1.0.INTRODUCTION

1. INTRODUCTION

According to recent UNAIDS estimates, in 2007 more than 33 million people were living with HIV and approximately 2.5 million peoples were newly infected.[1] Worldwide, nearly half of all individuals living with HIV (Human immune deficiency virus) are now women, who acquire the virus largely by heterosexual exposure[1-3]with an HIV vaccine likely to be year's away, topical microbicide formulations applied vaginally or rectally are being investigated as another strategy for HIV prevention. In most regions of the world, HIV is affecting women and girls in increasing numbers. As researchers, these statistics emphasize the responsibility and a challenge that include understanding personal risks and responsibilities of our young people to make healthy choices about their sexuality as well as a course for future action. Pursuing this 2nd objective, drug delivery across cellular barriers, such as vagina, is a challenging task. To date, most vaginal drug delivery systems (VDDS) have been formulated as conventional or traditional semi-solid formulations. There has been a great deal of interest in the design and application of different dosage forms via the vaginal route. Several studies have proven that the vagina is an effective route for drug administration intended mainly for local action, but systemic effects of some drugs also can be attained. The major advantages of this route include accessibility, good blood supply, the ability to bypass liver first-pass metabolism in liver and permeability to large molecular weight drugs, such as peptides and proteins. Among the delivery systems proposed for this route the use of vaginal gels, have been found to be potential vaginal drug delivery systems. The bioadhesives used in the formulation of gels, play a key role in the release of the drug through the attachment to the vaginal mucosa, where the drug diffuses from the gel to the mucus. Gels have been proven to be safe, effective, efficient, acceptable, nontoxic and easy to be administered via the vaginal route. Their continued use in research on drugs for STDs (Sexually transmitted disease) and HIV infections confirm their potential as a VDDS. The use of the gels in combination with condoms may be more effective and preferable, thereby markedly reducing the spread of STDs and HIV infections. Another area that was not investigated in detail in these studies is the cost-effectiveness of the gels compared with other VDDS, such as tablets, capsules, and rings. With the recent advent

of high-throughput screening of potential therapeutic agents, the number of poorly soluble drug candidates is increasing. Taking into account that the merits of the vaginal route are obvious, a challenge remains to design an appropriate formulation using the gels where the polymers offer most of the required release and absorption properties at the site of application.[4] Hussain *et al.* reviewed that the exhaustive efforts were made towards the administration of drugs, via alternative routes, that were poorly absorbed after the oral administration. The vagina as a route of drug delivery has been known since ancient times. In recent year, the vaginal route has been discovered as a potential route systemic delivery of both macromolecules and micromolecules[5]. However, a clear rationale exists for providing long-term, controlled release of anti-retroviral in order to provide continuous protection against heterosexually transmitted HIV infection and to improve user compliance, even during sexual activity. HIV microbicides are topical, self-administered products aimed at preventing or reducing HIV infection in women and may represent the most promising strategy for combating the HIV/AIDS epidemic at the present time. Although a safe and effective microbicide has yet to be identified, all products tested in Phase III trials to date have been vaginal gels containing non-specific compounds with modest potency that had to be applied close to the time of sexual intercourse.[6] New microbicide formulations in development are addressing many of the issues with the original gels such as coital dependency, frequency of use, acceptability, compliance, cost, and adaptability to large-scale production. All of these dosage forms are promising options for safe, effective, and acceptable microbicide products .[7] Keeping this in view, a new vaginal anti-HIV microencapsulated gel was engineered to coat vaginal tissue with a stable HIV protective layer, retention of this gel layer before intercourse and to release entrapped anti-HIV drug in a controlled fashion in presence of the main infecting agent semen. Most importantly, controlled releases of anti-HIV drugs form these microencapsulated in gel inactivate the viral load potentially introduced during sexual activity, due to increase in effective micro surface area of the therapeutics. The long-standing subordination of women and girls in Indian society takes on lethal dimensions with the rapid spread of HIV. The vagina is an ideal route for drug administration because it allows the administration of lower doses, steady state drug levels and less frequent administration compared to other routes. With vaginal drug

administration, absorption is unaffected by gastrointestinal and hepatic first-pass effect. VDDS is a class of novel drug delivery systems (NDDS), perhaps very less explored and newer area of research of its kind, world wide. Sexual contact is no doubt the major route of HIV transmission[8]. Greater understanding and acceptance by clinicians augmented the use of the vagina as a newer, potential route for drug administration[9]. The safety and efficacy of vaginal administration have been well established. Keeping in view the above uniqueness in mind, this research work is an attempt to design and evaluate a newer anti- HIV vaginal drug delivery system.

1.1.The sexual transmission of HIV and microbicide strategies

The sexual transmission of HIV is not uniformly efficient. The type of sexual activity and the phase of disease affect the risk of transmission. Initial estimates of transmission rates per coital act have ranged from 0·0003 to 0·008[10-14] with insertive vaginal intercourse associated with lower estimates and receptive anal intercourse associated with estimates as high as 0·01 or 1%.[15] The role of anal intercourse in heterosexual transmission is less well described and the frequency might be greater than previously thought.[16, 17]

Recent investigation has also shown that the rate of sexual transmission depends on co-factors such as circumcision status, genital ulcer disease, and phase of disease. High serum HIV-1 concentrations during the acute infection period increases the probability of male-to-female heterosexual transmission by up to eight to ten fold.[18] A study of Ugandan serodiscordantcouples found the rate of HIV-1 sexual transmission pre coital act within 2·5 months after seroconversion of the index partner to be 0·0082 or almost 1%.[19] Although these per-act estimates for HIV-1 transmission risk are not particularly high, the cumulative risk of sexual activity over an extended period of time-with prolonged viral shedding, frequent sexual contact, inflammation and ulcerative lesions of the genital tract, or having sex during a particularly high-risk period, such as acute infection-makes the sexual transmission of HIV-1 increasingly efficient. Male circumcision status also affects the efficiency of transmission. Uncircumcised men might acquire HIV at higher rates than circumcised men because of the presence of key target cells in human foreskin macrophages expressing CD4 receptors and dendritic cells expressing dendritic-cell C-specific intercellular adhesion molecular-3-grabbing non-integrin (DC-SIGN), a mediator of HIV entry into CD4 cells.[20-22] By contrast with human

foreskin, the intact vaginal epithelium and endocervix each present a different challenge to the entry pathway of the HIV virion. Although vaginal epithelial cells have limited permeability to particles greater than 30 nm (HIV virion is 80–100 nm),[23] HIV seems to enter the superficial layers of the squamous epithelium by diffusing across a concentration gradient,[24] and sequesters itself on the surface of epithelial cells until it can infect other cell types, particularly CD4+ helper cells and langerhans cells, both of which are found in mucosal epithelium.[25,26] The presence of normal commensal vaginal flora, particularly lactobacilli, and an acidic vaginal pH has been correlated with a decrease in HIV proliferation [27,28] as well as a decrease in HIV acquisition.[29–31] The development of compounds that protect the acidic vaginal milieu, either by buffering the neutralising effect of semen or maintaining sufficient lactobacilli production in the vaginal canal has been a second strategy in microbicide development. The subepithelial layer of the genital mucosa is a very favourable environment for HIV replication. Dendritic cells, macrophages, and T cells all densely populate the subepithelial stromal tissues of the male and female genital tract and the rectum. Each of these cell types expresses CD4, CCR5 and in lesser quantities, CXCR4 receptors, making them all vulnerable to HIV-1 binding and entry. The formulation of microbicide candidates as a protective gel is common to the development of many agents and has the theoretical benefit of minimizing mucosal breaks. Additionally, finding specific agents that block viral binding, entry, or viral replication have been other strategies. The single layer of columnar epithelium lining the endocervix is vulnerable to disruption,[33] and the cervical transformation zone between the squamous and columnar cells contains many HIV target cells near the surface.[34] The intact endocervix has the capacity to block infection of cell-associated and cell-free HIV and resists internalisation of viral particles, most likely because of a physical barrier created by cervical mucus. Additionally, antiviral proteins contained in the cervical mucus, such as secretory leucocyte protease inhibitor, and high levels of natural ligands to CXCR4 and CCR5 might block HIV-1 binding to local CD4+ cells.[35, 36] Mimicking or augmenting these natural ligands is a fourth modality under investigation. Microbicide development strategies have also had to account for the ways in which differences in the vaginal and rectal lumen might affect their success. The rectal mucosa seems to be less protective against HIV-1 than the vaginal mucosa. It consists of

one layer of columnar epithelium, and the subepithelial lamina propria contains many cell types to which HIV-1 typically binds.[37] Furthermore, rectal lymphoid follicles contain specialised M cells (microfold cells), which have been shown to bind and present HIV-1 to underlying lymphoid tissue.[38,39] Finally, unlike the lumen of the vagina, which is ultimately circumscribed at one end, the rectal space, as part of the colon, is open-ended so that vulnerability above the rectal vault might require additional coverage.[42] Because of the differences in the vaginal and rectal lumens, and the high rates of rectal transmission of HIV with unprotected anal intercourse, certain microbicide compounds are currently being assessed for rectal use in addition to vaginal use. The evolving conception of mucosal, submucosal, and lumenal vulnerability to HIV infection is informing a more targeted approach to microbicide development. Preclinical or non-clinical testing of microbicides before United State Food and Drug Administration licensing now includes a battery of at least nine study types, which include in-vitro assays, animal vaginal irritation tests, pharmacokinetic studies, genetic, general, reproductive toxicity studies, safety pharmacology studies, carcinogenic studies, hypersensitivity/photosensitivity studies and condom integrity studies.[42] Clinical testing includes phase I and phase II dosing, safety and acceptability studies, penile tolerance studies, and phase III trials for efficacy. Each preclinical and clinical phase of testing has its own set of limitations and it remains unclear which set of tests will best predict safety and effectiveness. In this context, we will describe the five broad classes of microbicides and some of their most important representative agents.

