NANOSCALE DELIVERY SYSTEMS OF TAMOXIFEN FOR THE
TREATMENT OF BREAST CANCER

Shivam U Upadhyay1*, Jayvadan K Patel2, Vishnu A Patel1, Ajay K Saluja1

1Department of Pharmaceutics, A R College of Pharmacy, Vallabh Vidhyanagar – 388 120, Gujarat, India
2Department of Pharmaceutics, Nootan College of pharmacy, Visnagar - 384 315, Gujarat, India.

ABSTRACT
Cancer is an abnormal mass of a tissue with extreme and uncoordinated growth which persists even after the cessation of the stimulus. Breast cancer – the malignancy of breast tissues is the first human tumor for which targeted therapies have been developed. Tamoxifen is the most extensively used drug in the hormonal treatment for all stages of breast cancer and has recently been approved for the prevention of breast cancer in high risk women. The use of nanoparticles as drug delivery vehicles for anticancer therapeutics has great potential to revolutionize the future of cancer therapy. The major goals to design nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the optimal rate and dose regimen. Polymeric nanoparticles show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size, and biocompatibility with tissue and cells. Solid lipid Nanoparticles – the lipid based colloidal carriers have emerged as a potential alternative to other colloidal systems like Polymeric Nanoparticles, Liposomes and Fat Emulsions as they have been stated to combine the advantages of the aforementioned systems and are able to overcome their drawbacks. Nanocrystals have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because of their versatile features and unique advantages. Nanocrystals are pure solid drug particles of Nano size consisting of Surfactants or Polymeric Stabilizers and a liquid dispersion medium.

Keywords: Breast cancer, Polymeric Nanoparticles, Solid lipid nanoparticles, Nanocrystals.

INTRODUCTION
CANCER
Etiology of cancer includes Geographical, Racial Factors, Environmental and Cultural Influences, Age, Heredity, Acquired Preneoplastic Disorders and Carcinogenic Agents which may activate Oncogenes or inactivate of Tumor Suppressor Gene. Pathogenesis of Cancer is diagrammatically represented in (Figure 1). Laboratory diagnosis of cancer includes Histological Diagnosis, Cytological Diagnosis, and Tumor Markers. Recent Techniques comprise of Immuno-cyto-chemistry, Immuno-
flourescence, DNA probe analysis, DNA flow cytometry, Ag NOR staining. Treatment of Cancer include Surgical Oncology, Medical Oncology, Endocrine Therapy, Immunotherapy, Gene Therapy, Photodynamic Therapy (PDT), Proton Therapy, Complementary and Alternative Therapy, Physical & Traditional Chinese Therapy, Psychological Therapy and Radiation Therapy. The proliferative effect of estrogens on breast epithelium has been acknowledged for decades. Most of determinants of hyperestrogenia are related to the modern, Western lifestyle including low parity, delayed age at first delivery, short duration of breastfeeding, overeating, limited exercise and so on [1-4].

**BREAST CANCER**

Breast cancer is the most common malignancy among females and affects approximately one in every ten women worldwide. Breast cancer is the first human tumor for which targeted therapies have been developed. Ageing of population in the industrialized world is the most obvious cause of increased breast cancer occurrence; indeed, the risk of developing breast cancer after 65 years of age is 5.8 times higher than before 65, and 150-fold higher than before 30 years of age. In addition to advanced age, a few dozens of other breast cancer predisposing factors have been identified; however, all these diverse risks can be assigned to either of two major categories: excessive exposure to estrogens and deficiency in maintenance of genomic integrity.

**Anatomy of the female breast** (Figure 2)

Breast cancer is a disease in which malignant (cancer) cells form in the tissues of the breast. The breast is made up of lobes and ducts. Each breast has 15 to 20 sections called lobes, which have many smaller sections called lobules. The lobes and lobules are connected by thin tubes called ducts. Each breast also contains blood vessels and lymph vessels. The lymph vessels carry almost colorless fluid lymph. The lymph vessels lead to small, bean-shaped organs called lymph nodes that help the body fight against infection and disease. Lymph nodes are found throughout the body.

**Clinical Manifestation (Signs and Symptoms):**

- A lump or thickening in or near the breast or in the underarm area.
- A change in the size or shape of the breast.
- A dimple or puckering in the skin of the breast.
- A nipple turned inward into the breast.
- Fluid, other than breast milk, from the nipple, especially if it's bloody.
- Scaly, red, or swollen skin on the breast, nipple, or areola (the dark area of skin that is around the nipple).

**Diagnosis**

**Ultrasound exam:** A procedure in which high-energy sound waves (ultrasound) are bounced off internal tissues or organs and make echoes. The echoes form a picture of body tissues called a sonogram.

**Mammogram:** An x-ray of the breast. A mammogram can be performed with little risk to the fetus. Mammograms in pregnant women may appear negative even though cancer is present.

**Biopsy:** The removal of cells or tissues by a pathologist so they can be viewed under a microscope to check for signs of cancer.

**Stages of Breast Cancer:**

Breast cancer staging using the TNM system is based on the size of the Tumor (T), whether or not the tumor has spread to the Lymph Nodes (N) in the armpits, and whether the tumor has Metastasized (M) (i.e. spread to a more distant part of the body). Larger size, nodal spread, and metastasis have a larger stage number and a worse prognosis.

**Stage 0 (carcinoma in situ)**
- Pre-cancerous or marker condition of two types:
  - Ductal carcinoma in situ (DCIS) - noninvasive condition in which abnormal cells are found in the lining of a breast duct.
  - Lobular carcinoma in situ (LCIS) - invasive cancer in which abnormal cells are found in the lobules of the breast.

