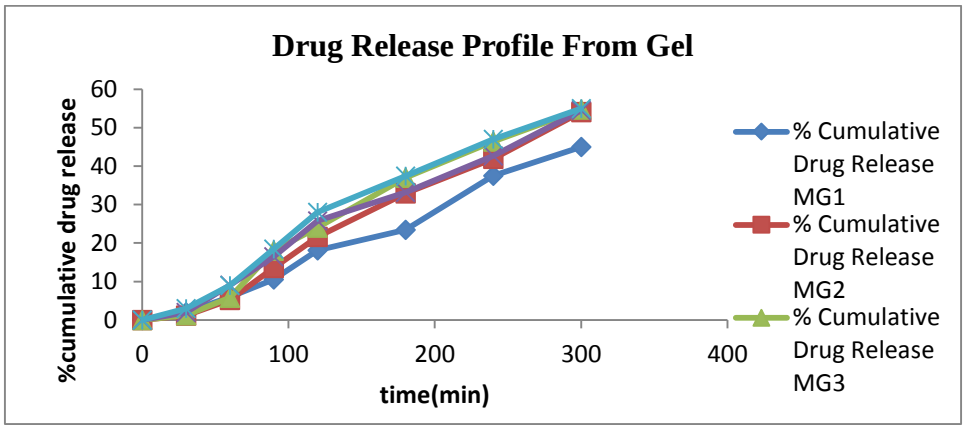


Fig 28: *In vitro* drug diffusion studies of microencapsulated vaginal gels

The percentage cumulative drug release of drug from prepared gel was sustained for longer duration. At the end of 5hrs, the percentage cumulative drug release from MG1 was found to be 45.04 %, MG2 showed 54.04%, MG3 showed 54.72%, MG4showed 54.45% and MG5showed 54.99% in Phosphate buffer pH 4.9. It was found that MG1 formulation was able to sustain the drug release for more period of time compared to other prepared formulations.

6.8.7.5 Vaginal bioadhesion measurements:

FORMULATION	Vaginal bioadhesion strength(g)
MG1	**46.4**
MG2	**8.3**
MG3	**6.8**
MG4	**17.1**
MG5	**34.8**

Table 21: Vaginal bioadhesion measurements

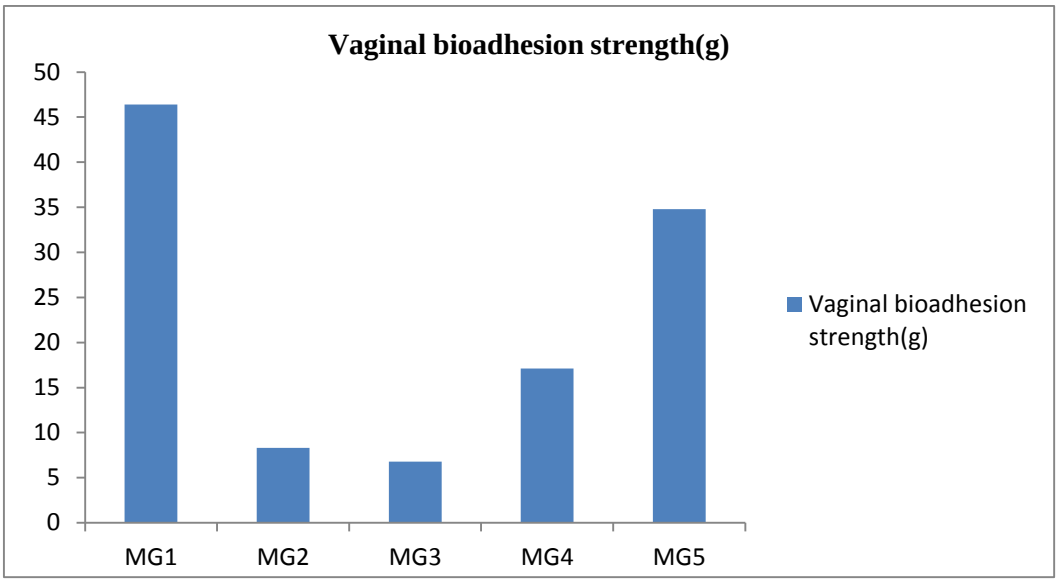


Fig 29: Vaginal bioadhesion strength

Figure 28and table 21 indicates the vaginal bioadhesive properties of the prepared gels in goat vagina and the result showed that all vaginal bioadhesive strengths were found in the following order MG5>MG4>MG1>MH2> MG3.

