# 2 Literature Review

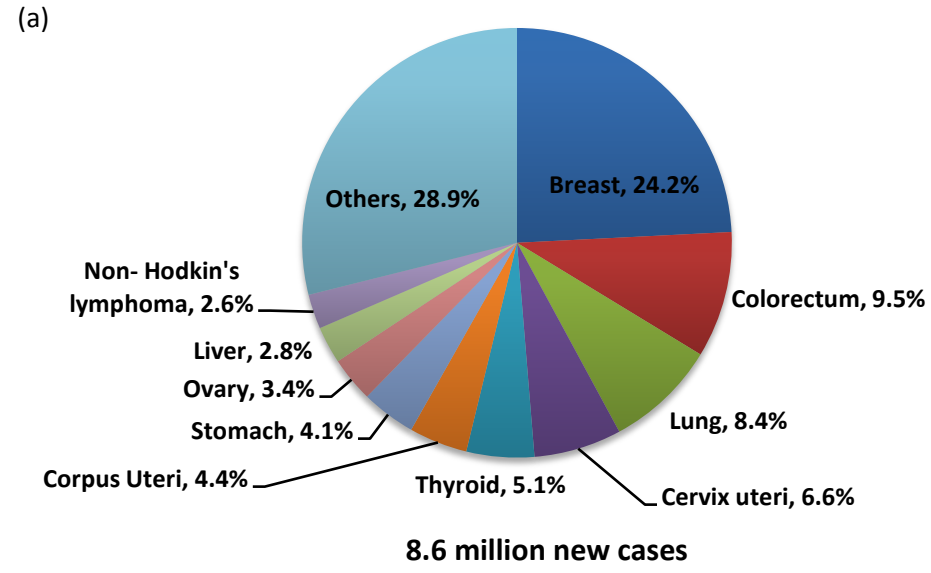
## 2.1 Breast cancer

Breast cancer can be described as the abnormal growth in the tissues of breast, usually ducts and lobules. Generally breast cancer developed in the cells of lobules or the ducts. Lobules are basically the milk-producing glands and the ducts comprise the passages which drain milk from lobules to the nipple. Occasionally, it can begin from the stromal tissues which consist of the fatty and fibrous connective tissues of the breast.

### 2.1.1 Breast cancer statistics

Breast cancer is the most commonly diagnosed cancer among females and the leading cause of cancer death worldwide representing nearly 25% of new cancer cases and 15 % of all cancer deaths. Worldwide, it was estimated to be 2.15 million newly diagnosed female breast cancer cases and 0.63 million deaths by the end of 2018 which accounts for almost 1 in 4 cancer cases among women. It is the most frequently diagnosed cancer and the leading cause of cancer death in the majority of the countries. The rates of cancer deaths in Asia and Africa are 57.3 % and 7.3 %, respectively. However the incidence rates are far higher than deaths i.e. 48.4% and 5.8%, respectively, for Asia and Africa. The reason can be attributed to the differences in distribution pattern in occurrence and fatality rates of different cancer types in these regions. It has been seen that women which resides in less developed regions predispose more incidences of cancer (883 000 cases) however the women residing in well developed regions displayed slightly less incidence (794 000). Although, the incidence rate of breast cancer is lower in India (25.8 per 100 000) when compared o data of United Kingdom (95 per 100 000) but mortality rates are similar in both regions (12.7 vs 17.1 per 100 000). Recently, significant rise in the incidence and cancer-related mortality was

reported in India (Bray et al., 2018, Malvia et al., 2017). The rates are so high that breast cancer has surpassed cervical cancer to becomes the leading cause of cancer death (Cronin et al., 2018, Smith and Desantis, 2018).



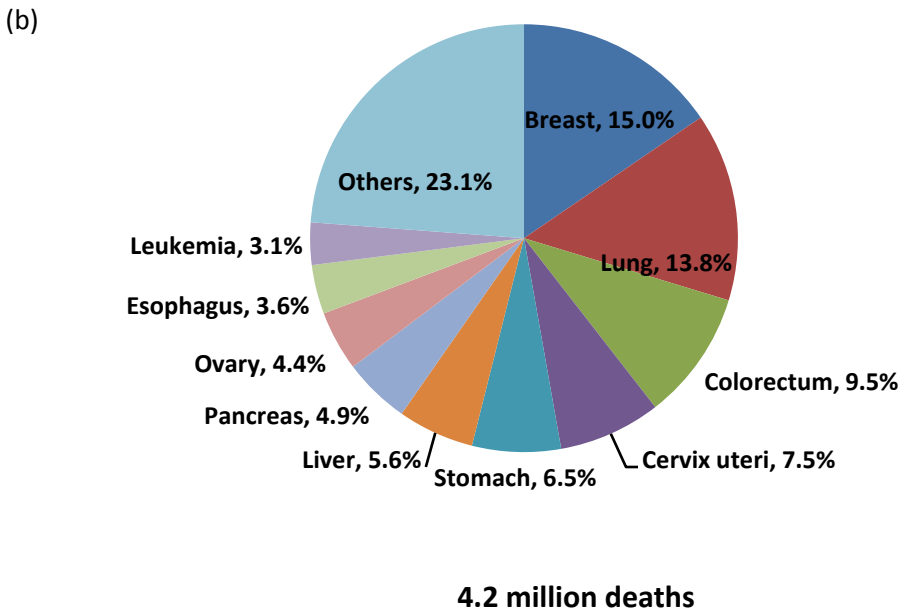




Figure 2.1: The distribution of breast cancer cases (a) and deaths (b) for the common cancers in world in the year 2018. Source: GLOBOCAN 2018.

### 2.1.2 Challenges

Breast cancer is a worldwide challenge. Although, major of causes and other characteristics of breast cancer are uniform, the severity and distribution differs region wise and every region has some unique characteristics for that cancer. The vulnerability of breast cancer has increase because of the following trends:

- The breast cancer cases are continuously increasing for a lesser age group.
- The cases are rising yearly in India
- The symptoms are displayed at a late stage which decreases the survival rates of patients.
- India, being a developing country lacked the awareness among females, which makes the screening more difficult.
- The cancer in young ladies was found to be of aggressive nature

## 2.2 Causes/Etiology

Multiple factors can contribute to human breast cancer such as hereditary, hormonal, and reproductive factors, environmental agents, chemical carcinogens etc. These can be modifiable risk factors or non-modifiable risk factors. Hopefully modifiable risk factors have contributed towards 70% of the total breast cancers cases. Mutations of drug-metabolizing enzymes from environmental chemicals, dietary agents, and endogenous steroids may enhance the risk of breast cancer. Other environmental factors include pollutants, occupational exposures, tobacco smoke, alcohol, and diet. Aromatic amines also act as potential mammary carcinogens. The compounds that are excreted into the urine after consumption of cooked meats have recently been detected in the breast milk of

lactating women. The precise causes of breast cancer are not well understood. However, several risk factors raise the chances of breast cancer. These factors can be modifiable or non-modifiable.