**Stage I**
Cancer has formed. No tumor is found in the breast, but small clusters of cancer cells.

**Stage II**
No tumor is found in the breast, but cancer is found in the axillary lymph nodes.

**Stage IIIA**
No tumor is found in the breast. Cancer is found in axillary lymph nodes that are attached to each other or to other structures, or cancer may be found in lymph nodes near the breastbone.

**Stage IIIB**

Tumor has spread to the chest wall and/or the skin of the breast; and Cancer that has spread to the skin of the breast is inflammatory breast cancer.

**Stage IIIC**

Tumor has spread to lymph nodes above or below the collarbone.

**Stage IV**

Metastatic Cancer that has a less favorable prognosis. Cancer has spread to other organs of the body, most often the bones, lungs, liver or brain.

**Treatment**

**Surgery**

Most pregnant women with breast cancer have surgery to remove the breast. Some of the lymph nodes under the arm are usually taken out and looked at under a microscope to see if they contain cancer cells.

**Breast Removal Surgery:**

- **Simple mastectomy:**
  A surgical procedure to remove the whole breast that contains cancer. Some of the lymph nodes under the arm may also be removed for biopsy. This procedure is also called a total mastectomy.
- **Modified radical mastectomy:**
  A surgical procedure to remove the whole breast that has cancer, many of the lymph nodes under the arm, the lining over the chest muscles, and sometimes, part of the chest wall muscles.

**Breast Conserving Surgery:**

- **Lumpectomy:**
  A surgical procedure to remove a tumor (lump) and a small amount of normal tissue around it. Most doctors also take out some of the lymph nodes under the arm.
- **Partial mastectomy:**
A surgical procedure to remove the part of the breast that contains cancer and some normal tissue around it. Some of the lymph nodes under the arm may also be removed for biopsy. This procedure is also called a segmental mastectomy.

**Radiation therapy**

Radiation therapy is a cancer treatment that uses high-energy x-rays or other types of radiation to kill cancer cells. There are two types of radiation therapy.

- **External radiation therapy** uses a machine outside the body to send radiation toward the cancer.
- **Internal radiation therapy** uses a radioactive substance sealed in needles, seeds, wires, or catheters that are placed directly into or near the cancer. The way the radiation therapy is given depends on the type and stage of the cancer being treated.

**Chemotherapy**

Chemotherapy is a cancer treatment that uses drugs to stop the growth of cancer cells, either by killing the cells or by stopping the cells from dividing. When chemotherapy is taken by mouth or injected into a vein or muscle, the drugs enter the bloodstream and can reach cancer cells throughout the body (systemic chemotherapy). When chemotherapy is placed directly into the cerebrospinal fluid, an organ, or a body cavity such as the abdomen, the drugs mainly affect cancer cells in those areas (regional chemotherapy).

The way the chemotherapy is given depends on the type and stage of the cancer being treated. Chemotherapy should not be given during the first 3 months of pregnancy. Chemotherapy given after this time does not usually harm the fetus but may cause early labor and low birth weight.

**Adjuvant and Neoadjuvant Therapy**

- Adjuvant therapy for breast cancer is any treatment given after primary therapy to increase the chance of long-term survival. Neoadjuvant therapy is treatment given before primary therapy.
- Adjuvant therapy for breast cancer can include chemotherapy, hormonal therapy, the targeted drug trastuzumab, radiation therapy, or a combination of treatments.
- Patients who have a higher risk of breast cancer recurrence are more likely to need adjuvant therapy. Doctors look at both prognostic and predictive factors to decide which patients might benefit from adjuvant treatments.
- Adjuvant and neoadjuvant therapies have side effects, but careful studies have shown that the risks of adjuvant therapy for breast cancer are outweighed by the benefit of treatment—that is, increasing the chance of long-term survival.
- Clinical trials of adjuvant and neoadjuvant therapies for breast cancer are testing new treatments, new combinations of treatments, and whether genetic information can be used to better tailor therapies to individual patients.

Tamoxifen

Tamoxifen is a standard endocrine therapy for the treatment of steroid receptor positive breast cancer \(^{(5)}\). Tamoxifen is the most extensively used hormonal treatment for all stages of breast cancer and has recently been approved for the prevention of breast cancer in high risk women \(^{(6)}\). Despite the knowledge that certain breast cancers responded to hormonal manoeuvres \(^{(7)}\), there was little general interest in the 1960s in advancing the development of antioestrogens as breast-cancer treatment. At that time, the reasons why some tumors responded to hormonal treatment and others did not were still unclear \(^{(8)}\). With the discovery of the oestrogen receptor (ER) \(^{(9)}\), Subsequently, tamoxifen was found to block oestrogen binding to the human ER and to prevent rat mammary carcinogenesis \(^{(10-11)}\), which suggested that it might have an expanded role in the treatment and prevention of breast cancer.

Studies of tamoxifen in advanced breast cancer showed response rates of about 30% in unselected patients and 40–60% in patients with ER positive tumors \(^{(12)}\). In patients with advanced disease whose breast tumors express both ER and progesterone receptors, response rates as high as 70% have been documented \(^{(13)}\). Studies showed that patients of all ages, with node-positive or node-negative tumors, benefit from adjuvant tamoxifen in terms of disease-free and overall survival \(^{(14,15)}\). The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 trial showed no further reduction in
breast-cancer recurrence in patients with node-negative breast cancer treated with tamoxifen for longer than 5 years, compared with those who discontinued tamoxifen after 5 years (16).