6.8.7.6 Selection of Optimised formulation:

Among the different clotrimazole microencapsulated mucoadhesive vaginal gel formulations, the formulation MG1 (microsphere formulation F4 loaded)was selected as optimised formulation, after considering its P^H,viscosity, Vaginal bioadhesion measurements, Drug content of prepared gel and drug release at sustained manner upto 5hrs. Further study was carried out on optimized formulation (MG1), as release kinetics study.

6.8.7.7 Release kinetics

Time (min)	Square root of time (min)	Log time (min)	% Cumulative Drug Release(Q_t)	Log Q_t	Log Q_o
30	5.47	1.47	1.98	0.29	1.99
60	7.74	1.77	5.94	0.77	1.97
90	9.48	1.95	10.53	1.02	1.95
120	10.95	2.07	18.09	1.25	1.91
180	13.41	2.25	23.49	1.37	1.88
240	15.49	2.38	37.53	1.57	1.79
300	17.32	2.47	45.04	1.65	1.74

Table22: Data for Release Kinetics of

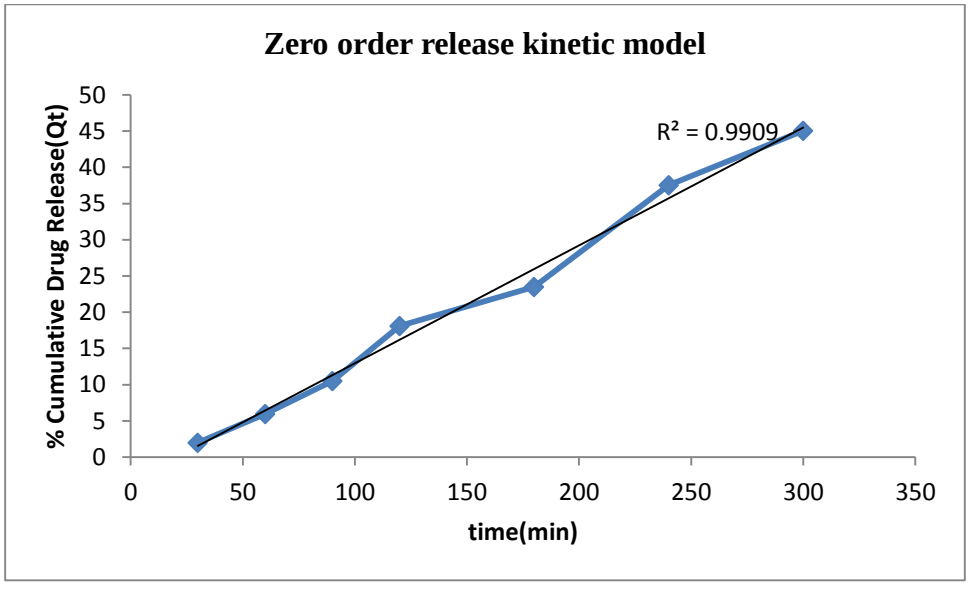


Fig30: Zero order release kinetic study of optimized formulation in phosphate buffer P^H4.9