1.2. Microbicide classes and key compounds

1.2.1. Surfactants/membrane disruptors

Surfactants are the earliest compounds to have been clinically evaluated as topical microbicides. These agents disrupt membranes non-specifically, offering contraceptive properties and activity against a wide range of potential STI pathogens. Nonoxinol 9(N-9; nonoxynol-9), an inexpensive and effective spermicide widely available in over-the counter preparations, disrupts the HIV envelope and early in-vitro efficacy against HIV was initially quite promising.[41] N-9 was the first microbicide to be formally tested for efficacy in preventing HIV transmission.[32,40] Toxicity to vaginal mucosal tissue at the higher doses was suggested as a possible cause for increased transmission among

frequent users. These disappointing results ended the development of N-9 as an anti-HIV microbicide. Sodium lauryl sulfate (Invisible Condom, Université Laval, Quebec, Canada) is a third surfactant compound that has been shown to disrupt both non-enveloped and enveloped viruses.[43] This agent has been formulated to act as an "invisible condom" in that it can cover the vaginal wall as a liquid at room temperature, and then transform into a gel at body temperature. In this form it can block HIV-1 and STI transmission.[44,45] Safety of sodium lauryl sulfate has been shown in a rabbit model and in at least two phase I clinical trials.[45-47]

1.2.2. Vaginal milieu protectors

The second broad class of microbicides in development, vaginal milieu protectors, works to maintain, restore, or enhance the natural protective mechanisms within the vaginal canal-the acidic pH maintained by lactobacilli. A pH between 4·0 and 5·8 has been shown to inactivate HIV.[48-50] However, a variety of situations, including the presence of semen or bacterial vaginosis, neutralise the baseline acidity of the vagina. The microbicide compounds in this class either operate as direct acidifying agents or as enhancers of lactobacilli production. Carbopol 974P (BufferGel, ReProtect, Baltimore, MD, USA) is a polyacrylic acid that buffers twice its volume of semen to a pH of 5 or less.[51] Buffer Gel has been shown to be spermicidal,[51] virucidal *in vitro* to HIV[49] and HSV,[27] and protective in mouse vaginal models against HSV and *C trachomatis*.[52] The gel also inhibits human papillomavirus (HPV) in animal models.[53] BufferGel was found to be safe in two phase I trials.[54,55] Acidform (Amphora, Instead Inc, Dallas, TX, USA) is currently approved as a sexual lubricant gel, but its acid-buffering and bioadhesive properties make it appealing for development as a candidate microbicide. A more recent "probiotic" strategy being developed to protect the vaginal milieu is the use of exogenous lactobacilli for colonisation since lactobacillus colonisation has been shown to correlate with decreased HIV proliferation.[28,29] Colonisation of macaque vaginal canals was safely achieved with *Lactobacillus crispatus* in one study and a pilot investigation of nine women also showed a 60% colonisation rate.[56,57] Bioengineered lactobacilli (or "live microbicides") are also being developed to express proteins that bind to HIV and block either viral-host cell fusion or viral entry into host cells. Three proteins expressed through

this type of system are CD4,[58] a derivative of gp41 and cyanovirin.[59] These live microbicides are all in preclinical development.

1.2.3. Entry inhibitors

Viral entry inhibitors form a third broad class of microbicide agents and bind sequences that block either the attachment of HIV-1 to host cells, the fusion of virus and host-cell membranes, or the entry of HIV-1 into host cells.

1.2.3.1. Anionic polymers-The first group of viral entry inhibitors to be investigated were anionic polymers.[95] Through their negative charge, anionic polymers interact with HIV's viral envelope proteins and interfere with the attachment of HIV to CD4+ cells.[61,62] The greater net positive charge on the gp120 protein of CXCR4-tropic viruses makes them particularly vulnerable to these compounds, but this is not always as reliably the case for CCR5-tropicviruses. For example, dextrin sulfate reduced in-vitro cell infectivity of a CXCR4 virus (HIV-1HSBc2) by 77%, but did not reduce infectivity of an CCR5 virus (HIV-1 JRCSF).[63]

1.2.5. Reverse transcriptase inhibitors

With the success of antiretroviral therapy in the treatment of HIV disease, as well as in the prevention of mother-to-child HIV transmission, interest has grown in using these more targeted drugs for prevention of the sexual transmission of HIV. Relying on compounds that interact with specific viral or cellular receptors, such as the CCR5 inhibitors and fusion inhibitors previously described, offers a more tailored approach than earlier microbicide formulations, with the promise of less toxicity and greater efficacy. The use of such targeted topical compounds has also been suggested as an adjunct strategy in preventing mother-to child transmission of HIV.[64] Reverse transcriptase inhibitors bind the HIV-1 reverse transcriptase enzyme and block the conversion of viral RNA into DNA-effectively halting viral replication. The nucleotide reverse transcriptase inhibitor tenofovir was the first antiretroviral drug to safely demonstrate in animal models both pre-exposure and post-exposure prophylaxis as proof of concept against the sexual transmission of HIV.[65] Unlike nucleoside analogues, tenofovir is active as a diphosphate, rather than a triphosphate, and does not act via HIV DNA chain termination. Both of these reasons, coupled with the limited phosporylation ability of macrophages, explain why the drug might be effective in macrophages and other non-dividing cells.[66,67]

1.3. Structure of HIV [68]

HIV has 0.1 m in diameter and is spherical in shape. The outer coat called viral envelop is composed of two layer of lipid. Projecting from this are around 72 little spikes, which are formed from the proteins gp120 and gp41. Just below the viral envelope is a layer called the matrix, which is made from the protein p17. The viral core (or capsid) is usually bullet-shaped and is made from the protein p24. Inside the core are three enzymes required for HIV replication called reverse transcriptase, in tegrase and protease. Also held within the core is HIV's genetic material, which consists of two identical strands of RNA.

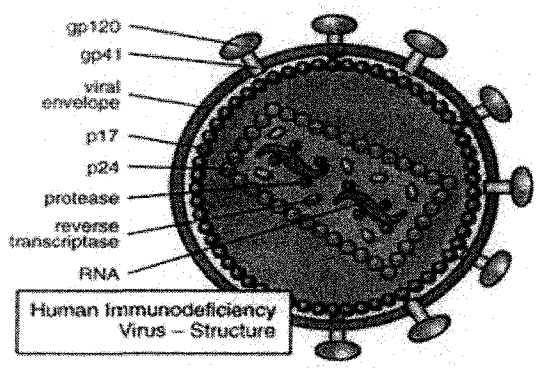

Figure 1.1 Structure of HIV

1.4. Life cycle of HIV

Virus gp120 molecule binds tightly to the CD4 molecule on the cell surface. Following CD4 binding, a center material change in the HIV gp 120/gp 41 complex is induced by interaction of gp 120 with the chemokine receptors CCR5 or CXCR4. This change in confirmation exposes gp 41 allowing it to initiate fusion of the membranes. Following CD4 binding, a center material change in the HIV gp 120/gp 41 complex induced by interaction of gp 120 with the chemokine receptors CCR5 or CXCR4. This change in confirmation exposes gp 41 allowing it to initiate fusion of the membrane. As the virus fuses with the cell, internalization of the viral core with the associated RNA occurs. Partial un coating of the viral core occurs to expose the viral RNA. Once in the cell cytoplasm, the conversion of the viral RNA

into double-stranded DNA commences as the viral reverse transcriptase becomes active. Uncoated HIV RNA serves as template from which complementary DNA strand are transcribed, catalyse by reverse transcriptase enzyme inhibitor mRNA is synthesized from the integrated DNA with the help of host cell enzyme regulating protiens are Vif, Vpr, and Vpu. mRNA transported to the cytoplasm for structural protein production. Newly made HIV core protein, enzyme and genomic RNA gather in sight the cell and an immature viral particle formed, buds off from the cell, acquiring an envelop that include both cellular and HIV protein from cell membrane. The gag and gag-pol, polyproteins associate with the inner surface of the plasma membrane and interact with gp41 present in the plasma membrane during budding, there is interaction between the HIV gag protein and molecules in the cell which directs accumulation of HIV components in the multivesicular bodies that carry protein out of body [68].

1.4.1. THE HIV REPLICATION CYCLE[68]

HIV Entry CD4 as a primary receptor for HIV: CD4 is a 58 kDa monomeric glycoprotein that can be detected on the cell surface of about 60 % of T-lymphocytes, on T-cell precursors within the bone marrow and thymus, and on monocytes and macrophages, eosinophils, dendritic cells and microglial cells of the central nervous system. The extracellular domain of the CD4 on T-cells is composed of 370 amino acids; the hydrophobic transmembrane domain and the cytoplasmic part of CD4 on T-cells consist of 25 and 38 amino acids, respectively. Within the extracellular part of CD4, four regions D1-D4 have been characterized that represent immunoglobulin-like domains. Residues within the V2 region of CD4 (amino acids 40-55) are important for the bonding of gp120 to CD4 and this region overlaps the part of the CD4 where its natural ligands, HLA class II molecules, bind. The identification of the gp120 binding site on the CD4 of CD4+ T-cells stimulated attempts to use soluble CD4 (sCD4) to neutralize the circulating virus in patients, the aim being the inhibition of viral spread. In contrast, sCD4 was able to induce conformational changes within the viral envelope that promoted the infection of target cell. CD4 attaches to the T-cell receptor complex (TCR) on CD4+ T-cells and binds to HLA class II molecules on antigen presenting cells. The binding of gp120 to CD4 is not only a crucial step for

viral entry, but also interferes with intracellular signal transduction pathways and promotes apoptosis in CD4+ T-cells. Interestingly, monoclonal antibodies against CD4 induced conformational (CD4i) epitopes to bind to the gp120 of CD4-independent viruses. This observation suggests that the gp120 of CD4-independent viruses already exposes the regions that are necessary for co-receptor recognition and binding and therefore binding to CD4 is not a prerequisite of entry for these viruses. CD4-independent viruses are easy to neutralize using the serum of HIV-infected patients, suggesting that the immune response selects against CD4-independent viruses.

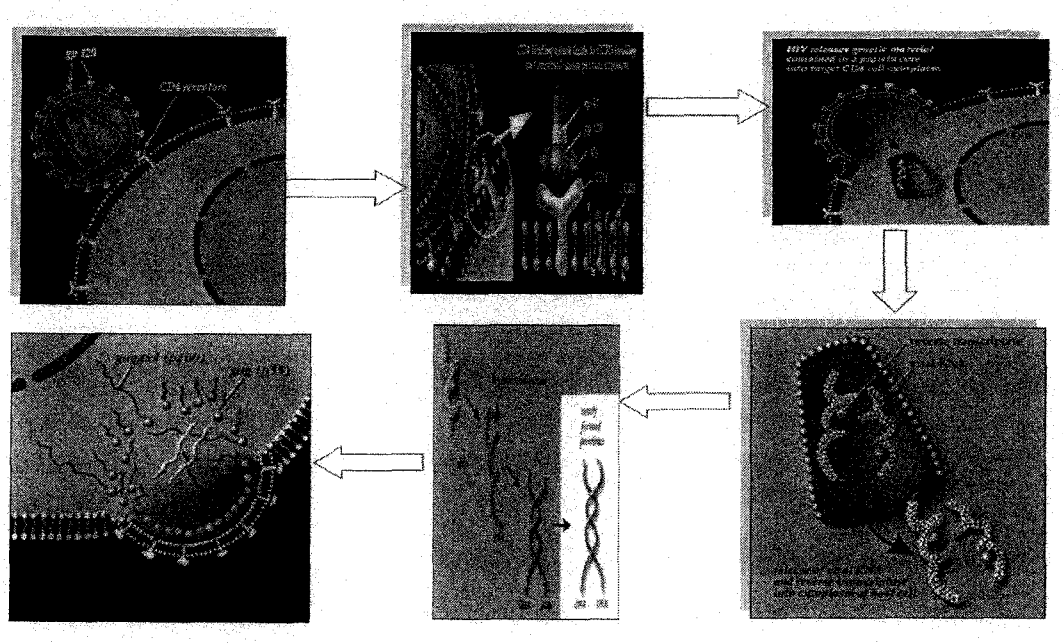

Figure 1.2 Replication cycle of HIV

1.5. Zidovudine(AZT):

Chemistry and Antiviral Activity[69] 1-(3-azido-2,3-dideoxy-b-D-*erythro*-pentofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione is a synthetic thymidine analog active against HIV-1, HIV-2, and human T- cell lymphotrophic virus (HTLV) I and II . Its *in-vitro* 90% inhibitory concentration (IC_{90}) against laboratory and clinical isolates of HIV-1 ranges from 0.03 to 0.3μg/ml.

