LITERATURE REVIEW

Ovarian Cancer:

Ovarian Cancer (OC) is a cancer that begins in the ovaries with the abnormal growth of the tissues of the ovary. Ovaries are the female reproductive gland each about a size of grape located on the either size of the uterus. There are more than thirty different types of ovarian cancers which are classified based on the type of cells from which they are originated. Most commonly occurring OCs are that occurs from the abnormal growth of epithelial cells, stromal cells and germ cells of the ovary. According to the American Cancer Society, ovarian cancer has four stages:

Stage I: Only ovary/ovaries are affected by cancer cells and the cells does not spread anywhere else in the body.

Stage II: Cancer cells spread to ovaries along with other genital organs such as fallopian tubes, uterus, pelvic etc.

Stage III: Cancer cells affected the ovaries and also spread to the inner lining of the abdomen or the lymph nodes present at the back of the abdomen.

Stage IV: The cancer may spread to other parts of the body.

From the data of American Cancer Society, in 2018, there will be approximately 22,240 new cases of ovarian cancer diagnosed and 14,070 ovarian cancer deaths in the US. Ovarian cancer accounts for just 2.5% of all female cancer cases, but 5% of cancer deaths because of the disease‟s low survival. This is largely because 4 out of 5 ovarian

cancer patients are diagnosed with advanced disease that has spread throughout the abdominal cavity. The treatments of the ovarian cancer include surgery, chemotherapy, immunotherapy and radiotherapy & targeted therapy. Chemotherapy for the OC usually includes treatment with two or three drugs together. Use of combination therapy instead of just single drug alone is conferring with better results. The approved drugs for the treatment of OC are: Paclitaxel, Docetaxel, Altretamine, Capacitabine, Cyclophosphamide, Etoposide, gemcitabine, Ifosfamide & Irinotecan.(12)

Nanocarriers in Drug Delivery:

Nanotechnology is being applied extensively to provide targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology. Since the beginning of 20th century, nanotechnology growing interested from the pharmaceutical technology research groups worldwide. It has practically made its influence in all technical fields. Industry estimates suggest that approximately 40% of lipophilic drug candidates fail due to solubility and formulation stability issues, which has been solved by various novel and advanced lipophilic drug delivery technologies. (13, 14). Nanocarrier systems, in their various forms, have been attracting ample recognition as a result of extensive investigations towards harnessing their potential and thereby providing a remarkable achievement in the field of medical sciences to combat with life threatening diseases. They provides everlasting opportunities to the drug delivery by virtue of their nano size range as well as their special characteristics for the delivery of lipopilic drugs, proteins, peptides, hormones, imaging agents etc. to their site of action to combat with deadly diseases like cancer. Nanocarriers fulfilled the demands of pharmaceutical industry by providing the various advantages to delivery systems like

sustained, prolonged release, improvement in solubility, encapsulation of lipophilic drugs, environmentally responsive drug release, prolongation of therapeutic half-life by surface functionalization, improvement in bioavailability, co-delivery of drugs for generation of synergistic effect as well as suppression of drug resistance, reduction of systemic side effects by site specific delivery of biomolecules and therapeutic entity which provides better efficacy and enhanced detection of multiple deadly diseases without causing toxicity to the healthy organs.(14-17)

Nanocarriers for anticancer therapy:

Conventional drug delivery systems suffer from the problem of their non specific biodistribution which affects the normal & healthy tissues/organs of the patient. Due to this non specific biodistribution, the therapeutic and bioavailable dose and efficacy of the drug molecules get reduces which makes the treatment unsuccessful. Due to these shortcomings of the conventional systems, the nano drug delivery systems had gains more attention of the pharmaceutical scientists. (13, 18-20). Nano delivering of the drugs can maximize the drug efficacy, reduces the doses of chemotherapeutic drugs, reduces toxicity and side effects, optimize drug loading and release properties, increase the specificity and drug bioavailability and reduces overall cost to the treatment. Various types of nanocarriers had been developed in the recent years for the delivery of anticancer drugs. The nanocarriers are: nanoparticles, nanosuspensions, Carbon nanotubes, Quantum dots, Nanodendrimers, Micelles, lipid based solid lipid nanoparticles (SLNs), Nanostructured lipid carriers, self micro emulsifying and nanoemulsifying drug delivery systems, nano gels, nanofibres and many more. As far as cancer therapy is concerned, the nanoparticles mediated cancer therapy can be better

