2.1. Anatomy and Physiology of Human Uterus

2.1.1. Structure of Uterus

The uterus is almost the shape and size of a pear and arranged in an inverted position within the pelvic cavity of the torso. It is positioned alongside the body's midline posterior to the urinary bladder and anterior to the rectum. The tapered inferior region of the uterus, known as the cervix, unites the uterus to the vagina below it and acts as a sphincter muscle to regulate the flow of material into and out of the uterus [1]. **Fig. 2-1** shows the structure of normal human uterus. The body (or corpus) of the uterus is the broader region of the uterus superior to the cervix. The body is an open and hollow structure where the fertilized egg, or zygote, implants and matures during pregnancy. The walls of the body are much more compact than those of the cervix as they provide for the protection and sustenance of the developing foetus and contain the muscles that push the foetus out of the mother's body during childbirth.

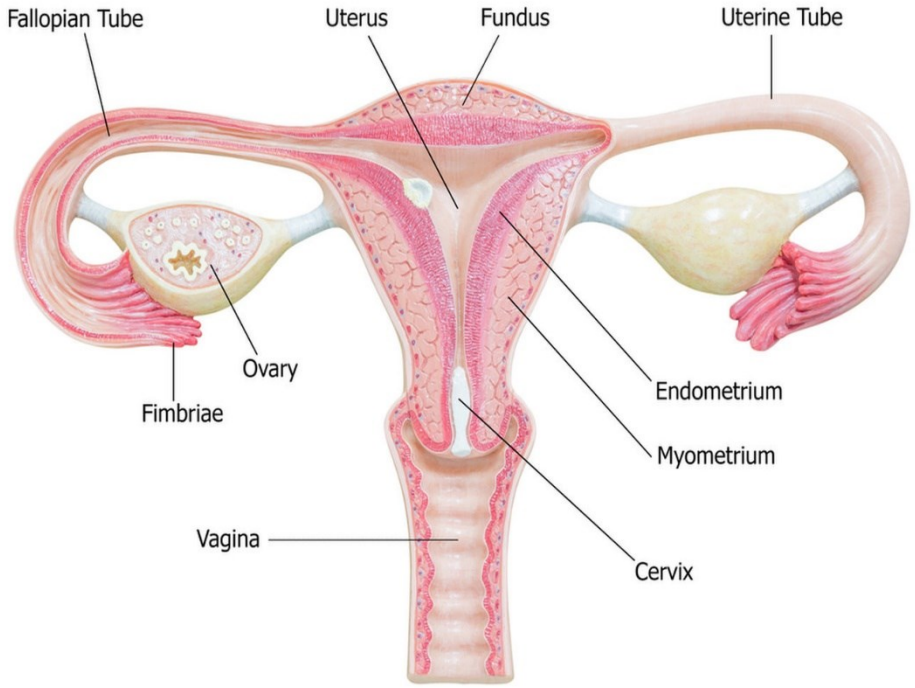

Fig. 2-1: Structure of Human Uterus

The dome shaped region located superior to the body of the uterus is known as the fundus. The fallopian tubes stretched laterally from the sides of the fundus.

Three different tissue layers build the walls of the uterus:

The perimetrium is the outer layer that builds the external skin of the uterus. It is a serous membrane continuing along with the peritoneum that covers the most organs of the abdomino-pelvic cavity. The perimetrium shelters the uterus from abrasion by forming a smooth layer of simple squamous epithelium along its surface and by secreting watery serous fluid to lubricate its surface [2].

Next to the perimetrium, the layer known as myometrium which forms the middle layer of the uterus and comprises many layers of visceral muscle tissue. At the time of pregnancy, the myometrium lets the uterus enlarge and then shrinks the uterus during childbirth.

Inner side of the myometrium, endometrium layer exists. It borderlines the hollow lumen of the uterus. The endometrium is constituted of simple columnar epithelial tissue with many associated exocrine glands and a highly vascular connective tissue that provides support to the developing embryo and foetus during pregnancy [3].

At the time of ovulation cycle, the uterus creates a thick layer of vascular endometrial tissue in readiness to receive a zygote, or fertilized egg cell. If the egg cell does not turns fertilized by the time it reaches the uterus, it will pass through the uterus and activate the blood vessels of the endometrium to atrophy and the uterine lining to be shed. The shedding of the egg cell and uterine linings is known as menstruation and happens approximately every 28 days for most women.

A zygote will implant itself into the endometrial lining, if the fertilization of the ova succeeds. At which, it begins to develop over many weeks into an embryo and finally a foetus. As the embryo develops into a foetus, it triggers changes within the endometrium that lead to the creation of the placenta. The placenta offers the developing foetus with vital nutrients and oxygen from the mother's blood, while

conveying carbon dioxide and metabolic waste products to the mother's blood for disposal.

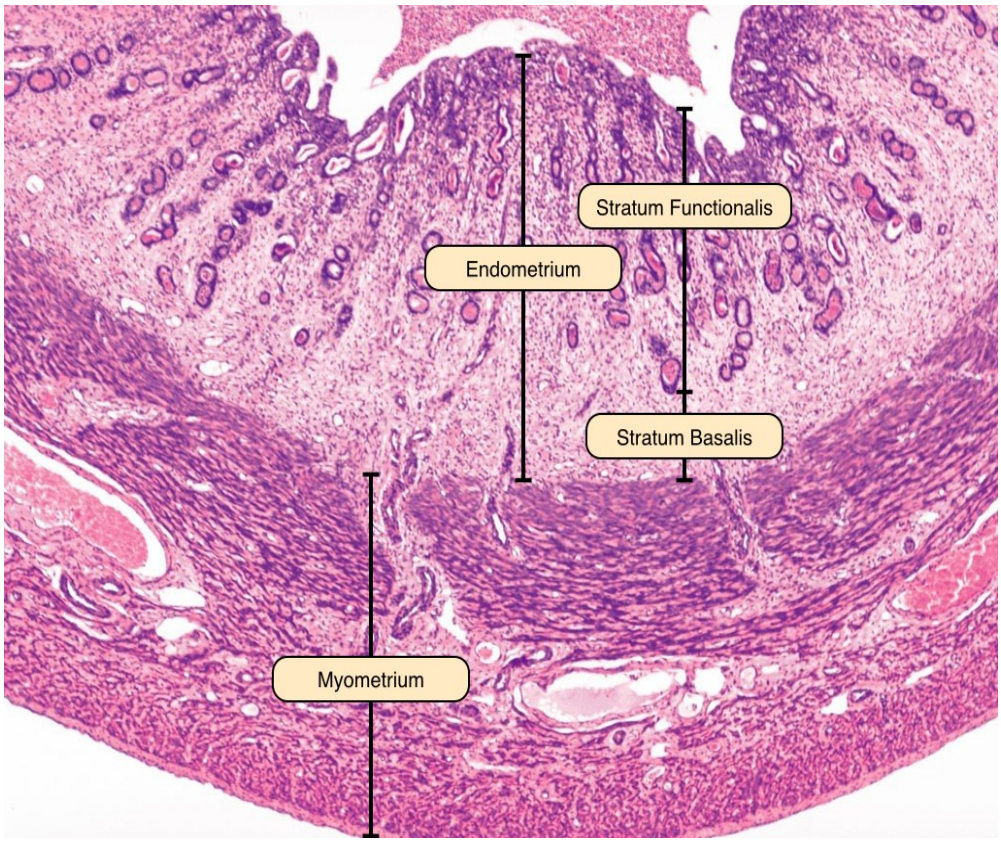

Fig. 2-2: Histology of Human Uterus

The uterus plays a critical role in the process of childbirth at the last stage of the pregnancy. Just before the delivery, hormones initiates waves of smooth muscle contraction in the myometrium that gradually improves in strength and frequency. Simultaneously, the smooth muscle tissue of the cervix starts to efface, or thin, and expand from less than a centimetre in diameter to about ten centimetres at full distention. Once the cervix is fully distended, the uterine contractions forcefully increase in intensity and duration until the foetus is pushed out of the uterus, through the vagina, and out of the mother's body [4].