Mechanism of action: After entering the host cell AZT is phosphorylated by thymidine kinase to a Monophosphate, then by thymidylate kinase to the diphosphate and finally by nucleoside diphosphate kinase to active zidovudine 5-triphosphate[70] (Furman *et al.*). High concentrations of the monophosphate may accumulate in the cell, and the intracellular half-life of zidovudine 5-triphospate is approximately 3hours. Zidovudine 5-triphosphate terminates viral DNA chain elongation by competing with thymidine triphosphate for incorporation in to DNA. Zidovudine 5-triphosphate also weakly inhibits cellular DNA polymerase-α and mitochondrial polymerase-γ, and the monophosphate competively inhibits cellular thymidylate kinase, an effect that reduces levels of thymidine triphosphate[70] (Furman *et al.*).

Absorption, Distribution, Metabolism and Elimination: AZT is absorbed rapidly from the gastrointestinal tract, with peak serum levels achieved within about one hour[71] (Dudley, *et al.*). The plasma half-life of the prodrug is considerably shorter than the intracellular half-life of active zidovudine 5-triphosphate. Plasma AZT concen- traitons do not correlate with intracellular triphosphate concentration or clinical efficacy. The rate-limiting step in intracellular activation is conversion to the monophosphate. Therefore higher plasma concentrations of AZT do not proportionately increase intracellular triphosphate concentration. AZT crosses the blood-brain barrier relatively well and achieves a cerebrospinal fluid (CSF) to plasma ratio of approximately 0.6.[72,73] (Gillet *et al.* & Watts *et al.*). AZT undergoes rapid first-pass hepatic metabolism by conversion to 5-glucuronyl AZT. The metabolite has an elimination half-life of 1 hour. Total urinary recovery of AZT and its major metabolite is approximately 90% (Watts *et al.*).[73]

Availability dosage form: Adult dose 500-600mg daily. Tablets (300mg), capsule (100mg), oral solution (50mg/5ml), oral solution (50mg/5ml), injection (10mg/ml), trans dermal patch.

Characters: A white or brownish powder, sparingly soluble in water, soluble in ethanol. It melts at about 124°C. It shows polymorphism and spectrophotometic range at 265 nm.

1.6. MICROCAPSULES

The term microcapsule is defined as a spherical particle with size varying from 50 nm to 2 mm, containing a core substance. Microcapsules are in strict sense spherical empty particles. However, the terms microcapsules and microcapsules are often used synonymously. In addition, some related terms are used as well. For example, essentially "microbeads" and "beads" are used alternatively. Spheres and spherical particles are also used for a large size and rigid morphology. The microcapsules are characteristically free-flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 μm.[74]

Biodegradable microsphere can be prepared from certain synthetic as well as natural polymers. An important requirement of such polymers is that the degradation products should be non-toxic because such as products eventually enter systemic circulation or result in tissue deposition. Long term toxicological evaluation of the degradation products therefore is important in determining the clinical suitability of such carriers. Biodegradable carrier matrices can be designed to deliver the therapeutic agent for periods ranging from a few days to a few years.[75] A microcapsule is an upcoming area of microparticulate drug delivery systems that contains a distinct core and a discrete envelope. In current revolutionary state, microparticulate systems were used in different areas of treatment in general the solid, liquid, or gas core is entrapped in the envelope, made up of continuous, porous or nonporous, polymeric phase. The drug can be dispersed inside the microcapsule as solid particulates with regular or irregular shapes.[76]

1.6.1. Methods of Microencapsulation

1.6.1.1. Solvent Evaporation:

The simplest method involves dissolving the polymer in an appropriate organic solvent and suspending this in an aqueous continuous phase that contains an appropriate surfactant. Continuous stirring then allows for evaporation of the organic solvent and hardening of the microcapsules. The key factors in the size as well as the size distribution of these particles are the polymer concentration in the solvent, the amount and type of

surfactant, and the stirring rate. This solvent evaporation method is most appropriate for incorporating drugs that are soluble in organic solvent. The solvents uses with this technique include dichloromethane, acetone, methanol, ethyl acetate, acetonitrile, chloroform, and carbon tetrachloride. The surfactants most commonly used are poly (vinyl alcohol), methylcellulose, gelatin, poly vinyl pyrrolidone and Tween-20 in concentration of 0.1-1.0% w/v polymer concentration of 125-1100 mg/ml will usually yield microcapsules in the size range of 10-15 µg decreasing the polymer concentration into 20 mg/ml can yield much smaller particles in the size range of 1.2-7.4 µm.[77,78] Behera *et al.* characterized glipizide loaded polymethacrylate microsphere prepared by using emulsion solvent evaporation method. Different amounts of Eudragit RS or Eudragit RS/RL combination were used for the preparation of microcapsules. Then these microcapsules were characterized by using different methods such as micromeritic analysis, particle size analysis using sieving method, drug content study, FTIR study, scanning electron microscopy (SEM), drug release behavior and kinetics of drug release.[78] Prakash *et al.* prepared and characterized lamivudine microcapsules using various cellulose polymers. The microcapsules were prepared by the solvent evaporation method. The drug and polymer (1:1) were dissolved or dispersed in acetone and added to liquid paraffin with stirring. Microcapsules were recovered by the treating with n – hexane. The prepared microcapsules were characterized for the percent drug content, entrapment efficacy, FTIR, DSC, scanning electron microscopy (SEM) and *in-vitro* dissolution studies. The reported microcapsules were spherical and free flowing.[77] (Saffari *et al.*) prepared and evaluated Eudragit L 100 microcapsules. Microcapsules were prepared by solvent evaporation technique using different amounts of Eudragit. After preparation and washing, the microcapsules were dried at room temperature for 24 hours. In the present work the microsphere were evaluated by using the different studies like percent yield, particle size and morphology, drug entrapment efficacy, Differential Scanning Calorimetry (DSC), Drug release studies and release kinetics.[79] Mandal *et al.* reported preparation of biodegradable microcapsules containing AZT using solvent evaporation technique. Sustained release biodegradable microcapsules of AZT were prepared using different concentrations of copolymer of poly (lactic/glycolic) acid

(PLGA 50:50 and PLGA 90:10) solid microcapsules was collected following the complete evaporation of the solvent. [80]

1.6.1.2. Emulsion Solvent Evaporation [86]

The polymer was dissolved in the organic solvent to form a homogeneous polymer solution. Core material, is added to the polymer solution and mixed thoroughly. The resulting mixture was added in a thin stream to 100 ml of 0.1 N HCl contained sodium CMC (0.5%w/v) contained in a beaker while stirring at 200 rpm to emulsify the added droplets. The solvent was removed by evaporation under reduced pressure into spherical microcapsules.

1.6.1.3 O/O Single Emulsion-Solvent Evaporation Method:

It is the emulsion based method which is used for the preparation of microcapsules. It is the method in which the dispersion phase is oil in nature and continuous phase is also oil in nature. In this method the solvent is evaporated by means of continuous heating. (Sahoo *et al,.*) Organized ethyl cellulose microcapsules containing anti-HIV drug was evaluated with the help of different studies. These microcapsules were formulated by using oil-in-oil (O/O) emulsion solvent evaporation method, using different ratios of drug:polymer. These microcapsules were evaluated using size distribution of micro-spheres, drug entrapment efficacy, scanning electron microscopy (SEM), FTIR, *in-vitro* drug release studies and release kinetics. [87, 88]

1.6.1.4. O/W Single Emulsion-Solvent Evaporation Method:

In this method the oleic solvents are act as the dispersion phase and aqueous solvents are the continuous phase. It is the method of solvent evaporation means the solvent evaporates during preparation of microcapsules by means of continuous heating.[87]

1.6.1.5. W/O Single Emulsion- Solvent Evaporation Method:

It is also the single emulsion solvent evaporation method. It is the method in which the aqueous phase acts as the dispersion phase, but the oleic solvents act as the continuous phase. In this method during processing the solvents evaporates by means of continuous heating.[87] Abu-Izza *et al.* Optimized already prepared and evaluated AZT loaded sustained release microcapsules. The effect of variables like emulsifier concentration, drug to polymer ratio and composition of the internal phase of the

emulsion on a number of response variables was systemically investigated. Entrapment efficacy, yield and percentage of loose surface study. [89] Garcia *et al.* reported preparation and evaluation of sustained release AZT-loaded microcapsules. Optimization of the release characteristics using response surface methodology the purpose of the study was to prepare and optimize a sustained release formulation of AZT. Ethyl cellulose microcapsules containing AZT were prepared using an emulsification/solvent evaporation technique. The microcapsules were characterized in terms of their particle size and surface morphology.[90]

1.6.1.6. W/O/W Double Emulsion-Solvent Evaporation Method:

In this method two emulsions are prepared basically. In first emulsion preparation the water act as the dispersion phase and the oil act as the continuous phase. In the preparation of second emulsion, the first emulsion acts as the dispersion phase and the aqueous solvents acts as the continuous phase. This method decreases the contrast between the organic phase and active substance.[87]

1.6.1.7. W/O/O Double Emulsion-Solvent Diffusion Technique:

In this method two emulsions are prepared. In first emulsion preparation the water act as the dispersion phase and the oil act as the continuous phase. In the preparation of double emulsion, the first emulsion act as the dispersion phase and the oleic solvents acts as the continuous phase.[87] Kumar et *al.* evaluated microcapsules of AZT prepared by W/O/O method using ethyl cellulose. AZT is widely used for the treatment of AIDS and related conditions, either alone or in combination with other antiviral agents. Ethyl cellulose, a non-biodegradable and biocompatible polymer one of the extensively studied encapsulating materials for the controlled release of pharmaceuticals was selected as the retardant material for AZT. All microcapsules were prepared by using the W/O/O double emulsion solvent evaporation method. These prepared microcapsules are evaluated using drug entrapment efficacy, differential Scanning Calorimeter (DSC), *in-vitro* release studies, release kinetics and microscopic studies. [91] Sahoo *et al.* organized ethyl cellulose microcapsules containing anti-HIV drug was evaluated with the help of different studies. These microcapsules were formulated by using oil-in-oil (O/O) emulsion solvent evaporation method, using different ratios of drug: polymer. These microcapsules were evaluated using size distribution of microcapsules, drug entrapment efficacy, Scanning

Electron Microscopy (SEM), FTIR, *in-vitro* drug release studies and release kinetics.[92] Das *et al.* has worked on microencapsulation of AZT by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion technique using ethyl cellulose polymer. The preparation of AZT-loaded ethyl cellulose microcapsules by w/o/o double emulsion solvent diffusion method with high entrapment capacity and sustained release is described. The prepared microcapsules were characterized. [93]

1.6.1.8. Interfacial Polymerization[81]

It is possible to encapsulate a wide range of products, including aqueous solutions, water immiscible liquids, solids, by this approach. Interfacial technique is suitable for encapsulating liquids rather than solids due to the fact that penetration of reactant into the polymerization zone is much more easily accomplished from a liquid than a solid state. A unique feature of this film forming technique is the polymerization of a monomer at the interface of two immiscible substances. The limitations of these methods are toxicity associated with the untreated monomer degradation caused by monomer reaction, high permeability of the coating and fragility of the microcapsules.

1.6.1.9. Simple Coacervation Separation[81]

The term coacervation was suggested for the first time by two Dutch Scientists, Bungenburg de Jong and Kruyt to describe the phenomenon of phase separation in colloidal systems. Simple coacervation can be accomplished by the addition of chemical compounds with a high affinity for water, such as salts and alcohols.

This process depends primarily on the degree of hydration produced. The added substances cause two phases to be formed, one rich in colloid droplets and the other poor. Its principal requirement is the creation of insufficiency of water in a part of the total system.