Table 2.1: Risk factors associated with breast cancer

| **Non-modifiable** | **Modifiable** |
|---|---|
| Age | Obesity |
| Gender | Alcohol intake |
| Breast Density | Exposure to radiation |
| Family History | Hormonal Replacement Therapy |
| Previous Breast cancer history | Exposure to Diethylstilbesterol |
| Exposure to estrogens | Breast cancer and breast implants |
| Height | |

## 2.3 Breast cancer treatments

Nowadays breast cancer treatments have been advanced to such a level in few decades that if the cancer can be detected early most women might survive a longer duration of life. Now a days there is a multidisciplinary team which can take care for breast cancer treatment. The team involves several specialists working together in order to provide better therapy to patients. The team usually include doctors from various disciplines like cancer specialist, oncologist, a radiologist and many more. The main treatment options followed recently are as follows:

- Surgery to remove cancer mass
- Radiotherapy to kill cancer cells
- Chemotherapy to kill or suspend the growth of cancer cells
- Hormonal therapy
- Biological therapy (targeted therapy)

These therapies may be used alone or in combination.

### 2.3.1 Chemotherapy

This therapy involves the use of anti-cancer drugs which can kill or destroy the cancer cells. Chemotherapy can be broadly classified as adjuvant therapy and neo-adjuvant therapy. When chemotherapy is started after surgery to eliminate any leftover cancer cells or that have escaped the surgery it is termed as adjuvant chemotherapy. However, if chemotherapy is given before surgery in order to shrink or reduce the tumor before surgery it is called neo-adjuvant chemotherapy. Chemotherapy can be easily provided on a day care basis. The drugs are usually administered via an intravenous infusion/bolus or tablets. Patients have to attend various chemotherapy sessions that may range from once in every two three weeks up to a period of four to eight months. The chemotherapy regimen often utilizes more than one drugs administered together. There are several side effects linked with chemotherapy such as anaemia, bone marrow depression, propensity for infections, bleeding predisposition, loss of appetite, nausea and vomiting, hair loss, mouth ulcers etc.

### 2.3.2 Drugs approved by FDA

Many FDA approved drugs (alone or in combinations) are being used for managing breast cancer. Although, the individual drugs used in the combinations for breast cancer treatment are FDA-approved the drug combinations themselves are not approved.

Table 2.2: List of drugs approved by FDA for treatment of breast cancer

| | |
|---|---|
| Abitrexate (Methotrexate) | Abraxane (Paclitaxel Albumin-stabilized |
| Nanoparticle Formulation) | Folex (Methotrexate) |
| Fluorouracil | Folex PFS (Methotrexate) |
| Ado-Trastuzumab Emtansine | Fulvestrant |
| Adriamycin (Doxorubicin Hydrochloride) | Gemcitabine Hydrochloride |
| Adrucil (Fluorouracil) | Gemzar (Gemcitabine Hydrochloride) |
| Herceptin (Trastuzumab) | Afinitor (Everolimus) |
| Anastrozole | Ixabepilone |
| Ixempra (Ixabepilone) | Ellence (Epirubicin Hydrochloride) |
| Epirubicin Hydrochloride | Kadcyla (Ado-Trastuzumab Emtansine) |
| Lapatinib Ditosylate | Everolimus |
| Exemestane | Letrozole |
| Megace (Megestrol Acetate) | Fareston (Toremifene)) |
| Faslodex (Fulvestrant) | Megestrol Acetate |
| Methotrexate | Methotrexate LPF (Methotrexate) |
| Fluoroplex (Fluorouracil) | Femara (Letrozole) |
| Mexate-AQ (Methotrexate) | Mexate (Methotrexate) |
| Paclitaxel Albumin-stabilized Nanoparticle | Neosar (Cyclophosphamide) |

| | |
|---|---|
| Formulation | Perjeta (Pertuzumab) |
| Pertuzumab | Nolvadex (Tamoxifen Citrate) |
| Novaldex (Tamoxifen Citrate) | Paclitaxel |
| Taxol (Paclitaxel) | Tamoxifen Citrate |
| Taxotere (Docetaxel) | Trastuzumab |
| Xeloda (Capecitabine) | Toremifene |
| | Tykerb (Lapatinib Ditosylate) |

## 2.4 Drug combinations used in breast cancer

| | |
|---|---|
| AC | Doxorubicin Hydrochloride +Cyclophosphamide |
| AC-T | Doxorubicin Hydrochloride + Cyclophosphamide + Paclitaxel |
| CAF | Cyclophosphamide + Doxorubicin Hydrochloride + Fluorouracil |
| CMF | Cyclophosphamide + Methotrexate + Fluorouracil |
| FEC | Fluorouracil + Epirubicin Hydrochloride + Cyclophosphamide |

### 2.4.1 Need of drug combinations

Most single drug based anticancer therapies are seldom effective owing to associated multiple genetic alterations and molecular abnormalities. The use of many drugs can target variety sub-populations, several targets, or several diseases simultaneously. The possible favorable advantages of synergism include:

1) Increased effectiveness of the chemotherapeutic effect,

2) Decreased dosage while maintaining the same activity and avoidance of toxicity,

3) Minimized or retardation of resistance development.

4) Provide selective synergism against target versus host (Chou, 2006).

### 2.4.2 Anticancer drug combination strategies

There is not any central dogma for combining anticancer drugs appropriate for every cancer patient deciphered, some strategies have been made. From ancient times, combination of anticancer drugs with biological therapies is good strategy. Several modules for combining anticancer drugs are categorized as follows:

- Combining anticancer agents must have different targeting or mechanisms of action
- The toxicities of on drug or both drugs should be reduced by addition of drug.
- Chemical cytotoxic anticancer agents can be combined with bio-therapeutic agents
- Cytotoxic anticancer agents can be combined with cytostastic anticancer drugs or less toxic adjuvant agents.
- Drugs can be combined if the combination provides benefits for resistance improvements.
- Anticancer agents which targets primary tumors can be combined with anti-metastatic agents or stem call modulators.
- Anticancer agents can be combined for personalized benefits after prediction of drug toxicity and responses, etc.

According to mathematical calculations there are many available combination options for clinical situations. We cannot practically compare these all combinations in lab or in animals. The following strategies can be considered in order to obtain solution to this problem.

- All combinational possibilities should be assessed with equal attention which might be time consuming and require manpower for completion.
- The better anticancer combinations can be discovered gradually followed by increasing the number of anticancer drugs used in combinations(Lu et al., 2015).

Since these researches require involvement of extensive labor work and money, it is not possible to assess all combinations during initial phases of drug developments. It was recommended to study these drug combinations by *in-vitro* proliferative studies on 1-3 cell lines. The results of in-vitro studies were taken as a basis to carry out higher levels of anticancer drug combinational studies. In these conditions it will be possible to achieve more information without involvement of extensive labor and money (Lu et al., 2015). Further, the estimation of synergy *in-vitro* follows same principle which works for animals. Many practical differences were encountered when these studies were carried out in animals:

(a) Animal drug combination studies are more expensive,

(b) Animal studies require more time and expertise,

(c) The variation in results also enhances, and

(d) The population size cannot be increased beyond a limit (i.e., smaller n).

