1 Introduction

Worldwide the cancer is the second most prominent cause of death, contributes high rate of morbidity and mortality. The new cancer case burden was 18.1 million in 2018 with estimation of 21.4 million cases in 2025 [Bray et al. 2018, WHO 2018]. Age standardized mortality showed that there were approximately 126 cancer deaths for every 100,000 men, and 83 for every 100,000 women in the world excluding non-melanoma skin cancer [Ferlay et al. 2015]. The most common cancers include are lung, liver, colorectal, stomach and breast cancer according to their order of prevalence. The breast cancer (BC) is most frequently detected form of cancer in women and has taken second place in women cancers and fifth place in all cancer. Standing on second rank, 2.08 million new BC cases were reported in 2018 and about 2.4 million cases are predicted in 2025. It is responsible for 6.53 % of total 9.6 million cancer deaths in 2018 [WHO 2018].

Breast cancer can be categorized on the basis of tumor size, a status of hormone receptor, patient age, histological characteristics (principally invasion in lympho-vascular tissues and histological status), status of axillary lymph node, and human epidermal growth factor receptor (HER2) status [Yersal and Barutca 2014]. The expression status of various molecular markers viz. progesterone receptor, HER2 and p53, estrogen receptor, etc. can be used for identification of BC type and selection of appropriate drug treatment [Bennett and Farah 2014]. HER2-positive BC, constituting about 30 % of BC cases, is highly metastatic and may spread to nearby organs like lungs, brain, etc. [Wei et al. 2015a].

Numerous therapies for BC management are available in the recent times. Conventional treatments are categorized as local therapies combined with either surgery or

radiotherapy, whilst systemic therapies entail hormonal therapy, gene therapy, and chemotherapy [Abdulkareem and Zurmi 2012, Kaliberov and Buchsbaum 2012]. Such treatments are either used as a single treatment or as a mix of two or more to treat BC effectively, depending upon progression stage of cancer [McDonald et al. 2016].

With the advent of new drugs and innovative drug delivery strategies, the paradigm is changing now from "same for everyone" care to the "targeted or customized" care era [Ligresti et al. 2008, Marmé and Schneeweiss 2012]. Targeted therapies follow the principles of, either passive targeting or active targeting. Drug targeting is achieved through the design of a receptor-complementary drug [Higgins and Baselga 2011, Munagala et al. 2011]. Certain drugs were developed to target the receptors like HER2, Epidermal Growth Factor Receptor (EGFR) are over expressed by BC cells [Wieduwilt and Moasser 2008]. They include kinase inhibitors like Lapatinib (LP), Gefitinib, Neratinib, monoclonal antibodies like Trastuzumab and Pertuzumab, etc.[Lee and Nan 2012]. For the present study, we used LP as an anti-cancer drug for formulation of nanocolliodal carriers.

Further, these drugs can be targeted to the tumor in the form of nanocarrier based drug delivery systems (DDS) by either passive or active targeting strategies to ensure enhanced concentration of drug at the desired site of action. Passive targeting of nanocarriers can be achieved by exploiting their nano size via enhanced permeation and retention (EPR) effect [Deshpande et al. 2013]. Whereas, active tumor targeting comprises of drug carriers that facilitate the drug delivery to cancerous cells by leveraging the concept of receptor-ligand interaction between unique receptors on the surface of a tumor cells and ligand harbored to the surface of DDS [Bazak et al. 2015]. It is well accepted fact that the cells lining the vasculature of the tumors lack tight intercellular junctions and the larger gaps are present. This leaky vasculature can allow

higher permeation of the nanocarriers (<200 nm size) into the tumor which results in the higher accumulation of nanocarriers locally in the tumor; the effect is called as EPR effect. Hence, the method of such targeting by the virtue of inherent characteristic DDS is known as passive targeting and used such passive targeting principle for targeting of nanocolloidal DDS to the tumors is used in this study

Lapatinib is a BCS Class II drug and dual tyrosine kinase inhibitor of both EGFR and HER2 receptors over expressed on surfaces of BC cells. Tykerb ® or Tyverb ® is a unique tablet medication form developed by GlaxoSmithKline (a pharmaceutical company). LP has also been demonstrated to suppress the activity of ABC carriers like P-gp, playing a significant role in drug resistance. [Dai et al. 2008]. It is better at drug resistance reversal and apoptosis induction of tumor cell as compared to monoclonal antibodies which have specificity to either EGFR or HER2 [Xia et al. 2002].

However, the clinical use of LP is still limited and loses patient compliance due to its few undesirable pharmaceutical characteristics. The major obstacle in formulating its delivery system is its poor aqueous solubility (7µg/ml) due to higher hydrophobicity (logP 5.45) [Bonde et al. 2018]. Therefore, the restricted dissolution of drug in gastro-intestinal fluids leads to poor and erratic absorption and low bioavailability after oral administration [Gao et al. 2014]. In turn, most of the part of total drug administered orally is primarily excreted unchanged via feces and get absorbed to the minor extent through GIT. To achieve desired effective drug concentration in blood, a high dose (1250 mg/day) of LP has to be administered, which results in multiple side effects like rashes, nausea and extreme diarrhea [Burris et al. 2009]. Unfortunately, about 99 percent binding ability of LP to alpha-1 glycoprotein and albumin in the blood stream was also recorded, which further decreases the amount of free drugs at the site of activity. [Medina and Goodin 2008]. The regardless oral route, LP is also failed as an

injectable therapeutics as the addition of surfactants like Cremophor EL, Tween 80, etc. unable to improve the aqueous solubility and is insoluble in mostly used solvents for injectables [Gao et al. 2014]. Therefore, LP throws a significant challenge before pharmaceutical fraternity to develop an appropriate dosage forms, enhance therapeutic value and enhance the patient compliance regarding daily higher dose for a long period.