1.6.1.10. Solvent Exchange Method[82]

Recently, a new microencapsulation technique called the solvent exchange method had been developed in an attempt to address the above problems associated with protein delivery. Most proteins undergo inactivation events such as degradation and aggregation within the microcapsules during the manufacturing process and release period. Briefly, the new method produces reservoir-type microcapsules by inducing collision between drug-loaded aqueous drops and polymer-dissolved organic solvent drops. The micro

scaled liquid drops can be generated by different equipment such as ink-jet nozzles and ultrasonic atomizers. In particular, a coaxial ultrasonic atomizer is able to produce micro drops and allow their collision in a simple and highly efficient manner. The potential advantage of the new methods is that it does not involve most sources that have been known to include activation of the encapsulated protein for the following features. First, in reservoir type microcapsules, the w/o interfacial area generated during the encapsulation process is relatively small as compared with conventional multinuclear microcapsules, so it is expected that there is less protein inactivation due to its exposure to the interface. Second, due to reduced contact area between the aqueous and polymer phases, the unfavorable interaction between encapsulated proteins and the hydrophobic environment can be minimized. Third, physical stress that the ultrasonic atomizer generates is relatively mild, so protein stability will barely be affected during the fabrication process. If these features successfully cooperate to maintain structural stability and biological activity of the encapsulated protein, the microcapsules generated by the new method will display improved release kinetics and stability profile as compared with conventional microcapsules.

1.6.1.11. Non Solvent Emulsion Addition Method[83]

Individual polymer or mixture of polymers is dissolved in acetone. The core material is dispersed in liquid paraffin by stirring. The polymer solution was then added slowly, using a burette, to the drug dispersion. The resultant mixture is agitated with paddle agitator at temperature of 35-40 ^{0}C. Then chloroform or cyclohexane (non-solvent) is also added. Then agitation of liquid paraffin containing core material, polymer solution and non-solvent was continued. The liquid paraffin was then decanted off and the microcapsuls were collected and washed twice with chloroform or cyclohexane to remove any remaining oily phase, and dried in vacuum oven at temperature of 50 ^{0}C for a period of 12hrs.

1.6.1.12. Air Stripping Method[84]

This method involves the following by Cells are suspended in a mixture of alginate and cellulose sulphate. Spherical constant size droplets are formed from the suspension in the droplet generator, the droplets are collected and hardened into beads by cross-linking with calcium ions in a first bath, the beads are dipped into a second bath where they react

during a short period of time with a polymethylene-co-guanidine solution, thus forming the semi-permeable membrane. The capsules are washed with buffer solution to stop the membrane formation at the desired thickness and the capsules are collected and stored in media buffer.

1.6.1.13. Thermal Change Method[85]

The polymer solution is dissolved in cyclohexane and the resulting solution is heated in a water bath. The temperature is gradually raised to 70 ^{0}C under constant stirring. The core material is then added to the hot polymer solution slowly and the temperature is further raised to 80 ^{0}C. The temperature is maintained at 80 ^{0}C for 30 minutes. It was then slowly cooled under continuous stirring and finally the temperature is dropped to 5^0 C to effect hardening of polymer coated microcapsules. The solution is to separate microcapsules. The microcapsules so obtained are to be collected and dried in a desiccator.

1.6.1.14. Orifice Ionic Gelation Method [86]

One of the most important and useful properties of alginates is the ability to form gels by reactive with calcium salts. These gels, which resemble a solid in retaining their shape and resisting stress, consist of almost 100% water (normally 99-99.5% water and 0.5-1.0% alginate). It had been suggested that the cross-links were caused either by simple ionic bridging of two carboxyl groups on adjacent polymer chain via calcium ions or by chelation of single calcium ions by hydroxyl and carboxyl groups on each of a pair of polymer chains. Although these bonds may play a role in the gelation mechanism, they are not sufficiently energetically favorable to account for the gelation of alginate. It has been shown that the shape of both polymannuronic acid segments and the polyguluronic acid segments of alginic acid is ribbon-like and extended, and that these extended ribbons can stack together in sheets. On the basis of these data and the properties of gels, it has been suggested that the cooperative association of either polymannuronic acid segments or polyguluronic acid segments is involved in the formation of cross-linked network of polymer chains.

1.6.1.15. Spray Drying Method:

In this technique the drug is dissolved or suspended in the organic polymer solution and the resultant mixture is spray dried to form microcapsules. The advantage of this technique is the water soluble and insoluble compounds can be incorporated into the

spheres in contrast to the single O/W emulsion evaporation system which is unsuitable for water soluble compound.[87] Elisabetta *et al.* were prepared mucoadhesive vaginal tablets as veterinary delivery system for the controlled release of an antimicrobial drug, Acriflavine. The tablets were prepared using drug-loaded chitosan microcapsules and additional excipients (methylcellulose, sodium alginate, sodium carboxymethylcellulose, or Carbopol 974). The microcapsules were prepared by a spray-drying method, using the drug to polymer weight ratios 1:1 and 1:2 and were characterized in terms of morphology, encapsulation efficiency, and *in vitro* release behavior.[95]

1.6.1.16. Phase Separation Coacervation Technique:

In this method microcapsules are produced by dispersing either the solid crystal particles or an aqueous solution of the drug in an organic solution of polymer, followed by a phase separation by adding a second organic solvent in which the polymer is not soluble. Karasulu *et al.* reported sustained release bioadhesive effervescent ketoconazole microcapsules tabletted for vaginal delivery. Microcapsules of ketoconazole with 1:1 and 1:2 core-wall ratios were prepared by means of the phase separation technique using sodium carboxymethylcellulose as a coating material. The microcapsules were mixed with effervescent granules and were tabletted. Dissolution studies of microcapsules, tabletted microcapsules and commercial ovules were carried out with a new basket method (horizontal rotating basket).[95]Tuncel *et al.* was performed *in vitro* and *in vivo* studies on microcapsules and tabletted microcapsules of cephradine. Cephradine was microencapsulated by coacervation. Ethyl cellulose was used as the polymer and a core/wall ratio of 1:1 was selected. The repose angle, apparent and tapped density, particle size distribution of cephradine microcapsules (CM) and of cephradine powder were examined.[96]

1.7. BIOADHESION AND BIOADHESIVE DRUG DELIVERY SYSTEMS (BDDS)

The term bioadhesions refers to either adhesion between two or more biological materials (including cells, cellular secretion, mucus, extra-cellular matrix, and artificial substrate such as metal, ceramics and so on) or adhesion between polymer samples, either synthetic or natural and soft tissue. Possible means of administration for BDDSs include the ocular, respiratory, GI, rectal, urethral and vaginal routes.[97-100] Mucoadhesives are synthetic or natural polymers that interact with the mucous layer covering the mucosal

epithelial surface and mucin molecules constituting a major part of the mucus.[101] Attachment of the natural and synthetic polymers to living tissue is a logical way to extend residence time of dosage forms for both local and systemic delivery of drugs. The specific time of contact will be limited by turnover time of the tissue (or surface coat) to which the polymer is attached in the case of water insoluble polymers or by dissolution rate of the polymer in the case of water soluble polymers.[102] At physiological pH, the mucous network may carry a significant negative charge due to the presence of acid residues, contributing to a significant bio adhesion. Maximum adhesion is observed from pH 5 to pH 6. On application of the gels formulated with mucoadhesive polymers to the vagina, bioadhesion occurs in successive stages. Carmen *et al.* synthesized hydrogel and performed the characterization by using the studies like FTIR spectroscopy, swelling studies, scanning electron microscopy, visco-elasticity, hardness and compressibility, *in-vitro* bioadhesive assay, drug loading and drug release studies. Bio adhesion study of hydrogel is also performed for determine the retention time.[103] Mishra *et al.* studied on developing safe and effective bioadhesive gelling systems of ketorolac tromethamine, a potent non-narcotic analgesic with moderate anti-inflammatory activity for nasal systemic delivery. Chitosan and pectin based gelling systems were prepared with variables like polymer concentration and type. These systems were characterized in terms of their physical properties, *in vitro* bio adhesion, *in vitro* drug release and long-term stability.[104] Ahmad *et al.* an acid buffering bioadhesive vaginal (ABBV) gel was developed for the treatment of mixed vaginal infections. Different bioadhesive polymers were evaluated on the basis of their bioadhesive strength, stability and drug release properties. Bio adhesion and release studies showed that guar gum, xanthan gum and hydroxypropyl methylcellulose K4M formed a good combination of Bioadhesive polymers to develop the ABBV gel. The developed bioadhesive gel was found to have prolonged *ex vivo* retention.[105] Kamel *et al.* reported chitosan and sodium alginate based bioadhesive vaginal tablets. Metronidazole was formulated in Mucoadhesive vaginal tablets by directly compressing the natural cationic polymer chitosan, loosely cross-linked with glutaraldehyde, together with sodium alginate with or without microcrystalline cellulose (MCC). Sodium carboxymethylcellulose (CMC) was added to some of the formulations. Swelling indices and adhesion forces were also measured for

all formulations.[106] Garg *et al.* reported development of novel sustained release bioadhesive vaginal tablets of povidone iodine. The present study was aimed at the development of rapidly disintegrating, bioadhesive and sustained release vaginal tablets of an iodophore, polyvinylpyrrolidone (povidone iodine), their evaluation and comparison with the marketed formulations. The formulation development included drug-excipient compatibility studies, optimization of performance parameters like disintegration time, bioadhesion and drug release profile and comparison of physical properties and performance parameters with the marketed formulation.[107]

First, there is mutual contact between the bioadhesive and the membrane. Then, there is interpenetration of the chains of the bioadhesive material with those of the mucus. The interaction between the bioadhesive and the mucus can be explained by the equilibrium of the attractive and repulsive forces. For the mucoadhesion to occur, the attractive forces must be higher than those of repulsive forces because the contact occurs as a result of the differences in their electrical structures. The electrical double layer at the interface determines the location where adhesion will take place. The factors that can affect the adhesive power are associated with or can be related to the polymer or the environment. The polymers-related factors include the molecular weight of the polymer, polymer concentration, flexibility of the polymer chains (important for interpenetration and entanglement), swelling characteristics of the polymer and spatial conformation. The environmental-related factors include pH of the mucosa, applied pressure, initial contact time, selection of the model substrate surface and physiological variables such as mucous turnover and disease states.[101]

An ideal polymer for mucoadhesive drug delivery should therefore have the following properties. Both polymer and its degradation products should be non-toxic and non absorbable from the vaginal tract. It should not be an irritant to the mucous membrane. It should preferably form a strong non-covalent bond with the mucin-epithelial cell surfaces. It should have good spreadability, wetting, swelling, solubility and biodegradability properties. Its pH should be biocompatible and should possess good viscoelastic properties. It should adhere quickly to the moist tissue, allow easily incorporation of the drug, should possess sufficient mechanical strength and offer no hindrance to its release. Polymer must be easily available and its cost should not be high.