It is often observed that the data generated under these conditions lack reproducibility and often resulted in vague conclusions. The translation of in-vitro results to animals is also another biomedical problem which cannot be solved by Chau Talalay Principle. Moreover, it is not possible to carry out synergism studies in human beings for clinical trials or in trials which required patients to be studied e.g. in case of cancer and HIV. Most of the clinical synergy claims lack the supported data when only single dose is used or single drug is utilized. Therefore, there is a need to carry out preclinical drug combination studies *in-vitro* cell line studies and in animals before starting clinical trials, to achieve the justification for carrying out human studies. The same principle FDA desires to put into effect (Breitinger, 2012, Chou, 2006, Chou, 2010).

### 2.4.3 Methodology

The natural phenomena occurring in human bodies are complicated due to complex mechanisms of enzyme action and receptor activity. Moreover, therapeutic involvement is not only limited to proteins, but at whole cascade of reactions and schemes like transcription factors, which initiate or control various processes of human body. Cancer chemotherapy on the other hand aims at cell damage, i.e. interference with whole individual. Mostly, either modes of action are unidentified, or are complicated which can be dealt. Experimental data with simple experiments also carries a significant error. Therefore, simplest possible mechanism has to be chosen to illustrate the experimental data. Therefore, study is constraint with the problems of either over-interpretation, or oversimplification of outcome – a suitable balance among these two limits is needed. Some of the main concepts for analyzing complex data are median effect analysis, combination index isobole method and response surface analysis (Breitinger, 2012).

The same mass-action law is applicable to the studies pertaining to combinational analysis. Mathematical modeling was applied to mass-action law to deduce the median-effect equation which can be inevitably used for the determination of combinational paradigms. The median effect equation has been emerged as a unified theory as it encompasses various equations utilized to explain the physical phenomena's such as Hill equation, Scatchard equation, Michaelis-Menten equation, and Henderson-Hasselbalch equation. This general equation is a comprehensive mean to analyze the multiple drug effects for n number of drugs. The median effect principle provides theoretical basis for calculation of combination index (CI)-isobologram equation that can be utilized for quantitative determination of synergism (CI < 1), additive effect (CI= 1), and antagonism (CI> 1) respectively. These algorithms are used to

develop computer software which allows simulation of drug interactions (synergism and antagonism) at all dose and effect levels. The software demonstrated the dose-effect curve, combination index plot, median-effect plot, isobolograms and dose-reduction index plot for *in-vitro*/ *in-vivo* combinational studies. The development of this theoretical software enables dose effect analysis for single drug and multiple drugs and opened opportunities for variety of disciplines of sciences. The integration of the mass-action law theory with arithmetical stimulation culminated as a exceptionally effective scientific tool (Breitinger, 2012).

However there are several drawbacks associated with combination studies. Synergism is occasionally confused by enhancement or potentiation. Thorough understanding of terms used in combination studies is necessary to avoid confusions at various levels. If only one of the combined drugs is having effect the enhanced effect is called potentiation. If each of the combined drugs has its individual effect, then the combination can result in synergistic, additive, or antagonistic effect. Therefore, synergism can be defined as an effect which is more that the additive effect whereas antagonism can be defined as an effect that is less than additive effect. Hence, clearly understanding the additive effect is the most fundamental measure for understanding synergism and antagonism. Many times authors reported the data which is associated with errors.

To minimize errors during combination studies, the basic scientific principles and experimental issues should be taken into account wisely.

### 2.4.4 Limitations associated with drug combination studies

A typical combination regime consists of more than one anticancer agents selected to interrupt tumor growth and several associated mechanisms simultaneously. The determination the optimal dose and dosing frequency of anticancer agents individually and in

combination displayed higher significances for combination regimens. Although, the combination treatments improved the outcomes for some cancers several drug combination still fail to elucidate the desirable effects. Several limitations are associated with combination therapy which can be as follows:

- Most often the combining agents predisposed drug interactions
- The combination can predispose toxicities to healthy cells
- The dissimilar drug pharmacokinetics limits the coordination among the combined agents for tumor exposure which can lead to drug resistance
- Variable drug ratios at tumor site
- Sub-optimal tumor exposure may result in reduced tumor cell death
- Synchronized target inhibition is required to be effective for activity of some drugs (Tolcher and Mayer, 2018)

The drug delivery technology can be utilized to address this problem by regulation of drug release from the combinations encapsulated in to the delivery systems so that synergistic ratios are maintained following administration. Applying this ratiometric approach to a range of drug combinations enabled the translation of *in-vitro* synergy information to *in-vivo*, which will finally result in fixed drug combination formulations displaying enhanced therapeutic efficacy.

### 2.4.5 Combination therapy

- Wang et al. (2014) studied the synergistic antitcancer effect with combination treatments of salinomycin and 5- fluorouracil for treatment of hepatocellular carcinoma. The results showed that the combination of resulted in a synergistic antitumor effect against liver tumors.

- Ashley et al. (2016) reported the development and evaluation of dual drug loaded nanoparticles co-encapsulating carfilzomib and doxorubicin for delivery to multiple myeloma cells. Various ratiometric combinations of two drugs were tested against myeloma cell lines and the most synergistic ratio was determined and then this ratio was included into liposomes. The results confirmed that the incorporation of synergistic ration as dual drug loaded liposomes showed higher efficacy and reduced systemic toxicity for tumor growth inhibition.
- Mayer et al. (2006) examined three combinations of drugs belonging to different anticancer drug categories (irinotecan plus floxuridine, cisplatin plus daunorubicin and cytarabine plus daunorubicin) for studying the dependency synergy on drug ratios. These studies reported that some rations showed synergistic interactions where other depicted antagonistic effects. Further liposomes were developed and loaded with combination of drugs. The liposomes were found to keep the synergistic drug ratios afterintravenous injections to animals and delivered the same ratio to tumor site.
- Pavillard et al. (2001) studied various combinations of topoisomerase-I and topoisomerase-II utilizing median effect principle. The studies reported the enhanced cytotoxicity of the combinations.
- Tardi et al. (2009) demonstrated that cytarabine and daunorubicin when combined together exhibits synergistic anticancer activities which are ratio dependent. Further, the encapsulation of both drugs in liposomes resulted in the same synergistic ratios in plasma after intravenous injection. The results indicated that *in-vitro* informatics

should be translated *in-vivo* which can enhance the anticancer efficacy of drug combinations as delivery systems.

## 2.5 Nanomedicines

Nanotechnology has been evolved as a promising carrier system for the delivery of many cytotoxic drugs and and can be applied efficiently to overcome several drug and formulation related toxicities (Agrawal et al., 2017, Blasi et al., 2007, Vijayakumar et al., 2016a, Vijayakumar et al., 2016b, Wong et al., 2007).