achieved by the concept of targeting which is of two types: Active targeting and passive targeting. Active targeting can be achieved by modification of the nanoparticle surface by various biomolecules, various alignments of atoms etc. Examples of active targeting include the modification by folic acid or folate targeting, hyaluronic acid targeting, pegylation etc. Passive targeting is nothing else the inherent property of nanoparticles for the treatment of cancer as the cancerous tissue develop leaky vasculature and nanoparticles, due to their nano ranges, can escape through the capillaries (through pores present near cancerous tissue) to the cancerous tissue directly and get distributed there. This phenomenon is known as Enhanced Permeation and Retention Effect (EPR). In this manner, the distribution of drug to the healthy tissues gets reduced thereby the related toxicity gets minimized. The choice of materials used for the formulation of the nanoparticles plays a vital role. The material chosen for the formulation should be biocompatible, non toxic, should be GRAS (Generally regarded As safe) certified, should be biodegradable etc. Generally, two types of materials are use for the formulation viz. lipids and polymers.So, lipid based drug delivery systems and polymer based drug delivery systems are the most common types among all nano drug delivery systems.

Lipid based Drug Delivery Systems:

From the past, lipids had gained more attention of the formulation scientist as compared to other synthetic and polymeric materials because of their biocompatible and biodegradable properties (4). The lipids employed to prepare lipid nanoparticles are usually physiological lipids (biocompatible and biodegradable) so, that drugs can be delivered at the required site of action with the controlled release with low acute and

chronic toxicity. Among the lipid formulation, the solid matrix containing nanoformulations are more popular. Two types of solid matrix formulations can be prepared namely, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). SLNs are composed of biocompatible solid lipids which are solid at room temperature. SLNs can serve the purpose in a better way but suffers from the problem of low entrapment efficiency owing to their unstable system and leakage of drugs from the nanoparticle system upon storage. Polymeric transitions of the lipid systems may occur with time due to the crystallinity of the solid lipids which leads to expulsion of the drugs from the matrix system. Sometimes modifications in the lipids occur after administration of the formulation which hinders the SLNs from performing their function of controlled release and protection of the drugs from biodegradation (21). So, in the process of improvement and reduction of the drawbacks of the SLNs, Nanostructured lipid carriers (NLCs) came into existence which had minimized the existing problems of the SLNs (4).

Nanostructured Lipid Carriers (NLCs):

NLCs had been introduced at the end of the 1990s by R.H. Muller (2, 3). They are the solid matrix lipid formulations, formulated by the blend of spatially different solid as well as liquid lipids which adds more imperfections in the molecule to accommodate more amount of drug. They were developed to overcome the disadvantages posed by SLNs. The various advantages of NLCs over SLNs are: they are more stable nanosystem, they have more entrapment efficiency and no drug leakage or drug expulsion during storage. NLCs matrix is solid at room temperature and the mixture remains solid matrix by controlling the amount of liquid lipid in lipid mix. A small

amount of liquid lipid is added to the lipid mixture which enhances the solubility of the lipophilic drugs and hence more entrapment if the lipophilic biomolecule is achieved with NLCs, also the presence of liquid lipid adds more imperfections to the crystal lattice which prevents the expulsion of the drugs and hence adds in improving shelf life of the delivery systems. A small amount of liquid lipid is added to the formulation usually 70:30 or 60:40, solid lipid to liquid lipid ratio(2-6).

Diameter of NLCs ranges from 500- 500 nm. They are the second generation SLN composed of liquid lipids along with solid lipids. The liquid lipids used for the formulations include Miglyol oil, Capmul MCM, Capmul oil, Captex 355, Capmul MCM10 and solid lipid include Glyceryl monosterate, Tristearin, GMO, Dynasan, Imwitor 900K etc depending upon their solubility with the drug molecule and their physical compatibility with each other.

Types of NLCs:

- Imperfect Type: Spatially different lipids are mixed together which adds imperfections in the order of crystal lattice. Small amount of chemically different liquid lipid can be added to the solid lipid to increase the drug payload.

