2 Literature Review

2.1 Breast cancer

Various heterogeneities have been reported in BC [Polyak 2011] and therefore, for achieving the right prognostic evaluations and for effective treatment, it has been categorized into various types by variety of criteria. These include tumor size, a status of hormone receptor, patient age, histological characteristics (principally invasion in lympho-vascular tissues and histological grades), status of axillary lymph node, and human epidermal growth factor receptor (HER2) status [Yersal and Barutca 2014].

Nowadays, classification, assessment of prognosis and responses to treatments for BC made easy with the development in the areas of molecular techniques, particularly gene expression profiling [Schnitt 2010]. Taking into account the histology of tumors, BC is widely categorized as *in-situ* and infiltrating or invasive carcinoma. Each of this type is further categorized into various subtypes as shown **Figure 2.1** [Ellis et al. 2003, Makki 2015]. This classification system served as a valuable tool for prognosis and treatment of breast cancer for several decades. With context to breast-conserving surgeries, it became mandatory to understand the classification of patients, to determine the relative risk of progression and recurrence, more accurately. Here comes, the evolution of classification system by incorporating molecular markers viz. progesterone receptor, HER2 and p53, estrogen receptor, etc., which are in practice for the cases of Invasive Ductal Carcinoma (IDC); **Figure 2.1(b)** [Bennett and Farah 2014]. Determining the status of these markers not only serves to suggest effective anti-cancer treatment, but also to determine how much the patient is likely to respond to the selected targeted treatment for cancer [Malhotra et al. 2010].

With the advancements in the targeted therapies, the present classification system was insufficient to predict response to recently developed targeted therapies. As a consequence, other molecular approaches such as objective hierarchical clustering and have been used to define and strategies microarray-based gene expression analysis, classify many newer inner molecular subtypes for proper BC molecular classification, unlike conventional negative / positive hormone receptor types [Dowsett and Dunbier 2008, Gusterson et al. 2005, Perou et al. 2000]. The molecular classification was summarized in **Figure 2.2(a)** [Dai et al. 2015b, Malhotra et al. 2010]. In addition to the molecular classification, functional classification system for BC depends on the cancer stem cells (CSC). Although the origin of CSC is not still completely understood, the frequently followed hypotheses are, CSCs either originate from common normal stem cells or arise from normal stem cells in stem cell hierarchy [Malhotra et al. 2010, Stingl and Caldas 2007]. The functional classification is depicted in **Figure 2.2(b and c)**.