2.1.2. Uterine Vasculature

The vagina has an extensive blood vascular supply. The blood supply of the uterus is resulting mostly from the uterine arteries. Uterus is supplied by branches of the uterine arteries (branch of the internal iliac artery). One branch travels within the broad

ligament of the uterus until the region close to the ovarian hilum, where it forms an anastomosis with the uterine branches of the ovarian artery (branch of the abdominal aorta). The second branch supplies the cervix and anastomoses with several branches of the vaginal artery. The uterine artery also gives several perforating branches within the uterine wall that form two surrounding systems around the uterus called the posterior and anterior arcuate arteries. The venous blood drains through the uterine venous plexus into the internal iliac vein [5].

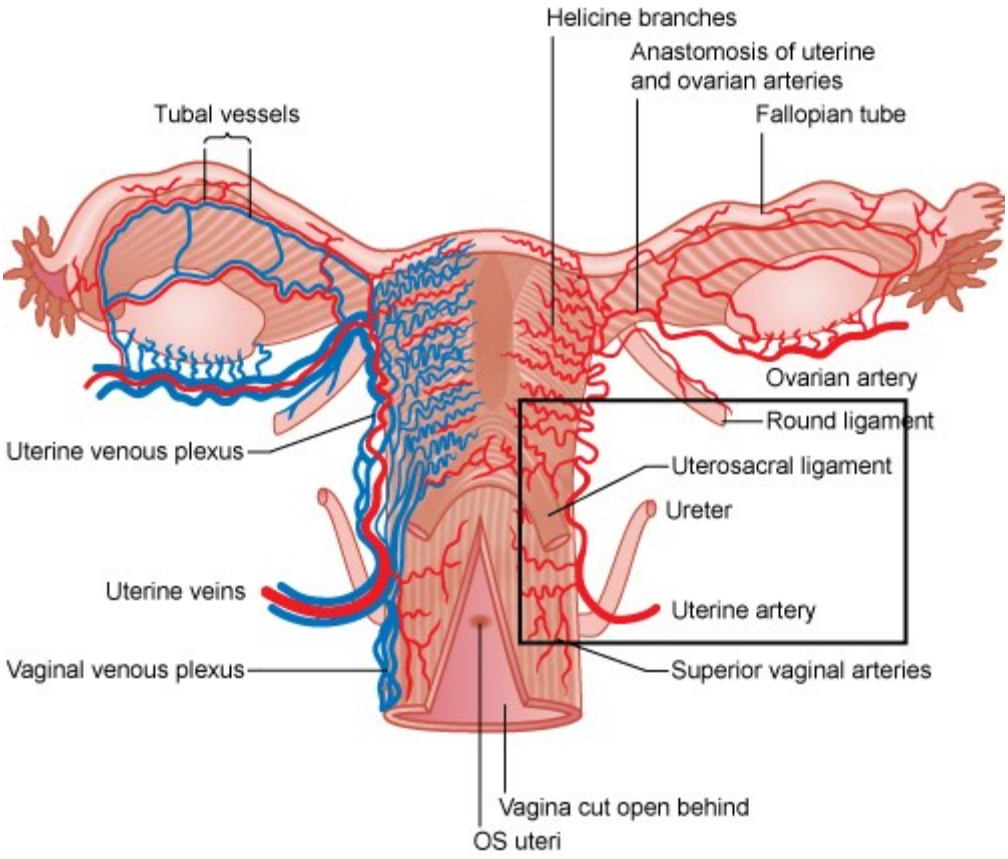

Fig. 2-3: Vascular Supply to the Uterus

Uterine veins parallel the arteries forming plexuses that end into the internal iliac vein; uterine veins merge with vaginal plexus (utero-vaginal venous plexus) downward and with the ovarian veins upward (utero-ovarian plexus). In recent years, interest has been focused on the close anatomical relationships between the venous and arterial wall in the female pelvic vessels. This makes possible the occurrence of a physiological exchange mechanism known as "counter-current exchange". A counter-current mode

of exchange exists with an upward vagina-to-uterus transport which creates a functional portal system linking the vagina to uterus which is also known as the "First Uterine Pass Effect" [6, 7].

2.2. First Uterine Pass Effect (FUPE)

Since long, the vaginal drug delivery has been thoroughly exploited with particular interest in the management of local genital conditions, such as infection, neoplastic lesions or atrophic vaginitis, or with contraceptive and labour inducing/prevention purpose. However, the effectiveness of the vaginal route of administration for various drug molecules preferentially to the uterus while minimizing its systemic distribution and side effects has not been explored thoroughly [8]. The potential of the vaginal route of administration for the treatment of uterus related disorders has been evidently supported by the experiments conducted for the delivery of hormones like progesterone.

The selective distribution of progesterone from vagina to uterus has been demonstrated by Miles et al in 1994 [9]. When comparing intramuscular and vaginal administration of progesterone, the researchers found that vaginal administration led to lower serum progesterone concentrations and higher tissue concentration in the endometrium when compared after intramuscular administration. The results were indicative of some form of direct transport mechanism through which the vaginally administered drugs are preferentially delivered to the uterus. According to this, vaginally administered drugs are passively targeted to the uterus and their tissue concentration is augmented and systemic absorption is reduced which confines the uterus specific distribution simultaneously reducing the circulating level and side effects. Four theoretical mechanisms have been proposed for this locally functional "portal" system by which drug can pass from vagina to uterus.

- Direct diffusion through tissues
- Intraluminal passage from the vagina to the uterus
- Transport via venous or lymphatic circulatory system

- Counter-current vascular exchange with diffusion between utero-vaginal veins and/or lymph vessels and arteries.

Drug substances are believed to be transferred to the uterus via passive diffusion through the vaginal and uterus tissues [10]. The transport of the substances directly from vagina to uterus has been demonstrated in literature. The results supported the view that the uterus and fallopian tubes represents a functional unit that is acting as a peristaltic pump [10]. The absorption of the drug placed in vagina is dependent upon transport in blood and/or lymph. Lymph originating from the mucous membrane and muscles in the cranial part of the vagina are collected by the small lymph vessels. The small vessels join to form two or three bigger vessels, which run on each side of the vagina passing through the plica urogenatalis. Finally these vessels merge with lymph vessels draining the posterior part of the uterus. Hence the lymphatic vessels of the upper part of the vagina being in direct communication with those of the uterus may represent a potential route for direct passage to the uterus for the substances applied to the vagina [11].

Counter-current transport is a physiological mechanism recognized to occur between two tubes or blood vessels if they have common or very close surfaces having their flows in opposite directions. The counter-current transport of minerals and metabolites in the kidney is a well acquainted system modulating the osmolality and concentration of urine. Consequently, more prominent concentrations of substances in venous blood as well as in the lymphatic vessels can transfer to the arteries so their concentration in local arterial blood may be higher than that in arterial blood supplying other organs. In women, the utero-ovarian veins also build a plexus on the surface of the ovarian artery, thus making the anatomical basis for counter-current transport. A number of experiments in animals as well as in humans substantiate the existence of counter-current exchanges between intra-pelvic vessels. Prostaglandins, peptide- and steroid hormones, have been found to be transferred between vessels in the ovarian adnexa in laboratory animals as well as in sheep, swine, cow and human. The transfer of substances was demonstrated to take place from vein blood to ovarian arterial

blood in the closely associated vessels in the ovarian adnexa but later, transfer was also indicated to take place from the lymph to the ovarian arterial blood. An anatomical correlation, which is analogous to that shown in the adnexa, also exists between utero-vaginal venous plexus and uterine arteries; in the mesometrium, the veins cover and writhe along the branches of the uterine artery. This results in a vast area of close surface contact between the veins and arteries and facilitate a direct passage of substances between the vessels.

