8. SUMMARY AND CONCLUSION

Systemic toxicity and elevated invasion of chemotherapeutic drugs into healthy and normal breast tissues & cells is paving for development of new drug delivery systems which can avoid these hurdles and turn for high clinical utility to treat breast cancer. Plants are ample source of clinically active phyto-constituents which possess variant pharmacological actions which can be used for treating several diseases including cancer. Amidst, DADS is an organosulphur compound and a well known anti-oxidant derived from allium species. Surveys and reports suggest that it exhibits several pharmacological actions which also include chemotherapeutic actions. Despite its pronounced cytotoxic action, lipophilicity and short biological half-life of DADS brings up challenge to the formulator in the designing a suitable drug delivery system. To excel the hurdles linked with delivery of DADS, lipid nanocarriers were employed for the drug delivery. And folic acid conjugation minimized the drug internalization into non-cancerous cells.

FA-DADS-SLNs were prepared by solvent emulsification method. Palmitic acid as lipid, Pluronic F-68 as surfactant and ethanol as solvent were selected for the preparation and conjugation was carried out by cross-linker EDC. To achieve tumor targeting drug delivery, nanoparticles should be sized with an average diameter <200 nm. Thereby, we had to select the nanoparticle size of ≤200 nm, which was optimized by statistical response surface method – Box behnken design and was confirmed by particle size analysis.

The size distribution of nanoparticles influences internalization and the drug release at cellular level. Developing small sized particle is necessary as it imparts enhanced permeability and retention (EPR) in tumor sites which are characterized by leaky vasculature. Further, EPR leads to high accumulation of drug at the tumor site. Both FA-DADS-SLN and DADS-SLN have exhibited the size of ≤200 nm feasible for the EPR enhancement. Furthermore, FA-DADS-SLNs exhibited low PDI (<0.4) indicating the homogeneity of nanoparticles. Zeta potential reports revealed the difference between FA-DADS-SLNs 3.6mv and DADS-SLNs -7.7mv that may be due to formation of amide groups. In vitro drug release study determined the behavior of FA-DADS-SLNs at the tumor site and site of administration. As per the drug release study, DADS-SLNs and FA-DADS-SLNs exhibited burst release at pH 4.5 and there was no burst release observed at pH 4.5. DADS is alkaline in nature due to presence of sulfide groups and its highly soluble at lower pH. Hence, the DADS encapsulated
in the lipid shell shows high tendency to relocate into the release medium of lower pH.

Dose dependent cytotoxicity of FA-DADS-SLNs against MCF-7 cell lines was demonstrated by SRB assay. FA-DADS-SLNs exhibited superior cytotoxic activity at lower doses when compared with DADS-SLNs and DADS. In accordance with drug release study, burst release characteristics of DADS from FA-DADS-SLNs at pH 4.5 which was previously postulated has close agreement with FA-DADS-SLNs 24 h treatment. And presence of folic acid showed active targeted delivery by the folate receptor mediated endocytosis. And the ROS levels were estimated which were in good agreement with the in vitro cytotoxicity studies. Confocal microscopic images of FA-DADS-SLNs indicated the prominent internalization and localization in tumor cells. Superior cytotoxic action of FA-DADS-SLNs might be due to active targeting and burst release of lipid shell in acidic tumor environment facilitating bystander killing effect. The SLNs acts as drug store house and exerts cytotoxic effect for longer duration.

DADS has been well documented as an anti-oxidant and chemotherapeutic. In this perspective, the underlying chemotherapeutic action of FA-DADS-SLNs was unraveled by flow cytometry and affirmed that elevated cytotoxic action of FA-DADS-SLNs was by pro-apoptotic mediated cell death. And western blot study revealed the significant findings about apoptosis cell death was by mitochondrial mediated pathway or intrinsic apoptotic pathway.

Verdict of the present study was developed formulation FA-DADS-SLNs has promising application. FA-DADS-SLNs were characterized by biomimetic, non-immunogenic, bio-degradable and high targeting potential features. The results clearly indicate the effect of folate receptor targeting for the active drug delivery which suggests the developed formulation as promising candidate in breast cancer treatment. Our results clearly demonstrated that DADS encapsulated in FA-DADS-SLNs exhibited cytotoxic action by apoptosis mediated cell death in MCF-7 breast cancer cell lines and witnessed the enhanced therapeutic efficacy by folate mediated delivery.

Future work: The developed formulations have to be subjected for pharmacokinetic and bio-distribution study in vivo models which may reveal the quantitative estimation and clear cut targeting effect of the developed formulations.
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### a. Publications

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<td>Lipid-based nanocarriers for breast cancer treatment—comprehensive review</td>
<td>Application of quality-by-design approach to optimize diallyl disulfide-loaded solid lipid nanoparticles</td>
<td>Development and efficacy evaluation of smart nanocarriers for targeting breast cancers</td>
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