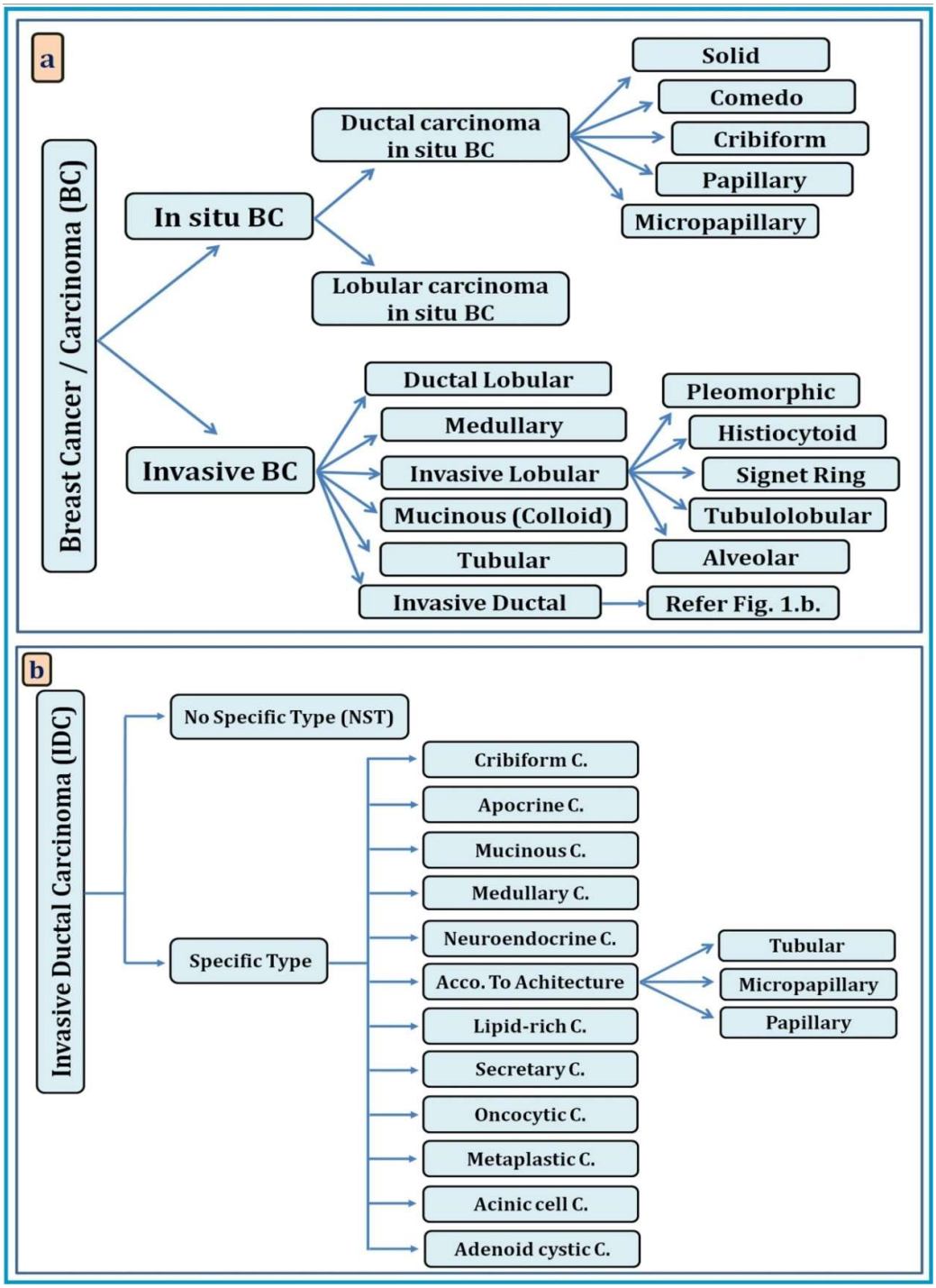

Figure 2.1 Histological classification of BC. (a) The classification of BC is based on the spread of malignant cells in the breast tissue. *In situ* BC remains confined to either duct or lobule whereas invasive BC spreads to adjacent tissues and lymph nodes. beyond the region of origin. Ductal cancer *in situ* is further classified on the basis of architectural features. **(b)** Classification of IDC. The poorly differentiated tumor cell mass of IDC is known as NST type and the other sub-types are properly identified by their peculiar features and classified according to architectural features [Bonde et al. 2018].

