1 Introduction

Breast Cancer, second leading cause of cancer death, is a major concern of health professionals in today's era (Bray et al., 2018). Though plethora of chemotherapeutics available for the treatment of breast cancer these often suffered from various drug and dosage form related problems. Vinorelbine bitartrate (VRL) is a semi-synthetic vinca alkaloid showing significant anticancer activity against various tumors such as breast cancer, non-small cell lung cancer (NSCLC), ovarian cancer and exhibits antitumor activity through disrupting microtubules. However, VRL is better tolerated among all vinca alkaloids owing to reduced neurotoxicity which can be attributed to its lesser affinity for axonal microtubules (Drummond et al., 2009, Vassilomanolakis et al., 2001, Wan et al., 2008, You et al., 2007, Zhang et al., 2008). VRL is a white to yellow amorphous powder with molecular weight of 1079.11 g and aqueous solubility more than 1 g mL^{-1} (Zhang and Ahmad, 2004). However, the associated toxicities like neutropenia (main dose-limiting toxicity), nausea, vomiting, diarrhoea, constipation, alopecia and peripheral neuropathy poses difficulties in its clinical translation. The marketed formulation, with the brand name Navelbine (i.v infusion), demonstrated serious venous irritation due to its vesicant nature and displayed injection site reaction, superficial phlebitis accompanied by erythema, pain, vein discoloration and tenderness along the vein (Emanuela et al., 2018, Kreidieh et al., 2016, Vassilomanolakis et al., 2001, You et al., 2007). An oral soft gelatin capsule encapsulating VRL was also developed which demonstrated low bioavailability (33 – 40 %) (Bougaret et al., 2005, Goa and Faulds, 1994). Moreover, the severity of the adverse effects with oral administration of soft gelatine capsule was found to be greater than that of *i.v.* counterpart (Li et al., 2012).

The major dose dependent toxicities associated with VRL can be overcome by reducing the dose and dosage frequency which can be achieved by formulating controlled release dosage forms or combining VRL with a non toxic anticancer drug or both. The single drug based anticancer therapies are seldom effective owing to associated multiple genetic alterations and molecular abnormalities(Mokhtari et al., 2017). The treatment of cancer can be made more effective by using different drugs in combination. The combination of drugs can also eliminate the drug resistance owing to non-overlapping mechanisms. Therefore, an attempt has been taken to combine VRL with another anticancer drug Resveratrol (RES) for better treatment of breast cancer in addition to reduce dose dependent side effects associated with VRL. Resveratrol (trans-3,4,5-trihydroxystilbene, RES), a naturally occurring polyphenol compound, is found in a variety of food sources such as grapes, mulberries and peanuts. RES is already proved to reduce VRL induced vascular endothelial cell injury by reduction in cell apoptosis, intracellular ROS generation and the intracellular SOD. RES has been demonstrated to possess a wide array of pharmacological properties, such as estrogenic, antiplatelet, anticancer and anti-inflammatory effects (Zhang et al., 2013). It was also reported to display anti-oxidative and chemopreventive effects via activation of Nrf2 and consequently GSH expression. Moreover, the poly-mechanistic resveratrol (blocks survival and anti-apoptotic mechanisms or cause DNA degradation, as a result of pro-oxidant action) can sensitize cancer cells, which may leads to synergistic anticancer activities when combined with other chemotherapeutic agents or cytotoxic compounds (Kou et al., 2013).