Most importantly, the recent NSABP chemoprevention trial showed that tamoxifen significantly reduces the frequency of invasive breast cancer and ductal carcinoma in situ by about 50% in high-risk women (17). Thus, a drug that was initially discovered in a fertility control programme is now the endocrine treatment of choice for all stages of breast cancer and the first preventive agent for this disease. However, tamoxifen has been pivotal for thousands of women with breast cancer. The intense pharmacological study of tamoxifen and its side-effects has opened the horizons to new antioestrogens that are proving to be valuable medicines in women’s health.

**Selective Oestrogen-Receptor Modulation (SERM)**

Tamoxifen and related non-steroidal compounds act as antioestrogens on the breast and mammary gland, while acting as partial antioestrogens on the uterus. Tamoxifen inhibits the growth of ER-positive MCF7 breast-cancer cells in vitro (18) and of dimethylbenzanthracene-induced mammary tumors in rats. However, the drug has oestrogenic activity in the uterus in mice and rats (19), and it is even classified as an oestrogen in mice. One possible explanation for this paradoxical activity is species-specific metabolism of the antioestrogens, but no species-specific metabolic routes have been identified (20).

However, the observations in mice provided the basis for the recognition of selective oestrogen-receptor modulator (SERM) action. Tamoxifen inhibits the growth of transplanted ER-positive human breast-cancer cells in athymic mice, while stimulating the uterus to grow. The same range of tamoxifen metabolites was found in both the mouse uterus and the human breast tumor, which led to the idea that the tamoxifen–ER complex is oestrogenic at one site and antioestrogenic at another. Furthermore, when athymic mice were given two transplants—a human breast tumor and a human endometrial tumor—tamoxifen inhibited growth of the former while stimulating growth of the latter (21). This observation led to the investigation of endometrial-cancer risk in patients treated with tamoxifen (22).
There are several divergence points that ultimately will determine the biological response to a SERM (Figure 3). There are two structurally related ERs, α and β (23), which have some degree of homology. A SERM can bind to either receptor molecule. Both ERs have a ligand-binding domain and a DNA-binding domain, and they can bind directly to DNA to activate gene transcription. There are, however, differences in the activating functions (AF) that can alter the SERM–ER complex, resulting in greater or lesser oestrogenicity. Tamoxifen seems to be more antioestrogenic when complexed with ER β than with ER α (24).

The ligand programmes the shape of the ER complex so that coactivators or corepressors can bind to the external surface of the SERM–ER complex (25). Coactivators aid signal transduction, whereas corepressors block transduction. At the time that a transcriptional complex is to be formed, the SERM–ER complexes have to form homodimers or heterodimers before initiating gene transcription. Lastly, there is evidence that SERM agents can modulate gene transcription through two mechanisms, either through an activating protein (AP)-1 pathway, when a protein–protein interaction occurs with fos and jun, or the SERM–ER complex can activate or silence an oestrogen-response element directly on DNA (26).

NOVEL DRUG DELIVERY SYSTEMS
Cancer and Nanotechnology

In the search for successful cancer treatment is the quest for the ultimate cancer therapeutic. Although conventional treatment options such as chemotherapy and radiation have experienced many advances over the past decades, cancer therapy is still far from optimal. Effectiveness of cancer therapy depends on a fine ratio that is determined by the ability of the therapeutic to eradicate the tumor while affecting as few healthy cells as possible. In this case, systemically administering bolus doses of powerful chemotherapeutics often results in intense side effects due to the action of the drugs on sites other than the intended target. With such nonspecific drug action, the concentration of drug rendered available at the tumor site itself is potentially beneath the minimal effective concentration, entering the patient into a vicious predicament between choosing a near-toxic effective dose and a comfortable ineffective dose. To alleviate this difficulty,
decades of research have focused on developing cancer-specific drugs or delivery systems that can preferentially localize existing agents to the tumor site. Recent advances in nanotechnology promises further developments in target-specific drug delivery systems.

Nanotechnology is considered to be an emerging, disruptive technology that will have significant impact in all industrial sectors and across-the-board applications in cancer research. There has been tremendous investment in this area and an explosion of research and development efforts in recent years, particularly in the area of cancer research. At the National Institutes of Health, nanomedicine is one of the priority areas under its Roadmap Initiatives. Moreover, in 2005 the National Cancer Institute alone committed $144.3 million over 5 years for its Alliance for Nanotechnology in Cancer program. Much research and development is progressing in the areas of cancer diagnostics, devices, biosensors, and microfluidics, but this review will focus on therapeutics (27).

So, we will discuss the aspects of Nanotechnology in Cancer and Tamoxifen onwards in this paper.

Introduction

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix (28). The advances in high speed and high storage capacity computers, as well as accurate instruments for measuring and manipulating at the nanoscale, have accelerated the development of nanoscale structures and devices into reality (29).

Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained (30). Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed (31). In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a
prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes (32,33).

The major goal in designing nanoparticles as a delivery system is to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen by controlling:

- Particle Size
- Surface Properties
- Release of pharmacologically active agents

Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties (34,35). The advantages of using nanoparticles as a drug delivery system include the following:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

In spite of these advantages, nanoparticles do have limitations. For example,
- Small size and large surface area lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.
- Small particles size and large surface area result in limited drug loading and burst release.

**Role of Nanotechnology in Cancer**

The use of Nanoparticles as drug delivery vehicles for anticancer therapeutics has great potential to revolutionize the future of cancer therapy. As tumor architecture causes nanoparticles to preferentially accumulate at the tumor site, their use as drug delivery vectors results in the localization of a greater amount of the drug load at the tumor site; thus improving cancer therapy and reducing the harmful nonspecific side effects of chemotherapeutics. In addition, formulation of these nanoparticles with imaging contrast agents provides a very efficient system for cancer diagnostics.