It should demonstrate local enzyme inhibition and penetration enhancement properties. It must not be decomposed on storage. It should have required spatial conformation. It should have optimum molecular weight.[108] Adhesion can be defined as the bond produced by the contact between the pressures sensitive and a surface. American Society of Testing and Materials has defined it as the state in which two surfaces are held together by interfacial forces which may consist of valence forces, inter locking action or both.[109]

1.7.1. ADVANTAGES OF BIOADHESIVE SYSTEM

Bioadhesive drug delivery systems have four distinct advantages when compared to conventional dosage forms. First one is the enhanced bioavailability and effectiveness of drug due to targeted delivery to a specific localized region such as vagina. Second one is the maximized absorption rate due to intimate contact with the absorbing membrane and decreased diffusion barriers. Thirdone, drug protection is improved by polymer encapsulation and direct contact with absorbing cell layers. Fourthone, longer residence time resulting in extended periods for absorption.[76].

1.7.2. Theories of Bioadhsion[110-112]

A number of researchers worked out theories that explain the mechanisms with which mucoadhesive adhere to the mucous layer. The theories of mucoadhesion are based on the classical theories of metallic and polymer adhesion. There are six main theories that have been adapted to study mucoadhesion.

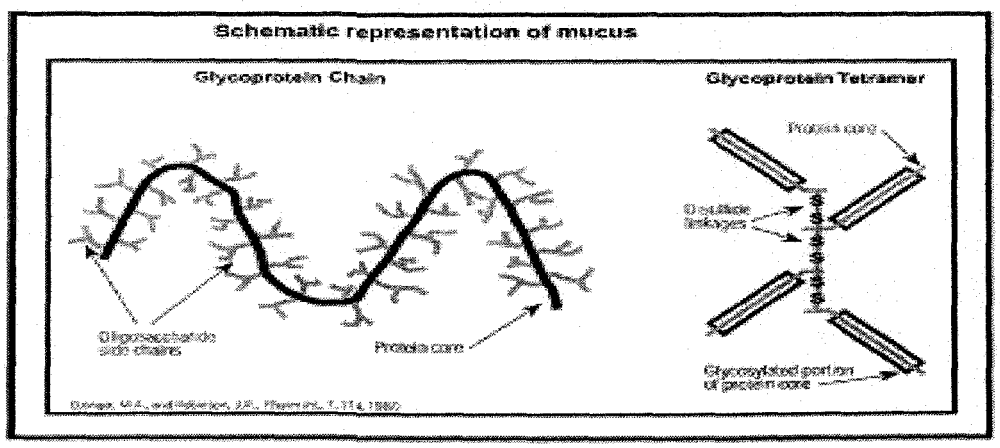

Figure 1.3 Schematic representation of mucus

1.7.2.1. The Electronic Theory

The hypothesis of the electronic theory was on the assumption that the bioadhesive material and the target biological material have different electronic structures. On this assumption when mucus and mucoadhesion comes in contact with each other, electron transfer occurs in an attempt to levels, causing the formation of a double layer of electronic charge at the bioadhesive-biologic material interface. This system is analogous to a capacitor. The system is charged when the adhesive and substrate are in contact and discharged when they are separated. The electronic theory has produced some controversy regarding whether the electrostatic forces are an important cause of the result of the contact between the bioadhesive and the biological comportment.

1.7.2.2. The Adsorption Theory[110-112]

The adsorption theory describes the attachment of adhesives on the basis of formation of hydrogen bonding and vender wall forces and related forces between an adhesive substrate and tissue or mucosa. It has been proposed that these forces are the main contributors to the adhesive interaction. Although these forces are individually weak, the sheer number of interactions can as a whole, produce intense adhesive strength. The resulting attractive forces are considerably larger than the forces described by the electronic theory. The adsorption theory is the most widely accepted theory of adhesion.

1.7.2.3. The Wetting Theory[110-112]

The wetting theory is primarily applied to liquid systems and considers surface and interfacial energies. It involves the ability of a liquid to spread spontaneously onto a surface as a prerequisite for the development of adhesion. The affinity of a liquid for a surface can be found using techniques such as contact angle to measure the contact angle of the liquid on the surface, with the general rule being that the lower the contact angle, the greater the affinity of the liquid to the solid. The wetting theory is significant, since spreading of the mucoadhesive over the mucus is prerequisite for the validity of all the other theories.

1.7.2.4. The Diffusion Theory[110-112]

The concept that interpenetration and entanglement of bioadhesive polymer chains and mucous polymer chains produce semi permanent adhesive bond is supported by the diffusion theory. It is believed that bond strength increases with the degree of penetration

of the polymer chains into the mucous layer. Penetration of polymer chains into the mucus network and vice versa, is dependent on concentration gradient, diffusion coefficients, molecular weight and the degree of crossliking, chain length, spatial conformation and flexibility. For diffusion to occur it is important to have good solubility of one component in the other. The bioadhesive and mucous should form between biomaterials whose solubility parameters are similar to the target mucus glycoprotein. Thus, the diffusion theory states that interpretation and entanglement of polymer chains are responsible for mucoadhesion. The more structurally similar a mucoadhesive is to its target, the greater the mucoadhesive bond will be.

1.7.2.5. The Fracture Theory[110-112]

The fracture theory differ a little form the other five in that it relates the adhesive strength to the force required for the detachment of the two involved surface after adhesion. This assumes that the failure of the adhesive bond occurs at the interface. However, failure normally occurs at the weakest component, which is typically a coadhesive failure within one of the adhering surfaces.

1.7.2.6. The Mechanical Theory[110-112]

The mechanical theory assumes that arise from an interlocking of a liquid adhesive into irregularities on a rough surface. However, rough surfaces also provide an increased surface area available for interaction along with an enhanced viscoelastic and plastic dissipation of energy during joint failure, which are thought to be more important in the adhesion process than a mechanical effect. None of these theories give a complete description of the mechanical of mucoadhesion. The total phenomenon of mucoadhesion is a combined result of all these theories.

1.8. BIOADHESIVE POLYMERS

From current scientific literatures, two classes of polymers appeared to be of interest for bioadhesion hydrophilic polymer and hydrogel. Recent research has suggested that in the large class of hydrophilic polymers, those containing carboxyl groups exhibit the best bioadhesive properties. Other promising bioadhesive polymers have included sodium alginate (SA), methylcellulose (MC), hydroxy propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC), hydroxy methyl cellulose (HMC) and cationic hyhrogel such as chitosan. In general, hydrogel have most often been used for bioadhesive drug delivery

because of polymer-mucin chain entanglement, an essential component in bioadhesive bond formation. Other factor includes surface energy, surface texture, electrical charge and hydrophilic functional groups may be equally important. It was also found from literatures, non-hydrogel polymers which are having hydrophilic functional groups can also produce intense bioadhesive interactions.[76]

1.8.1. CLASSIFICATION OF BIOADHESIVE POLYMERS

Polymers can be classified as following category such as hydrogel polymer, natural polymer and natural modified polymer synthetic polymer. Hydrogel are water swell able material, usually a cross link polymer with limited swelling capacity. Some time their aqueous dispersion is very mildly acidic. Upon neutralization with an alkali, the resins expand extensively like a gel- like structure and their viscosity increases drastically, thus forming aqueous mucilage. As a result, the dispersion become thickened e.g Carbopol-974P, Carbopol-934, Carbopol 974P NF resin, Carbopol 980 NF etc. Various biocompatible natural polymer which are useful in bioadhesive vaginal formulation, includes pectin, guar gum etc. Another way to achieve desirable polymer properties is the modification of preformed in natural polymer. This modification may take place on the reactive sites of the polymer chain through alkylation, hydrolysis, sulfonation, esterification etc. Examples of natural polymer and their modifications are useful in bioadhesive formulation, e.g. chitosan and chitin. Various synthetic polymer which are useful in bioadhesive vaginal preparation system are hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose(NaCMC), hydroxy methyl cellulose(HMC), sodium alginate and sodium polystyrene sulfonate (PSS), polyacrylic acid (PAA) or polycarbophil polyacrylate.[76]

1.9. VAGINAL GEL

The U.S.P. defines gels as semisolids, either suspension of small inorganic particles or large organic molecules interpenetrated with liquid. The inorganic particles, such as bentonite form a three-dimensional structure throughout the gel. This is a two phase system where large organic molecules tend to exist in solution or randomly coiled flexible chains. These molecules either natural or synthetic polymers, tend to entangle with each other because of their random motion. Such systems are actually single phase.

It is the interaction between the units of the colloidal phase, inorganic or organic that sets up the structural viscosity immobilizing the liquid continuous phase.[113]

Gels are transparent or translucent semisolid formulations containing a high ratio of solvent/gelling agent. When dispersed in an appropriate solvent, gelling agents merge or entangle to form a three-dimensional colloidal network structure, which limits fluid flow by entrapment and immobilization of the solvent molecules. The network structure is also responsible for gel resistance to deformation and hence, its viscoelastic properties.[114]

Vaginal gel increases the bioavailability of drug as compared to oral route. Intra-vaginal gel acts as the transparent condom so it prevents the STDs. Intra-vaginal gel prevents the first-pass metabolism of drug and also prevents the gastric side-effects.[114]

1.9.1. Classification of gel

Gels can be classified on the basis of colloidal phases, nature of solvent used consistency & viscosity.

1.9.1.1 Hydrogels:

The term hydrogel is composed of "hydro" (=water) and "gel" and it refers to aqueous (water-containing) gels, or to be more precise to polymer networks that are insoluble in water, where they swell to an equilibrium volume but retaining their shapes.

The hydrophilicity of the network is due to the presence of chemical residues such as hydroxylic (-OH), carboxylic (-COOH), amide (-CONH-), primary amidic ($-CONH_2$), sulfonic ($- SO_3H$), and others that can be found within the polymer backbone or as lateral chains.

Classification of hydrogels[113]:

Classifications of Hydrogels are as follows:

1. Chemical Hydrogel.
2. Physical Hydrogel.

This contains non-aqueous solvent as a continuous phase. For example plastibase (low molecular weight polyethylene dissolved in mineral oil and shock cooled) and dispersion of metallic stearate in oil.

1.9.1.2. Based on colloidal phases:

Gels are classified into inorganic (two phase system) & organic (single phase). In a two phase system, if the particle size of the dispersed phase is relatively large & forms the tree dimensional house of card structure throughout the gel, then the gel mass sometime is referred to magma (e.g. bentonite magma). Both gel & magmas may be thixotropy forming semisolid on standing & become liquid on agitation. Single phase gel consists of large organic molecule existing on the twisted matter strands, dissolved in continuous phase, such that no apparent boundaries exist between dispersed macromolecule & the liquid. These large organic molecule either natural or synthetic polymer often referred to as gel formers, tend to be entangled with each other due to their random motion or stronger type of Vander Waal force so as to form crystalline or amorphous region throughout the entire system.

1.9.1.3. Based on the nature of solvent

Gel may be classified even as the hydrogel (water based) or an organo gel (with a nonaqeous solvent) based on the type of solvent used as continuous liquid phase. Carbomers are the example of hydrogel. Example of organogel are plastic based (low molecular polyethylene dissolved in mineral oil & shock cooled). Olga (aerosol) gel is dispersion of metallic stearate in oil xerogel. Solid gel with low solvent concentration is known as xerogel. These are often produced by evaporation of solvent, freeze drying, leaving in the gel framework.

1.9.1.4. Based on rheological properties

Gels are considered to exhibit Non-newtonian flow properties. Gels may be classified based on rheological properties as plastics, pseudo plastic & thixotropic gels.