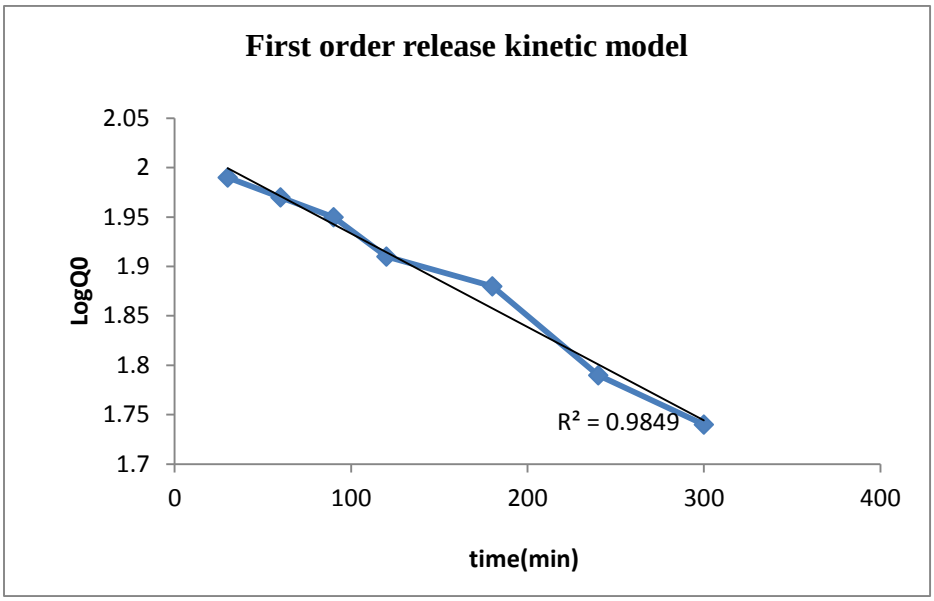


Fig31: First order release kinetic study of optimized formulation in phosphate buffer P^{H}4.9

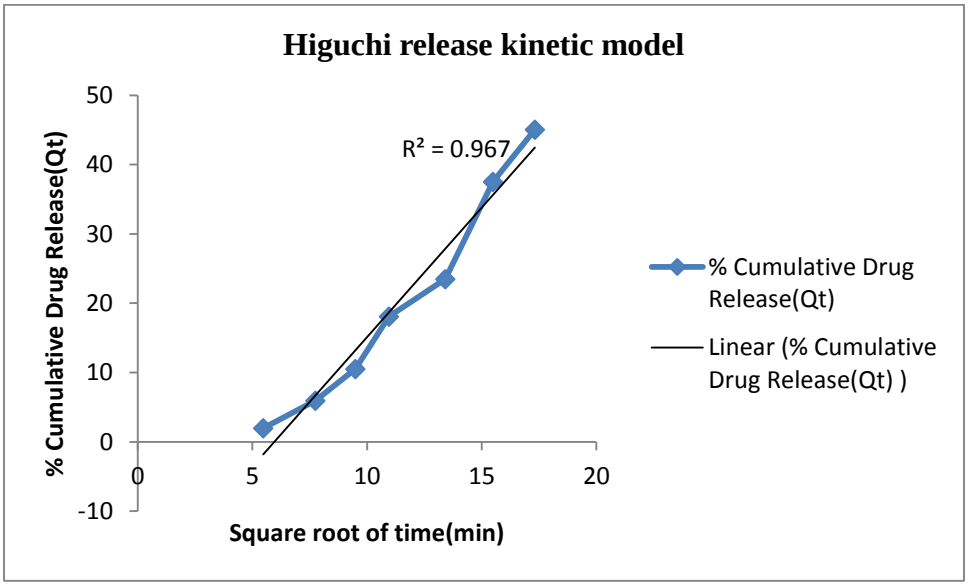


Fig32: Higuchi release kinetic study of optimised formulation in phosphate buffer P^{H}4.9