### 2.5.1 Solid lipid nanoparticles (SLNs)

Recently, solid lipid nanoparticles (SLNs) are seeking more importance over other carrier systems owing to their unique advantages (e.g. low toxicity, biocompatibility, physical stability, excellent tolerability, ability to accomodate hydrophilic and lipophilic drugs, protection of incorporated labile drugs from degradation and controlled release of the active drugs) and minimized associated problems (Bhalekar et al., 2017, Jain et al., 2014, Kushwaha et al., 2013, Singh et al., 2014, Wong et al., 2007). The prospect of improved cancer chemotherapy using SLNs as a drug delivery system is promising. In tumors vasculature becomes leaky and causes enhanced permeation and retention (EPR effect) in the cell leading to accumulation of SLNs in the tumor cell (Grobmyer et al., 2010, Iyer et al., 2006, Yin et al., 2014). Though SLNs are largely implicated for lipophilic drugs water soluble drugs can also be encapsulated efficiently owing to emulsifying nature of various lipids such as glyceryl monooleate and glyceryl monostearate (Grobmyer et al., 2010, Purvin et al., 2014, Wong et al., 2007).

### 2.5.2 Aqueous core nanocapsules (ACN)

Combination therapy further requires carriers which can encapsulate drugs with different physicochemical properties. Moreover, encapsulation of both hydrophilic and lipophilic moieties into single carrier predisposes wide applications in anticancer therapy which necessitates combination therapies. In this regard aqueous core nanocapsules (ACNs) displaying a core-shell structure, where the core acts as a reservoir while shell a protective membrane, can be considered suitable. ACNs exhibit a core-shell carrier where the core is composed of hydrophilic drugs and the shell is a polymeric or lipid capsule (Anton et al., 2009, Cosco et al., 2015, Kothamasu et al., 2012, Vrignaud et al., 2013). Due to various advantages like high drug encapsulation, reduced polymer content, protection of core against degradation and reduction of tissue irritation, these can be applied as potential drug delivery carriers. ACNs can be prepared by polymerization or synthesized using preformed polymers. Various methods utilizing polymerization includes interfacial polymerization, water in oil microemulsions and in- situ polymerization. Synthesis with preformed polymers comprises of nanoprecipitation, emulsion-coacervation, emulsion–diffusion, double emulsification, polymer-coating and layer-by-layer surface modification (Anton et al., 2009, Carbone et al., 2015, Dos Santos et al., 2015, Fuchs and Thurecht, 2015, Kansal et al., 2013, Mora-Huertas et al., 2010).

## 2.6 Drug and excipient profile

### 2.6.1 Vinorelbine bitartrate

Vinorelbine bitartrate (VRL) is a semi-synthetic vinca alkaloid showing significant anticancer activity against various tumors such as breast cancer, non-small cell lung cancer (NSCLC), ovarian cancer and exhibits antitumor activity through disrupting microtubules.

However, VRL is better tolerated among all vinca alkaloids owing to reduced neurotoxicity which can be attributed to its lesser affinity for axonal microtubules (Drummond et al., 2009, Vassilomanolakis et al., 2001, Wan et al., 2008, You et al., 2007, Zhang et al., 2008). VRL is a white to yellow amorphous powder with molecular weight of 1079.11 g and aqueous solubility more than 1 g $mL^{-1}$ (Zhang and Ahmad, 2004). However, the associated toxicities like neutropenia (main dose-limiting toxicity), nausea, vomiting, diarrhoea, constipation, alopecia and peripheral neuropathy poses difficulties in its clinical translation. The marketed formulation, with the brand name Navelbine (i.v infusion) demonstrated serious venous irritation due to its vesicant nature and displayed injection site reaction, superficial phlebitis accompanied by erythema, pain, vein discoloration and tenderness along the vein (Emanuela et al., 2018, Kreidieh et al., 2016, Vassilomanolakis et al., 2001, You et al., 2007). An oral soft gelatin capsule encapsulating VRL was also developed which predisposed low bioavailability (33 – 40 %) (Bougaret et al., 2005, Goa and Faulds, 1994). Moreover, the severity of the adverse effects with oral administration was found to be greater than that of *i.v.* counterpart (Li et al., 2012).

*2.6.1.1 Physicochemical properties (National Center for Biotechnology Information. Pubchem Compound Database; Cid=45055483)*

Table 2.3: Physicochemiccal properties of Vinorelbine bitartrate

| **Property** | **Value** |
|---|---|
| Appearance | White to pink crystalline. |
| Assay | ≥99% |
| Melting point: | 219°C |
| Water solubility | 1.22 g $L^{-1}$ |
| logP | 4.39 |
| pka (strongest acidic) | 10.87 |
| pka (strongest basic) | 8.72 |

*2.6.1.2 Structure of Vinorelbine bitartrate*

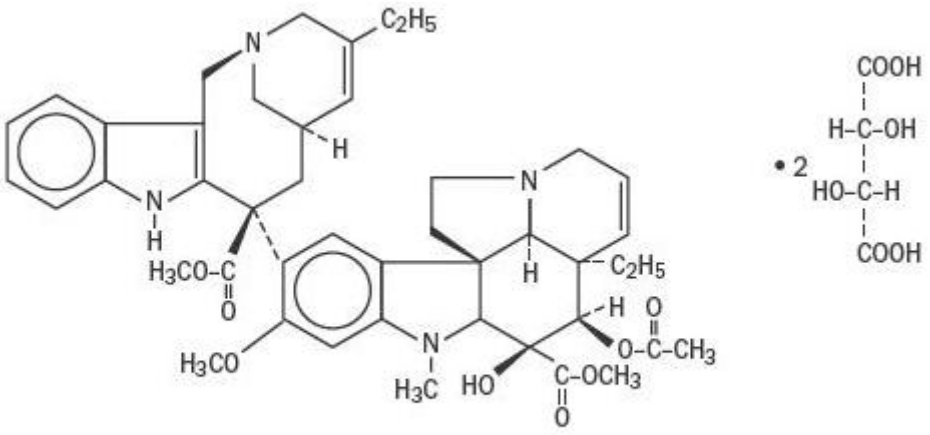



Figure 2.2: Chemical structure of Vinorelbine bitartrate

*2.6.1.3 Molecular formula:*

$C_{53}H_{66}N_4O_{20}$

*2.6.1.4 Chemical name/ IUPAC name:*

5'-Noranhydrovinoblastine tartrate; R-(R*,R*)-2-3-dihydroxybutanedioate (1:2) salt; Didehydro-4'-deoxy-C'-norvincaleukoblastine

*2.6.1.5 Molecular weight:*

1079.11g $mol^{-1}$

*2.6.1.6 Mode of action:*

The anticancer activity of vinorelbine is primarily due to microtubule arrest during the mitosis phase of cell cycle. VRL binds to the microtubular proteins which induces crystallization in the proteins of microtubules and kill the cells. vinorelbine may also interfere with the following proteins present in the cell

1) Amino acids, cyclic AMP (cAMP), and glutathione metabolism,

2) Calmodulin-dependent $Ca^{+2}$ transport, ATPase activity

3) Cellular respiration, and

4) Nucleic acid and lipid biosynthesis

*2.6.1.7 Pharmacokinetics:*

Volume of distribution (Vd) is found to be 25.4 - 40 L $Kg^{-1}$ and metabolism is reported by Hepatic metabolism. Renal Excretion accounts for 18 %.

*2.6.1.8 Dosage and administration*

General Advice

- Vinorelbine should be diluted upto a certain tolerable concentration before administration.
- VRL should be administered by IV infusion only, intrathecal or i.p may lead to death.
- Infusion should be administered IV over a period of 6 to 10 min followed by 75 mL to 125 mL dextrose of sodium chloride infusion.