A. **Plastic gels**- it exhibit a plastic flow & the plot of rheogram gives the yield value of the gel above which the elastic gel distorts & begins to flow. Examples are bingham bodies, flocculated suspension of aluminium hydroxide.
B. **Pseudo plastic gels**- the liquid dispersion of natural gums like tragacanth, sodium alginate, methyl cellulose exhibit pseudo plastic flow. The viscosity of pseudo

plastic gels decreases with increasing rate of shear, with no yield value. The rheogram for the pseudo plastic material results from the shearing action on the long chain molecule of the linear polymer. As the shearing stress is increased, the disarranged molecules being to align their long axis in the direction of flow with release of solvent from gel matrix.

C. **Thixotropic gels**- the bonds between the particles in these gels are very weak & can be broken down by shearing. It is likely to occur in colloidal system with non spherical particle to build up a scaffold like structure, e.g. Kaolin & agar 0.5%.

1.9.1.5. Based on physical nature

A. **Elastic gel**- gels of agar, guar gum and alginates exhibit an elastic behavior. The fibrous molecules linked at the point of junction by weak bonds such as hydrogen bond.

B. **Rigid gel** – this gel can be formed from macromolecule in which the framework linked by primary valence bond, e.g. in silica gel silicic acid molecule are held by Si-O-Si-O bond to give a polymer structure possessing a network of pores.

1.9.2. Properties of gels[113,114]

1.9.2.1. Swelling

If a xerogel is placed in contact with liquid that solvent it then an appreciable amount of a liquid is often taken up & the volume of the xerogel increases. This is referred to swell & the pressure developed is known as swelling pressure.

1.9.2.2. Syneresis

Gel will often contact spontaneously & exude some amount of the fluid medium. This effect is known as syneresis & the degree to which it occurs usually decreases as the concentration of gelling agent increases. The occurrence of syneresis indicates that the original gel was thermodynamically unstable.

1.9.2.3. Ageing:

Colloidal system usually exhibit slow spontaneous aggregation. This is referred to as ageing. In gels, ageing result in the gradual formation of a denser network of the gelling agent.

1.9.2.4. Rheological properties:

Gels exhibit the mechanical properties of rigidity, tensile strength & elasticity that are characteristic of solids. In the thixotropic gels, this effect is only apparent below the yield value. Solution of gelling agents & dispersion of flocculated solids are typically pseudo plastic, exhibiting a non-Newtonian flow behavior characterized by a decreasing viscosity with increasing shear rate. Such behavior is due to progressive breakdown of structure of the system. The structure of inorganic particle dispersed in water is disturbed by an applied shear stress

1.9.3. Structure of Gel

The rigidity of a gel arises from the presence of network formed by the interlinking of particle of gelling agent. The nature of particles and the type of force that is responsible for the linkages determines the structure of the network & the property of gel. The individual property of a hydrophilic colloid may consist of either spherical or an isometric aggregates of a small molecule or single macromolecule. In linear macromolecule the network comprised of entangled molecules, the points contacts between which either be relatively small or consist of several molecules aligned in a crystalline order. The cross linking of macromolecule by primary valency bonds provides further a mechanism for the formation of a gel network. The force of attraction responsible for the linkages between gelling agent particles may range from strong primary valences, as in silicic acid gel, to weaker hydrogen & Vander Waal's force. The weaker nature of this latter force is indicated by the fact that slight increases in temperature often caused liquefaction of a gel. Systems that exhibit this type of transition, such as agar & gelatin gels, are termed as thermal gels. In addition, the transition from gel state to a colloidal dispersion may in some cases be brought about by mechanical agitation. System, such as bentonite, alluminium hydroxide gels, exhibit this type of transition termed as thixotropic gel.

1.9.4. Preparation of Gels:[113,115]

In the industrial scale gels are normally prepared under the room temperature however few of the polymers need special treatment for processing. The gel preparation can be categorized under the following heading.

1.9.4.1. Gel Prepared By Thermal Changes:

The solubility of most lipophilic colloids, e.g. gelatin, agar is reduced on lowering of the temperature thus cooling of a concentrated hot sol often produce a gel, just contrast to this, some materials such as the cellulose ether having water solubility due to the hydrogen bonding. Rising the temperature of this will disturb the hydrogen bonding & the reduced solubility will cross gelatin.

1.9.4.2. Gels Prepared By Flocculation with Salt & Non-Solvent:

Gelation is produced by adding just sufficient precipitant to produce the gel state but insufficient to bring about complete precipitation. It is necessary to ensure repeated mixing to avoid local high concentration of precipitant. Solution of ethyl cellulose polystyrene in the benzene can be gelled by repeated mixing with suitable amount of petroleum ether

1.9.4.3. Gel Prepared By Chemical Reaction:

In the preparation of sols by precipitation from solution, e.g. aluminium hydroxide sol prepared by interaction in aqueous solution of an aluminium salt & sodium carbonate, increased concentration or reactants will produce a gel structure.

1.10. Gel As Vaginal Drug Delivery System:[115]

The united state pharmacopeia (USP) defines gel as semisolid being either suspension of small inorganic particles or large organic molecule interpenetrate with liquid. The inorganic particles such as bentonite form a three dimensional "house of cards" structure throughout the gel. This is two systems, as the inorganic particles are not soluble but merely dispersed throughout the continuous phase. Gels are semisolids system in which a liquid phase is constrained within a three dimensional polymeric matrix (natural or synthetic gums) in which a high degree of physical sometime or chemical cross linking has been introduced.

1.11. Advances in Intra-Vaginal Drug Delivery System:

To date the greatest number of intra vaginal drug delivery systems for microbicides, by far, are in the form of creams or gels. Although commonly used for the topical intravaginal delivery of microbicides, these systems are messy, uncomfortable and may

not provide an exact dose due to non-uniform distribution and leakage. To evaluate the efficacy of a 3-day course of clindamycin vaginal cream in the treatment of bacterial vaginosis, Valence *et al.* performed a randomized, placebo controlled trial in pregnant women and found schematic depicting the various mechanisms of preventing the transmission of sexually transmitted infections and HIV by employing microbicide delivery systems. That the clindamycin cream was well tolerated and more efficacious than placebo.[116] Masaki *et al.* has studies in a release study of alginate gel beads, swelling and erosion of the beads were observed at pH 6.8.The decrease in bead size caused a rapid release of PL.[117] Shivakumar *et al.* has discussed on hydrogels as controlled drug delivery system. Hydrogels are presently under investigation as a delivery system for bioactive molecules, because of their similar physical properties as that of living tissue, which is due to their high water content, soft and rubbery consistency, and low interfacial tension with water or biological fluids.[118] This investigation describes the formulation and characterization of rheologically structured vehicles (RSVs) designed for improved drug delivery to the vagina. Interactive, multicomponent, polymeric platforms were manufactured containing hydroxyethylcellulose (HEC, 5% w/w) polyvinyl pyrrolid one (PVP, 4% w/w), Pluronic (PL, 0 or 10% w/w), and either polycarbophil (PC, 3% w/w) or poly(methylvinylether-co-maleic anhydride) (Gantrez S97, 3% w/w) as a mucoadhesive agent. It was considered that these semisolid drug delivery systems may be useful as site-retentive platforms for the sustained delivery of therapeutic agents to the vagina reported by[119] Andrews *et al.* Chatterjee *et al.*, has studied on bioadhesive vaginal microparticulatd gel for HIV treatment for womens.[120] Caroline *et al.* has studied the contraceptive properties of a gel formulation containing sodium lauryl sulfate were investigated in both in vitro and in vivo models.[121] Metters *et al.* Over the past few decades, advances in hydrogel technologies have spurred development in many biomedical applications including controlled drug delivery. Many novel hydrogel-based delivery matrices have been designed and fabricated to fulfill the ever-increasing needs of the pharmaceutical and medical fields. Mathematical modeling plays an important role in facilitating hydrogel network design by identifying key parameters and molecule release mechanisms.[122] Dimitrov *et al.* has reported the chemically cross-linked hydrogels containing various drugs have been investigated. It can be summarized that the basic

factors influencing the drug release are the type and the molecular mass of the cross linking agents, the mass ratio PAA: MDI, drug concentration and the pH of the eluent. The studied systems provide retarded drug release and appear to be potential candidates for use in the pharmaceutical practice.[123] Saleem *et al.* formulated and evaluated topical gel of gatifloxacin. The gels were prepared by using Carbopol, sodium hydroxide, HPMC, sodium alginate, glycerin, methyl paraben, proply paraben and distilled water. These prepared gels were evaluated with the help of different studies like that of appearance of gel, pH, drug content uniformity, viscosity, spreadability, extrudability, *in-vitro* diffusion studies, clarity, colour homogeneity, presence of particles and fibers.[124] Mutimer *et al.* comparative evaluation of bases. Selection of the base for the preparation of ointment should be made only after comparative evaluation of many factors, among which are drug release characteristics, consistency and body as defined by viscosity, tube extrusion pressure, penetrometer readings, spreadability and effect of increased solids, physical and chemical stability, handling and manufacturing characteristics. Methods for measuring some of these properties are described and commonly used ointment bases are compared. Introduced about the methods and techniques used for the study of semisolid dosage forms on laboratory scale.[125] Reddy *et al.* studied four minoxidil gels were prepared using carbopol, hydroxypropyl cellulose, hydroxypropyl methylcellulose and combination of hydroxypropyl cellulose, hydroxypropyl methylcellulose for the treatment of alopecia. The gels were evaluated for some *in vitro* drug content, viscosity determination, in vitro permeation across dialysis membrane and mouse skin, skin irritation and stability at 4, 25 and 37° tests. [126] Zeljka *et al.* developed evaluated and *in-vitro* liposomal vaginal delivery system for acyclovir. Vaginal route of drug administration is commonly used for the local therapy of specific gynaecological diseases. Design of a liposome delivery system for vaginal administration of acyclovir, able to provide sustained release and improved bioavailability of the encapsulated drug for the local treatment of genital herpes was investigated. All liposome preparations were characterized and compared for particle size, entrapment efficacy and tested for *in-vitro* in different media chosen to simulate human vaginal conditions. Incorporation of liposomes in bioadhesive hydrogel further improved their stability.[127] Disouze *et al.* has performed the *in-vitro* antibacterial and skin irritation studies of microsponges of benzoyl

peroxide. In the present work skin irritation study was performed using the Albino rabbits. Formalin 8.5 %v/v was used as a standard irritant and score was recorded by comparing with standard irritant.[128] D'Cruz et al a micro-emulsion gel formulation of compound WHI-05 had no acute or sub-chronic toxicity under the conditions investigated. Bioadhesive gels have been reported to lower perceived vaginal odor because the incorporated polyamines are converted to their non-volatile salt forms. These salts are not antimicrobial, but they can create an un-favorable environment for microbial growth.[129] Valenta *et al* studied an important area of the reproductive tract as vagina and serves as a potential route of drug administration. Besides locally acting drugs it is also of importance for systemic drug delivery, uterine targeting or even vaccination. Currently available dosage forms have several limitations, therefore novel concepts and dosage forms are needed. In this field mucoadhesive polymers will play a major role.[130] Chi *et al.* reported the release mechanisms and the diffusion coefficients of salicylic acid loaded polyacrylamide hydrogels were investigated experimentally by using a modified Franz-Diffusion cell at the temperature of 37^0C to determine the effects of crosslinking ratio and electric field strength. The release profile was found to follow Q vs. t1/2 linear relationship.[132] Ramadan *et al.* has reported miconazole nitrate is an imidazole derivative characterized by longer half-life and higher efficacy in the treatment of the protozoal and anaerobic bacterial infection of the vagina. Over 90% is reported to be bound to plasma proteins. Since miconazole is not available as gel formulation, miconazole was formulated into buffered gels (pH 4.75) using different hydrophilic gel bases, including hydroxypropylmethyl cellulose (HPMC), carbopol 934 and sodium alginate. Determination of drug content, pH, viscosity, percent adhesion and *in vitro* release through both artificial and natural diffusion barrier were investigated for all the prepared bioadhesive gels alone and incorporated with enhancers namely tween 80 (T80) and taurocholic acid.[132] Panigrahi *et al.* studied lincomycin hydrochloride is a systemic antibiotic, which is active against most common gram positive bacteria. It has proved to be excellent for infectious diseases like acne, anthrax, pneumonia, and also for the treatment of furunculosis, carbuncles, impetigo, burns and wounds, carrying gram positive bacteria. Gels were prepared using carbopol 940 as gelling agent, and isopropyl myristate and dimethyl sulfoxide as permeation enhancer. The formulations were