It has recently been demonstrated that the extent of the first uterine pass effect might be dependent on the exact location within the vagina of the administered formulation. A study performed showed preferential vaginal to uterine distribution of estradiol when a single estradiol tablet was placed in the upper third of the vagina, whereas no effect was observed for placement in the lower third [12].

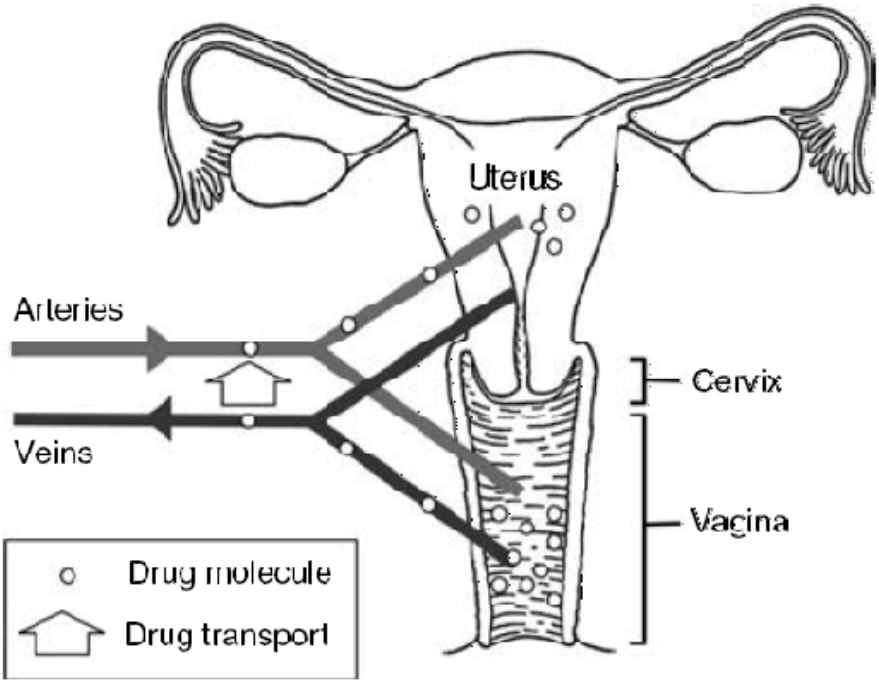

Fig. 2-4: Representation of First Uterine Pass Effect

Although there is evidence for the first uterine pass effect after vaginal drug application, the mechanism of this has not yet been elucidated. Whether this is due to

absorption in to rich venous or lymphatic vaginal system and/or counter current transfer between utero vaginal lymph vessels or veins and arteries or due to direct diffusion through the tissues or through intraluminal transfer from the uterus to vagina. Nevertheless, the vaginal route might as a result of FUPE be a valuable route of drug delivery to the uterus. Targeted drug delivery to uterus through vaginal administration is particularly appealing for substances destined to exert their primary action on the uterus itself.

2.3. Endometrial Cancer

Endometrial carcinoma -a tumour originating in the endometrium- is the most common gynaecologic malignancy and will be encountered by almost every gynaecologist. Globally, endometrial cancer is merely the second to cervical cancer for the frequency among gynaecological cancers. A thorough understanding of the epidemiology, pathophysiology, and management strategies for endometrial carcinoma allows the obstetrician-gynaecologist to identify women at increased risk, contribute toward risk reduction, and facilitate early diagnosis of this cancer. Although a serious public health problem, endometrial cancer has long been a neglected disease, receiving less attention from researchers than cancers of other organ systems.

Globally, endometrial cancer is the sixth most common cancer in women [13]. The incidence of endometrial cancer is highest in North America and Western Europe. In 2015, the number of new cancers diagnosed in the U.S. was almost 55,000, while in 2012, Europe showed close to 100,000 new cases. Endometrial cancer has, in general, a favourable prognosis. For example, in the U.S, its incidence (25.1/100,000) far exceeds the mortality rate (4.4/100,000). Endometrial cancer mortality rates throughout 12 European countries are also generally low. Endometrial cancer is a disease linked to a high standard of living and, thus, the majority of cases are diagnosed in developed countries. Endometrial cancer is predominantly a disease of postmenopausal women, with a median age of 63 years at presentation and less than 10% occurring in women younger than 50 years of age [14, 15]. In the United States, cancer of the endometrium (the lining of the uterus) is the most common cancer of the female reproductive

organs. The American Cancer Society estimates for cancer of the uterus in the United States for 2020 are about 65,620 new cases of cancer of the body of the uterus (uterine body or corpus) will be diagnosed. Also about 12,590 women will die from cancers of the uterine body [16]. Incidence rates are rising, with approximately 8500 new cases reported in the UK in 2015. Most EC cases occur in postmenopausal women; and the age-specific incidence increases steeply from 50 years. However, up to 25% of women are pre-menopausal at the time of diagnosis. A major contributing risk factor to this increase in incidence is the current obesity epidemic and EC ranks highest amongst all cancers in its association with obesity. If diagnosed and treated at an early stage whilst confined to the uterus, EC carries an excellent prognosis with high curability. A recent integrated molecular classification drawing on proteomic, genomic and transcriptomic analyses performed by The Cancer Genome Atlas (TCGA) resulted in new insights into EC subtypes. This molecular classification represents a paradigm shift in the understanding of ECs and heralds a move towards a more targeted and personalized approach to patient management [17].

2.3.1. Pathology of the Endometrial Cancer

The majority of EC arise from endometrial epithelial cells and are adenocarcinomas. ECs have traditionally been classified into Type 1 and Type 2 cancers based upon their clinical, metabolic and histological features. Type 1 ECs incorporate most of tumours seen and include the endometrioid adenocarcinomas, which account for >80 % of all ECs. These tumours are oestrogen-dependant and are frequently associated with endometrial hyperplasia (EH). EH represents a spectrum of morphological endometrial alterations, whereby abnormal proliferation of the endometrial glands results in an increase in the endometrial gland-to-stroma ratio. The presence of cytological abnormality is associated with a high risk of progression to malignancy, such that atypical endometrial hyperplasia (synonymously known as Endometrioid Intraepithelial Neoplasia, EIN) carries a 40 to 60 % risk of future EC. Endometrial biopsy samples reported as EIN carry a significant risk of a co-existing underlying associated EC that has not been detected by endometrial sampling. Type 1 ECs are strongly

associated with conditions contributing to unopposed oestrogen exposure (e.g. obesity, polycystic ovarian syndrome (PCOS), oestrogen only hormone replacement therapy (HRT), chronic anovulation, tamoxifen therapy, granulosa cell ovarian tumours, etc.), in addition to nulliparity, hypertension and insulin resistance. Furthermore, emerging evidence suggests a role for endocrine disrupting chemicals (EDCs) in EC development. At a molecular level, type 1 ECs frequently harbour mutations in the genes PTEN, KRAS, CTNNB1 and PIK3CA. Type 1 ECs are assigned a grade depending on the degree of differentiation and nuclear features. G1 are slow growing tumours with low metastatic potential whilst G3 represent tumours with poor differentiation and an aggressive phenotype and are therefore sometimes considered like Type 2 tumours. Type 2 ECs tend to be oestrogen-independent and include the serous', 'clear cell' and 'mixed-cell' histological subtypes and other rare sub-types. Type 2 ECs are high-grade by definition. Type 2 ECs are not associated with the same risk factors as type 1 ECs and are more often associated with endometrial atrophy in the postmenopausal woman rather than with EH. They are associated with a poorer clinical prognosis and have a high tendency for extra-uterine spread. At a molecular level type 2 ECs are associated with HER2 amplification and recurrent TP53 mutations. The division into type 1 and type 2 ECs is not rigid and it is recognized that many ECs have overlapping features of both tumour types, such that 10 - 19 % of endometrioid ECs are deemed high-grade and have clinical, histopathological and molecular features that are more akin to Type 2 ECs. Rarer subtypes of EC also exist and include; endometrial carcinosarcomas, neuroendocrine tumours and dedifferentiated tumours [17].