- Amorphous Type: They are the structure less solid matrix containing NLCs. They can be achieved by mixing solid lipids with special lipids eg. Hydroxy octacosnyl hydroxyl stearate, isopropyl myristrate and medium chain triglycerides like miglyol etc. So, the NLC formulated will be in amorphous form.

- Multiple types: These type of NLCs contains multiple oil in lipid phase in water type emulsions. These type of NLCs can be achieved when higher amount of liquid lipid has to be added to have the more solubilization of the lipophilic drug.

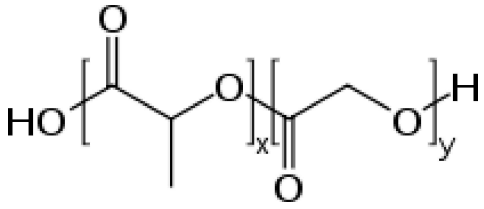
Polymeric Nanopartciles:

Among the various nanoforms, the drug delivery by using polymeric nanoparticles had been proved to be highly efficient so has attracted the attention of whole manufacturers (9, 22, 23). They can be the best solution to the chemotherapeutic engineering as due to their biodegradable and non toxic nature, they can be easily administered through intravenous (i.v) route and after the sustained release of the therapeutic agent, and they leave the human body by virtue of phagocytosis (24). Polymeric nanoparticles are defined as polymeric entities in nano range which contains the matrix architecture of biodegradable and biocompatible, synthetic, semi synthetic or natural polymers. The methods used for the formulation of polymeric nanoparticles include Nanoprecipitation method, Solvent evaporation method, Emulsification and solvent evaporation method, Copolymerization and Solvent diffusion methods depending upon the properties of polymer and solvent used. The drug release pattern, pharmacokinetics and release kinetics solely depends on the properties of polymers, methods for the entrapment of drugs and the particle size obtained. Size of the nanoparticles get varied depending upon the method used for the manufacturing of nanoparticles. Nanocapsules are formulated when the drug is entrapped inside the core of the polymer coat and nanospheres are obtained if the drug is absorbed on the surface of the polymeric matrix or entrapped inside the polymeric matrix. A wide variety of biodegradable polymers

have been used for the manufacturing of the nanoparticles include natural polymers, such as chitosan, alginate, gelatin, among others (Park et al., 2010), and the synthetic ones, such as polylactic acid (PLA), polylactide-co-glycolic acid (PLGA), poly(ε-caprolactone) (PCL), polymethyl methacrylate (PMMA), and polyglycolic acid (PGA).

Excipients profile

Poly (lactic co glycolic) acid (PLGA):

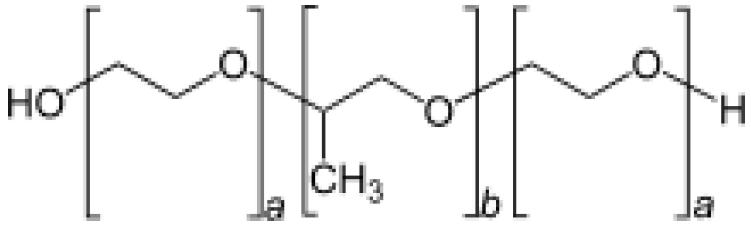


Poly (lactic co glycolic) acid (PLGA) is one of the most widely used biodegradable, non toxic polymer which is certified as safe by GRAS (Generally regarded as safe) (7-9) and is FDA approved polymer. PLGA is synthesized by ring opening co polymerization method of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. Different types of PLGA are based on the different ratios of lactide to glycolide used for the polymerization process. Different types of PLGA used in the market are PLGA 75:25 whose composition is 75% lactic and 25% glycolic acid and PLGA 50:50 has 50% lactic and 50% glycolic acid. The properties of PLGA vary with its different forms as crystallinity of PLGAs varies from amorphous to fully crystalline form. Glass transition temperature for PLGA varies from 40-60°C. PLGA can be dissolved in wide variety of solvents depending upon its composition like Acetone, chlorinated solvents etc. PLGA undergoes hydrolysis in the body to produce the original monomers: lactic acid and glycolic acid which are also the byproducts of

various metabolic pathways so they metabolize in the body and get excreted though kidney. As they metabolize in the body, the toxicity produced is minimal and they as regarded as safe and biocompatible polymers.

