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It is certified that the work contained in the thesis titled ***"DEVELOPMENT AND EVALUATION OF TARGETED NANOMEDICINE FOR LUNG CANCER THERAPY"*** by ***MATTE KASI VISWANADH*** has been carried out under my supervision and that this work has been not submitted elsewhere for a degree.

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Place

Date (Matte Kasi Viswanadh)

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List of Abbreviations and Symbols

%	Percentage
4-ATP	4-aminothio phenol
℃	Temperature on the Celsius scale
AD	Adenocarcinoma
AFM	Atomic force microscopy
ANOVA	Analysis of variance
ATR	Attenuated total internal reflectance
AUC	Area under the curve
A549 cells	Human lung cancer cells
B(a)P	Benzo(a)pyrene
Cmax	Maximum plasma concentration
C6	Coumarin-6
CQA	Critical quality attributes
CPP	Critical process parameters
CS	Chitosan
CTX	Cetuximab
DMEM	Dulbecco's modified eagle’s medium
DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOE	Design of experiments
Docel™	Marketed docetaxel preparation
EDC	1-ethyl-3-(3-dimethylaminopropyl)-N-carbodiimide hydrochloride
EE	Entrapment efficiency
EPR	Enhanced permeability and retention
FBS	Fetal bovine serum
FD	Factorial design
FDA	United States Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
GNP	Gold nanoparticle

h	Hour(s)
HPLC	High-performance liquid chromatography
i.p.	Intraperitoneal
i.v.	Intravenous
kDa	Kilodalton
MDR	Multi drug resistance
mV	Milli volts
NIR	Near infrared
NMR	Nuclear magnetic resonance
mAb	Monoclonal antibody
mg	Milligram
min	Minute
ml	Milliliter
MRT	Mean residence time
MTT	3-(4,5-dimethylthiazolyl-2-yl)-2, 5 diphenyl-tetrazolium-bromide
NaOH	Sodium hydroxide
Na-TPP	Sodium tri poly posphate
NHS	N-hydroxy-succinimide
nm	Nanometer
NP/NPs	Nanoparticles
NSCLC	Non-small cell lung cancer
NT	Non-targeted
PBD	Plackett–Burman design
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
PDI	Polydispersity index
P-gp	P-glycoprotein
PS	Particle size
QbD	Quality by design
RES	Reticuloendothelial system
rpm	Revolutions per minute
S.D.	Standard deviation
SCLC	Small cell lung carcinoma

sec	Seconds
Tmax	Time to reach maximum plasma concentration
TEM	Transmission electron microscopy
TPGS	D-α-tocopheryl polyethylene glycol 1000 succinate
TPGS-COOH	Acid functionalized TPGS
µg	Microgram
µl	Microliter
µM	Micromole
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction spectroscopy
ZP	Zeta potential

Preface

The application of nanotechnology to medicine is the basis for the development of nanomedicine. It is a technology in which the drug-loaded nanomedicine of 1-1000 nm exhibit strong interaction between drugs and their targets. Recent advancements in nanotechnology have contributed to the development of nanomedicine systems that enabled specific delivery of several drugs and/or macromolecules including drugs, antibodies, protein, targeting ligands and imaging agents. Anti-cancer drugs usually suffer from low solubility, rapid *in-vivo* degradation, poor pharmacokinetics, undesirable biodistribution and poor permeability across biological barriers. During chemotherapy, large doses are recommended for treatment, which may induce adverse effects on normal cells and the surrounding healthy organs. Thus, the objective of this study was to design and develop targeted delivery systems with the aim of restricting high dose administration and reducing the dose-related adverse side effects and also the frequency of dosing.

Chitosan is a nontoxic, semicrystalline, biodegradable and biocompatible linear polysaccharide of randomly distributed N-acetyl glucosamine and glucosamine units. The amino as well as carboxyl groups of the chitosan molecule usually form a hydrogen bond by lipoprotein interaction with the cell membrane, bringing out an ideal adhesive effect. Docetaxel is a second-generation taxane derived from the needles of the European yew tree. Unlike paclitaxel, docetaxel exhibits linear pharmacokinetics and, due to differences in drug efflux, is retained intracellularly for a longer period. D-α- tocopherol glycol 1000 succinate (TPGS) is a surfactant used for pharmaceutical dosage form preparations. It is a water-soluble derivative of natural Vitamin E, which is formed by esterification of vitamin E succinate with PEG. The TPGS can be used as an absorption enhancer, emulsifier, solubilizer, additive, permeation enhancer and stabilizer. The novelty of this work thus lies in the development of low-dose, bioadhesive and EGFR targeted chitosan nanosystem and redox sensitive nanosystem of docetaxel for the advanced therapy of non-small cell lung cancer. The redox sensitive nanomedicine has high efficacy, specificity and sensitivity and facilitates *in vivo* imaging in lung cancer applications when loaded with an imaging material. The high levels (>20 mM) of glutathione (GSH, a cysteine-containing tri-peptide) in cancer cell microenvironment, compared to that of in blood circulation (2–20 μM),

facilitates for quicker release of the anti-neoplastics from redox-responsive NP that are composed of redox-sensitive disulfide (S–S) bonds. These S–S bonds will be cleaved to trigger the drug delivery from NP in the vicinity of cancer cells.

The design, development, and optimization of nanoformulations were done by employing systematic design of experiments (DoE). DoE involves stepwise assessment of critical quality attributes, screening of factors, experimental design and optimization with minimal consumption of time and resources. PBD (Placket-Burman design) was employed to evaluate the effect of independent factors on the dependent responses and Pareto chart was employed to select the most important factors that highly influence the selected responses. The effect of independent variables on the responses was illustrated by 3D response surface methodology. A graphical and numerical optimization procedure was carried out to obtain the predicted value of various factors and responses. The final optimized batch of the nanoformulation was evaluated and validated.

Further, the prepared nanoformulations were subjected to detailed *in-vitro* evaluations for solid-state characterization, physicochemical characterization, stability studies, *in-vitro* drug release, stability, *in-vitro* cellular uptake, cytotoxicity, wound-healing and apoptosis studies in A549 cell lines. Also, *in-vivo* pharmacokinetic and histopathology studies in Wistar rats, *in-vivo* anticancer efficacy studies in Swiss albino mice were performed and the results are discussed in detail. These results indicate that the newly developed nanoparticulate systems could prove to be promising drug delivery systems for prolonging the drug release and achieving the drug concentration at the tumor site at desired rate and amount for longer duration resulting in improved therapeutic efficacy of the drug in the treatment of lung cancer.