2.4. Management of endometrial cancer

2.4.1. Surgical Principles in Endometrial Cancer

Surgery is the mainstay of treatment for EC and is usually total hysterectomy with bilateral salpingo-oophorectomy. There has been a progressive move towards the use of minimally invasive surgical (MIS) techniques. It is now accepted that for women with grades 1 - 2 EC apparently confined to the uterus (based on physical examination, with

or without pelvic imaging), MIS techniques (laparoscopic or robotic) should be considered the optimal approach. MIS is associated with a lower rate of severe post-operative morbidity and shorter hospital length of stay compared with laparotomy and it is more cost-effective with comparable oncologic outcomes. Laparoscopic surgery is not inferior to open surgery in terms of overall survival. Robotic surgery has been shown in the short term to be non-inferior to laparoscopy. It appears to be associated with lower conversion to laparotomy rates in high-BMI patients, although overall it has a higher cost association. Hysterectomy is the removal of uterus by surgical means. However, for women who desire pregnancy or who wish to avoid surgery, hysterectomy is not a viable therapeutic option. Morbidity after a hysterectomy can include bleeding, infection, injury to adjacent organs, and vaginal shortening. Hysterectomy with ovarian conservation is also associated with some loss of ovarian function and a decreased age of menopause. Ovarian failure chances also increases upon hysterectomy. Women who undergo hysterectomy have been shown to have an increased risk of blood loss with increasing uterine size [17, 18].

2.4.2. Chemotherapy in Endometrial Cancer

Management of endometrial cancer has become more complex during the past 10 years for several reasons: changes in histological classification (type 1 vs. type 2) have affected surgical management, adjuvant therapy strategies deeply modified based on data from randomized trials and indications and modalities of lymphadenectomy have changed, even if therapeutic or simply prognostic role of lymphadenectomy remains to be defined. The estimated cumulative life-time risk to develop endometrial cancer is 0.96%, the corresponding mortality risk is 0.23%, and the mortality to incidence ratio is 0.24 (lower with respect to breast cancer (0.32), ovarian cancer (0.63), and cervical cancer (0.55)) [13, 15, 19]

The International Federation of Gynaecology and Obstetrics (FIGO) staging systems, classified advanced endometrial cancer in class III and IV. As the tumour of endometrium progresses to malignant form, the chemotherapy is used as the most effective treatment option, either as an adjuvant or main therapy for stage III and IV

EC [20-23]. Different treatment regimens containing various combinations of chemotherapeutic agents to treat EC are in practice. The combination therapy of PTX and CBP is most widely used treatment considering its advantages over other combination chemotherapy [24]. The results of clinical studies suggest that PTX and CBP has significant activity in advanced endometrial cancer with minimal side effects. 3 year progression free survival and overall survival rates were 50% and 75%, respectively, in PTX and CBP receiving group and 37.5% and 50%, respectively, in the cisplatin, doxorubicin, and cyclophosphamide receiving group [25-28].

2.4.2.1. Paclitaxel (PTX)

PTX was discovered as part of the new cancer drugs screening and discovery program of the National Cancer Institute in the 1960s. In this program many plant extracts were screened for anticancer activity, which included a crude extract from the bark of Taxus brevifolia (Pacific or Western yew). This crude extract showed antitumour activity against several cancer cell lines and the chemical structure of the active ingredient of the extract was identified as PTX [29]. No other chemotherapeutic agent other than penicillin has generated so much interest as PTX since its unique mode of action was discovered in 1979 [30]. PTX is one of the most important lead compounds to emerge from a natural source. After about two decades, since its identification as an anticancer agent from Taxus brevifolia [31, 32], PTX has been an approved drug for ovarian and breast cancer in the USA. PTX is effective against several murine tumours and is one of the most exciting anticancer molecules currently available [33].

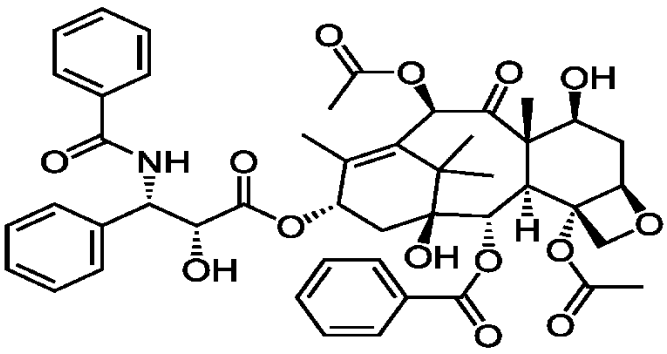

Fig. 2-5: Chemical Structure of PTX

2.4.2.1.1. Physicochemical Properties of PTX

PTX is a monomeric diterpene compound extracted from Chinese yew bark and is a complicated secondary metabolite. Stage II-III clinical studies show that PTX is most suitable for ovarian and breast cancer, and has certain efficacy in treating prostate cancer, lung cancer, colorectal cancer, melanoma, head and neck cancer, oesophageal cancer, germ cell tumours, endometrial cancer, lymphoma, brain tumours, bladder cancer, upper gastrointestinal cancer, small cell and non-small cell lung cancer.

Table 2-1: Physicochemical Properties of PTX

Description	White powder
CAS No.	33069-62-4
Molecular Formula	$C_{47}H_{51}NO_{14}$
Molecular Weight	853.91 g/mol
Melting point	216-217 °C
Density	0.200 g/cm^3
pKa	11.90±0.20
Water Solubility	0.3mg/L (37 °C)
Air & Water Reactions	sensitive to prolonged exposure to moisture

The physicochemical properties of PTX are tabulated in **Table 2-1** [34]. PTX is a white crystalline powder that melts at a temperature of 216-217 °C [35]. It is a non-ionic molecule and is practically insoluble in aqueous mediums. However, PTX is soluble in several non-aqueous solvents such as methylene chloride (~17.1 mg/mL), ethanol (~39.4 mg/mL), methanol (~50 mg/mL) and dimethyl sulfoxide (~50mg/mL) [35-37]. The high lipophilicity (theoretical log P = 3.20) and the elevated net energy of PTX results in a limiting aqueous solubility that has been informed as approximately 0.3 - 0.5 µg/mL [38]. Studies have indicated that an intact taxane ring and an ester side-chain are essential for cytotoxic activity [39]. In addition, it was shown that the presence of an accessible hydroxyl group at position 2" of the ester side-chain enhances the cytotoxic activity of the drug [40]. These structure–activity relationships are very important in the development of paclitaxel analogs.

2.4.2.1.2. Mechanism of Action of PTX

Microtubules are cylindrical structures (made up of proteins, mainly tubulin) that are involved in various cellular functions such as movement, ingestion of food, controlling the shape of cells, sensory transduction and spindle formation during cell division [41, 42]. Paclitaxel has a unique mechanism of action and differs from that of other currently available anticancer agents [43]. It aids polymerization of tubulin dimers to form microtubules, even in the absence of factors that are normally required for microtubule assembly (e.g. guanine triphosphate, GTP), and then stabilizes the microtubules by preventing depolymerization [30, 44]. Paclitaxel mainly binds to microtubules, rather than to tubulin dimers [45]. The binding site for paclitaxel is the N-terminal 31 amino acids of the b-subunit of tubulin in the microtubule [46], unlike the binding sites of colchicine, vinblastine and podophyllotoxin for GTP [45]. The microtubules formed due to paclitaxel action are not only very stable but are also dysfunctional, leading to cell death [47]. While the precise mechanism of action of the drug is not understood fully, paclitaxel disrupts the dynamic equilibrium within the microtubule system and blocks cells in the late G2 phase and M phase of the cell cycle, thereby inhibiting cell replication [33].