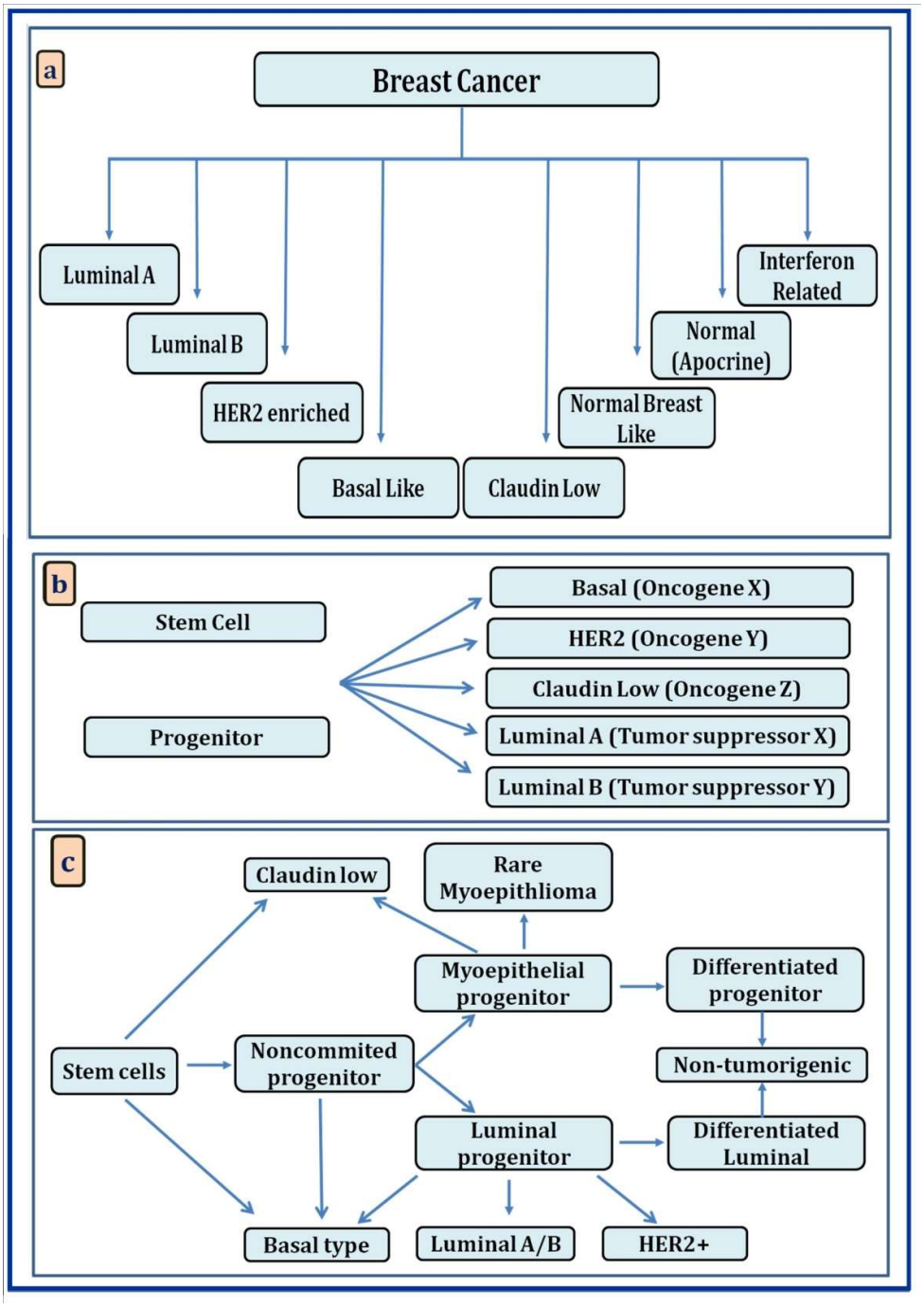

Figure 2.2 (a) Molecular classification of BC on the basis of presence or absence of various molecular markers such as Estrogen Receptor (ER), Progesterone Receptor (PR), Human Endothelial Growth Factor Receptor 2 (HER2), vimetin, E-adherin, etc. forms the basis of molecular classification.; **Functional classification of BC** on the basis of type of tumor initiating cells or the level of stem cells proliferation hierarchy **(b)** BC subtypes originate from a single mammary stem cell or progenitor cell after activation by different oncogenes; **(c)** BC heterogeneity initiate from different mammary stem cells or progenitor cells at distinct levels of stem cells proliferation hierarchy [Bonde et al. 2018].

2.2 Drug and Excipients profile

2.2.1 Lapatinib

It was approved on March 13, 2007 by the United States Food and Drug Administration (USFDA), In conjunction with capecitabine (Xeloda®), for the therapy of mature and metastatic BC, the tumors of which were overexpressed with HER2 and treated with anthracycline, a taxane, and trastuzumab (Herceptin®) [Ryan et al. 2008]. On 29 January 2010, LP was approved for use in conjunction with letrozole in BC patients, particularly postmenopausal women with HER2 overexpressing metastatic BC and for whom hormonal therapy is recommended. [National Cancer Institute, Ryan et al. 2008].

2.2.1.1 Pharmacokinetics

LP is classified as Biopharmaceutical Classification (BCS) Class II drug owing to its extreme hydrophobicity [Custodio et al. 2008, Gao et al. 2014]. LP has been documented to have approximately 99 percent binding preference to plasma proteins like alpha-1 glycoprotein and albumin [Medina and Goodin 2008]. Its poor bioavailability is due to limited dissolution after oral administration. However, approximately 325 percent improvement in bioavailability was observed by concomitant administration with a high-fat diet [Ratain and Cohen 2007][30]. In Phase I trials with 1200 mg oral dose, the pharmacokinetic parameters were noted to be 14.3 h.g/mL (AUC), 4h (t_{max}), 1.22 g/mL (C_{max}) and 0.30 g/mL (C_{min}) in metastatic carcinoma patients [Burris III et al. 2005]. LP metabolizes mainly in the liver, although less than 2 per cent and nearly 27 per cent of the oral dose was discarded by the urine and feces, respectively [van Erp et al. 2009]. The other relevant information is as follows.

Synonym	: Lapatinib ditosylate, Tyverb®, Tykerb®
Chemical Formula	: $C_{29}H_{26}ClFN_4O_4S$
Chemical Structure	: Illustrated in **Figure 2.3**
Molecular weight	: Average molecular weight 581.058 g/mol
Solubility	: Poorly soluble in water (7µg/ml), soluble in methanol and dimethyl formamide
Metabolism	: Primarily in liver
Excretion	: Primarily via feces followed by urine
Clinical use	: Hormone receptor-negative metastatic cancer, Postmenopausal women with hormone receptor positive metastatic cancer.

2.2.1.2 Pharmacodynamics

Lapatinib is one of the members of TKIs family having 4-anilinoquinazoline moiety. It specifically and reversibly binds to the intracellular TK domains of both receptors, HER2 and EGFR resulting in interruptions in PI3K and MAPK signaling pathways. These mechanisms leads to the inhibition of proliferation of cells [Tevaarwerk and Kolesar 2009]. Additionally, LP showed an multidrug resistance reversal activity by inhibiting ABC transporters like P-gp [Dai et al. 2008]. Therefore, LP in combination with drugs showing resistance were investigated and had been shown to be effective in the MDR cancer treatment [Dehghan Kelishady et al. 2015, Hu et al. 2015, Li et al. 2011a, Ravar et al. 2016, Vergara et al. 2012, Wang et al. 2014, Wei et al. 2015b]. LP or its combination with imatinib has also shown to have inhibitory action on EGFR and used in treatment of triple-negative breast cancer (TNBC) as few recent studies have documented an overexpression of EGFR in TNBC defined by all negative ER / PR / HER2 [Hurvitz and Mead 2016, Wang et al. 2015]. The higher dosage contributes to harmful effects such as rashes, extreme diarrhea, and nausea. [Burris et al. 2009] The

increased risk of hepatotoxicity was documented by combination of LP with paclitaxel and capecitabine evidenced by enhanced total bilirubin (more than two folds) and aspartate transaminase (more than three folds) than their upper limit of normal value [Castellino et al. 2011, Dai et al. 2015a]

