

## List of Figures

Figure Number	Details of Figures	Page Number
1	Hallmarks to effective treatment of cancer and possible approaches	3
2	Advantages of combinatorial drug treatment over single drug treatments against cancer	5
3	Hypothesis of the study.	8
4	Possible mechanism of simultaneous action of Doxorubicin Vincristine combination on the tumor cells	13
5	Advantages of Combinatorial approach over Single drug regimens	22
6	The lipid and polymer based nanocarrier approaches against NSCLC and TNBC	36
7	Antitumor effect of Paclitaxel injection and nanoparticles in A549 tumor bearing mice. (A) tumor volume of various treatment groups (control, injection and nanoparticles at 15HALO); time dependent comparison of tumor volume of injection (B) and nanoparticle (C); Inhibition of tumor growth (%) of injection and nanoparticles	41
8	Comparative evaluation of in-vivo efficacy of octa-arginine modified pegylated liposomal doxorubicin against unmodified liposome: (A) tumor volume and tumor weight, (B) apoptosis assay, (C) Caspase 3/7 assay as indicator of apoptosis.	49
9	Evaluation of the anti-tumor effect of cyclic peptide conjugated polyerosome loaded docetaxel in A549 tumor in mice (dose 10 mg/Kg administered on days: 0,4,8,12). (A) change in tumor volume with time (B) Mean values of tumor inhibition of the treatments (C) Changes in bodyweight (D) Survival curves with treatments (Kaplan–Meier curves)	50

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
<b>10</b>	The need and potential advantages of combinatorial nanocarriers over single nanocarriers as well as free drug cocktail for chemotherapy in clinic. The use of ratiomimetic dose of synergistic combination of drugs through nanocarriers results in co-ordinated PK-PD profiles of the agents to site of action with reduced toxicity (A). Combinatorial nanocarriers on intravenous administration presents synergistic temporospatial presence of agents at tumor site resulting in improved chances of effective treatment (B).	65
<b>11</b>	Synergistic combination of Doxorubicin and Vincristine co-loaded in single liposome: (A) Acute toxicity at various concentrations (B) Tumor regression against MDA-MB 231 (TNBC); Tumor regression study against A549 (NSCLC).	67
<b>12</b>	Antitumor efficacy of active targeted RPV peptide modified epirubicin-dioscin liposome in NSCLC (A) changes in bodyweight (B) Changes in tumor volume (C) Pathological images of neoplastic tissue (H&E staining) (D) Apoptosis pictographs of tumor cells by TUNEL assay (E) Quantitative estimation of apoptotic index (1- control, 2- free drug epirubicin, 3- free drug epirubicin+dioscin, 4-liposomal epirubicin, 5- dual (epirubicin+dioscin) liposome, 6- RPV modified dual liposome.	70
<b>13</b>	Important characteristics and properties to be considered for design of nanocarriers (a) including charge (A), drug location (B), hydrodynamic size (C) and targeting moieties (D). Different types of passive/ active targeted, single drug/combinatorial polymeric and hybrid lipid/polymer nanocarrier (b); polymeric micelles (c) and liposomes (d).	82
<b>14</b>	Chromatogram of Adaptation of USP method of assay for both drugs	125

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
15	Chromatogram of experiment with change in buffer system and elution technique	127
16	Chromatogram of experiment with change in pH of buffer system and column	129
17	Chromatogram of experiment with change in composition of mobile phase B	131
18	Chromatogram of experiment with change in gradient elution program and mobile phase composition	134
19	Chromatogram of experiment with modulations in chromatographic conditions: study samples	136
20	Chromatogram of experiment with modulations in chromatographic conditions: standard solutions	137
21	Chromatogram of method of assay of both drugs as acquired during analysis of optimised dual drug liposomal batch DV6	141
22	Chromatogram of method of free drug content for both drugs as acquired during analysis of optimised dual drug liposome batch DV6	145
23	Chou-Talalay diagram and the combination effect diagram. (A) Schematic diagram of Chou-Talalay method to determine the combination index of two drugs in combination used in the treatment of cancer. Combination index values of <math><0.85</math> are synergistic, 0.9–1.1 are nearly additive and >1.1 are antagonistic. The synergistic combinatorial index values can be further subdivided into very strong (<math><0.1</math>), strong (0.1-0.3), synergistic (0.3-0.7) and moderate (0.7-0.85); (B) Schematic diagram representing hypothetical tumor inhibition (cell killing) by synergistically acting drug combinations targeting multiple key pathways	152