It has been proved that combination of anticancer drugs can act synergistically, additively or antagonistically depending on the ratio of the agents being combined therefore ratiometric combination of drugs have gained wide attention in recent times (Ascierto and Marincola,

2011, Ashley et al., 2016, Chou, 2010, Lu et al., 2015, Mayer et al., 2006, Pavillard et al., 2001, Saputra et al., 2018, Tolcher and Mayer, 2018, Zacharchuk, 2018). However, it is not feasible to analyze all combinations in human subjects so the current scenario utilizes *in-vitro* synergy rather than *in-vivo* anti-proliferative studies. The synergy among the drugs can be estimated with the help of Chou Talalay method which is based on median effect principle. The combination index (CI) can be determined with the help of CalcuSyn software (Biosoft, Ferguson, MO, USA). According to Chou Talalay principle CI <1, CI=1 and CI>1 represented synergistic, additive and antagonistic activity of combination of two drugs (Mayer et al., 2006, Tardi et al., 2009, Wang et al., 2014). Although, this relationship can be established easily under *in-vitro* controlled conditions, clinical translation of such information is complicated by the uncoordinated pharmacokinetics of free-drug combinations. The dissimilar or independent pharmacokinetics of individual drugs, in combination, may lead to exposure of tumor cells to antagonistic or suboptimal ratios with corresponding loss in therapeutic activity (Ashley et al., 2016, Chen et al., 2016, Greco and Vicent, 2009, Kashif et al., 2015, Lu et al., 2015a, Mayer et al., 2006, Tardi et al., 2009, Wang et al., 2014).

Hence, there is a need to circumvent the effect of uncoordinated pharmacokinetics of the combined drugs in such a manner so as to attain the most synergistic combination at the targeted site (Mayer et al., 2006, Tardi et al., 2009). This can be attained by encapsulating the desired ratios of therapeutics in drug delivery systems which can reach the tissues. Novel drug delivery systems like liposomes, polymeric nanoparticles, solid lipid nanoparticles, carbon nanotubes and nanocapsules are already reported to enhance the anticancer efficacy of drugs, modulate pharmacokinetics and can efficiently overcome toxic effects of anticancer

agents (Agrawal et al., 2017, Singh et al., 2016, Vijayakumar et al., 2016a, Vijayakumar et al., 2016b, Vijayakumar et al., 2016c).

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

Combination therapy further requires carriers which can encapsulate drugs with different physicochemical properties. Moreover, encapsulation of both hydrophilic and lipophilic moieties into single carrier predisposes wide applications in anticancer therapy which necessitates combination therapies. In this regard aqueous core nanocapsules (ACNs) displaying a core-shell structure, where the core acts as a reservoir while shell a protective membrane, can be considered suitable. ACNs exhibit a core-shell carrier where the core is composed of hydrophilic drugs and the shell is a polymeric or lipid capsule (Anton et al., 2009, Cosco et al., 2015, Kothamasu et al., 2012, Vrignaud et al., 2013). Due to various

advantages like high drug encapsulation, reduced polymer content, protection of core against degradation and reduction of tissue irritation, these can be applied as potential drug delivery carriers. ACNs can be prepared by polymerization or synthesized using preformed polymers. Various methods utilizing polymerization includes interfacial polymerization, water in oil microemulsions and in- situ polymerization. Synthesis with preformed polymers comprises of nanoprecipitation, emulsion-coacervation, emulsion–diffusion, double emulsification, polymer-coating and layer-by-layer surface modification (Anton et al., 2009, Carbone et al., 2015, Dos Santos et al., 2015, Fuchs and Thurecht, 2015, Kansal et al., 2013, Mora-Huertas et al., 2010). Moreover, it can be assumed that direct contact with the shell offers rapid release of the poorly soluble drug as compared to matrix nanoparticles which can be solid lipid nanoparticles or polymeric nanoparticles.

Further, the development and optimization of nanocarriers involves the choice and vital understanding of several formulation as well as process variables. The choice of suitable formulation and process variables depends upon the physicochemical properties of drug and the method utilized to prepare the nanocarriers. With thorough understanding of the effect of various variables, it is possible to obtain nanocarriers depicting desired critical quality attributes. Design of Experiments (DOE) is a test or series of tests for improvement in the product and process quality by introducing changes in input variables. The experiments are conducted systematically and efficiently which reduces development time as well as cost. Owing to many advantages it has gained wide applications in development of various drug delivery systems (Gupta et al., 2017, Mishra et al., 2017, Montgomery, 2017, Patel et al., 2016, Patel et al., 2014, Srinivas et al., 2017, Vardhan et al., 2017, Yuangyai and Nembhard,

2010). DOE approach can be applied for development of nano-carriers utilizing various statistical designs.