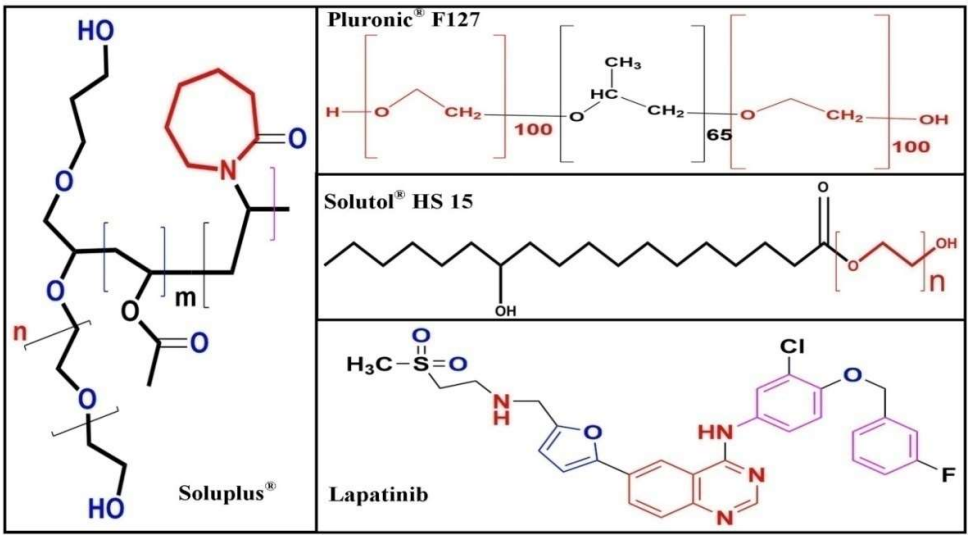

Figure 2.3 Chemical structure of drug and excipients

2.2.2 Soluplus®

Soluplus® is a FDA approved novel amphiphilic polymer [Kou et al. 2018]. Beyond CMC, amphiphilic polymers self-assemble to reduce the interaction of their hydrophobic portion with aqueous fluids and improves their thermodynamic stability decreasing free energy [Alvarez-Rivera et al. 2016]. Through their surfactant behavior, SOL was demonstrated to improve aqueous solubility and bioavailability [Varela-Garcia et al. 2018]. Additionally, SOL, being a surfactant, reduces the surface energy of nanocolloids and avoids the aggregation as well as crystal growth of insoluble drug [Hou et al. 2016]. The information regarding SOL is as follows

General : Novel amphiphilic polymer developed by BASF. It is water soluble free flowing yellowish or white granules [Kou et al. 2018].

Description : It is a triblock grafted polymer consisting of polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol 6000 (57:30:13). Chemical Structure is illustrated in **Figure 2.3.**

Molecular weight: 90,000 to 140,000 g/mol (average)

Solubility : Soluble in water, ethanol (<25%), methanol (<45%), acetone and dimethylformamide (<50%).

CMC : 7.6×10^{-3} mg/mL in water [Singh et al. 2018, Yu et al. 2013].

Glass transition temperature: 70 °C

Pharmaceutical application: Solubilizer, surfactant, excellent excipient for hot-melt extrusion and solid dispersion [Alam et al. 2012, Kou et al. 2018, Nasr et al. 2018, Pignatello and Corsaro 2019, Sarabu et al. 2019]

2.2.3 Kolliphor^{TM} HS 15

It is known as Solutol HS 15. It is used as a co-biomaterial for micelles preparation due to its ability to provide sustained release, to improve stability in aqueous dispersion owing to its long polyethylene oxide (PEO) chains and circulation time of drug [Meng et al. 2017, Senthilkumar and Dash 2019] and its use in anticancer therapy was also identified [Pellosi et al. 2017]. The information regarding SHS is as follows [BASF]

Synonym : Ployoxyl 15 Hydroxystearate, Macogol 15 Hydroxystearate

General : Novel amphiphilic polymer developed by BASF.

Description : It is a synthesized from monomers, ethylene oxide and 12-hydroxy stearic acid (15:1 molar ratio) and its chemical Structure is illustrated in Figure. It is yeelowish white paste at room temperature and liquefies above 30 C. Chemical Structure is illustrated in **Figure 2.3**.

Molecular weight: average molecular weight of 90,000 to 140,000 g/mol

Solubility : Soluble in water, 2-propanol and ethanol

CMC : 50-200 mg/mL in water

Pharmaceutical application: Solubilizer, emulsifier.

2.2.4 Kolliphor® 407

It is commonly known as Pluronic® F127 (PF127) is a member of poloxamer family of polymers developed by BASF. It is made up of approximately 70% ethylene oxide that imparts hydrophilicity to the polymer. Its dispersion in aqueous medium (20 - 30% w/v) possess the significant feature of reverse thermal gelation i.e. they are transformed into gels upon warming while remains liquid in lower temperature range (4-5°C) [Srivastava et al. 2017]. PF127 is perhaps more soluble in cold water than that in warm water owing to enhanced hydrogen bonding and salvation at lower temperatures. The observed inhibition of MDR proteins, other efflux transporters expressed on cancer cell surface and the inhibition of ATP production which made them suitable for the MDR cancer cell targeting therapy. Poloxamers also possess cancer cell targeting properties due to the differences in the membrane properties of cancerous and non-cancerous cells. Recently, it is been reported to use as a co-material for the formulation of binary micelles for the enhancement of drug solubility and controlled / sustained drug delivery of hydrophobic anticancer drugs [Baidya et al. 2019, Manjappa et al. 2019]. The information regarding PF127 is as follows [BASF]

Synonym : Pluronic® F127. Lutrol® F127, Poloxamer 407

General : Novel nonionic amphiphilic surfactant

Description : It is triblock co-polymer comprising of polyoxyethylene-polyoxypropylene-polyoxyethylene monomer in ratio 101:56:101. It is

in the form of waxy white flakes. Chemical Structure is illustrated in **Figure 2.3**.