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
24	Combinatorial index of varying molar ratios of free drugs VCR and DOX in MDA-MB-231 and A549 cell lines	156
25	Ishikawa diagram linking the effect of independent variables on co-loading of two drugs to dependent responses.	166
26	Effect of the variation in the Phosphatidylcholine chain length (HSPC and DAPC liposomes): on the drug release profiles at (A) pH 7.4 (B) pH 5.5; the cryo-TEM images of HSPC liposomes (C) and DAPC liposomes (D).	174
27	Schematic representation of the dual drug loading using ammonium sulphate transmembrane gradient	181
28	Cryo-TEM images of Pegylated liposomal suspensions of (A) Blank liposomes; Dual drug loaded containing different transmembrane salt gradients: (B) Ammonium Sulphate; (C) Ammonium Phosphate; (D) Ammonium citrate; (E) EDTA Ammonium salt; (F) Sulphobutylether Beta-Cyclodextrin ammonium salt; (G) Copper sulphate; (H) Copper gluconate/TEA	182
29	Cryo-TEM images of Pegylated liposomal suspensions with active dual drug loading at (A) 45°C; (B) 55°C; (C) 65°C; (D) 75°C.	184
30	Cryo-TEM images of Pegylated liposomal suspensions with different molar concentrations of cholesterol in the lipid composition (A) 0 mole% cholesterol (B) 38 mole% cholesterol (C) 45 mole% cholesterol (D) 55 mole% cholesterol. Effect of variation in cholesterol content on the drug release profiles at (E) pH 7.4 (F) pH 5.5.	186
31	Effect of the variation in the drug loading pH (5.5 and 6.5): on the drug release profiles at (A) pH 7.4 (B) pH 5.5; the cryo-TEM images of dual drug formulations loaded at pH 5.5 (C) and pH 6.5 (D).	189

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
<b>32</b>	Effect of the variation in the ammonium sulphate concentration: cryo-TEM images- (A) 250 mM (B) 350 mM (C) 450 mM; drug release profiles at (D) pH 7.4 (E) pH 5.5.	194
<b>33</b>	Contour plots of responses- Particle Size (A-C), Zeta potential (D-F), Entrapment efficiency of DOX (G-I) and Entrapment efficiency of VCR (J-L) as a measure of the factors (pH of drug loading, Ammonium sulphate concentration, PC: Cholesterol ratio)	202
<b>34</b>	Morphological evaluation of DOE batches using Cryo-TEM: (A) DV4, (B) DV6, (C) DV7 and (D) DV8; in-vitro drug release profiles: cumulative release from formulations (E) DOX at pH 5.5, (F) DOX at pH 7.4, (G) VCR at pH 5.5, (F) VCR at pH 7.4.	205
<b>35</b>	Attenuated total reflection-Fourier transform infrared spectroscopy spectrum for Doxorubicin (a), l-Histidine (b), Sucrose (c), Ammonium sulphate (d), Cholesterol (e), HSPC (f), mpeg-2000-DSPE (g) and Vincristine (h).	232
<b>36</b>	Attenuated total reflection-Fourier transform infrared spectroscopy spectra for drug-free liposomes, VCR liposome, DOX liposome and dual drug liposome-labeled peaks (P) of HSPC (P1: 2958/cm; P3: 2853/cm; P5:1472/cm; P6:1375/cm; P7: 1252/cm; P8: 1173/cm; P9: 1093/cm; P11: 1019/cm; P12: 970/cm); cholesterol (P2: 2922/cm; P10: 1049/cm) and mPEG-2k-DSPE (P4: 1738/cm).	233
<b>37</b>	Microcalorimetry- Differential scanning calorimetry of drug free liposomes (A), VCR liposome (B); DOX liposome (C) and dual drug liposome (D)	235
<b>38</b>	Cryogenic Transmission electron microscopy of drug free liposomes (A), VCR liposome (B); DOX liposome (C) and dual drug liposome (D)	237

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
39	Evaluation of morphology of the dual drug liposomes using AFM (A) and its 3D reconstruction (B)	238
40	Evaluation of morphology of the dual drug liposomes using FESEM	239
41	(A) %CF retention in various formulations (B) Percentage carboxy-fluorescein (CF) retention at different time points for dual loaded liposomes with and without cholesterol (L: Liposomes; WC: Without Cholesterol).	244
42	Characterization plots using Small Angle X-ray scattering: A) Double log plot B) paired distribution profile C) Kratky plot D) Porod-Debye Plot. The uniform density models of drug free liposomes (E), DOX liposomes (F), VCR liposomes (G) and Dual drug liposomes (H). The colour coding for each plots: dual drug liposomes- green (A, B, C), red (D); drug free liposomes- red (A, B), cyan (C), brown (D); VCR liposome- cyan (A, B), red (C), blue (D); DOX liposome- magenta (A, B, C, D).	248
43	Release profile for drug solution, single and dual loaded liposomal formulations at pH 7.4 (A), pH 6.4 (B), pH 5.5 (C), on dilution with plasma (D), on dilution with bovine serum albumin (E).	251
44	Optical microscopy images of formulation induced Haemolysis for various tested formulations at 0.5 hour at 500 µg/ml concentration of the drugs (A: Blank liposomes; B: Dox Liposomes; C: VCR liposomes; D: Dual loaded liposomes).	256
45	(i) Optical microscopy images of formulation induced Haemolysis for various tested formulations at one hour at 500 µg/ml concentration of the drugs (A: Blank liposomes; B: Dox Liposomes; C: VCR liposomes; D: Dual loaded liposomes). (ii) % haemolysis for various carrier free drug solutions, drug free liposomes, single liposomes and dual liposomal formulations at varying concentrations.	257