However, it has been reported that the acidic degradation of PLGA reduces the local pH low enough to create an autocatalytic environment. It has been shown that the pH inside a microsphere can become as acidic as ~2.8 In the past decade, PLGA nanoparticles had shown their potential in improving the non specific biodistribution and thus improved the oral bioavailability of therapeutic agents of different classes. In the view of this, their potential in intravenous formulations was also studied by a class of workers and they were found to be working well (25).

Poloxamer 188:



A= 2-130; b = 15-67

Poloxamers are the triblock, copolymers which are non ionic in nature. They are composed of a central hydrophobic chain of polyoxypropylene flanked by two hydrophilic chains of polyoxyethylene (poly (ethylene oxide)). They are also known as Synperonics/ Pluronics and Kolliphor. Depending upon the length of their polymeric blocks, their physicochemical properties varies. These copolymers are generally named with the letter P (for poloxamer) followed by three digits: the first two digits multiplied by 100 give the approximate molecular mass of the polyoxypropylene

core, and the last digit multiplied by 10 gives the percentage polyoxyethylene content (e.g. P407 = poloxamer with a polyoxypropylene molecular mass of 4000 g/mol and a 70% polyoxyethylene content). Temperature dependent self assembling and thermo gelling properties are the two main peculiar properties of the poloxamer solutions. The concentrated solution of poloxamer exhibit the liquid form at low temperature and became gel at higher temperatures in a reversible process. These transitions depend on the polymer composition (molecular weight and hydrophilic and hydrophoiobic molar ratio). At low temperatures, below the CMC (Critical Micelle Concentrations), the unimers are present in the solutions and above CMC, aggregation of unimers occurs and the process is known as micellization. They can be used as surfactants as they offer amphiphilic nature. They are used in industries as a surfactant to increase the water solubility of hydrophobic substances and also to enhance the miscibility of two substances by reducing their interfacial tension. They are also used as drug delivery systems for various pharmaceuticals and cosmeceuticals. Poloxamers also posses cancer cell targeting properties due to the differences in the membrane properties of cancerous and non cancerous cells. They also inhibit MDR proteins and other drug efflux transporters present on the surface of cancer cells which made them suitable for the cancer cell targeting therapy. Another effect of the polymers upon cancer cells is the inhibition of the production of ATP in multi-drug resistant (MDR) cancer cells.

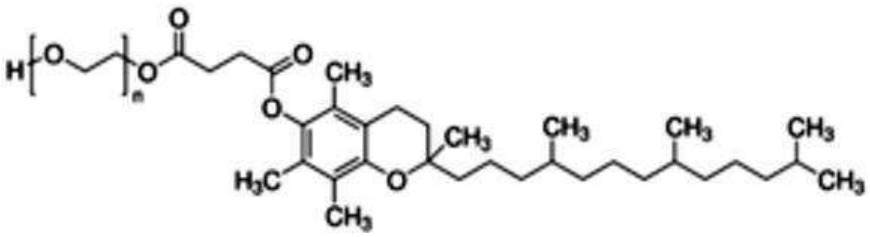
Due to their anticancer potential, they were selected as one of the excipients in the formulation of polymeric nanoparticles of anticancer drugs.

Capmul MCM (Glyceryl Caprylate/Caprate):

They are a mono-diglyceride of medium chain fatty acids (mainly caprylic and capric acid). It acts as an excellent solvent for many organic compounds, including steroids. It is a mixture of mono acylglycerols which are obtained by direct esterification reaction of glycerol with caprylic (octanoic) and capric (decanoic) acids.

- Appearance/Form: colorless or slightly yellow oily liquid or soft mass at 25°C.
- It is also a useful emulsifier for water-oil (w/o) systems.
- This is certified by GRAS, USFDA & US-NF.
- It is used as liquid lipid for various lipid based nanoformulations e.g. SMEDDS & SEDDS & NLCs.