Molecular weight: average molecular weight of 12,600 g/mol

Solubility : Soluble in water, methanol and ethanol.

CMC : 5.43 g/mL in water

Pharmaceutical application: Solubilizer, emulsifier, viscosity builder, thickening agent, excipients for *in-situ* thermoreversible gels.

2.3 Nanocarrier drug delivery systems

Developments in nanotechnology have culminated in various forms of nanocarriers with certain excellent characteristics that are intended to resolve pitfalls of conventional DDS such as safety, decreased bioavailability, low aqueous solubility, limited targeting efficiency, etc. [Gothwal et al. 2016]. **Table 2.1** enlists the peculiar characteristics of nanocarriers that have attracted significant attention for drug delivery of hydrophobic drugs [Bazak et al. 2015, Bozzuto and Molinari 2015, Deshpande et al. 2013, Fang et al. 2004, Gao et al. 2014, Gao et al. 2012, Hu et al. 2015, Huo et al. 2015, Li and Huang 2009, Wei et al. 2015b]. The nanocarriers encompass nanocapsules, nanoparticles (NPs), nanocolloids like polymeric micelle (PM), binary micelles (BMs), etc.; schematic structures of different nanocarriers are illustrated in **Figure 2.4.** The surface of nanocarriers can be decorated with specific ligands for active targeting of tumors. The concept is based on implementation the ligand-receptor interaction between receptor on cell surface and ligands, complementary to receptors, contained on the surface of DDS [Bazak et al. 2015]. Nano sizes of these carriers, on the other hand, allow them to easily cross the leaky vasculature of tumors for passive targeting.

[Deshpande et al. 2013, Fang et al. 2004]. 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. In the light of such benefits various nanocarrier systems like nanoparticles, nanocpasules, liposomes, nanocomposites, PMs were investigated for the delivery of LP [Bonde et al. 2018]. However, PMs and BMs were chosen as DDS for LP for the present research.

Table 2.1 Characteristics of nanocarrier systems and purpose to use for drug delivery [Bonde et al. 2018]

S. No.	Characteristics	Purpose
1.	Size (approx. upto 200-250 nm)	For passive targeting via enhanced permeation and retention effect [Deshpande et al. 2013, Fang et al. 2004, Wei et al. 2015b]
2.	Surface Charge induction	For higher uptake in cancer cell [Huo et al. 2015], to escape from RES [Li and Huang 2009] and for prolonging systemic circulation time [Bozzuto and Molinari 2015]
3.	Surface engineering with ligands or antibodies	For active targeting [Bazak et al. 2015] and minimize side effects to un-intended organs [Wei et al. 2015b]
4.	Core-Shell Structure especially in nanocapsules, micelle and some Nanoparticles	For achieving higher drug loading and enhance solubility of hydrophobic drugs particularly BCS Class II drugs [Gao et al. 2014]
5.	Sustained or controlled delivery	For minimizing total drug dose [Gao et al. 2012], increasing therapeutic efficacy with less side effects of drug [Hu et al. 2015]
6.	Encapsulation of drug	For avoiding systemic drug loss due to first pass metabolism and plasma protein binding leading to enhanced bioavailability [Huo et al. 2015]