Nanoparticles are excellent tumor-targeting vehicles because of a unique inherent property of solid tumors. Due to the rapid growth of solid tumors, many tumors present with fenestrated vasculature and poor lymphatic drainage, resulting in an enhanced permeability and retention (EPR) effect (36), which allows nanoparticles to accumulate specifically at the tumor site (Figure 4). Although nanoparticles protect the drug from rapid metabolism and clearance, as well as nonspecific recognition and distribution, stealth-shielding nanoparticles (using PEG surface modification), in addition, will help to avoid uptake by the reticuloendothelial system (37) and mononuclear phagocytes (38).

Altogether, this results in the property of nanoparticles to circulate for prolonged periods of time, allowing them to eventually reach the tumor vasculature where, guided by the EPR effect, they specifically extravasate through the fenestrated capillaries to accumulate drugs at the tumor mass. It has been shown that nanoparticle and polymer conjugate delivery can allow concentrations of the drug near the vicinity of the tumor to reach 10- to 100-fold higher than when administering free drug (39). Beyond the passive tumor-targeting properties by the EPR effect, oral localization of nanoparticles can be further improved by active targeting through conjugation of the particle with tumor-specific recognition of small molecules, such as folic acid (40), thiamine (41) and even antibodies or lectins (42). In addition, at the tumor site, nanoparticles offer one further
advantage: they can be endocytosed / phagocytosed, enhancing cell internalization of the drug, and leading to delivery of the drug closer to the intracellular site of action.

Polymeric Nanoparticles

The field of polymer nanoparticles (PNP) is quickly expanding and playing a pivotal role in a wide spectrum of areas ranging from electronics to photonics, conduct in materials to sensors, medicine to biotechnology, pollution control to environmental technology, and so forth, during the past decades (43). Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled- and sustained-release properties, subcellular size, and biocompatibility with tissue and cells (44). Biodegradable nanoparticles have been used frequently as drug delivery vehicles due to its grand bioavailability, better encapsulation, control release and less toxic properties. Nanomedicine formulation depends on the choice of suitable polymeric system having maximum encapsulation (higher encapsulation efficiency), improvement of bioavailability and retention time.

The performance of nanoparticles in vivo is influenced by

- Morphological characteristics
- Surface chemistry
- Molecularweight
Method of Preparation of Polymeric Nanoparticles

- PREPARATION OF NANOPARTICLES
  - FORMATION OF NP BY POLYMERISATION PROCESS
    - DISPERSION POLYMERISATION
  - FORMATION OF NP FROM PREFORMED POLYMERS
    - EMULSIFICATION POLYMERISATION
      - SPONTANEOUS FORMATION
      - EMULSIFICATION TECHNIQUES
BIODEGRADABLE POLYMERS FOR POLYMERIC NANOPARTICLES (45)  
(Figure 5)

Poly-d,l-lactide-co-glycolide (PLGA)

PLGA is approved by the Food and Drug Administration for drug delivery use. Owing to their excellent biocompatibility, the biodegradable polyester called poly (D, L-lactide-coglycolide) (PLGA) is the most frequently used biomaterial and is already commercialized for a variety of drug delivery systems (blends, films, matrices, microspheres, nanoparticles, pellets, etc.) (46). The effect of the oral administration of PLGA nanoparticles on the efficacy and toxicity of tamoxifen was assessed by Jain et al (47). The effect of manufacturing process parameters on particle size and entrapment efficiency was proved by Sahana et al in PLGA Nanoparticles (48) 192 nm mean size and 33 w/w% loading capacity of Tamoxifen Citrate was established by using 3 mg.mL-1 PLGA/dichloromethane ratio to prepare Nanoparticles (49). PLGA Nanoparticles for Oral administration were successfully prepared to improve bioavailability and sustained release of Estradiol by varying the molecular weight and copolymer composition of PLGA (50).

Hydrolysis of PLGA nanoparticles: PLGA nanoparticles are biologically hydrolyzed in acidic medium into lactic and glycolic acid. These hydrolysis products have been metabolized in TCA cycle.
Polylactic acid (PLA)

PLA (polylactic acid) polymer is biocompatible and biodegradable material which undergoes scission in the body to monomeric units of lactic acid as a natural intermediate in carbohydrate metabolism. PLA nanoparticles have been mostly prepared by Solvent evaporation and solvent displacement.

Poly-ε-caprolactone (PCL)

PCL (poly-ε-caprolactone) is degraded by hydrolysis of its ester linkages in physiological conditions (such as in the human body) and has therefore received a great deal of attention for use in drug delivery. In particular, it is especially interesting for the preparation of long-term implantable devices, owing to its degradation slower than that of polylactide. PCL nanoparticles have been prepared mostly by nanoprecipitation, solvent displacement and solvent evaporation. To increase the local concentration of tamoxifen in estrogen receptor (ER) positive breast cancer nanoparticle formulation using PCL was prepared by solvent displacement method (51).

Chitosan

Chitosan is a natural polymer obtained by N-deacetylation of chitin. After cellulose chitin is the second most abundant polysaccharide in nature. It is biologically safe, non-toxic, biocompatible and biodegradable polysaccharide. Chitosan nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method and providing versatile routes of administration (52). Methods of Preparation are Ionotropic gelation, Microemulsion Method, Emulsification solvent diffusion method, Polyelectrolyte complex (PEC).

Gelatin

Gelatin is extensively used in food and medical products and is attractive for use in controlled release due to its nontoxic, biodegradable, bioactive and inexpensive properties. It is a polyampholyte having both cationic and anionic groups along with hydrophilic group. It is known that mechanical properties, behavior and thermal properties depend significantly on the crosslinking degree of gelatin.