evaluated for drug content, viscosity, pH, extrudability, homogeneity, skin irritation test, spreadability, and gel strength.[133] Sved *et al.* study demonstrated that 5-Fluorouracil (1%) gel is tolerable, safe and significantly more effective than placebo to cure intravaginal warts in HIV-positive women, who were already on treatment with AZT 250 mg and the regimen can be considered as a reliable dual treatment modality to cure intra vaginal warts.[134] Bilensoy *et al.* studied therapeutic efficacy and patient compliance in the treatment for vaginitis of clotrimazole vaginal gel using the thermo sensitive polymer Pluronic F127 together with mucoadhesive polymers such as Carbopol 934 and hydroxypropylmethylcellulose.[135] Sonderfan *et al.* studied about heterosexual intercourse as the major route of HIV-1 transmission worldwide. In the absence of an effective vaccine or consistent male condom use, there is a clear need for female-controlled prevention technologies. He has identified an antiviral agent (PRO 2000) that is well suited for use as a vaginally applied microbicides to prevent HIV-1 transmission.[136] Lewis *et al.* Background. PRO 2000 Gel is a candidate vaginal microbicides designed to prevent the sexual transmission of HIV and other STD pathogens.[137] Chatterjee *et al.* shows formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel *in vitro* and stability study of vaginal gel.[138]

1.12. PHARMACOKINETIC ANALYSIS OF VAGINAL DRUG DELIVERY SYSTEMS:

Pharmacokinetic is the study of the time course of drug and metabolite levels in different body fluids during the absorption, distribution, metabolism and elimination phases of the drug. Sharma *et al.* Showed *in-vitro* and *in-vivo* released study of AZT liposome gel in rabbit model. Liposome preparation was characterized and compared for the particle size mean dispersion, entrapment efficacy, DSC and tested for *in-vivo* release in rabbits. *In-vitro* release studies also performed to compare the *in-vitro* and *in-vivo* of drug.[139] Tapash *et al.* investigated the bioavailability of drug by using the HPLC method. In the present work patches were prepared and then evaluated by using the skin permeability study and *in-vivo* study using the animal model male hairless rates. In the *in-vivo* study the blood samples firstly centrifuged and plasma was separated for the analysis of drug presence by using the HPLC method.[140] Abu-Izza *et al.* reported *in-vivo* evaluation of AZT loaded ethyl cellulose microcapsules after oral administration in

beagle dogs. The purpose of this study was to evaluate the *in-vivo* performance of sustained release AZT microcapsules after oral administration in beagle dogs, and to establish an *in-vitro* and *in-vivo* correlation. Two AZT microsphere formulations as well as AZT powder were administered to four beagle dogs. Plasma samples were analyzed by HPLC. The plasma concentration - time data was analyzed by both compartmental and noncompartmental pharmacokinetic analyses.[141] Levy *et al* reported pharmacokinetics of natural progesterone administered in the form of a vaginal tablet, study was conducted to assess the pharmacokinetics of natural progesterone administered in the novel formula of an effervescent vaginal tablet.[142] Vibhuti *et al* has develop a sensitive and rapid reverse phase high performance liquid chromatography (HPLC) method for the measurement of the levels of AZT and nevirapine (NVP) in human plasma. It would also be potentially useful in the determination of pharmacokinetic profiles and in bioequivalence studies in HIV research.[143]After administration by any route; a drug will reach the blood stream. This process is known as "absorption". The drug in the blood distributes rapidly between the plasma and blood cells and also between plasma proteins. This process of transferring a drug from blood to various tissues is called "distribution". A drug is eliminated either directly through an excretory route such as urine, bile, etc. which is known as "elimination", or indirectly through enzymatic or biochemical transformation by the liver. The latter path of elimination is called "metabolism". The study of this whole process of absorption, distribution, metabolism and elimination of a drug is presented schematically as shown in Figure1.5.

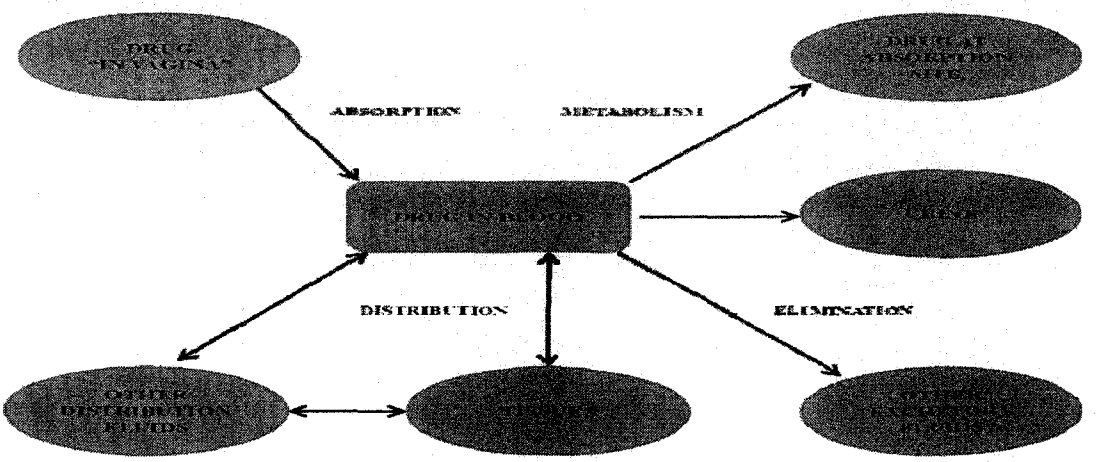

Figure 1.4: Schematic Representation of Drugs Path from Blood

1.12.1. PHARMACOKINETIC PARAMETERS:

1.12.1.1. Volume of Distribution (V_d):

This is the volume into which a drug appears to have been dissolved after administration to an organism, symbolized by V_d. Drug has been completely absorbed from its site of application, has reached an equilibrium in its distribution among the several tissues of the body and no biotransformation in its distribution among the several tissues of the body and no biotransformation or excretion of the drug has occurred. If one knew the mass (dose) of drug administered and the average concentration of the drug in the body.[144]

The clearance and apparent volume of distribution are two separate and independent pharmacokinetic characteristics of the drug. Since they are closely related with the physiologic mechanism in the body, they are called as primary parameters. Mathematically, apparent volume of distribution.[145]

V_d = Amount of drug in the body /Plasma drug concentration = X/C.

1.12.1.2. Total Clearance (C_L):

The clearance is defined as the theoretical volume of body fluid containing drug (i.e. that fraction of apparent volume of distribution) from which the drug is completely removed in a given period of time.[145]

Like that of V_d is needed to relate plasma drug concentration with amount of drug in the body, clearance is a parameter to relate plasma drug concentration with the rate of drug elimination according to following equation:

Clearance = Rate of elimination / Plasma drug concentration

$Cl = (dX/dt)/C.$

The C_L is that hypothetical volume of distribution of un-metabolized drug that is cleared per unit time by any pathway of drug removal. The value of C_L can be determined from the dose administered D, absolute bioavailability and AUC.[147]

$C_L = D.F/AUC$

Where, F is absolute bioavailability.

In addition C_L, V_d, and $t_{1/2}$ are interrelated, whereas C_L and V_d are the independent variables and $t_{1/2}$ is the dependent variable.[147]

$C_L = (0.693.V_d)/t_{1/2}$

The C_L is the key to estimating the dose rate R for controlled release dosage forms and is related to the mean steady state concentration C_{ss}.[147]

1.12.1.3. Half Life ($t_{1/2}$):

This is the time required by the body, tissue or organ to metabolize or inactivate half the amount of a substance taken in. This is an important consideration in determining the proper amount and frequency of doses of drug to be administered.[147] Mathematically, it can be described by the following equation.[144]

$t_{1/2} = 0.693/K$

Where, K is the elimination rate constant.

1.12.1.4. Blood (or Serum or Plasma) Concentration Time Curve:

Following the topical administration of a medication, if blood samples are drawn from the patient a specific time intervals and analyzed for drug content, the resulting data may be plotted on ordinary graph paper to yield the type of drug blood level curve. The vertical axis of this type of plot characteristically presents the concentration of drug present in the blood (or serum or plasma) and the horizontal axis presents the time of samples were obtained following the administration of the drug. When the drug is first administered into the body (time zero), the blood concentration of the drug should also be zero. As the drug crosses the cell membrane (vaginal mucosa), it releases the drug from the dosage form, eventually dissolves and is absorbed. As the sampling and analysis continue, the blood samples reveal increasing concentrations of drug until the maximum (peak) concentration (C_{max}) is reached. Then, the blood level of the drug progressively decreases and if no additional dose is given, eventually falls to zero. The diminished blood level of drug after the peak height is reached, indicates that the rate of drug elimination from the blood stream is greater than the concentration to be found in various tissues and cells for which it has an affinity until ultimately it is excreted as such or as such drug metabolites in the urine or *via* some other route.[144]

1.12.1.5. Area under the Blood (or Plasma or Serum) Concentration Time Curve (AUC):

It represents the total integrated area under the plasma level-time profile and expresses the total amount of drug that comes into the systemic circulation after its administration. AUC is expressed in mcg/mL X hours. It is the most important parameter

in evaluating the bioavailability of a drug from its dosage form as it represents the extent of absorption.[144] The AUC is a measure of the quantity of drug in the body. If curve fitting is done, which assumes a specific model, the AUC can be determine from the coefficients and constants.[147]

1.12.1.6. Peak plasma concentration (C_{max}):

The point of maximum concentration of drug in plasma is called as the peak and the concentration of drug at peak is known as peak plasma concentration. It is also called as peak height concentration and peak maximum drug concentration. C_{max} is expressed in µg/ml.[145]

1.12.1.7. Time of Peak Concentration (T_{max}):

The time for drug to reach peak concentration in plasma (after extra-vascular administration) is called as the time of peak concentration. It is expressed in hours and is useful in estimating the rate of absorption.[145]

1.12.1.8. Mean Residence Time (MRT):

The MRT is defined as the average amount of time spent by the drug in the body before being eliminated.[145] The MRT is the mean time a drug molecule resides in the body; it is the time corresponding to 63.2% elimination from the body. It is calculated from AUC (area under curve) and AUMC (area under the first-moment curve).[147]

MRT = AUMC/AUC

Where, AUMC = Area under the first-moment curve, AUC = Area under the zero-moment curve. MRT = Mean residence time, AUMC is obtained from a plot of product of plasma drug concentration and time (i.e. C.t) versus time t from zero to infinity.[145]

AUC is obtained from a plot of plasma drug concentration versus time from zero to infinity.[145]

1.12.1.9. Mean Steady State Concentration (C_{ss}):

The mean C_{ss} is not the numeric mean between peak (C_{ssmax}) and trough (C_{ssmin}) at steady state but an integrated concentration. With constant rate infusion and in ideal controlled release delivery system, no fluctuations occur at steady state, hence

$C_{ss} = C_{ssmax} - C_{ssmin}$.