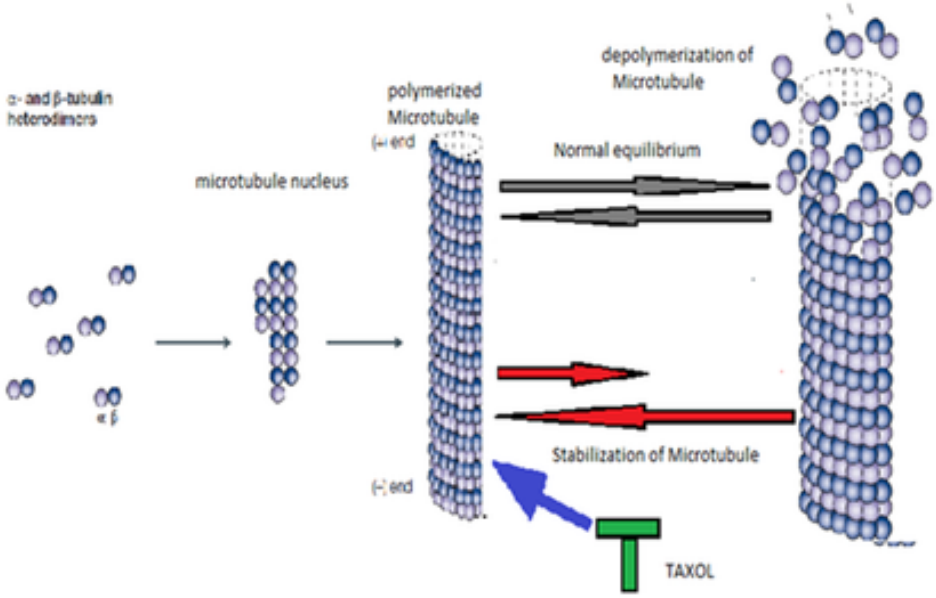

Fig. 2-6: Mechanism of Action of PTX

2.4.2.1.3. Work done on PTX

PTX is one of the most useful and effective antineoplastic agents for treatment of many forms of advanced and refractory cancers. The success of PTX in these diseases has been due to its singular properties: a broad spectrum of antitumour activity, effectiveness on both solid and disseminated tumours and a unique mechanism of action. However, significant side effects produced by the vehicle of conventional formulation of PTX have limited its optimal clinical utility as an anticancer agent [35]. Recently, alternative PTX nano-formulations have been developed to minimize or overcome these limitations [48]. Work done on PTX is summarized in **Table 2-2**.

Table 2-2: Work done on PTX

Author	**Technique**	**Outcome**
Tao Yang et al [49]	PTX incorporated liposome has been constructed to improve solubility and physicochemical stability	5% (v/v) of PEG 400 in the hydration medium of liposome significantly increased the solubility -up to 3.39 mg/mL- as well as the EE and the PTX content in the liposome formulation composed of 10% w/v of SPC with cholesterol (cholesterol-to-lipid molar ratio = 10:90)
C Holvoet et al [50]	The solubility of paclitaxel was enhanced using liposomes	Solubility enhancement of almost 85 times the native solubility of PTX.
J Allen Zhang et al [51]	Developed lyophilized liposome-based PTX formulation that is sterile, stable	0.25mg/ml diluted formulation without drug precipitation or change in particle size. In vitro drug release study in PBS, pH 7.4 showed that less than 6% of the entrapped PTX was released after 120 h
P Crosasso et al [52]	Prepared different conventional and	Conventional liposome formulation was composed of

	PEGylated liposomes containing PTX	ePC/PG 9:1, while for PEGylated liposomes the best composition was ePC/ PG/ CHOL/ PEG(5000)-DPPE 9:1:2:0.7. PEGylated liposomes were found to be less stable during storage than the corresponding conventional liposomes and to have lower drug release in human plasma
Jun Wu et al [53]	Liposomal formulation of PTX targeting the folate receptor was developed to overcome vehicle toxicity associated with the traditional Cremophor EL-based formulation. (DPPC/DMPG/mPEG-DSPE/folate-PEG-DSPE) at molar ratios of (85.5:9.5:4.5:0.5) and a drug-to-lipid molar ratio of 1:33	FR-targeted liposomes containing PTX showed 3.8-fold greater cytotoxicity compared to non-targeted control liposomes in KB cells. Plasma clearance profiles of PTX in the liposomal formulations were then compared to PTX in Cremophor EL formulation. The liposomal formulations showed much longer terminal half-lives (12.33 and 14.23 h for FR-targeted and non-targeted liposomes, respectively) than PTX in Cremophor EL (1.78 h).
Meng-Xia Chen et al [54]	Paclitaxel (PTX) loaded multi-layered liposomes were prepared using layer-by-layer assembly in an effort to improve the stabilization of the liposomal compositions for PTX delivery	Optimized formulation was found to have particle size of 215 ± 17 nm, zeta potential of +27.9 ± 3.4 mV and encapsulation efficiency of 70.93 ± 2.39%. Formulation exhibited sustained release behaviours in vitro and enhanced PTX induced cytotoxicity in human cervical

		cancer cell culture experiments.

2.4.2.2. Carboplatin (CBP)

Scientists from the Institute of Cancer Research, London went on to test around 300 derivatives of cisplatin throughout the 1970s to see if they could produce a milder form, leading to the discovery of carboplatin - an extremely effective anti-cancer drug but with dramatically reduced side-effects [55]. Carboplatin, cis-diammine(cyclobutane-1,1-dicarboxylato)platinum(II), is a platinum anticancer drug used for treating many types of human cancer. Since carboplatin was found to be much less oto-, neuro-, and nephrotoxic than cisplatin [56-58], it was ultimately approved as a drug in 1989 under the brand name Paraplatin. Carboplatin contains a bi-dentate di-carboxylate chelate leaving ligand, a structural feature that makes the compound much less chemically reactive than cisplatin [58, 59].

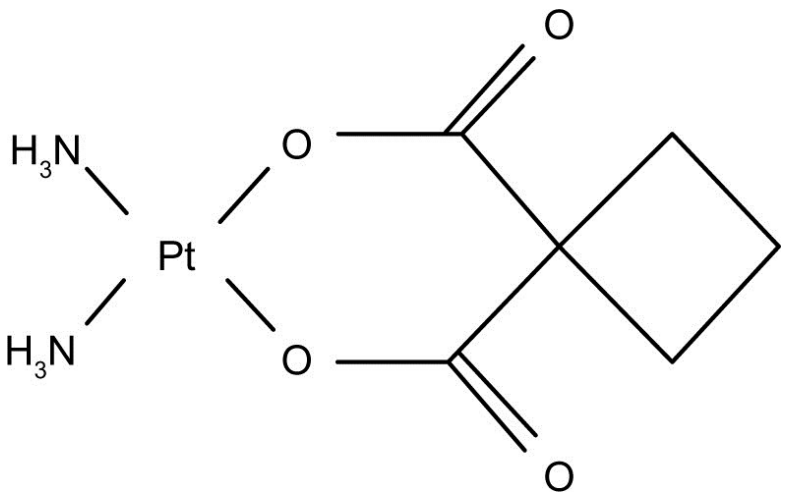

Fig. 2-7: Chemical structure of CBP

2.4.2.2.1. Physicochemical Properties of CBP

CBP belongs to the class of organic compounds known as dicarboxylic acids and derivatives. These are organic compounds containing exactly two carboxylic acid groups. Summarises the physicochemical properties of the molecule.