All of this factual information necessitates an uprising in drug delivery technologies with regard to

1. Bypass adsorption of LP to plasma proteins via encapsulation
2. Develop the parenteral DDS for systemic delivery
3. Target the cancer tumors precisely either through passive or active targeting
4. Enhance the bioavailability.

For the present research, attempts had been made for encapsulation of drug, targeting the tumors by passive methods and its propriety for intravenous route.

The design and development of LP nanocarrier systems is a less studied field. Nonetheless, over the last few years, there have been few nanocarrier systems viz. polymeric microstructures, polymeric micelles (PMs), nanoparticles, nanocapsules, etc. have been investigated for encapsulation of LP and its targeted and sustained delivery to tumors [Bonde et al. 2018, Jeong et al. 2015, Ravar et al. 2016]. The core-shell structure of PMs consists of hydrophilic shell while core is hydrophobic in nature [Dehghan Kelishady et al. 2015]. Hydrophobic drug gets enveloped inside the core of PMs. Various benefits provided by PMs, like improving bioavailability, higher stability,

hemocompatibility, etc. have attracted the attention of pharmaceutical arena to employ them as a nanocarrier for hydrophobic anti-cancer drugs [Bonde et al. 2018, Dian et al. 2014]. Consequently, LP was predicted to be contained within the hydrophobic interior of the micelle and thereby facilitate its aqueous solubility and achieve passive targeting post-intravenous administration. The above-said facts form the basis of selection of delivery strategy for LP for this research work.

Amphiphilic polymers, consisting of both hydrophobic and hydrophilic parts, have a characteristic to form micelles. Nowadays, various novel amphiphilic polymers were synthesized with higher molecular weight, e.g. multiple grades of poloxamers, soluplus®, solutol®, etc. These polymers self-assemble on contact with aqueous fluids to diminish the interaction with the surrounding aqueous environment and achieve thermodynamic stability. Soluplus® is an FDA approved novel amphiphilic triblock polymer consisting of polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol [Kou et al. 2018]. Recent evidence proved them as a promising nanocolloidal/micellar nanocarrier for hydrophobic drugs to exhibit both improved bioavailability and dissolution profiles [Grimaudo et al. 2019, Hou et al. 2016, Hu et al. 2017]. Over the critical micellar concentration (CMC) Soluplus® appears to self-assemble forming nanocolloidal micelle instantly in an aqueous fluids to decrease free energy [Alvarez-Rivera et al. 2016]. Investigations in the past decade have demonstrated their higher ability for the improvement in aqueous solubility [Varela-Garcia et al. 2018] and sustained release of poorly water soluble drugs [Xia et al. 2016, Yu et al. 2013, Zeng et al. 2017]. Additionally, Soluplus®, being a surfactant, avoids the aggregation as well as crystal growth of insoluble drug [Hou et al. 2016].

Beyond PMs prepared by using one polymer, the combination of two polymers for the preparation of binary micelles (BMs) has been shown to offer better kinetic and

thermodynamic stability with enhanced drug encapsulation and stability [Cagel et al. 2017]. For the purpose, we selected Solutol® HS15 (SHS) as co-biomaterial 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].

During the formulation development, conventional methods of optimization are time consuming and require huge number of trials. Therefore, for the efficient use of time and resources advanced principles of design of experiments (DoE) are used to reduce the number of trials with efficient optimization of formulation in view of required characteristics and performance. The optimization of experiment is based on proper selection of various attributes and their levels. Necessary steps of experimentation includes objective definition, identification of critical parameters, screening and selection of influencing factors, design of experiments, analysis and interpretation of results and optimization of the product as per desired attributes (10, 11). For the purpose of present research, optimization of LP-loaded nanocarriers was performed by response surface methodology (RSM) based Box-Behnken Design (BBD) preceded by desirability approach based on numerical optimization technique.

By considering all these evidence, the present research was aimed to exploit the promising benefits of the micellar nanocolloidal platform for the efficient delivery of LP, and fulfilling both pharmaceutical and clinical needs for efficient LP delivery for effective treatment of breast cancer. Preformulation experiments involved the determination of the thermodynamic stability of nanocolloidal PMs dispersion through the measurement of CMC of the polymer and its mixture. The compatibility of drug and

excipients was evaluated by Fourier Transform Infra-Red (FT-IR) analysis. Micelles were fabricated by employing thin-film hydration method.

Further, this research work was divided in two parts; first part comprises of development of PMs by using only Soluplus® whereas second part consists of preparation of binary micelle (BMs) using combination of Soluplus and Solutol HS 15. The optimization of PMs was performed by using Quality-by-Design (QbD) approach based on appropriate choice of dependent and independent variables. Both the formulations were then subjected to various physico-chemical investigations, encapsulation efficiency, drug loading and *in*-vitro drug release studies. Further, various *in-vitro* studies were carried out to demonstrate *in-vitro* anti-cancer potency and hemocompatibility of prepared formulations. Pharmacokinetic and pharmacodynamic experiments were performed in the last part to examine the *in-vivo* efficiency of prepared LP-loaded nanocolloidal DDS.