Poly-alkyl-cyano-acrylates (PAC)

The biodegradable as well as biocompatible pol alkyleyanoacrylates (PAC) are degraded by esterases in biological fluids and produce some toxic products that will stimulate or damage the central nervous system. Thus this polymer is not authorized for application in human.

SOLID LIPID NANOPARTICLES (Figure: 6)
Despite almost 30 years of research, nanoparticulate products do practically not exist on the pharmaceutical market. To overcome problems of polymeric nanoparticles (e.g. lack of large scale production), nanoparticles based on solid lipids were developed (53). Solid lipid nanoparticles (SLN) introduced in 1991 represent an alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles. SLNs are rapidly developing nanotechnology with several applications in drug delivery system, clinical medicine and other science. The solid lipid nanoparticles are submicron colloidal carriers (50-100 nm) which are composed of physiological lipid, dispersed in water or in aqueous surfactant solution. SLNs as colloidal drug carrier combines the advantage of polymeric nanoparticles fat emulsions and liposomes. In order to overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by solid lipid which eventually transformed into solid lipid nanoparticles (54).

SLN will carry the adequate dose of the drug at the tumor site by Passive Targeting through EPR (Enhanced Permeability and Retention) Effect for a known period of time and reduce the adverse effects on normal organs.

The main goals of Solid Lipid Nanoparticles (SLN) in terms of drug delivery are:

- To increase the bioavailability and efficacy of drugs,
- To control nonspecific toxicity, immunogenicity, pharmacokinetics and pharmacodynamics of drugs.

NANOSTRUCTURED LIPID CARRIERS (NLC): (55)

NLC often referred to as the second generation of SLN, were first developed by Müller et al. in the late 1990s. In contrast to the lipid crystal matrix of SLN, the lipid matrix of NLC has an imperfect crystal or amorphous structure, which allows for drug loading in both the molecular form and in clustered aggregates at lattice imperfections. As a result, NLC show enhanced drug loading and less pronounced drug expulsion by avoidance of a crystal structure. NLC, similar to SLN, are colloidal particles that typically range in size from 100 to 500 nm, depending on production parameters. A blend of solid- and liquid-phase lipids, NLC are generally solid at temperatures above 40°C. They have been successfully multifunctionalized to capture a payload of drugs, to target specific cells, and to release entrapped drugs in a controlled manner.
NLC have been mostly researched for oral or dermal drug delivery applications, with little focus on parental administration; however, recent literature has demonstrated their potential as attractive candidates for the delivery of anticancer agents, as well as therapeutic proteins and peptides. Nano- and microparticles made of solid lipids and suspended in water offer an option for formulating both BCS class II and IV APIs as well as biologics that may overcome the issues of shelf-life stability and the cost and toxicity associated with the use of organic solvents. In effect, the concepts of nanoparticles and solid solutions are being combined. The shelf-life stability of SLNs can be very good. Lipids can be chosen that do not hydrolyze in aqueous suspension (56).

LIPID DRUG CONJUGATES (LDC):

A major problem of SLNs is the low capacity to load hydrophilic drugs due to partitioning effects during the production process. Only highly potent low dose hydrophilic drugs may be suitably incorporated in the solid lipid matrix. In order to overcome this limitation, the so called LDC nanoparticles with drug loading capacities of up to 33% have been developed.

METHOD OF PREPARATION OF SLN

SLN are produced by using several methods extensively described in the literature:

1. High pressure homogenization (cold and hot homogenization) (57-62) (Figure: 7)
2. Breaking of o/w microemulsion (63-66) (Figure: 8)
3. Solvent emulsification-evaporation (67-69) or solvent emulsification–diffusion (70,71) (Figure: 9)
4. Solvent injection (72) (Figure: 10)
5. Preparation by water-in-oil-in-water double emulsion (w/o/w) (73,74) (Figure: 11)
6. High shear homogenization (75) and/or ultrasound dispersion (76-78) (Figure: 12)
7. Preparation by using membrane contactor as a new reported technique for SLN production (79)
8. SLN preparation by using supercritical fluid (80)

DRUG INCORPORATION MODELS AND TYPES OF SLN

Factors affecting loading capacity of a drug in lipid are:

- Solubility of drug in lipid melt,
- Miscibility of drug melt and lipid melt,
• Chemical and physical structure of solid matrix lipid,
• Polymorphic state of lipid material.

In particular, there is an inverse relationship between solubility of the drug and loading capacity. Enhancement in aqueous solubility of drug leads lower to entrapment efficiency. For this reason, Müller et al reported a cold homogenization technique which is performed at room temperature or below (0 °C). Therefore, solubility of the drug is also an important factor for choosing the production method of SLN. While the hot homogenization technique is much more suitable for lipophilic drugs, the cold homogenization technique is employed for hydrophilic drugs in order to reach the highest payload and to prevent drug partition to the aqueous phase during SLN production.

- Drug incorporation models of SLN are as follows:
  - Solid solution model
  - Core-shell model (drug-enriched shell and drug-enriched core)

**Models of drug incorporation into SLN:**

In the case of the solid solution model, the drug is molecularly dispersed in the lipid matrix when the particles are produced by the cold homogenization technique and using no surfactant or no drug-solubilizing surfactant. The drug has strongly pronounced interactions with the lipid. According to the drug-enriched shell model of drug incorporation, a solid lipid core forms when the recrystallization temperature of the lipid is reached. On reducing the temperature of the dispersion, the drug concentrates in the still liquid outer shell of the SLN.

According to the drug-enriched core model of drug incorporation, cooling the nanoemulsion leads to a supersaturation of the drug which is dissolved in the lipid melt at
or close to its saturation solubility and the drug precipitates prior to lipid recrystallization. Further cooling finally leads to the recrystallization of the lipid surrounding the drug as a membrane.