Table 2-3: Physicochemical Properties of CBP

Description	White crystalline powder
CAS No.	41575-94-4
Molecular Formula	$C_6H_{12}N_2O_4Pt$
Molecular Weight	371.25 g/mol
Melting point	235-245 °C
Log P	0.14
Water Solubility	14 mg/mL

2.4.2.2.2. Mechanism of Action of CBP

CBP (1,1-cyclobutyldicarboxylate) is one of the main platinum-based drug used as an antitumour drug. While details of its molecular mechanism of action are not completely known, CBP is believed to exert its biological effects by interacting with cellular targets such as genomic DNA, tubulin, and other cellular proteins [59]. The main target of CBP is DNA, to which it binds efficiently, thereby inhibiting replication and transcription and inducing cell death. The nature of these DNA adducts affects a number of transduction pathways and triggers apoptosis or necrosis in tumour cells. Adducts formed by this compound can be mono-adducts or intra and inter-chain di-adducts [60].

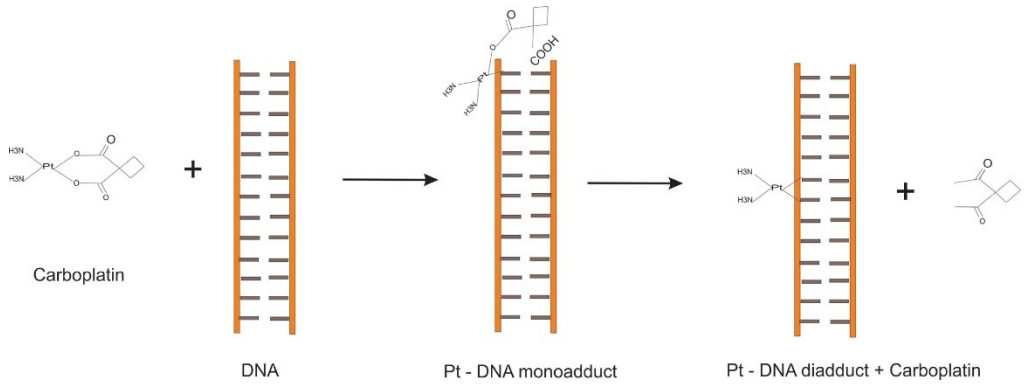

Fig. 2-8: Mechanism of action of CBP

2.4.2.2.3. Work Done on CBP

Carrier-based delivery of anticancer drugs has received much attention in recent years because of its potential for improving drug efficacy, reducing unwanted side effects and circumventing cellular accumulation mediated drug resistance. Such delivery approaches often exploit differences between normal tissues and tumours to increase the selectivity of the drug towards its intended target. Specifically, the enhanced permeability and retention effect (EPR effect) is based on the increased permeability of macromolecules in the tumour containing tissues coupled with poor lymphatic clearance and slow venous return in these tissues [61, 62]. While most clinically used anticancer drugs have low molecular weight and rapidly pass through the membranes of both normal and cancerous tissues, the drugs coupled to liposomes, lipid particles, micelles and various other polymeric carriers selectively accumulate in tumours [63]. Work done on CBP is summarised in **Table 2-4**.

Table 2-4: Work done on CBP

Author	**Technique**	**Outcome**
Chaudhury, A. et al [64]	Stable lyophilized cholesterol free PEGylated liposome- Drug loading using passive equilibration method	The lyophilized liposomes had low water content of 2.59 ± 0.18%. Lyophilized cholesterol-free, PEGylated liposomes exhibited two-fold increase in drug content when carboplatin was loaded via the passive equilibration method, and the in vitro drug release profile of these liposomes were not different from that of the non-lyophilized counterparts.
Wehbe, M. et al [65]	A passive equilibration method which relies on addition of candidate drugs to pre-formed liposomes is used as an	Maximum CBP encapsulation is achieved within 1 h after the CBP solution is added to pre-formed DSPC/Chol liposomes in the presence of

	alternative method for preparing liposome encapsulated drugs.	30% (v/v) ethanol at 60 °C. When the pre-formed liposomes are mixed with ethanol (30% v/v) at or below 40 °C, the encapsulation efficiency is reduced by an order of magnitude. The cytotoxic activity of CBP was unaffected when prepared using this method and the resultant formulations exhibited good stability in vitro and in vivo.
Chaudhury, A. et al [66]	Folate receptor-targeted liposomes for carboplatin were developed and evaluated	Significant enhancement in carboplatin potency and intracellular drug accumulation was observed in KB cells when treated with Folate receptor-targeted liposomes. Study suggested a novel mechanism by which Folate receptor-targeted liposomes could sensitize cancer cells to drug treatment via modulation of ERK-related cell survival signals.
Poy, D. et al [67]	Encapsulated anticancer drug CBP into liposomal nanoparticles by reverse-phase evaporation technique and evaluated its efficacy on lung cancer in vitro environment.	Nanoscale particles with 67% drug encapsulation efficiency were prepared. Also, high retention capability (drug release equal to 25% after 72 h) of the nanodrug was confirmed. In addition, results of the nanodrug cytotoxicity indicated nanoparticles increased potency of the drug by approximately 90%. Findings of the study

		indicated liposome can be used for CBP delivery to lung cancer.
Khan, M.A. et al [68]	Developed chitosan nanoparticles by an ionic interaction procedure. The nanoparticles were characterized by physicochemical parameters and cytotoxicity in vitro.	The average particle size of chitosan and CBP nanoparticles was found to be 277.25 ± 11.37 nm and 289.30 ± 8.15 nm and zeta potential was found to be 31 ± 3.14 mV and 33 ± 2.15 mV respectively with low PDI. The encapsulation and loading efficiencies of CBP were obtained to be 58.43 % and 13.27 % respectively. The cytotoxic effects of the CBP loaded chitosan nanoparticles were tested in-vitro against breast cancer (MCF-7) cell lines.
Nanjwade, B.K. et al [69]	Natural biodegradable polymer sodium alginate nanoparticles were prepared by the ion gelification technique	Drug encapsulation efficiency was about 52.24–68.70%. In vitro release profile showed more than 12 h release by nanoparticulate formulation as compared to pure drug (up to 3 h). In case of free drug, less amount of drug was found in liver, lungs and spleen as compared to drug encapsulated nanoparticles in laca mice.

2.5. Vaginal Route for Drug Delivery

The vaginal route of drug delivery has been recognized since ancient Egyptian times, with the use of various substances as vaginal contraceptives. Agents commonly delivered vaginally include antimicrobials, spermicides and agents used for

contraception, hormone replacement, cervical ripening/ labor induction and pregnancy termination. Commonly utilized dosage formulations for these indications include solid dosage forms such as suppositories and tablets and semi-solid forms such as creams and gels. Intravaginal rings (IVRs), vaginal films, and foams are also utilized. Woolfson et al. [70] also expressed that choice of vaginal delivery platform depends upon multiple variables, spanning from drug properties to clinical requirements to user acceptability. Several advantages of the vaginal drug delivery route have been defined [70-73]. The vagina can provide an accessible route of delivery, due to ease of self-insertion. Vaginal delivery represents a non-invasive route of delivery, by avoiding pain, tissue damage and possible infections associated with the parenteral route. Drugs delivered vaginally are able to avoid hepatic first-pass metabolism, and the vagina has a well-developed blood supply for drugs absorption. Furthermore, the vagina provides great permeability for drugs with certain physicochemical characteristics. Drug absorption depends on the physicochemical properties of the agent in question, specifically molecular weight, dissolution characteristics, and ionization properties. Vaginal drug absorption may occur by diffusion and/or active transport. This delivery route also allows for avoidance of the incidence and severity of various side effects, such as gastrointestinal and hepatic effects, associated with oral and parental drug delivery [74].

