1. INTRODUCTION

The targeted delivery of anticancer agents to the cancer cells is one of the major challenges and an active field of research in the development of cancer chemotherapeutic agents. PEGylated and metallic nanoparticles have garnered increasing attention in recent years as a novel tool for therapeutic delivery of active agents to tumor-specific cells. This research attempts to unveil the multidimensional therapeutic aspects of nanoparticles as passive targeting (PEGylated lipidic nanocapsules) and active targeting (SPIONs: superparamagnetic iron oxide nanoparticles) approaches including the RES escaping strategies to target the microenvironment of solid tumor. Conventionally, anticancer drugs are designed with a relatively low molecular weight having an agreement between hydrophilic and lipophilic balance (HLB), hence allowing partition across the lipid membranes easily. Therefore, drugs rapidly get distributed throughout the body including the non-target tissue/organ and rapidly get metabolized by liver and/or excreted by the kidneys. Ideally, for effective drug targeting, it is essential that a drug should not eliminate quickly and the drug carrier should provide a pharmacokinetic profile that will allow the drug to interact with its target (Yvonne and Thomas, 2010).

Paclitaxel (PAC), isolated from Taxus brevifolia, is one of the most potent and commonly-prescribed chemotherapeutic agent for the treatment of various cancers such as lung, ovarian, bladder, breast, and head-and-neck cancers (Crown and O'Leary, 2000). By stabilizing microtubule structure, it is involved in apoptosis, whereas by down-regulating the production of vascular endothelial growth factor (VEGF) and expression of matrix metalloproteinase (MMP), it also inhibits tumor-angiogenesis (Stearns and Wang, 1992; Toiyama et al., 2009; Xiao et al., 2006). Restriction in the use of higher dose of PAC is due to its dose-limiting toxicity, whereas, lower dose leads to multifactorial chemo resistance by activating nuclear factor (NF)-kB, and upregulating multidrug resistance-1 (MDR-1) gene, Akt and mitogen-activated protein kinase (MAPK) (Aggarwal et al., 2005; Mabuchi et al., 2004). To encounter multidrug resistance (MDR) of PAC, curcumin (CUR) was judicially selected to combat the most challenging aspects of cancer chemotherapy (Akhter et al., 2015). Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione) (CUR) is a polyphenol, extracted from the...
rhizome of *Curcuma longa*, belonging to the family Zingiberaceae. Apart from its traditional use as a spice, natural colorant and flavoring agent, recent preclinical and clinical studies have proved its therapeutic benefits as an antispasmodic, anticoagulant, antimicrobial, anti-oxidant, anti-inflammatory, anti-tumor, anti-HIV and hypocholesterolemic for the treatment of various chronic disorders, such as neoplastic, neurological, hepatic, cardiovascular, pulmonary, metabolic and psychological diseases (Duvoix et al., 2005). CUR shows its pleiotropic therapeutic effect in cancer by modulating intracellular signaling pathways as it: (i) inhibits the activity of transcriptional factor nuclear factor kappa B (NFκB) (Aggarwal et al., 2005); (ii) down regulates the intracellular levels of three major ATP-binding cassette (ABC) drug transporters; P-glycoprotein (P-gp), multi-drug resistance associated protein 1 (MRP-1) and breast cancer resistance protein (ABCG2) (Chearwae et al., 2006; Limtrakul et al., 2007). Its antiproliferative, antiangiogenic, antimetastatic and proapoptotic properties set it as a potent chemopreventive molecule.

CUR reported its synergistic chemotherapeutic effects on promoting the apoptotic response of PAC (Bava et al., 2011; Hossain et al., 2012; Sreekanth et al., 2011). Thus, by co-administering PAC and CUR, various formulations were successfully developed to improve the bioavailability and therapeutic efficacy of PAC by enhancing the cytotoxicity in wild-type and resistant cells (Ganta and Amiji, 2009; Ganta et al., 2010; Sreekanth et al., 2011). However, both the anticancer agents, PAC and CUR are poorly soluble in water and, thus show very less oral bioavailability (Rachmawati et al., 2013; Yoshizawa et al., 2011). To line the burden associated with less solubility, marketed formulation of PAC is composed of Cremophor® EL (polyethoxylated castor oil) and dehydrated alcohol. Despite this, we entangle in unavoidable hypersensitivity reactions associated with Cremophor® EL. Regardless, cancer nanotechnology ceaselessly chases the search for the development of Cremophor® EL-free formulations (Gelderblom et al., 2001; Zhang et al., 2005).

Eventually, over the past few decades, significant advances in various lipidic nanoparticles (NPs) establish them as one of the promising delivery systems for poorly aqueous soluble drugs used for cancer therapy (Fundarò et al., 2000; Meidan et al.,
2006; Roger et al., 2009; Yang et al., 2007b; Yoshizawa et al., 2011; Zara et al., 2002; Zeng et al., 2012). But, rapid clearance by reticular endothelial system (RES) upon parenteral administration of lipidic NPs put another challenge to navigate researchers for the discovery of novel formulation strategies. To confront this problem, PEGylated sterically-stabilized NPs were developed with hydrophilic polymer “polyethylene glycol (PEG)”, which can prolong systemic circulation due to its stealth character and accumulate effectively in solid tumor microenvironment via the enhanced permeability and retention (EPR) effect through the passive targeting mechanism (Tsukioka et al., 2002; Yuan et al., 1995). Surface modification of NPs with Food and Drug Administration (FDA)-approved polyethylene glycol-2000-diastearoyl-phosphadityl ethanolamine (PEG_{2000}-DSPE) with methoxy terminal (MPEG_{2000}-DSPE) can retard the mononuclear phagocytic system (MPS) uptake and increase biological half-life and tumor bioavailability by EPR effect (Matsumura and Maeda, 1986; Torchilin and Trubetskoy, 1995; Yang et al., 2007b). Additionally, by using poloxamers, we can make stealth NPs to support a long blood circulation time (Salmaso and Caliceti, 2013). Based on this evidence, few studies reported enhanced tumor bioavailability along with reduced toxicities, and improved immunological profiles and therapeutic responses of loaded anticancer molecule formulated as lipidic NPs (Bally et al., 1998; Lundberg, 1994; Lundberg et al., 2004).