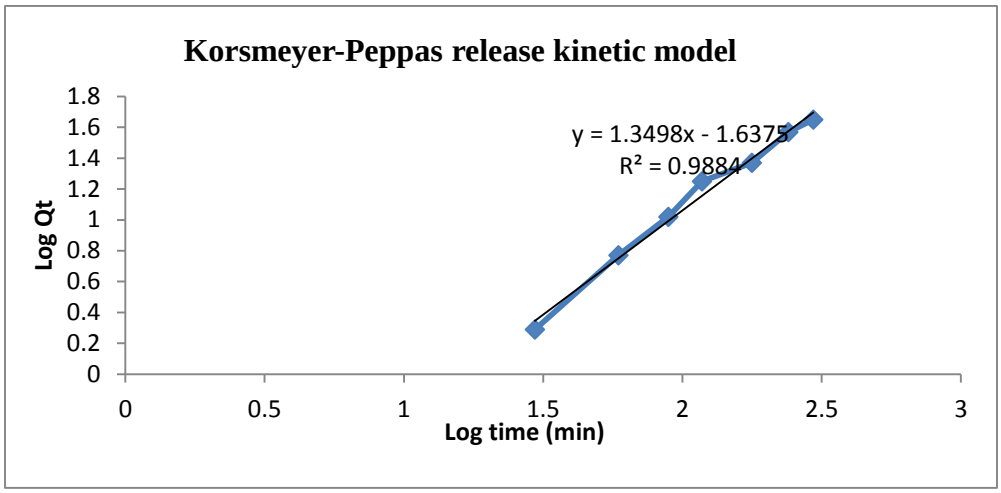


Fig33: Korsmeyer-Peppas release kinetic study of optimised formulation in phosphate buffer P^H4.9

The value of resulting regression coefficient for each model was calculated (Fig 30 to 33). The data was also fitted into Korsemeyer-Peppas model in order to obtain the 'n' value to describe the mechanism of drug release. The 'n' value of 1.349 in Phosphate buffer pH 4.9 indicates that the drug release follows Super case-II transport mechanism.

CHAPTER 7

CONCLUSION

Clotrimazole which was supplied for the study is a standard drug which was evaluated by IR spectroscopy as well as determining the melting point. The drug-excipients compatibility study revealed that drug is compatible with ethyl cellulose and carbopol934P which was evaluated by IR spectroscopy and DSC.

Based upon the Preformulation studies, 5 different batches of microsphere of clotrimazole was prepared using the Solvent evaporation method. The prepared microsphere were evaluated in terms of % microsphere yield, % entrapment efficiency, particle size and *in vitro* drug release study. The microsphere yield was found to be in the range between 49% to 73.33% w/w, entrapment efficiency was found to be in the range between 54.37% to 97.87%, particle size of clotrimazole was found to be in the range of 52.12 µmto 80.34 µm. The *in vitro* study upto 5hrs shows that the percentage cumulative drug release from from F_1 was 61.65%, F_2 showed 59.85%, F_3 showed 57.10%, F_4 showed 68.58% and F_5 showed 68.44% in Phosphate buffer pH 4.9 respectively.

Among the different clotrimazole microsphere formulations, the formulation F_5 (drug-polymer ratio 1:5) was selected as ideal formulation, after considering its optimum mean particle size, better entrapment efficiency and also drug release at sustained manner upto 5 hrs; for the study of Release kinetics analysis.

Another objective was to further incorporation of selected microcapsules in gel by using different carbopol polymers for prolonging the bioadhesion and release of representative drug. The prepared gel was further evaluated in terms of viscosity, P^H , Vaginal bioadhesion measurements, Drug content, *In vitro* drug diffusion studies . The *in vitro* study of prepared gel upto 5hrs shows that the percentage cumulative drug release from from MG1 was 45.04%,

MG2showed 54.04%, MG3 showed 54.72%, MG4 showed 54.45% and MG5showed 54.99% in SVF pH 4.9 respectively.

The evaluation reports of microencapsulated gel explained MG1 gel (containing 1.0 % w/w of drug loaded microcapsules and 1.0% w/w of carbopol 934P) was found to be the best, releasing the drug in a sustained manner up to 5hrs SVF successfully.

The novel formulation design facilitated the optimization and successful development of MBVG formulations for enhanced vaginal drug delivery by optimum vaginal bioadhesion and longer retention. Our data concluded that MBVG protocol may be an effective strategy for the development of easy, reproducible and cost effective method to prove its potential for safe and effective vaginal delivery therapy.

CHAPTER 8

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