The vagina is a distensible muscular tube which extends postero-superiorly from the external vaginal orifice to the cervix which plays major role in reproduction. It is positioned between the rectum, bladder and urethra. The vagina is a slightly S-shaped fibromuscular collasible tube and its dimensions range from 8.4 to 11.3 cm in length and 2.1 to 5.0 cm in diameter. The shape of the vagina is not a round tunnel. In the transverse plane it is more like an “H” lying on the side. At the upper ending, the vagina surrounds the cervix, creating two domes (fornices or vaults): an anterior and a (deeper) posterior one.

The vagina is composed of four histological layers (internal to external):

Stratified squamous epithelium – this layer provides protection and is lubricated by cervical mucus (the vagina itself does not contain any glands).

Elastic lamina propria – a dense connective tissue layer which projects papillae into the overlying epithelium. The larger veins are located here.

Fibromuscular layer – comprising two layers of smooth muscle; an inner circular and an outer longitudinal layer.

Adventitia – a fibrous layer, which provides additional strength to the vagina whilst also binding it to surrounding structures.

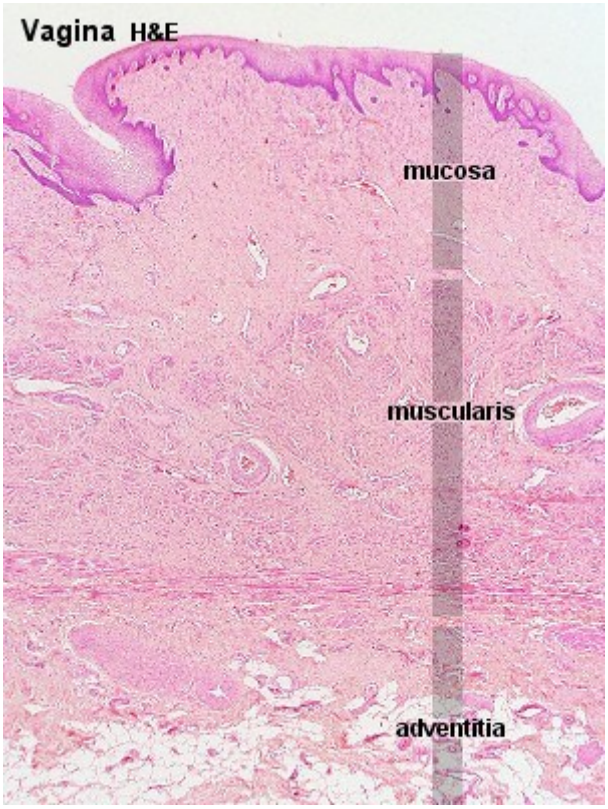

Fig. 2-9: Vaginal Histology

The arterial supply to the vagina is via the uterine and vaginal arteries – both branches of the internal iliac artery.

Venous return is by the vaginal venous plexus, which drains into the internal iliac veins via the uterine vein.

Lymphatic drainage is divided into three sections:

Superior – drains to external iliac nodes

Middle – drains to internal iliac nodes

Inferior – drains to superficial inguinal lymph nodes.

Innervation is predominantly from the autonomic nervous system. Parasympathetic and sympathetic nerves arise from the uterovaginal nerve plexus (in turn a subsidiary of the inferior hypogastric plexus). Only the inferior 1/5th of the vagina receives somatic innervation. This is via a branch of the pudendal nerve, the deep perineal nerve [75].

Intra Vaginal Rings (IVRs)

The vaginal route has been extensively studied as a site for drug administration. As a consequence, a novel controlled release system known as intra vaginal ring was developed. Vaginal rings are torus shaped devices formed by a polymeric material (e.g. poly-dimethylsiloxane/silicone, ethylene vinyl acetate copolymer (EVA) etc.) which holds the active ingredient [76]. The ring is simply inserted into the vagina and it releases the drug in a controlled manner. The rate of release is controlled by several factors like the relationship between the characteristic dimensions, the initial load of active ingredient, the presence of excipients, the design of the IVRs etc [77]. The use of vaginal rings presents numerous advantages [76] like,

- It permits the controlled release of drug
- Avoids daily administration
- Allows the use of low drug dose and the simultaneous administration of several drugs by the same device
- It is user controlled

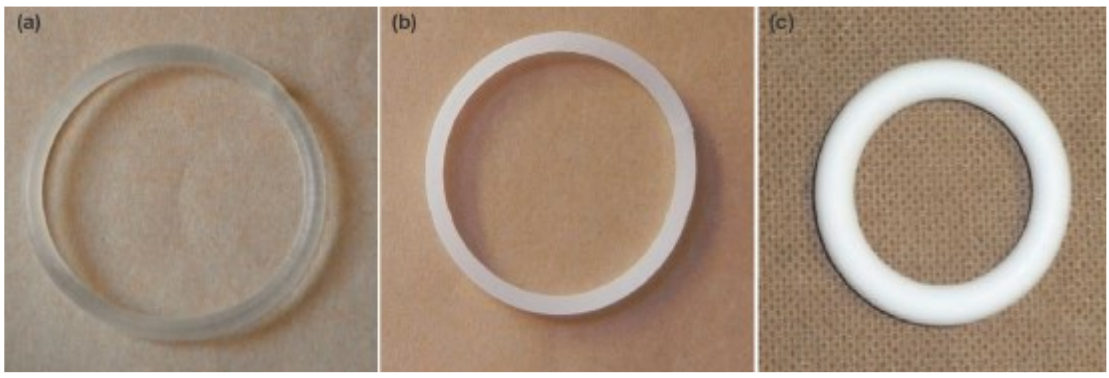

Fig. 2-10: Intra Vaginal Rings (Matrix type)

Fig. 2-11: Intra Vaginal Rings (Reservoir type)

Intravaginal ring delivery systems are usually based upon silicone elastomers with an inert inner ring, which is coated with another layer of elastomer containing drug. An outer rate controlling elastomer layer may be added as a third to prevent an initial burst release in various rings. The rings may be upto 6 cm in diameter and 4-7 mm in cross-section. The rings are left for several days and can deliver drugs at a consistent ratio with approximately zero-order kinetics. The marketed rings loaded with hormones are Nuvaring®, Nesterone® and Femring®.

2.6. Ultra deformable nano vesicles (UDNVs)

UDNVs comprising phospholipids and an edge activator (EA) are a new approach in vesicular drug delivery. UDNVs, by the virtue of their enhanced elasticity compared with standard liposomes, are more amenable to the transport of therapeutic agents across the biological membranes [78]. Elasticity in these vesicles is generated by the presence of an EA, which is generally a single chain surfactant with a high radius of curvature, capable of weakening the lipid bilayers of the vesicles and increasing their deformability. During destabilization and owing to their affinity for curved configurations, surfactants attempt to gather at the location of enhanced pressure and consequently lead to a decrease in the energy needed for the change of shape. The maximum ultradeformability is obtained when the vesicular membrane attempts to

optimize its local composition in response to an external, spatially anisotropic stress (**Fig. 2-12**) [79].

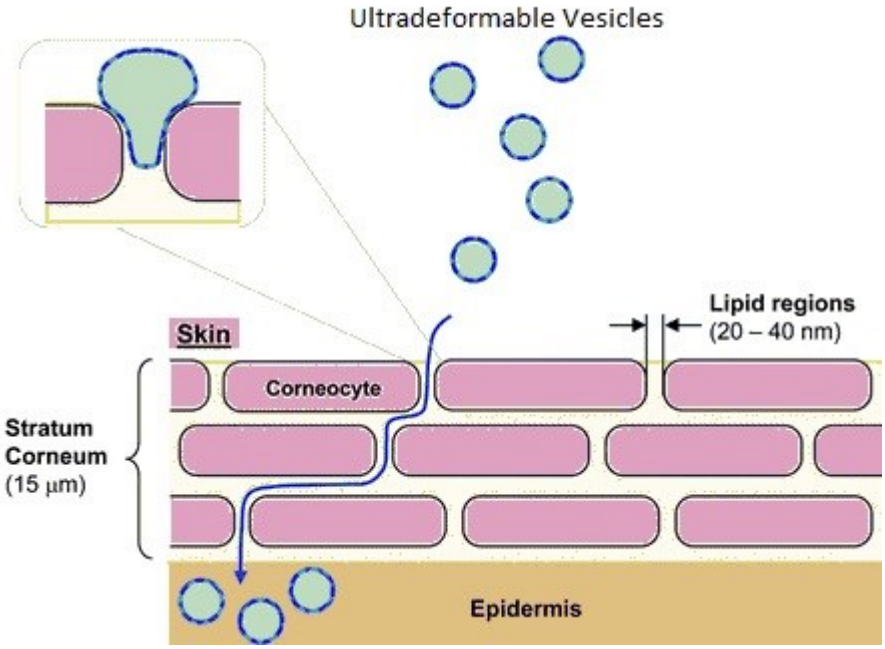

Fig. 2-12: Mechanism of ultradeformability for penetration

Properties and applications of UDNVs summarized below [80].