The value of C_{ss} can be estimated from the dose ratio R and C_L.[145]

$C_{ss} = R / C_L$.

1.12.1.10. First-Pass Effect (FPE):

Pre-systemic loss of drug can occur regardless of whether the drug is administered systemically or locally, except when given intravenously. It is that fraction of drug which is degraded, inactivated, or metabolized after its release from the dosage form. Most prominent is the pre-systemic loss of drug upon per oral administration, namely degradation by the intestinal contents and enzymes, biotransformation by the intestinal microbial flora and first-pass effect, the metabolism in the gut wall, mesenteric veins, portal vein and liver. In the case of FPE upon peroral administration an estimate can be obtained from intravenous data of the drug.[147]

$f_{EPE} = 1 - D_{i.v.} / LBF. AUC_{i.v.}$

Where, LBF is Lower Blood Flow.

1.12.1.11. BIOAVAILABILITY:

The bioavailability of a drug is defined as its rate and extent of absorption. Rapid and complete absorption is usually desirable for drugs used on an acute or "as needed" basis for pain, allergic response, insomnia or other conditions.[146]

Estimating the extent of absorption:

The total area under the drug level-time curve for drugs eliminates by first-order kinetics is given by:

AUC = Amount of drug reaching the bloodstream/ k.V

Where, k is the apparent first-order elimination rate constant.

It follows that the bioavailability (F) of a drug from a drug delivery product may be determined from the expression:

$F = (AUC)_{Drug\ product} / (AUC)_{Standard}$

When equal doses are administered if different doses of the product and standard are given the area estimates should be scaled appropriately to permit comparison under conditions of equivalent doses, assuming AUC is proportional to dose.[146]

The amount of drug excreted unchanged in the urine (A_u) after administration is given by:

$A_u = F. Dose. (k_u/k)$

Where, k_u = Urinary unchanged excretion rate constant, k = Overall elimination rate constant.

1.13. STABILITY STUDIES:

One of the major characteristics for a quality analytic method for pharmaceuticals is its ability to determine distinctively the parent compound from the degradation products. The current trend in stability-indicating methods is based on direct chromatography or derivatization chromatography. These approaches are used extensively in stability evaluation of pharmaceutical products. When it is not possible to determine the intact drug directly because of interfering substances, it is desirable to procede the final analysis with a separation procedure.[148] Sanjay *et al.* Formulated and evaluated gel of fluconazole. Gels were prepared by the HPMC, methyl cellulose, carbopol, triethano- lamine, sodium alginate, calcium gluconate, ethanol, methyl paraben, propyl paraben and evaluated with the view to develop localized drug delivery system of fluconazole. The gels were evaluated for various physicochemical parameters like pH, viscosity, rheology, drug content and spreadability. The rheological behavior and apparent viscosity values for different gel bases were measured before and after storage under freezing conditions at 2-8°C and were taken as measure for stability of gel network structure. Accelerated stability testing at 45 ±2°C and 75 % ± 5 % R.H. for three months was performed.[149]

Stability of a pharmaceutical preparation can be defined as "the capability of a particular formulation (dosage form or drug product) in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf-life." The term pharmaceutical stability encompasses several concepts. The first one among of them is chemical stability of the drug in the dosage form. But, the performance of a drug given as tablet, capsule, gel, syrup or injection depends not only on drug content, but also on the pharmaceutical properties of dosage form such as hardness, friability, disintegration, dissolution, viscosity, etc.[150]

Stress testing is defined as the stability testing of the drug substances and drug products under conditions exceeding those used for the accelerated testing. Although it is an integral part of the information providing to regulatory authorities in registration application dossiers, details regarding the design of such studies are not covered by regulatory guidance documents.[151]

Stress testing is generally carried out on a single batch of a drug substance, covering the influences of temperature, humidity, pH, light and oxidation. [152] Stress tests are generally not required when

a. The drug substance is covered by the pharmaceutical monograph and the degradation products are covered under purity or impurity tests.
b. Relevant data is available in public domain to support the proposed degradation pathways.

Purpose of stability testing[152]

Stability testing of pharmaceutical products is done for the following purposes:

- To ensure the efficacy and safety of active drug substance and dosage forms.
- To ensure the quality of active drug substance and dosage forms.
- To establish shelf-life or expiration period.
- To support label claim.

1.13.1. Guidelines For Stability Testing[151,152]

Now-a-days, many useful guidelines are available to address most of the stability-associated problems. These guidelines include:

1) ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for human use).
2) WHO (World Health Organization).
3) FDA (Food and Drug Administration).
4) CPMP (Committee for Proprietary Medicinal Products).

Types of stability testing[152]

As per ICH guidelines the stability testing for pharmaceutical is of two types:

- Accelerated (short term) stability testing.
- Long term stability testing.

1.13.2. Accelerated Stability Studies:

US FDA guidelines give the following definition for accelerated stability testing: accelerated Testing: Studies designed to increase the life of chemical and physical degradation of a, drug substance or drug product by using exaggerated (accelerated) stability conditions. The purpose is to determine kinetic parameters to predict the tentative expiration dating period (i.e shelf-life of the product).[29]

Accelerated stability testing gives the advantage over evaluating the stability of a new formulation in a short period of time, e.g. 12 weeks to 6 months and establishing to the extrapolated shelf-life (expiry date of the product), thereby marketing the product at the earliest, instead of waiting for the actual ambient room temperature stability for a long period of time e.g. 18 months to 2 years.[153]

1.13.3. Stability of Semi-Solid Dosage Forms:

Some quantitative indicators of chemical degradation are the development of colour (or a change in colour) and the development of disagreeable odor. Often, products turn yellow or brown with age, indicating oxidative changes in the constituents of the base. This can be overcome by addition of antioxidants.[153] Changes in particle size or particulate nature of a suspension phase must be viewed as extremely serious physical alteration. Any crystalline alteration can lead to a pronounced reduction in the drug delivery capabilities and therapeutic utility of the drug. Another commonly enhanced change in creams and gels is the evaporation loss of water or other volatile phases upon long storage due to either an inappropriate choice of package (some plastic materials) which allows diffusive loss of low-molecular-weight molecules through the container walls, themselves or to improper sealing of the container closure, allowing diffuse leakage around the seams of tubes or the caps of tubes and jars. This process causes a formation to stiffen or become puffy, noticeably changing its application characteristics. Change in the rheological behavior of a formulation can indicate physical or chemical instability. Semi-solids may undergo a progressive loss of viscosity or consistency, changing from semi-solids to viscous liquids, which could be due to storage at warm temperature.[153]

1.14. Vaginal Irritation Study:

A porcine model was established to test the mucosal toxicity potential of a thiophene thiourea (PHI-443)-based anti-HIV microbicide and a vanadocene-based spermicide, vanadocene dithiocarbamate (VDDTC) in comparison to benzalkonium chloride (BZK). Nine domestic pigs (Duroc) in nonestrus stage received a single intra vaginal application of 2% BZK, 2% PHI-443, or 0.1% VDDTC-containing gel. Osmond *et al.,* was reported that key cervico vaginal inflammatory cytokines are useful *in vivo* biomarkers for predicting the mucosal toxicity potential of vaginal products in the physiologically relevant and sensitive porcine model.[154] By using rabbit vaginal irritation test, none of the 6 rabbits given daily intra vaginal application of spermicidal GM-144 for 10 days developed epithelial ulceration, edema, leukocyte influx, or vascular congestion characteristic of inflammation. Therefore, GM-144has the potential to become a clinically useful safe vaginal contraceptive and a vehicle for formulating lipophilic drugs used in reducing the risk of heterosexual transmission of sexually transmitted diseases.[155]

1.15. Other Perspective of Vaginal Delivery Systems:

Gupta *et al* developed and characterized bioadhesive vaginal films of sodium polystyrene sulfonate (PSS), a novel contraceptive antimicrobial agent. Film were prepared by solvent evaporation and optimized for various physical, mechanical and aesthetic properties. Vaginal film contain 300 mg PSS per unit have been developed, using generally regarded as safe (GRAS) listed excipients. Films were prepared by solvent evaporation by using film forming polymer and plasticizer by stirring mechanically at room temperature. They found film were colorless, transparent,thin, soft and tough, dissolve rapidly in physiological fluid to form a smooth, viscous and bioadhesive solution that could be retained in vagina for a prolong time[156]. Perioli *et al.,* developed new formulation for topical administration of ibuprofen in the oral cavity has been developed using several film-forming and mucoadhesive polymers. The films have been evaluated in terms of swelling, mucoadhesion and organoleptic characteristics. The best film, containing polyvinylpyrrolidone (PVP) as film-forming polymer and carboxymethylcellulose sodium salt (NaCMC) as mucoadhesive polymer, was loaded with ibuprofen as a model compound and in vitro and in vivo release studies were performed.[157] Allemande *et al.,* were carried out a pre-formulation studies concerning the

design of novel mucoadhesive films have been carried out. The rationality of the design is based on the utilization of mucoadhesive polymers (carbomer and carboxymethyl-cellulose), a plasticizer (polyethyleneglycol 400, PEG400) and a surfactant (ascorbyl palmitate, ASC16). In the gel preparation, the casting method using water as a solvent was employed[158]. Chun *et al.,* developed a polymeric film composed of Carbopol, Poloxamer and hydroxypropyl methylcellulose was prepared to develop a buccal patch and the effects of composition of the film on adhesion time, swelling ratio, and dissolution of the film were studied. The effects of plasticizers or penetration enhancers on the release of triamcinolone acetonide (TAA) were also studied.[159] Khiang *etal.,* investigate the suitability of an SCMC (sodium carboxymethyl cellulose/ poly ethylene glycol 400/carbopol 934P) and an HPMC (hydroxypropylmethyl cellulose/ poly ethylene glycol 400/carbopol 934P) films as drug vehicle for buccal delivery.[160] Chatterjee *et al.,* reported that prolong release anti-retroviral microparticulate gel for treatment of HIV infection in women.[161] Solanki *et al.,* shows that preparation and characterization of AZT vaginal microcapsules.[162] Chatterjee *et al.,* shows that use of vaginal AZT microcapsules for treatment of heterosexually transmitted HIV infection.[163] Awashti *et al.*, reported that formulation and evaluation of AZT bio adhesive vaginal film for treatment of AIDS.[164] Rahangdale *et al.,* studies of antiretroviral Intra- Vaginal Microcapsules for HIV treatment. [165] Thakuria *et al* shows that drug release from polymeric vaginal film for antiretroviral therapy with different *in vitro* evaluations.[166] Bhowmik *et al* reported that controlled release of anti-HIV biomimetic microdevice in gel for intra-vaginal lubrication and aids therapy.[167] Lalit *et al* shows that vaginal microcapsulated tablet and preclinical bioadhesive testing for prevention of transmission and treatment of HIV and different *in vitro* evaluation procedures. [168]

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