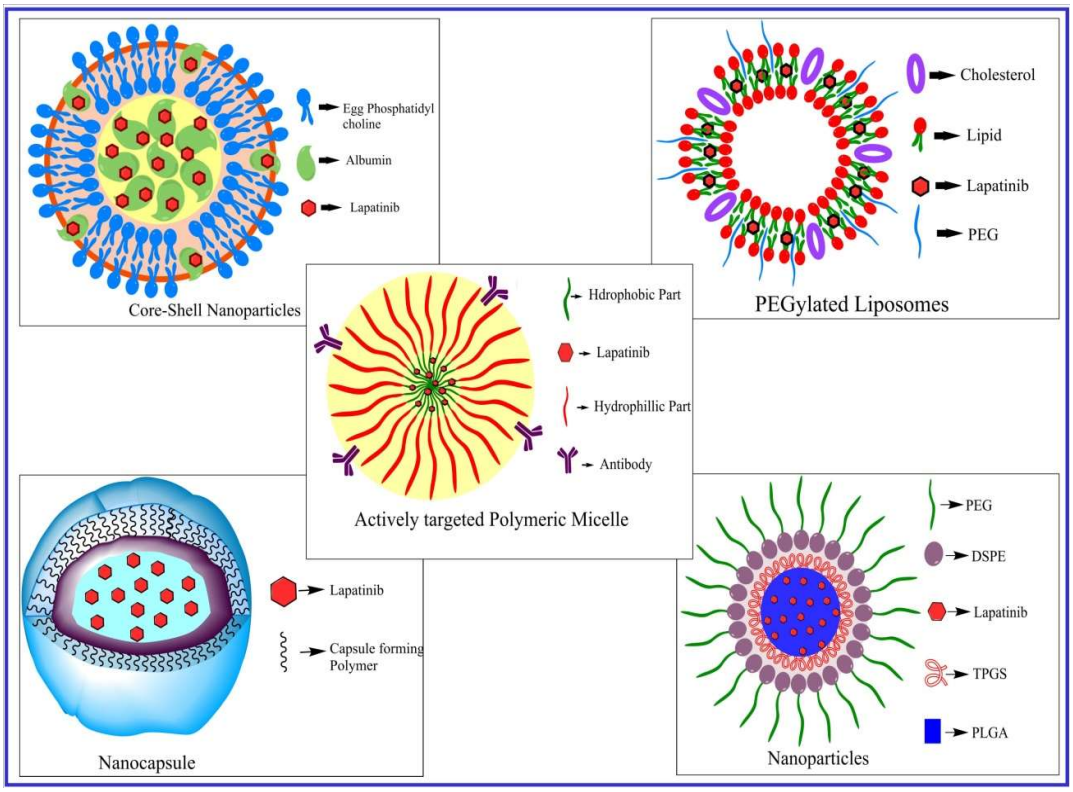

Figure 2.4 Schematic diagrams depicting different nanocarrier drug delivery systems used for delivery of Lapatinib [Bonde et al. 2018]

2.3.1 Nanocolloidal Micelles as DDS

PMs is one of the example of nanocolloidal systems that have been widely investigated and shown to be most attractive nanocarrier for the improvement of bioavailability and solubility of hydrophobic drugs, especially anti-cancer drugs, by the virtue of their peculiar core-shell architecture [Guo et al. 2016, Kandekar et al. 2019]. The core-shell formation of PMs consists of hydrophilic shell while core is hydrophobic in nature [Dehghan Kelishady et al. 2015]. Hence, hydrophobic drug seats inside the corona whereas hydrophilic corona that isolates the drug from surrounding aqueous environment. This peculiarity of PMs promotes the entrapment of hydrophobic drugs through solubilization, whereas the hydrophilic corona establishes hydrogen bonding with the aqueous surroundings and increases durability of the PMs [Yoncheva et al.

2012]. Moreover, encapsulation of drug inside PMs core provides protection against protein binding and stability over instant dilution draws significant attention of research arena for their systemic administration [Dian et al. 2014]. In fact, the exterior hydrophilic layer allows PMs to prolong blood circulation time circumventing the detection and phagocytosis by a reticuloendothelial system (RES) [Bonde et al. 2018, Majumder et al. 2020, Zhang et al. 2014]. In addition to stability, hemocompatibility and decreased toxicity are primary factors to decide the employability of parenteral products [Jafarzadeh-Holagh et al. 2018, Sun et al. 2015]. On the other side, their nano-size (< 200 nm) reveals their outstanding therapeutic ability for cancer therapy owing to their tumor-targeting capability by enhanced permeability and retention (EPR) behavior [Deshpande et al. 2013, Fang et al. 2004, Huo et al. 2015, Majumder et al. 2020]. In this way, the delivery of drugs to healthy tissues is reduced and the resulting risk is decreased. Through literature search, it had been noted that various research groups are investing the employability of PMs for the delivery of plethora of drugs for the treatment of diabetes, autoimmune diseases, cancer and many more.

Beyond conventional PMs, some research groups prepared micelles by combination of two or more polymers for the preparation of binary micelles (BMs) or mixed micelles. These have been shown to offer better kinetic and thermodynamic stability with enhanced drug encapsulation and stability [Cagel et al. 2017]. Further, BMs possess the ability to provide sustained release, to improve stability in aqueous dispersion [Meng et al. 2017, Senthilkumar and Dash 2019] and its use in anticancer therapy was also identified [Pellosi et al. 2017]. Some of the recent uses of PMs and BMs as DDS are discussed below.

Recently, Cao et al. exploited polymeric mixed micelles for intracellular drug delivery and for reversing the multi drug resistance in breast cancer. The research group

prepared mixed micelles of hyaluronic acid–deoxycholic acid–histidine and Pluronic F127 (PF127) and further functionalized with endocytosis mediated via CD44 receptor. The mixed micelles were loaded with DOX. The drug loaded mixed micelles showed pH triggered drug release in an endosomal acidic environment. Further, the better anticancer activity was evidenced by enhanced cytotoxicity and superior MDR reversion performance against drug-resistant MCF-7/Adr tumor cells. In conclusion, the functionalized mixed micellar system might serve as a better intracellular delivery of DOX and better treatment of MDR of breast cancer [Cao et al. 2019].