**COATING OF SOLID LIPID NANOPARTICLES (SLN) WITH HYDROPHILIC SUBSTANCES (81)**

In case of systemic use, ideal drug delivery is selective uptake by the target organ or at the site of action with a low systemic level of drug. It is very difficult to provide this ideal situation in practice because anatomical barriers limit and govern the distribution of drugs. SLN which are hydrophobic, are exposed to phagocytic uptake by macrophages. Studies on systemic use of SLN have focused on improving their presence in the blood circulation since macrophages in RES recognize them as foreign substances and quickly remove them due to their physicochemical properties, mainly particle size, surface charge and surface. Uptake by phagocytic cells is mediated by blood components which are called opsonins and specific cell receptors on macrophages that operate independently. The opsonic factors include proteins such as immunoglobulin G, Complement C3b and fibronectin. These factors are adsorbed by nanoparticles, then the particles are immediately cleared by the macrophages of the mononuclear phagocytic system. The surface characteristics of the particle determine whether or not opsonization will take place and which component will be involved. As a consequence, the mechanism of particle-cell interaction will also depend on the nature of the opsonic component and the relevant receptor-mediated process.

To avoid phagocytic uptake and to modulate biodistribution parameters of drugs for their long blood circulation, surface properties of colloidal drug carriers can be modified by using various techniques.

**ADVANTAGES OF SLN (82,83)**

- No toxic metabolites are produced as compared to some polymeric nanoparticles since use of physiological and biocompatible lipids.
- Protecting the labile and sensitive drugs from chemical, photochemical or oxidative degradation, due to immobilization of drug molecules by solid lipids and reduce drug leakage as commonly observed in liposomes.
- Ease of industrial scale production by hot dispersion technique.
- Low cost of solid lipids as compared to phospholipids and biodegradable polymers.
Avoidance of organic solvents.  
Both lipophilic and hydrophilic compounds can be encapsulated and delivered by SLN with modification in the formulation.  
SLN have been proposed as a colloidal drug carrier therapeutic system for different administration routes such as oral, dermal, ophthalmic, pulmonary, rectal and parenteral administration.  
Small size and relatively narrow size distribution which provide biological opportunities for site-specific drug delivery by SLNs.  
Controlled release of active drug over a long period can be achieved.  
Possible sterilization by autoclaving or gamma irradiation.  
SLNs can be lyophilized as well as spray dried.  
Avoidance of organic solvents.  
Relatively economic and stable.  
Incorporation of drug can reduce distinct side effects of drug, e.g. Thrombophlebitis that is associated with i.v. injection of diazepam.  
Surface modification can easily be accomplished and hence can be used for site-specific drug delivery system.

DISADVANTAGES OF SLN (84)

- Poor drug loading capacity because of poor solubility of drug in the lipid melt  
- Drug expulsion after polymeric transition during storage and relatively high water content of the dispersions (70-99.9%) has been observed.  
- The structure of the lipid matrix and the polymeric state of the lipid matrix. If the lipid matrix consists of especially similar molecules (i.e. tristearin or tripalmitin), a perfect crystal with few imperfections is formed. Since incorporated drugs are located between fatty acid chains, between the lipid layers and also in crystal imperfections, a highly ordered crystal lattice can not accommodate large amounts of drug. Therefore the use of more complex lipids is more sensible for higher drug loading.

SLN VERSUS OTHER COLLOIDAL CARRIERS

- SLNs versus 0/w Emulsion

If protection of drug against chemical degradation is required. Incorporation of drug in the solid lipid matrix surely offer a better protection than can be achieved in the oily internal phase of emulsion and liposomes.
Prolonged release of drug from emulsion is not feasible which can be achieved to a certain extent from SLN.

**SLNs versus Polymeric Nanoparticles**
- Lower cytotoxicity due to the absence of solvents.
- Low cost of excipients.
- Large scale production is possible by the simple process of high-pressure homogenization.

**SLNs versus Liposomes**
- In comparison with liposomes SLNs offer better protection to drug against chemical degradation there is no or little access of water to the inner core of lipid particles.
- Depending upon the nature of the drug higher payload might be achieved.

**APPLICATIONS OF SLN**
- SLNs as gene vector carrier
- SLNs for topical use
- SLNs as cosmeceuticals
- SLNs for potential agriculture application
- SLNs as a targeted carrier for anticancer drug to solid tumors
- SLNs in breast cancer and lymph node metastases
- Oral SLNs in antitubercular chemotherapy
- Stealth nanoparticles

**NANOCRYSTALS**

The number of poorly soluble drugs (e.g. Tamoxifen) in classical and pharmabiotech New Chemical Entities (NCE) is steadily increasing. A poor solubility is generally associated with poor bioavailability. Nanocrystals are a novel formulation approach for these compounds which produce patient- convenient oral dosage forms. These nanoformulations offer increased dissolution rates for drug compounds and complement other technologies used to enhance bioavailability of insoluble compounds (BCS Class II and IV) such as solubility enhancers (i.e. surfactants), liquid-filled capsules or solid dispersions of drugs in their amorphous state. Nanocrystalline API has been shown to dramatically increase the rate of dissolution in vitro, improve bioavailability, reduce variability and alleviate positive food effects for orally administered drug molecules.
Drug nanocrystals are pure solid drug particles with a mean diameter below 1000 nm. A nanosuspension consists of drug nanocrystals, stabilizing agents such as surfactants and/or polymeric stabilizers, and a liquid dispersion medium. The dispersion media can be water, aqueous solutions, or nonaqueous media (86). With the introduction of combinatorial chemistry and high throughput screening, the properties of new chemical entities shifted towards higher molecular weight and increasing lipophilicity that results in decreasing aqueous solubility (87). It is estimated that 40% or more of active substances are poorly soluble in water.