The thesis embodies development and characterization of two types of nanocarriers i.e. matrix type and core shell type for improving entrapment of VRL. Firstly, the polymeric nanoparticles were developed for entrapment of hydrophilic VRL. Preliminary studies showed that the drug loading of VRL in polymer (poly (lactic-co-glycolic acid), PLGA is very low therefore we tried formulations with lipids i.e. glyceryl monooleate (GMO) and glyceryl monostearate (GMS). The high drug loading with these lipids compared to and PLGA guided us for the development of SLNs for VRL. So, the first formulation of the present research work was the VRL loaded solid lipid nanoparticles (TPGS-VRL-SLNs and PL-VRL-SLNs) developed by solvent diffusion technique. GMO (Glyceryl mono-oleate) was utilized as core forming solid lipid material, TPGS (α-tocopheryl polyethylene glycol succinate) and poloxamer were explored as surfactants. Lipids like GMO with long triglyceride chain and short fatty acid chain are proved to emulsify highly water soluble drugs (Chen, 2011, Chen, 2012). Although, the entrapment inside the hydrophobic core was still a challenge, owing to lower affinity for hydrophobic core and more affinity towards hydrophilic dispersed phase, appreciable entrapment (≈50 %) of VRL in SLNs was achieved. However, the fusogenic nature of GMO renders it unsafe for intravenous administration. Therefore, our approach was to formulate ACNs. The lipid based ACNs (GMS-VRL-ACNs) were developed utilizing double emulsion solvent evaporation technique. Further, polymeric ACNs (PLGA-VRL-ACNs) were also developed utilizing same technique replacing GMS with FDA approved polymer PLGA (poly (lactic-co-glycolic acid)). The ACNs were carried forward to develop dual drug loaded nanocapsules (dd-ACNs).

There were very few studies for developing nanoformulations co-encapsulating synergistic combinations during conceptualization of this research work. Previous researchers have reported that combination of drugs may result in synergistic, additive or antagonistic effect depending on the ratio of drugs combined (Ashley et al., 2016, Mayer et al., 2006, Mokhtari et al., 2017). The drugs when delivered as physical combination may follow uncoordinated pharmacokinetics and result in sub-optimal treatment. Therefore, it is necessary to deliver the synergistic ration of drugs to the tumor site. The delivery of drug combinations as control drug delivery systems may resolve these issues. Some researchers developed liposomal formulation ((Ashley et al., 2016, Tardi et al., 2009, Tolcher and Mayer, 2018). Recently a novel delivery technology (CombiPlexR) enabled efficient and sustained delivery of combination treatments at a synergistic ratio (Tolcher and Mayer, 2018). But the advantages of SLN and core-shell nanocarriers over liposomes directed us towards development of these nanocarriers.

Therefore, the main objective of this study was to develop nanoformulations co-encapsulating synergistic ratio of Vinorelbine bitartrate and resveratrol for improved anticancer efficacy. Firstly, VRL loaded nano-formulations were developed in order to achieve maximum entrapment of VRL. Once higher entrapment of water soluble drug was achieved, VRL was combined with RES in different ratios and evaluated for synergistic activity. Further, the best formulation was selected for encapsulation of the most synergistic combination of VRL and RES. The selected formulation was characterized for physicochemical characteristics and evaluated *in-vitro* for safety and anticancer efficacy. Moreover, the systemic toxicity and *in-vivo* anticancer efficacy of the selected formulation

were evaluated in Sprague dawley rats and 4T1 breast cancer cells grafted Balb/c mice model, respectively.