Jiang et al. synthesized a novel diblock copolymer and conjugated it with DOX which were further loaded with PTX. The high drug loading, reported in the study, supports the fact hydrophobic interactions of core with the hydrophobic molecules like PTX could efficiently enhance their encapsulation and loading in micellar form during the process of self-assembly of micelle formation. The micelles showed high serum stability pH dependent drug release evidenced by the pH-triggered drug release profiles. The size of the micelles were approximately 110 nm that are suitable for EPR effect and passive targeting. The research group investigated the anti-cancer efficacy of the prepared micelles against several cell lines. Their research findings indicated high cytotoxicity of the prepared micelles containing DOX and PTX as compared with free drugs and control. Conclusively, the study showed the high efficiency of polymeric micelles for high drug loading of hydrophobic drugs and implications for their use in enhancing therapeutic efficacies of anti-cancer drugs [Jiang et al. 2020].

Recently, Lu et al. also demonstrated the higher encapsulation, anti-cancer efficacy with low systemic toxicity of docetaxel by encapsulating it in a micelles prepared from a novel polymer, PEGylated triacontanol. PMs had a size of approx. 93.7 nm and drug encapsulation efficiency of approx. 90%. The drug release studies showed the

sustained release and prolonged systemic circulation time. Furthermore, results of in vivo tumor inhibition studies indicated that PMs exhibited better anti-tumor activity with low systemic toxicity. The research findings implied that PMs could efficiently be exploited to enhance encapsulation efficiency of hydrophobic drugs and can serve as a promising carrier for them [Lu et al. 2020].

The discussion henceforth throws light on some of the nanocolloidal PMs based formulation approaches that were investigated for the successful delivery of LP.

2.3.2 Polymeric Micelles:

Lapatinib was also reported to benefit for reversal of multidrug resistance. Wang *et al*. prepared PMs for entrapment of doxorubicin (DOX) and LP combination, poorly-water soluble drugs, for the treatment multidrug resistant (MDR) BC. They synthesized Poly(ethylene glycol)-block-poly (2-methyl-2-benzoxycarbonylpropylene carbonate) (PEG-PBC) polymer and prepared PMs by using film dispersion method. The low CMC value (1.5 mg/L) of a polymer suggests good dynamic stability of drug loaded PMs. The PMs were of 100 nm size with spherical shape. The results of *in-vivo* anticancer efficacy studies, in drug resistant MCF-7/ADR cells tumor model, showed that the combination of LP and DOX was very potent in inhibition of cancer tumors than DOX free and DOX loaded PMs. Hence, It may be inferred that LP greatly improved the anticancer function as well as the intracellular aggregation of DOX alluded to in the literature for inhibition of P-gp and BCRP by LP [Wang et al. 2014].

Similar finding were reported Wei *et al*. where they investigated combination of Paclitaxel (PTX) and LP. The higher resistance against PTX was due to of HER-2 overexpression in cencer cells [Yu et al. 1996]. LP effectively enhances tumor sensitivity to PTX, evidenced in phase II and III clinical trials [Dai et al. 2008, Di Leo

et al. 2008, Jagiello-Gruszfeld et al. 2010]. For improved anticancer effectiveness against HER2-positive breast cancer by synergistic activity, they synthesized a polymer with poly(ethylene glycol) (PEG) and poly(lactic acid) (PLA) and conjugated LP to it, to improve LP entrapment in micelle. Successful conjugation was confirmed by matrix-assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF MS) and proton nuclear magnetic resonance (PNMR). The drug loaded PMs was of 25.80±0.47 nm size and had zeta potential -3.17±0.15 mv indicating partial neutralization of the surface negative charge as compared to PTX loaded PMs. The EE was found to be 84.21±9.83%. The synergistic effect of both the drug was demonstrated by significant decrease in IC_{50} values of combination than any single drug. Further, they found enhanced apoptosis when the cancer cell lines treated with combined micelle as compared individual drug loaded micelles. So, it can be corroborated that polymeric micelle of combined drugs could serve the better DDS for HER-2 positive breast cancer treatment [Wei et al. 2015b].

Similar approach was used by Li *et al*. 2011 and they synthesized the diblock PEG polymer with poly(2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol) (PEG-PCD). They prepared LP and PTX-loaded PMs for MDR-prostate cancer treatment. Surprisingly, PMs showed almost 100 % EE and had 60 nm diameters. The significant reduction cell viability (nearly 35%) and IC_{50} value in cell cytotoxicity assays indicated the better reversal of MDR for PTX when treated with combination with LP, inferring that LP can inhibit MDR transporters and sensitize the cancer cell the other chemotherapeutic agent. The fact was again confirmed by 60 fold improvement of intracellular intensity of fluorescence, in Calcein AM assay, achieved only in combination of LP and PTX micelles, showing inhibition of P-gp in presence of LP. Furthermore, the increased sub-G1 cell population indicated the effectiveness of

combination therapy for induction of apoptosis in cancer cells. The results were supported by significant inhibition in tumor growth and angiogenic activity post-treatment with combination (5mg/kg each of LP and PTX) in comparison to PTX (10 mg/kg) monotherapy. [Li et al. 2011a].