The absorption of such compounds when presented in the crystalline state to the gastrointestinal tract is typically dissolution rate-limited, and the drugs are typically BCS class II or class IV compounds. (Figure: 13) The rate and extent of absorption of class II compounds is highly dependent on the performance of the formulated product. These drugs can be successfully formulated for oral administration, but care needs to be taken with formulation design to ensure consistent bioavailability. Essentially the options available involve either reduction of particle size (of crystalline drug) or formulation of the drug in solution, as an amorphous system or lipid formulation (88).

Solubility in different solvents is an intrinsic material characteristic for a defined molecule. To achieve a pharmacological activity, the molecules must in general exhibit certain solubility in physiological intestinal fluids to be present in the dissolved state at the site of absorption. The aqueous solubility is a major indicator for the solubility in the intestinal fluids and its potential contribution to bioavailability issues (89). So, there is tremendous work is going on after Nanocrystals. (Figure: 14)

**INCREASING DISSOLUTION RATE THROUGH NANOSIZATION — THEORETICAL ASPECTS** (90)

The solid API dissolution rate is proportional to the surface area available for dissolution as described by the Nernst–Brunner/Noyes–Whitney equation:

\[
\frac{dX}{dt} = \frac{A \cdot D}{h} \left( C_s - \frac{X_d}{V} \right)
\]

Where

\(\frac{dX}{dt}\) = dissolution rate,
\(X_d\) = amount dissolved,
\(A\) = particle surface area,
D = diffusion coefficient,
V = volume of fluid available for dissolution,
Cs = saturation solubility,
h = effective boundary layer thickness.

Based on this principle, API micronization has been extensively used in the pharmaceutical industry to improve oral bioavailability of drug compounds. It is evident that a further decrease of the particle size down to the sub-micron range will further increase dissolution rate due to the increase of the effective particle surface area. For example in the case of aprepitant, the nanocrystal dispersion of 120-nm particle size exhibits a 41.5-fold increase in surface area over the standard 5 μm suspension. Furthermore, as described by the Prandtl equation, the diffusion layer thickness (h) will also be decreased thus resulting in an even faster dissolution rate.

SPECIAL FEATURES (91)

- Drug nanocrystals are particles made from 100% drug. No Carrier.
- Increased saturation velocity
- Increased adhesiveness to surfaces/cell membranes
- Increased dissolution velocity
- Crystalline or amorphous structure
- Amorphous particle state offers advantages
- Size below 1 μm

Methods of Preparation (Figure 16)

⇒ Pearl Milling

The method is first developed by liversidge et.al (92). In this method the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. The milling medium is framed of glass, zirconium oxide or highly cross-linked polystyrene resin. The milling chamber is charged with the milling media, water, drug and stabilizer, and the milling media or pearls are then rotated at a very high shear rate.

Homogenisation

Homogenization involves the forcing of the suspension under pressure through a valve having a narrow aperture. (Figure 15) The most commonly used homogenizer in the
preparation of nanosuspension is the APV micron LAB 40 (APV Deutschland GmbH, Lubeck, Germany). However, other piston-gap homogenizers from Avestin (Avestin Inc., Ottawa, Canada) and Stansted (Stansted Fluid Power Ltd, Stansted, UK) can also be used. The instrument can be operated at pressures varying from 100 to 1500 bars. In some instruments, a maximum pressure of 2000 bars can be reached. Most of the cases require multiple passes or cycles through the homogenizer, which depends on the hardness of the drug, the desired mean particle size, and required homogeneity. High-pressure homogenizers are available with different capacities ranging from 40ml (for laboratory purposes) to a few thousand litres (for large-scale production). Before subjecting the drug to the homogenization process, it is essential to form a presuspension of the micronized drug in a surfactant solution using high-speed stirrers (93).

**Nanoprecipitation**

Precipitation has been applied for years to prepare submicron particles within the last decade (94, 95) especially for the poorly soluble drugs. Typically, the drug is firstly dissolved in a solvent. Then this solution is mixed with a miscible antisolvent in the presence of surfactants. Rapid addition of a drug solution to the antisolvent (usually water) leads to sudden supersaturation of drug in the mixed solution, and generation of ultrafine crystalline or amorphous drug solids. This process involves two phases: nuclei formation and crystal growth. When preparing a stable suspension with the minimum particle size, a high nucleation rate but low growth rate is necessary. Both rates are dependent on temperature: the optimum temperature for nucleation might lie below that for crystal growth, which permits temperature optimization.

Comparison of these Methods of Preparation is specified in Table 1. Marketed Examples of Nanocrystal Preparations are collected in Table 2.
Figure 1: Pathogenesis of Cancer

Acquired / Environmental Factors

Genetic Factors

Changes in the Genome of Somatic cells

Activation of Oncogenes cells

Inactivation of Tumor Suppressor Gene

Expression of altered gene products and loss of regulatory gene products cells

Benign Tumor

Malignant Tumor

Clonal Expansion

Additional Mutations

Heterogeneity
Figure 2: Anatomy of the female breast.
The nipple and areola are shown on the outside of the breast. The lymph nodes, lobes, lobules, ducts, and other parts of the inside of the breast are also shown.

Figure: 3: Selective Estrogen Receptor Modulator
Divergence points that a SERM must pass to modulate oestrogen-like actions in a target tissue. A SERM can bind to either ER α or ER β, and the complexes can then recruit coactivators or corepressors. The complexes may form homodimers or heterodimers and modulate genes by a non-traditional pathway of protein–protein interactions or a traditional pathway of ER–DNA interaction.