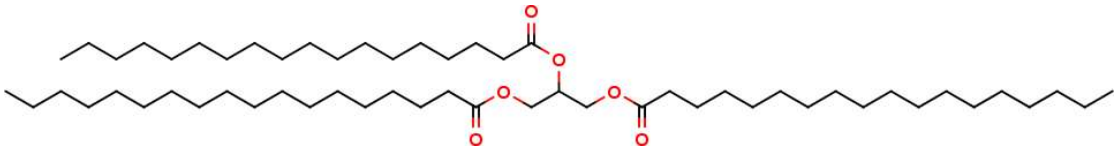
Vitamin E TPGS (D-α- tocopherol polyethylene glycol 1000 Succinate)



D-α-Tocopheryl polyethylene glycol 1000 succinate (simply TPGS or Vitamin E TPGS) is synthesized by the esterification reaction of Vitamin E succinate with polyethylene glycol 1000. As a nonionic surfactant, it has amphipathic properties and results in stable micelles in aqueous vehicles at concentration as low as 0.02 wt%. Tocopherols (TCP) (vitamin E) are a sequencing (α, β, γ and δ) of chiral organic molecules that vary in their degree of methylation of the phenol moiety of the chromanol ring. It is widely explored for its emulsifying, dispersing, gelling, and solubilizing effects on poorly water-soluble

drugs. It is a P-glycoprotein (P-gp) inhibitor and served the purpose of an excipient for overcoming multidrug resistance (MDR) and for increasing the bioavailability of many anticancer drugs. It is approved by FDA as a safe pharmaceutical adjuvant, many TPGS-based drug delivery systems (DDS) have been developed. They are lipid soluble anti-oxidants that protect the cell membranes from oxidative damage. α-Tocopherol is the form of tocopherol mainly absorbed by humanbeings. Tocopherol polyethylene glycol 1000 succinate (TPGS) may be used to create biodegradable polymers and antioxidant surfactants. Due to their antioxidant and anticancer properties, they are used in the formulation of nano drug delivery systems for anticancer medicines.

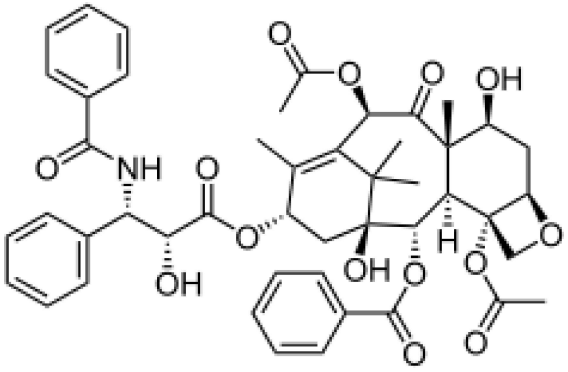
Tristearin:



It is a triglyceride derived from three units of stearic acid. Most of the triglycerides are obtained from at least two and more commonly three different fatty acids. Like other triglycerides, they can crystallise and form three polymorphs. The melting points of these forms are: 54 (α-form), 65, and 72.5 °C (β-form). Stearin is obtained from animal fats created as a byproduct of processing beef. They can also be obtained for tropical plants such as palm. It can be partially purified by dry fractionation by pressing tallow or other fatty mixtures, which leads to separation of the higher melting stearin-rich material from the mother liquid,. It can be obtained by inter esterification, again exploiting its higher melting point which allows the higher melting tristearin to be removed from the equilibrated mixture.

Drugs profile:

Paclitaxel (PTX):



Paclitaxel (PTX), a naturally occurring diterpenoid isolated from *Taxus bravifoliais* a first line drug for the treatment of wide panel of solid tumors including urothelial, breast, lung and ovarian tumors (1). It acts by stabilizing the microtubules of the cell where it binds to tubulin, and interfere with normal function of microtubule growth. It inhibits late G2 or M phase of the cell cycle and thereby inhibits cell replication. It is a first line drug for the treatment of all forms of ovarian cancer but suffers from the disadvantage of poor water solubility and nonspecific biodistribution which causes serious side effects to the human body. Owing to its low water solubility, it was formulated in a mixture of cremophore EL and ethanol (50:50% v/v), a marketed combination known as “Taxol” which suffers from serious side effects like allergic reactions, neutropenia, and neuropathy to the patients. Common side effects include hair loss, bone marrow suppression, numbness, allergic reactions, muscle pains, and diarrhea. Other serious side effects include heart problems, increased risk of infection, and lung inflammation.

Average molecular weight: 853.9 a.m.u