*2.6.1.9 Adverse effects, treatments and precautions*

Table 2.4: Adverse effects associated with Vinorelbine bitartrate

| | |
|---|---|
| Cardiovascular | Chest pain. |
| Fatigue | mild to moderate peripheral neuropathy |
| Dermatologic | Mild alopecia; rash and injection site reactions; pain at injection site. |
| Gastrointestinal | Transient elevations in LFTs; constipation; paralytic ileus; mild stomatitis; anorexia; diarrhea. |
| Hematologic | Dose-limiting granulocytopenia occurs with a nadir of 7 to 10 days. |
| Hypersensitivity | Anaphylaxis; angioedema; pruritus. |
| Musculoskeletal | Jaw pain; myalgia; arthralgia |
| Respiratory | Dyspnea. |

*2.6.1.10 Precautions*

IV use only: Intrathecal use of vinca alkaloids has been fatal.

Granulocytopenia: May be severe and can lead to infection.

Avoid extravasation: Proper placement of needle/catheter prior to administration. Extravasation can cause severe local necrosis.

*2.6.1.11 Storage/Stability*

The vials must be stored in refrigerator at a temperature ranging 2 - 4°C, secluded from light. The vial if unopened vials are reported to be stable up to 72 h when kept at room temperature (25°C (77°F)). Diluted VRL solutions do not contain preservatives and can be stored at room temperature or in refrigerated conditions for 24 h in polypropylene syringes or PVC bags.

*2.6.1.12 Analytical methods*

Vinorelbine is widely estimated Spectrophotometrically and Liquid chromatography.

*2.6.1.13 Research envisaged*

- Yamada et al. (2010) studied the effects of oxidative stress in developing VRL induced endothelial cell injury. The results showed that VRL have tendency to enhance oxidative stress by decreasing glutathione, GSH and increasing reactive oxygen species generation. Hence oxidative stress plays crucial role in VRL induced vascular cell injury.
- Zhang et al. (2008) developed a lipid microsphere carrier loading vinorelbine and studied pharmacokinetics, stability and safety issues. The VRL encapsulated lipid microspheres reduced the irritation and toxicity that was associated with the conventional injection. The developed microspheres were found to be stable and scalable with very less venous irritation. The pharmacokinetics was however found to be similar to the VRL aqueous injection.

- Cao et al. (2005) developed a simple HPLC method coupled with electrospray ionization mass spectrometry (HPLC/ESI-MS) which was found to be sensitive enough for detection and separation of VRL and impurities associated with VRL. The analytical instrument composed of a reverse phase C8 coloumn. 0.02M ammonium formicate buffer (pH 4.2) and methanol (46:54, v/v) were taken as mobile phase at 0.8 mL min-1 flow rate at room temperature. The estimation of VRL was done at 267 nm. Further the separated impurities were characterized by 1D and 2D NMR and structure elucidation was discussed afterwards.
- Ochiai et al. (2002) studied the effect of vinorelbine on the proliferation rates of human myeloma cell lines. Trypan blue exclusion test and Alamar blue assays were carriedout for the estiation of the same. Results indicated that VRL aqueous solution (Navelbine) reduced the cellular proliferation of myeloma in a dose dependent manner and the effect was markedly increased when dexamethasone was combined with VRL solution. Flow cytometry further confirmed the apoptosis induced by VRL in all cell lines. Therefore, VRL was supposed to e better treatment for the benefit of myeloma patients.
- Webb et al. (2007) designed and characterised an intravenous formulation that consists two drugs among which one is cytotoxic agent i.e. VRL and another is a lipid which can induce apoptosis i.e phosphatidylserine which showed synergistic in-vitro activity. The results displayed the potential of drug combination studies in therapeutics. The resuts further demonstrated the importance of utilizing drug delivery systems for loading of drug combinations for improved treatment.

- Van Heugen et al. (2001) developed and validated a new HPLC method coupled with mass spectrometry for the quantitation of vinorelbine and its metabolites. The method was found to be superior in quantifying VRL with all metabolites in human plasma for up to 4-5 elimination half lives. Therefore, this method improved the analysis of pharmacokinetic data of VRL.
- Sorio et al. (2009) analyzed the pharmacokinetics of VRL in eldery patients. Moreover, VRL tolerance studies were also conducted for estimation of safety of VRL in elderly patients. The results suggested that there is no correlation between toxicity, age and drug exposure. However, the study does not elucidate any justification for dosage reduction of VRL in tested elderly patients.
- Yoh et al. (2007) carried out potential randomized trial in order to establish occurrence of venous toxicity by a bolus injection (1 min bolus) of VRL and with an infusion (6 min infusion). The results suggested that reducing the time of administration does not have any significant effect on reducing the VRL associated venous toxicity.
- Udom, et al. (2000) conducted a clinical trial on patients suffered from advance breast cancer. VRL was administered for two weeks and the response and toxicity of VRL administrations was studies in patients who have already received two prior chemotherapeutic treatments. The study involves less number of patients and the results cannot be generalized but the preliminary data suggested that two week treatment is effective in patients who have already taken some sort of treatments. The response generated in such studies was found to elucidate similar effects as generated by conventionally treated patients.

### 2.6.2 Resveratrol

Resveratrol (trans-3,4,5-trihydroxystilbene, RES), a naturally occurring polyphenol compound, is found in a variety of food sources such as grapes, mulberries and peanuts. RES is already proved to reduce VRL induced vascular endothelial cell injury by reduction in cell apoptosis, intracellular ROS generation and the intracellular SOD. RES has been demonstrated to possess a wide array of pharmacological properties, such as estrogenic, antiplatelet, anticancer and anti-inflammatory effects (Zhang et al., 2013). Resveratrol exerts anti-aging effect in animals. Various *in-vitro* and animal studies reported that resveratrol has potent antioxidant and ant-inflammatory effects, promotes vascular endothelial function, enhances lipid metabolism and has anticancer activity (Monograph, 2010).

#### *2.6.2.1 Physicochemical properties (National Center for Biotechnology Information. Pubchem Compound Database; Cid=445154, Zur Mühlen et al., 1998)*

Table 2.5: Physicochemical properties of RES

| **Property** | **Value** |
|---|---|
| Appearance: | Off white powder |
| Assay: | ≥99% |
| Melting point: | 254 ℃ |
| Water solubility | 30 mg $L^{-1}$ |
| logp | 3.06 |
| pka (strongest acidic) | 10.64 |
| pka (strongest basic) | 8.99 |

*2.6.2.2 Structure of Resveratrol*

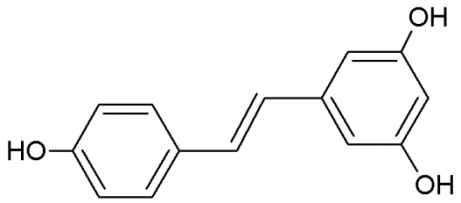



Figure 2.3: Chemical structure of Resveratrol

*2.6.2.3 Molecular formula*

$C_{14}H_{12}O_3$

*2.6.2.4 Chemical name*

Resveratrol; Trans-resveratrol; 3,4',5-Trihydroxystilbene; (E)-5-(4-Hydroxystyryl)benzene-1,3-diol; 3,5,4'-Trihydroxystilbene, 5-((~{E})-2-(4-hydroxyphenyl)ethenyl)benzene-1,3-diol

*2.6.2.5 Molecular weight*

228.247 g $mol^{-1}$

*2.6.2.6 Mechanism of action*

RES was reported to display anti-oxidative and chemopreventive effects via activation of Nrf2 and consequently GSH expression. Moreover, the poly-mechanistic resveratrol (blocks survival and anti-apoptotic mechanisms or cause DNA degradation, as a result of pro-oxidant action) can sensitize cancer cells, which may leads to synergistic anticancer activities when combined with other chemotherapeutic agents or cytotoxic compounds (Kou et al., 2013).