Figure: 4: Tumor Site

A schematic representation of the nanoparticle localisation in solid tumors by the EPR (Enhanced Permeability and Retention) Effect.

Long-circulating nanoparticles, shielded by water-soluble polymer such as poly(ethylene glycol), preferentially accumulate in the tumor mass by extravasation through the fenestrated tumor interstitium.
Figure 5: Biodegradable Nanoparticles

Type of biodegradable nanoparticles: According to the structural organization biodegradable nanoparticles are classified as nanocapsule, and nanosphere. The drug molecules are either entrapped inside or adsorbed on the surface.
Figure 6: Trends in SLN Research

Figure 7: Method of Preparation of Solid-Lipid Nanoparticles:

High Pressure Homogenization (Cold and Hot Homogenization)
Figure 8: Method of Preparation of Solid-Lipid Nanoparticles:

Breaking of o/w microemulsion
Figure 9: Method of preparation of Solid-Lipid Nanoparticles:
Solvent Emulsification-Evaporation or Solvent Emulsification–Diffusion

Figure 10: Method of Preparation of Solid-Lipid Nanoparticles: Solvent Injection
Figure 11: Method of Preparation of Solid-Lipid Nanoparticles:

Preparation via Water-In-Oil-In-Water Double Emulsion (W/O/W)
Figure 12: Method of Preparation of Solid-Lipid Nanoparticles:
High Shear Homogenization And Ultrasound Dispersion

Figure 13: BCS Classification
A typical representation of the biopharmaceutical classification system indicating that absorption of a class II drug can be markedly improved by attention to the formulation. If a class II drug can be maintained in a solublized state in the lumen of the gut one can achieve an absorption profile more like that of a class I drug.

Formulation strategies can do little to improve the absorption of classes I and III drugs which are limited by poor membrane permeability. These are candidates for improvement at the chemical level (i.e. lead optimization).

Figure: 14: Nanocrystal Publication

Figure 15: High Pressure Homogenizer – Piston Gap

Figure 16: Method of Preparation of Nanocrystals
# TABLE 1: COMPARISON OF METHODS OF PREPARATION OF NANOCRYSTALS

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Precipitation | ✅ Finely dispersed drug  
               | ✅ Good control of desired size  
               | ✅ Needs to be stabilized  
               | ✅ Organic solvent residue  
               | ✅ Not universally applicable, only drugs with certain properties are possible (e.g., soluble in at least one solvent) |
| Milling     | ✅ Low energy technique  
               | ✅ Proven by 4 FDA approved drugs  
               | ✅ Residue from milling media  
               | ✅ Can be a slow process (several days)  
               | ✅ Needs to be stabilized  
               | ✅ Large batches difficult to produce due to size of milling chamber |
| Homogenization | ✅ Universally applicable  
               | ✅ No problem with large batches  
               | ✅ Fast method (several minutes possibly)  
               | ✅ Water free production possible  
               | ✅ High energy technique  
               | ✅ Great experience needed |

TABLE 2: MARKETED EXAMPLES OF NANOCRYSTAL PREPARATIONS

<table>
<thead>
<tr>
<th>TRADE NAME</th>
<th>DRUG</th>
<th>INDICATION</th>
<th>APPLIED TECHNOLOGY</th>
<th>COMPANY</th>
<th>STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamune®</td>
<td>Rapamycin</td>
<td>Immunesuppressive</td>
<td>Nanocrystal®élan</td>
<td>Wyeth</td>
<td>marketed</td>
</tr>
<tr>
<td>Emend®</td>
<td>Aprepitant</td>
<td>Anti-emetic</td>
<td>Nanocrystal®élan</td>
<td>Merck</td>
<td>marketed</td>
</tr>
<tr>
<td>Tricor®</td>
<td>Fenofibrate</td>
<td>Hypercholesterolemia</td>
<td>Nanocrystal®élan</td>
<td>Abbott</td>
<td>marketed</td>
</tr>
<tr>
<td>Megace ES®</td>
<td>Megestrol</td>
<td>Anti-anorexic</td>
<td>Nanocrystal®élan</td>
<td>Par Pharmaceutical Companies</td>
<td>marketed</td>
</tr>
<tr>
<td>Triglide®</td>
<td>Fenofibrate</td>
<td>Hypercholesterolemia</td>
<td>IDD-P®Skyepharma</td>
<td>Sciele Pharma Inc.</td>
<td>marketed</td>
</tr>
<tr>
<td>Semapimod®</td>
<td>Guanylhydrazone</td>
<td>TNF-α inhibitor</td>
<td>own</td>
<td>Cytokine Pharmasciences</td>
<td>Phase II</td>
</tr>
<tr>
<td>Paxceed®</td>
<td>Paclitaxel</td>
<td>Anti-inflammatory</td>
<td>unknown</td>
<td>Angiotech</td>
<td>Phase III</td>
</tr>
<tr>
<td>Teralux®</td>
<td>Thymectacin</td>
<td>Anti-cancer</td>
<td>Nanocrystal®élan</td>
<td>Celmed</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

CONCLUSION

Tamoxifen is the drug of choice as an adjuvant therapy for the treatment of breast cancer. Researchers all over the Globe are putting efforts for the Targeted Drug Delivery utilizing Nanoscale Delivery Systems. It is our endeavor to assemble the information concerning the subject till date.

REFERENCE


24. Hall JM, McDonnell DP. The estrogen receptor beta-isofrom (ER beta) of the human estrogen receptor modulates ER alpha transcriptional and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology, 1999; 140: 5566–78.


For Correspondence:
Shivam U Upadhyay
Email: shivampharmacy@gmail.com