- They are able to transport therapeutic agents through very narrow pathways between most cells in the biological membranes (e.g. Stratum Corneum).
- UDNVs are biocompatible, biodegradable and are capable of protecting the encapsulated drug from metabolic degradation.
- UDNVs have also been used as transporters for various macromolecules, including peptides, proteins (insulin albumin, gap junction protein (GJP)), DNA, antigens, nutraceuticals, analgesics, anaesthetics, corticosteroids and sex hormones, and have been proven to significantly augment amount of drug permeated.
- With their great penetration abilities, UDNVs offer a non-invasive means of drug delivery.

Past work done on ultradeformable vesicle formulation is summarized in **Table 2-5**.

Table 2-5: Work done on ultradeformable vesicles

Author	Technique	Outcome
Maghraby, G.M.E. et al [81]	5-fluorouracil loaded ultradeformable liposomes were developed	The ultradeformable formulation was superior to standard liposomes in the skin delivery of 5-FU. Of the traditional liposomes, the non-rigid preparation was the best. However, stabilization of the liposome membrane with cholesterol abolished the benefit of this non-rigid preparation. It was concluded that ultradeformable vesicles are promising agents for skin delivery of drugs.
Jain, S.K. et al [82]	Elastic liposomes bearing acyclovir sodium were prepared for its enhanced transdermal delivery by conventional rotary evaporation method and characterized.	The elastic liposomal formulation for transdermal delivery of acyclovir sodium provides better transdermal flux, higher entrapment efficiency, ability as a self-penetration enhancer and effectiveness for transdermal delivery as compared with conventional liposomes. In vivo studies showed that on transdermal application of elastic liposomes, the concentration of acyclovir sodium in plasma was found to be 105 ± 9.4 ng/ml after 24 hr which is about 4.2 times compared with conventional liposomes.

Paul, A. et al [83]	Ultradeformable immune-liposomes developed using an ethanol solution of soybean phosphatidyl-choline (SPC) combined with edge-active bile salt (BS)	The non-invasively, epi-cutaneously administered ultradeformable liposomes shown to be at least as successful in inducing antigen-specific antibodies as subcutaneously injected vesicle suspensions. ultradeformable liposomes offer at least two advantages: first, they are applicable without injection and second, they give rise to rather high antibody titres and, possibly, to relatively high IgA levels. Each of these factors provides an interesting basis for the development of improved vaccines.
Mishra, D. et al [84]	Elastic liposomes bearing propranolol hydrochloride were prepared by conventional rotary evaporation method and characterized.	Results indicate that the elastic liposomal formulation for transdermal delivery of propranolol hydrochloride provides better transdermal flux, higher entrapment efficiency, ability as a self-penetration enhancer and effectiveness for transdermal delivery as compared to liposomes.
Cosco, D. et al [85]	Resveratrol- and 5-fluorouracil-loaded ultra-deformable liposomes were investigated for the potential treatment of non-melanoma skin cancer.	Co-encapsulation of resveratrol and 5-fluorouracil (multi-drug carrier) in ultradeformable liposomes improved their anticancer activity on skin cancer cells as compared to

		both the free drug form and the single entrapped agents.

2.7. Quality by Design (QbD) in Formulation

The concept of QbD is usually used in pharmaceutical development and manufacturing to enhance the quality of the obtained products. QbD is a systematic approach that starts with predefined objectives (also known as "Target Product Profile" or product specifications) and emphasizes on product critical quality attributes (CQAs), understanding of critical process parameters (CPPs) and process control. This can be improved by building quality standards into the process of development and manufacturing and not only testing the product at the end of the process [86, 87].

This concept was introduced in the 2000s by the pharmaceutical industry combined with support from the Food and Drug Administration (FDA). Even though, the quality of the final products was usually ensured, a high percentage of the production was wasted because the quality control only took place at the end of the process, leading to higher costs associated with medicine manufacturing. Taking into account the high cost and complex production associated with nanomedicines, the optimisation of the manufacturing process is key to ensure that advance formulations, reach the market [88].

According to the 21st Century Initiative, more controls were necessary to be implemented in manufacturing in order to improve efficiency and safety in the process leading to the establishment of GMP (Good Manufacturing Practices). Since the FDA-GMP Initiative, new documents have been published by the International Conference on Harmonization (ICH) focused on the concept and implementation of QbD in pharma companies: Q8 Pharmaceutical Development, Q9 Quality Risk Management and Q10 Quality Systems Approach to Pharmaceutical GMP Regulations. In pharmaceutical R&D (research and development), QbD, specifically design of experiments (DoE), is commonly used to obtain optimised formulations taking into

consideration a wide range of factors that can affect the Target Product Profile, as experiments are set up in an efficient and precise way [89, 90].

2.7.1. Statistical Designs in Formulation Optimization

Formulation optimisation usually takes place in several steps, starting with a pre-screening design (also known as ruggedness testing) in order to identify the critical main factors of the process utilising the minimum number of experimental runs to be performed to ensure cost and time efficiency. Pre-screening is performed by using different types of factorial models, among which Plackett-Burman and Taguchi are the most utilised. These models are useful to determine which factors have higher or lower, positive or negative influence in the development of the formulation (eg. amount of components or parameters in the manufacturing process). Once, the most influential factors have been identified with the pre-screening designs, response surface models are commonly employed to find the optimal design space. Mixture design spaces are also utilised where the suitable ratio between excipients needs to be identified but there is no investigation on the process parameters. Plackett-Burman design can evaluate between 2 to 47 factors, where each factor is set to 2 levels (higher and lower). This design can be applied to investigate up to N-1 variables with N experiments. This design is particularly useful to test ruggedness when the aim is focused on finding a small or non-existent effect due to the factors. A ruggedness test determines the sensitivity of a procedure to small changes in operational factors. The Taguchi model is an orthogonal array design which evaluates two-level factorial designs (higher and lower). This type of DoE reduces effectively the number of experiments required in a final optimization design process, instead of having to test all possible combinations. Taguchi emphases on the concept of robust design methodology where alterations due to noise factors beyond the control of the design are considered and the obtained responses are only affected by controllable factors. Response surface methodology consists on a group of mathematical and statistical techniques based on the fit of experimental models to the empirical data obtained in relation to DoE [91].

As a modern DoE technique intended to aid the better understanding and optimisation of the responses, it is often used to refine models once the main factors have been previously identified with a pre-screening test. There are two main types of response surface designs: the central composite and the Box-Behnken. A central composite design is a 2-full-factorial design that includes both the central and star points allowing estimation of curvature. The number of central point runs the design and the star points represent the extreme values (low and high) for each factor in the design. This design estimates efficiently first- and second-order terms and is especially useful in sequential experiments as previous factorial experimental data can be fed in the design. In contrast, Box-Behnken design requires fewer design points and hence is less expensive to run with the same number of factors. Box-Behnken design always have three levels per factor unlike central composites which can have up to five [92].

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