Kelishady *et al*. attempted to prepare PF127 micelles and load them with deliver both, LP and PTX. The optimized micelle had a diameter of approx. 64.81 nm and shown to have high EE for LP (70.1%) and PTX (68.3%). The prolongation of circulation time was attributed to the smooth surface and spherical shape of the micelles due to escape from RES. Further, biphasic *in-vitro* release profile for both the drugs was observed. The slower LP release was thought to be due to more hydrophobicity of LP than PTX, and therefore more hydrophobic interactions of LP with the hydrophobic core of the micelle reducing its release than PTX. The higher cytotoxicity of the combination was deduced to be due to: First- the sensitizing action of LP on reflux transporters increasing intracellular PTX concentration and Second- direct uptake of micellar systems via endocytosis avoiding contact of bare drugs to transporters in cellular domain. In addition to this, PF 127 also facilitates the cytotoxicity of the drug owing to its established ATP depletion function in cancer cells, which prevents P-gp efflux development. [Alakhova and Kabanov 2014, Guan et al. 2011]. Thus, the developed PF127 micelle may provide efficient and safe nanocolloidal drug delivery for hydrophobic drugs like LP and PTX [Dehghan Kelishady et al. 2015].

Recently, Guo *et al.* synthesized a novel a polycarbonate-doxorubicin conjugate to prepare polymeric micelles that can be loaded with LP. The prepared micelles were spherical with 112 nm diameter. The micelles showed a pH triggered enhanced internalization of micelles and tumor penetration at acidic tumor environment. Further,

enhanced release of DOX and LP from the pH-sensitive core of micelles in the acidic tumor environment and evidenced the enhanced inhibition of the proliferation and migration, of 4T1 cells in vitro. The findings were then supported by the 90.2% inhibition rate of tumor growth in 4T1 xenograft-bearing mice and showed significant reduction in pulmonary metastatic nodules, without significant systemic toxicity. Therefore, the research findings of the study supports the use of micelles as an efficient nanocarrier system to enhance the antitumor efficacy, significant inhibition of tumor metastasis with minimum systemic drug toxicity [Guo et al. 2020].

2.3.3 Mixed / Binary Micelles

Two or more polymers can be used for the fabrication of micelles to add on the addition of their individual characteristics. These are generally referred as hybrid micelles or mixed micelles or binary micelles (using combination of two polymers) in literature. Hybrid micelles were reported to be one of the efficient methods for enhancing the stability and solubility of hydrophobic drugs [Chen et al. 2020].

Chen *et al.* formulated hybrid micelles, based on this principle, using combination of SOL, D-a-tocopherol acid polyethylene glycol 1000 succinate (TPGS) and dequalinium. The group attempted the delivery of Hinokiflavone (HF), a natural anti-cancer drug, having poor solubility and bioavailability. Prepared hybrid micelles showed high EE due to formation of hydrogen bonds between hydroxyl group of HF and carbonyl group of SOL which further could explain the enhanced stability of micelles. Higher in vitro cytotoxicity and in-vivo ani-cancer efficacy was attributed to the prologation of circulation of time, high EE of hybrid micelles. Little toxicity was observed to the other organs in histo-chemical investigations compared with free HF.

Therefore, the group suggested the hybrid micelles could be better nanocarrier for hydrophobic drugs [Chen et al. 2020].

Similar attempt was done by Feng *et al.* where they employed binary micelles prepared from SOl and TPGS. Surprisingly, the EE was almost 99% for Amentoflavone, a poor soluble and bioavailable drug candidate. The BMs had size of 67.33 ± 2.01 nm and found to be stable for 60 d. Further, results concluded that prepared BMs had higher bioavailability and cytotoxicity as compared to the drug. The metabolites of drug were detected mainly in rat feces and absent in bile and plasma, post-administration of only the drug. However, administration of BMs showed the presence of some metabolites of drug in blood and urine, indicating their enhanced distribution through blood, indicating the enhanced bioavailability by using BMs [Feng et al. 2020].

Solutol® HS15 is one of the amphiphilic polymer used for the preparation of mixed / binary micelles. Yan et al. employed the BMs of SOL and lecithin for the delivery Baohuoside I, a potent but a poorly soluble anti-cancer drug, to non-small cell lung cancer (NSCLC) A549 cells. BMs had approximately 62.54 nm diameter with narrow size distribution. The enhanced cellular uptake and more potent anti-proliferative action of BMs were evidenced from decrease in IC_{50} value by one fold on A549 cancer cell line than drug itself. Moreover, in-vivo imaging and anti-tumor activity in nude mice demonstrated higher targeting and anti-tumor efficacy of BMs. Therefore, the study indicated not only improving the solubility and targeting but also efficient anti-tumor activity [Yan et al. 2016].

On the similar basis, combination of SHS and Pluronic® F68 was used to fabricate baicalein-loaded BMs by Shen *et al.* to improve oral bioavailability of drug. The formulation was optimized by QbD approach and observed to have size about 23.14 nm

and EE of 93%. BMs showed the sustained release of drug over the duration of *in-vitro* release studies. After *in-vitro* permeability studies in Caco-2 cell showed higher permeability of drug after encapsulation it into BMs which is further supported by 3.02 fold improvement in relative oral bioavailability compared with post-oral administration of drug itself. Conclusively, BMs prepared from SHS and Pluronic® F68 has demonstrated great potential for enhancing solubility and oral bioavailability [Shen et al. 2019].

A search through literature reveled that, in spite of various known advantages, very few attempts were done for development of LP loaded PMs/BMs using novel amphiphilic polymers that are already available in the market these days with established excipient profiles. The observed lacuna inspired the theme of research.