Nanoformulations like SLNs, nanoemulsion, nanostructured liquid carriers etc. are attractive for their potential to improve biopharmaceutic properties of herbal drugs (Parveen et al., 2008). SLNs are colloidal carriers made up of lipids that remain solid at room temperature and body temperature and also offer unique properties such as small size (50-500 nm), large surface area, smooth and spherical shape, favourable zeta potential (i.e. responsible for its stability properties) and high drug loading (Cavalli et al., 1993). Moreover, SLNs are less toxic than other nanoparticulate systems because of their biodegradable and biocompatible nature. These are capable of encapsulating hydrophobic as well as hydrophilic drugs, and provide protection against chemical, photochemical or oxidative degradation of drugs, as well as the possibility of a sustained release of the incorporated drugs (Estella-Hermoso de et al., 2009).

Some other properties of SLNs are the drug pseudo-zero order kinetics, the prolonged/sustained/ release obtained in vitro for drugs incorporated in SLN (depending upon surface properties), their rapid uptake (internalisation) by cell lines (5-10 mins), and also the possibility of preparation of stealth SLN by using PEG molecules so as to avoid reticuloendothelial system (RES) (Manjunath et al., 2005). Moreover, the possibility of loading drugs with differing physico-chemical and pharmacological properties and no requirement of specialized instruments/ apparatuses make SLNs a highly versatile delivery system (Gasco, 2007).

AD is a labdane diterpenoid, obtained from the herb Andrographis paniculata and is responsible for most of its pharmacological properties including anti-cancer activity (Jarukamjorn & Nemoto, 2008). The compound induces a G0/G1 cell-cycle arrest in various kinds of cancer cell, activates the death receptor pathways, induces TRAIL (TNF-related apoptosis-inducing ligand)-mediated apoptosis and causes inhibition of NF-kB transcriptional factors and various angiogenic factors (Varma et al., 2011). But the effectiveness of the drug is hampered by its low aqueous solubility (3.29 ± 0.73 µg/ml), high lipophilicity having log P value = 2.632 ± 0.135 and low bioavailability (Chellampillai & Pawar, 2008).

Attempts have been made to increase its bioavailability, i.e. encapsulation of AD into liposomes (Sinha et al., 2000), alginate pellets (Shariff et al., 2007), polymeric nanoparticles (Chellampillai and Pawar, 2011), preparation of β-cyclodextrin (Ren et al., 2009) and hydroxy-β-cyclodextrin (Zhao et al., 2003) complexes of AD. SLNs have
turned out a better carrier than the above-reported delivery systems like polymeric nanoparticles, liposomes, and microemulsion etc. in few aspects like lower cytotoxicity, higher API loading capacity, better production scalability, low cost of excipients, no or little access of water to the inner core of lipid particles and better protection of drug in comparison with liposomes and microemulsions etc.

The present study makes at conception, design and characterization of a stable and biodegradable nanoparticulate formulation of AD (AD-SLNs), its characterization, aimed at increasing its aqueous solubility, stability and thereby enhancing its oral bioavailability.

4.1. OBJECTIVES OF THE STUDY

The specific objectives of the study were:

- To develop a nanoparticulate drug delivery system for herbal anticancer drug (AD)
- To develop a novel analytical method for the analysis of the drug
- To evaluate the solubility and drug release in in vitro dissolution study
- To investigate the efficacy of formulation ex vivo (cell line studies)
- To determine the pharmacokinetic parameters of developed SLNs in vivo
- To evaluate anticancer activity of the developed SLNs on an animal model

In order to meet the aforementioned objectives, the following plan of work (as approved by BRS) was envisaged and executed.
4.2. PLAN OF WORK (as in the PhD synopsis)

1. Literature survey and patent survey

2. Analytical method development
   - For routine analysis like solubility studies, dissolution studies, and stability studies using UV
     - Determination of maximum absorption ($\lambda_{\text{max}}$).
   - For plasma analysis using HPLC / HPTLC
     - Development of HPLC / HPTLC method
     - Validation of HPLC / HPTLC method

3. Formulation development and optimization
   - *Methods of preparation of nanoparticles*
     - Melt emulsification
     - Self-emulsification solvent evaporation
     - Solvent injection
   - *Optimization of formulation and process variables*
     - Optimization of lipid: drug ratio
     - Optimization of lipid concentration
     - Optimization of stabilizer concentration
     - Optimization of solvent type
     - Optimization of surfactant: stabilizer ratio
     - Optimization of aqueous: organic phase ratio
     - Optimization of process parameters (rpm, temperature, stirring time and filtration)

4. Characterization of the prepared formulations using different physical parameters
   - Drug loading and encapsulation efficiency
   - Surface morphology
   - Particle size and size distribution
   - Zeta potential

5. *In vitro* dissolution studies and assessment of the drug release from the optimized formulation

6. *Ex vivo* studies on cancer cell line

7. *In vivo* studies
CHAPTER - 3

RATIONALE & OBJECTIVES

- Pharmacokinetic study
- Antitumor activity

8. Stability studies of the optimized formulation
9. Data compilation and manuscript writing