*2.6.2.7 Pharmacokinetics*

Despite of several advantageous pharmacological effects the therapeutic applications of RES are limited due to its rapid metabolism, short biological half life ($t_{1/2}$), and rapid elimination. Plasma half life following oral administration and intravenous administration was found to be 15 min and 33 min, respectively. It undergoes rapid metabolism by glucuronidation and sulphonation and about 22-44% of the administered dose was excreted in urine with in 12 h. The short half life and rapid metabolism of RES requires higher dose and frequent administration for achieving therapeutic effect.

*2.6.2.8 Indications*

Cardioprotection, Anti platelet aggregation, Anticancer activity, Anti angiogenesis, Vasodilation, Anti oxidant activity and Prolongation of lifespan,

*2.6.2.9 Adverse effects*

In short term, repeated dose study no serious adverse effects were reported. No biochemical, neurological, electrocardiographical, or other objective adverse effects were reported.

*2.6.2.10 Storage/Stability*

The vials must be stored in refrigerator at a temperature range of -20 °C, protected from light. Ethanolic solutions are stable up to 12 h if stored in refrigerator at 2-8 °C.

*2.6.2.11 Analytical methods*

Resveratrol is widely estimated Spectrophotometrically and by liquid chromatography.

*2.6.2.12 Research envisaged*

- Vijayakumar et al. (2016) developed RES loaded TPGS and DSPE PEG coated nanoformulations to improve pharmacokinetics and chemotherapeutic application of

RES against glioma. All nanocarriers showed better phycochemical properties, improved pharmacokinetics and enhanced cytotoxicity towards brain cancer.

- Sinha et al. (2016) published a review focussing on the role of resveratrol and its associated analogue due to their efficacy against induction of apoptosis, tumor cell proliferation, metastasis, and sensitization by cytotoxic drugs in several *in-vitro* and *in-vivo* breast cancer models. The roles of resveratrol as a aromatase inhibitor, phytoestrogen, and stem cell modulator and adjuvant treatment were also taken into account. Hence, this paper explores the prospectives of resveratrol in prevention and treatment of breast cancer with existing limitations, challenges and future directions of research.
- Zhang et al. (2013) studies the effect of RES in reducing the vascular injury induced by VRL. Human vascular endothelial cell line were exposed to VNR and then the cells were cultured with or without RES for 24 h. MTT assay was used to identify the cell viability. ROS and SOD were assessed with 20, 70-dichlorofluorescein diacetate (DCFH-DA) and SOD detection kit, respectively. VRL was found to decrease the proliferation of endothelial following a dose-dependent manner. RES pretreatment further increased the cell viability in a dose-dependent manner. Cellular apoptosis, reduction of intracellular SOD, increase in the intracellular ROS induced by VRL were also attenuated. These results demonstrated that RES would protect against vascular endothelial cell injury induced by VRL.
- Summerlin et al. (2015) published a review focussed on the various challenges and opportunities related to development of resveratrol loaded nanoformulations.

### 2.6.3 Glyceryl monooleate (GMO)

#### *2.6.3.1 Description*

Glyceryl monooleate is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate

- Nonproprietary Names — Glycerol Mono-oleate, Glyceryl Monooleate, Glyceryl Monooleate
- Synonyms — GMO; glycerol-1-oleate; glyceryl mono-oleate; mono-olein;
- Chemical Name — 9-Octadecenoic acid (Z), monoester with 1,2,3-propanetriol
- Empirical Formula — $C_{21}H_{40}O_4$
- Molecular Weight — 356.55 (for pure material)

#### *2.6.3.2 Chemical structure*

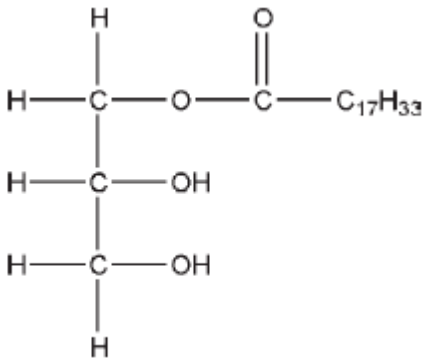



Figure 2.4: Chemical structure of GMO

#### *2.6.3.3 Functional category*

Bioadhesive material; emulsion stabilizer; gelling agent; sustained-release agent, emollient; emulsifying agent; mucoadhesive; nonionic surfactant

### *2.6.3.4 Applications in pharmaceutical formulation and technology*

GMO, a polar lipid, has the tendency to swell in water and reforms several different types of phases having different rheological properties. It occurs in two grades i.e. non-emulsifying grade (ne) and self-emulsifying grade (se). The ne grade is widely utilized as an emollient for topical formulations and it also used as an emulsifying agent and stabilizer for water-in-oil type and oil in water type emusions, respectively. The se grade GMO is also widely used as emulsifier for oil-in-water emulsion systems. Glyceryl monooleate forms a gel when it is mixed with excess water, resulting in a highly well-organized cubic phase. The highly organized cubic structured gel structure can be utilized to sustain the release of various water soluble drugs. The same gel structure can be utilized in developing mucoadhesive delivery systems, transdermal and buccal penetration.

### *2.6.3.5 Typical properties*

Table 2.6: Typical properties of GMO

| | |
|---|---|
| Appearance | Amber coloured oily liquid, partially solidified at room temperature |
| Solubility | Soluble in chloroform, ether, ethanol (95%), mineral oil and vegetable oils; practically insoluble in water. |
| Boiling point | 238–240 °C |
| Density | 0.942 g $cm^{-3}$ |
| Flash point | 216 °C |
| HLB value | 3.3 (non-emulsifying grade); 4.1 (self-emulsifying grade). |
| Melting point | 35 °C |
| Refractive index | 1.4626 |

*2.6.3.6 Stability and storage conditions*

GMO has to be stored airtight containers away from sunlight in a cool and dry place.

*2.6.3.7 Safety*

GMO is used in oral and topical formulations and is regarded as nonirritant and nontoxic.

*2.6.3.8 Regulatory status*

GRAS listed. It is included in FDA Inactive Ingredients Guide, nonparenteral medicines in the UK and the Canadian List of Acceptable Non-medicinal Ingredients.