In one part of our present study (chapter 3), we have formulated and characterized PAC and CUR-loaded PEGylated lipidic nanocapsules (LNCs) by utilizing anticancer therapeutic benefits of CUR and poloxamer 407 to improve poor aqueous solubility of PAC along with the expectation of enhanced therapeutic response of PAC in vivo by passive targeting mechanism based on above-discussed literatures. In other part of our research, our goal was to develop CUR and PAC-loaded SPIONs as an active targeting strategy for solid tumor therapy. But, we encountered a formulation problem during CUR loading into SPIONs related to its solubility in our stabilizer of interest. So, we first attempted to nanosize CUR through supercritical antisolvent (SAS) process to enhance its solubility in our stabilizer of interest.
Various formulation approaches (for example, polymeric prodrugs (Anwar et al., 2011; Li et al., 2014), co-crystallization (Hickey et al., 2007; Shan and Zaworotko, 2008), solid dispersion (Jang and Kang, 2014; Sarode et al., 2014; Sinha et al., 2010), salt formation (Guzman et al., 2007; Li et al., 2005), cyclodextrin complexation (Brewster and Loftsson, 2007; Muankaew et al., 2014), micronization (Mosharraf and Nyström, 1995; Vogt et al., 2008), nanocrystallization (Fakes et al., 2009; Tran et al., 2014), etc.) have been studied for various molecules to enhance their solubility. Recently, the use of a supercritical fluid as a solvent (rapid expansion of supercritical solutions; RESS) or an anti-solvent (supercritical antisolvent; SAS) for poorly-aqueous soluble drugs has been set up as a feasible technique for their size reduction to micro or nano level. Supercritical carbon dioxide (ScCO₂) is commonly used as a supercritical fluid in SAS and RESS process due to its advantages, such as, mild critical temperature and pressure (T_c = 31.1°C, P_c = 7.38 mPa), low viscosity, low surface tension, low cost, non-toxicity, non-flammability, strong solvent power and safety in environmental considerations.

Due to low solubility of most drug molecules in ScCO₂ use of RESS process is mostly limited, whereas, amorphization (Kim et al., 2008b; Kim et al., 2008c), recrystallization (Chen et al., 2010; Park et al., 2007), micronization (Cardoso et al., 2008; Kim et al., 2007; Zhiyi et al., 2009) and nanonization (Kim et al., 2008b; Kim et al., 2008c; Mezzomo and Ferreira, 2013) through SAS process has been successfully achieved. SAS is a one-step efficient process leading to completely dry, smaller particles with narrow size distribution, controlled crystals with preferred morphology and better flowability, and organic solvent-free product justifying their industrial applications. By reducing the size and increasing the surface area, SAS process can improve a drug’s solubility, dissolution rate, and stability in physiological fluid, which may ultimately reduce the dose, dosing frequency and associated toxic effects, along with bioavailability enhancement of the drug. CUR also undergoes photo degradation when exposed to light, in solution as well as in solid form (Setthacheewakul et al., 2010), placing difficulty in its handling during formulation development process in an open environment. As SAS process operates in a closed environment, it can solve the above-mentioned formulation problems. Our research aimed to improve the solubility of CUR by reducing its particle size to the nanometric range using SAS process. In this study (chapter 4), the effect of
different organic solvents and process variables of SAS process on the particle size and size distribution of CUR NPs were investigated.

In the last section of our research work (chapters 5 and 6), we have developed CUR and PAC-loaded superparamagnetic iron oxide nanoparticles (SPIONs) for the evaluation of targeting and anti-cancer potential towards solid tumor. Among various smart drug delivery strategies, SPIONs comprising of magnetite (Fe₃O₄) and maghemite (γ-Fe₂O₃) have potential therapeutic as well as theranostic efficiencies as targeted drug delivery systems. Properties, such as non-toxicity, biocompatibility, high degree of saturation magnetization, small size and an ease of appropriate surface modification with different polymers, make SPIONs the best choice for site-specific drug delivery systems (Mahmoudi et al., 2011; Veiseh et al., 2010a). Different stabilizers, such as oleic acid, lauric acid, alkane sulphonic acids, and alkane phosphonic acids, have been used significantly for the stabilization of small-sized SPIONs (Sahoo et al., 2001). Among the stabilizers, an exception within the monosaturated fatty acids is the oleic acid, which exerts anti-tumorigenic effects by suppressing the over-expression of human epidermal growth factor receptor-2 (HER2) without any chronic adverse effects and toxicity (Colomer and Menéndez, 2006; Dai Tran et al., 2010; Simopoulus, 2001). In recent years, there are an increasing number of research publications on the use of SPIONs for tumor targeting applications by loading anticancer agents onto them. Anticancer drug-loaded SPIONs can be guided to a target site by the application of an external magnetic field, commonly known as magnetic drug targeting (MDT). Thus, PAC and CUR-loaded SPIONs, along with oleic acid as a stabilizer, provide a novel, innovative approach for use in cancer treatment (Khopde et al., 2000; Kurien et al., 2012).