2.6.4 Glyceryl monostearate (GMS)

*2.6.4.1 Description*

Glyceryl monostearate consisted of mono-glecerides, di-glycerides and several esters of long-chain fatty acids, these esters can further categorized in two grades i.e. 40–55 percent mono-glycerides and 90 percent mono-glycerides According to the USP32–NF27 GMS consist of not less than 90% of mono-glycerides of saturated fatty acids, chiefly glyceryl monostearate and glyceryl monopalmitate.

- Nonproprietary Names Glyceryl Monostearate, Glycerol Monostearate
- Synonyms GMS; 2,3-dihydroxypropyl octadecanoate; glycerine monostearate; glycerol stearate; glyceryl stearate; Myvatex; 1,2,3-propanetriol octadecanoate;
- Chemical Name Octadecanoic acid, monoester with 1,2,3-propanetriol
- Empirical Formula $C_{21}H_{42}O_4$
- Molecular Weight 358.6

*2.6.4.2 Structural formula*

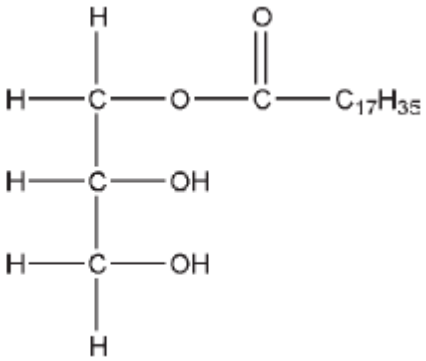



Figure 2.5: Chemical structure of GMS

*2.6.4.3 Functional category*

Emollient; solubilizing agent; stabilizing agent; tablet and capsule lubricant, emulsifying agent; sustained-release agent;

*2.6.4.4 Applications in pharmaceutical formulation and technology*

GMS is widely used as stabilizer, nonionic emulsifier, emollient and plasticizers in a several cosmetics, food and pharmaceutical applications. It is also utilized in hot melt granulation technique. It is a lubricant and also used to develop sustained release dosage forms. Hence it is a vital excipient in tablets and capsules industry. However, the GMS exhibit polymorphism and show two polymorphs i.e. α-form and β-form. The α-form is foamy in nature hence easily dispersible however the β-form is denser in nature, more stable and suitable for waxy matrices. Hence the polymorphism should be considered while developing dosage forms with GMS.

### *2.6.4.5 Typical properties*

Table 2.7: Typical properties of GMS

| | |
|---|---|
| Appearance | white or cream-colored, wax-like solid in the form of beads, flakes, or powder. |
| Solubility | Soluble in chloroform, ethanol, ether, hot acetone, mineral oil, and fixed oils |
| Flash point | 240 °C |
| HLB value | 3.8 |
| Melting point | 55–60 °C |
| Specific gravity | 0.92 |
| Polymorphs | The α-form is converted to the β-form when heated at 50 °C. |

### *2.6.4.6 Stability and storage conditions*

GMS has to be stored in tightly closed containers and kept way from light in a cool and dry place.

### *2.6.4.7 Safety*

GMS is widely used in cosmetics, foods, and oral and topical pharmaceutical formulations and is generally regarded as a nontoxic and nonirritant material.

### *2.6.4.8 Regulatory status*

GRAS listed. It is included in FDA Inactive Ingredients Guide, nonparenteral medicines in the UK and the Canadian List of Acceptable Non-medicinal Ingredients. If the source of GMS is animal fat additional regulatory requirements of the source to be free of contamination.

### 2.6.5 Poly (lactic-co glycolic acid), PLGA

#### *2.6.5.1 Description*

PLGA can be described as a glassy material

- Nonproprietary Names None adopted.
- Synonyms DL-Dilactide homopolymer; DL-dilactide polymer; lactic acid homopolymer; D,L-lactic acid polymer; D-lactic acid-L copolymer; DL-lactide polymer; D-lactide-Llactide copolymer; D,L-polylactic acid; poly(dl-lactic acid); polylactide; poly(DL-lactide); poly-DL-lactide;
- Chemical Name Poly (oxy(1-methyl-2-oxo-1,2-ethanediyl))
- Empirical Formula $(C_3H_4O_2)_n$
- Molecular Weight varies according to the intended application.

#### *2.6.5.2 Structural formula*

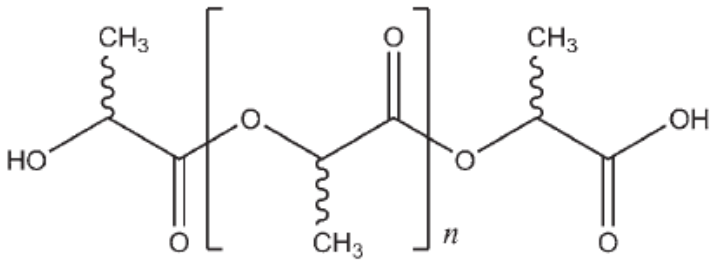



Figure 2.6: Chemical structure of PLGA

#### *2.6.5.3 Functional category*

Biodegradable material; coating agent; controlled-release agent

### *2.6.5.4 Applications in pharmaceutical formulation and technology*

PLGA is widely used polymer in manufacturing various drug delivery systems such as oral solid dispersions, implants and injections. It has utility as coating agent.

### *2.6.5.5 Typical Properties*

Thermal and mechanical properties of PLGA are directly affected by the molecular weight and composition of the polymer.

Table 2.8: Typical properties of PLGA

| | |
|---|---|
| Appearance | glassy material which occurred as white to golden-yellow pellets or granules. |
| Solubility | Soluble in dichloromethane, tetrahydrofuran, ethyl acetate, chloroform, hexafluoroisopropanol, and acetone. |
| Boiling point | 238–240 °C |
| Density | 1.21–1.28 g $cm^{-3}$ |
| Glass transition temperature | 40–69 °C |
| Elongation (%) | 2.5–7.0 (according to molecular weight) |
| Melting point | Amorphous (some sources quote a melting point in the range 165–180 °C) |
| Tensile strength | 35–85MPa |

### *2.6.5.6 Stability and Storage Conditions*

PLGA is almost stable under dry conditions and biodegrades over a period of 10–15 months depending upon the molecular weight. Increased moisture and temperature enhances the rate biodegradation; it starts to degrade in 6 months in contact with water at 25 °C. In contrast to other biodegradable polymers, PLGA degrades following a two-step mechanism. The first

degradation step involved hydrolysis of the ester bonds to produce a low molecular-weight polymer. When the molecular weight drops below 10,000 KD microorganisms digest the polymer into carbon dioxide and water. PLGA should be stored in a dry inert environment at a temperature of 15 – 20 °C.

#### *2.6.5.7 Safety*

PLGA degrades to produce lactic acid, which is considered a well-tolerated nontoxic material. Several *in-vitro* and *in-vivo* studies reported that poly (lactic acid) in general (including poly (DL-lactic acid)) is well tolerated.

#### *2.6.5.8 Regulatory status*

Included in the FDA Inactive Ingredients Database (IM, powder, for injection, suspension, and lyophilization) and considered non-hazardous.

### 2.6.6 d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS)

#### *2.6.6.1 Description*

TPGS is a synthetic product. It is chemically a mixture of mono-esterified polyethylene glycol 1000, the di-esterified polyethylene glycol 1000, free polyethylene glycol 1000, and free tocopherol.

- Nonproprietary Names — Vitamin E Polyethylene Glycol Succinate
- Synonyms — TPGS, tocofersolan; tocopherol polyethylene glycol succinate; D-α-tocopheryl polyethylene glycol 1000 succinate; vitamin E TPGS
- Chemical Name — 4-O-(2-Hydroxyethyl)-1-O-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydrochromen-6-yl]butanedioate

- Empirical Formula $C_{33}O_5H_{54}(CH_2CH_2O)_{20–22}$

*2.6.6.2 Structural formula*

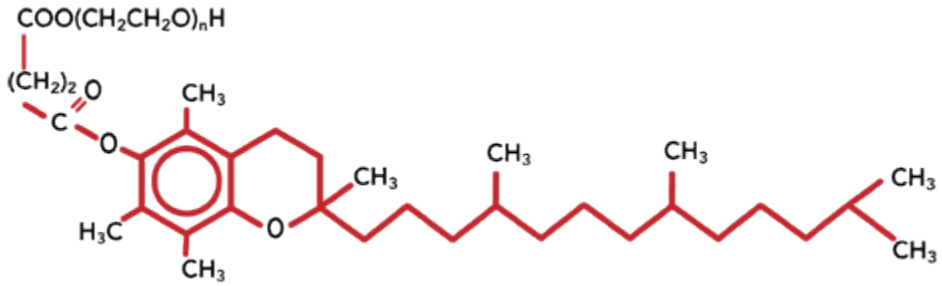



Figure 2.7: Chemical structure of TPGS

*2.6.6.3 Functional category*

Absorption enhancer; antioxidant; emulsifying agent; granulation aid; ointment base; solubilizing agent; surfactant; suspending agent; tablet binder.

*2.6.6.4 Applications in pharmaceutical formulation and technology*

TPGS is an esterified vitamin E (tocopherol) derivative primarily used as a solubilizer or emulsifying agent because of its surfactant properties. Structurally, it is amphipathic and hydrophilic, unlike the tocopherols, and therefore it is a water-soluble derivative that can be used in pharmaceutical formulations. One of the most important applications is its use as vehicle in lipid-based drug delivery formulations.

### *2.6.6.5 Typical properties*

Table 2.9: Typical properties of TPGS

| | |
|---|---|
| Appearance | white to light-brown, waxy solid and is practically tasteless. |
| Solubility | Miscible in water in all parts |
| Acid value | 41.5 |
| HLB value | 13.2 |
| Melting point | 37–41 °C |
| Specific gravity | 1.06 (at 45 °C) |
| CMC | 0.02% by weight (37 °C) |

### *2.6.6.6 Stability and storage conditions*

TPGS is stable at room temperature for up to 4 years. Aqueous solutions of TPGS are stable over a pH range of 4.5–7.5 and can be further stabilized with propylene glycol.

### *2.6.6.7 Safety*

TPGS is considered safe after clinical trials conducted in animals and humans. TPGS is also a safe compound for transportation, storage and handling. It showed no-observed-adverse-effect-level (NOAEL) in rats of 1000 mg/kg/day

### *2.6.6.8 Regulatory status*

GRAS listed. FDA includes TPGS in the list of inactive and has approved products containing TPGS.

## 2.6.7 Poloxamer-188 (PL-188)

### *2.6.7.1 Description*

Poloxamers generally constituted a wide category comprising of various poloxamers some of them are solid and some even occur as liquid. PL-188 is a white, waxy, free-flowing prilled granules or cast solids. They are practically odorless and tasteless.

- Nonproprietary Names: Poloxamers, Poloxamer
- Synonyms Lutrol; Monolan; Pluronic; poloxalkol; poloxamera; polyethylene– propylene glycol copolymer; polyoxyethylene–polyoxypropylene copolymer; Supronic; Synperonic.
- Chemical Name α-Hydro-o-hydroxypoly(oxyethylene)poly(oxypropylene) poly- (oxyethylene) block copolymer
- Empirical Formula $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$

### *2.6.7.2 Structural formula*

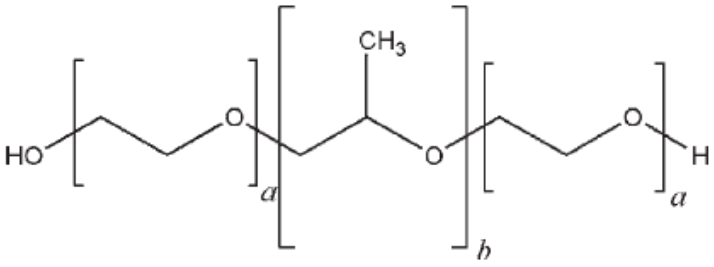



Figure 2.8: Chemical structure of Poloxamer-188

### *2.6.7.3 Functional category*

Dispersing agent; emulsifying agent; solubilizing agent; tablet lubricant; wetting agent.

| Use | Concentration (%) |
|---|---|
| Fat emulsifier | 0.3 |
| Flavor solubilizer | 0.3 |
| Fluorocarbon emulsifier | 2.5 |
| Gelling agent | 15–50 |
| Spreading agent | 1 |
| Stabilizing agent | 1–5 |
| Suppository base | 4–6 or 90 |
| Tablet coating | 10 |
| Tablet excipient | 5–10 |
| Wetting agent | 0.01–5.8 |

### *2.6.7.4 Typical properties*

Table 2.10: Typical properties of Poloxamer-188

| | |
|---|---|
| Appearance | white, waxy, free-flowing prilled granules, or as cast solids |
| Solubility | Freely soluble in ethanol and water. |
| Acidity/alkalinity | pH = 5.0–7.4 for a 2.5% w/v aqueous solution. |
| Cloud point | >100ºC for a 1% w/v aqueous solution, and a 10% w/v aqueous solution of poloxamer 188. |
| Density | 1.06g $cm^{-3}$ at 25°C |
| Flash point | 260 °C |
| HLB value | 29 |
| Melting point | 52–57 °C |
| Moisture content | poloxamers generally contain less than 0.5% w/w water |

*2.6.7.5 Stability and storage conditions*

PL-188 is a stable material. The aqueous solutions containing Pl-188 are stable when kept with alkalis, acids and metal ions. However bulk solutions should be kept in well close containers in a cool and dry place in order to retard mold growth.

*2.6.7.6 Safety*

Poloxamers are widely employed in oral, parenteral, and topical formulations and regarded as nontoxic and nonirritant materials. These are not metabolized in the body. Toxicity studies performed in various animals such as dogs and rabbits have reported poloxamers to be nonirritating and nonsensitizing when applied in 5% w/v and 10% w/v concentration to the eyes, gums, and skin. Moreover, poloxamers (0.001–10% w/v) are found to be haemocompatible when blood cells were incubated for 18 hours at 25℃.

*2.6.7.7 Regulatory status*

GRAS listed. It is included in FDA Inactive Ingredients Guide, nonparenteral medicines in the UK and the Canadian List of Acceptable Non-medicinal Ingredients.