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
46	Stability study: variation in the assay of the drugs (DOX, VCR) with storage at temperature (2-8°C and 25±2°C/65±5%RH) with time	260
47	Stability study: variation in the free drug content (DOX, VCR) with storage at temperature (2-8°C and 25±2°C/65±5%RH) with time	260
48	Stability study: variation in the particle size and zeta potential with storage at temperature (2-8°C and 25±2°C/65±5%RH) with time	261
49	Confocal microscopic fluorescent images of MDA-MB 231 with free DOX, Liposomal DOX, FITC liposomal VCR and dual drug liposome. For each panel, the images from top to bottom showed the bright field image of cells, cell nuclei stained by using DAPI solution(blue), Dox fluorescence in cells (red)/ FITC fluorescence in cells (green) and merged image	275
50	Confocal microscopic fluorescent images of A549 with free DOX, Liposomal DOX, FITC liposomal VCR and dual drug liposome. For each panel, the images from top to bottom showed the bright field image of cells, cell nuclei stained by using DAPI solution (blue), Dox fluorescence in cells (red)/ FITC fluorescence in cells (green) and merged image.	276
51	Cellular uptake by flow cytometry of MDA-MB 231 incubated with free DOX, Liposomal DOX, FITC liposomal VCR and dual drug liposome. The histogram and Geometric mean fluorescence intensity data plotted as bar graph for the cellular uptake quantification.	278
52	Cellular uptake by flow cytometry of A549 incubated with free DOX, Liposomal DOX, FITC liposomal VCR and dual drug liposome. The histogram and Geometric mean fluorescence intensity data plotted as bar graph for the cellular uptake quantification.	279

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
53	Results of Cell viability (%) at various concentrations of DOX solution, VCR solution, DOX liposome, VCR liposome, DOX liposome+ VCR liposome and DOX+VCR liposome after 24 hours (11.5 A), 48 hours (11.5 B), and 72 hours (11.5 C) of treatment in MDA-MB 231 cell line. Cell viability of free drug solutions after 24, 48, 72 hours is indicated (11.5 D)	282
54	Results of Cell viability (%) at various concentrations of DOX solution, VCR solution, DOX liposome, VCR liposome, DOX liposome+ VCR liposome and DOX+VCR liposome after 24 hours (11.6 A), 48 hours (11.6 B), and 72 hours (11.6 C) of treatment in A549 cell line. Cell viability of free drug solutions after 24, 48, 72 hours is indicated (11.6 D)	284
55	Results of cell cycle analysis in MDA-MB 231 (A) as well as in A549 (B) cell lines.	286
56	Quantitative assessment of apoptosis in A549 cells induced by formulations using Annexin V assay (R5 – Necrotic cells (red), R4 - late apoptosis (orange), R3 – early apoptotic cells (yellow), R2 – Live cells (green)).	289
57	Quantitative assessment of apoptosis in MDA-MB 231 cells induced by formulations using Annexin V assay (R5 – Necrotic cells (red), R4 - late apoptosis (orange), R3 – early apoptotic cells (yellow), R2 – Live cells (green)).	290
58	Wound recovery study results as a function of A) various concentrations of free drugs in MDA-MB 231 and A549 (B) effect of various formulations at 50 $\mu$ M effective concentration.	291
59	Results of acute toxicity study of Liposomal Doxorubicin (DOX-L), Liposomal Vincristine (VCR-L) and dual drug liposome at various concentrations	303

<b>Figure Number</b>	<b>Details of Figures</b>	<b>Page Number</b>
<b>60</b>	In-vivo efficacy study in A549 xenograft model in nude athymic mice (A) Body weights (B) Mean tumor volumes (C) % Test/Control (D) Kaplan–Meier percent survival (graphs are offset for clarity) for various liposomal formulations	309
<b>61</b>	In-vivo efficacy study in MDA-MB 231 xenograft model in nude athymic mice (A) Body weights (B) Mean tumor volumes (C) % Test/Control (D) Kaplan–Meier percent survival (graphs are offset for clarity) for various liposomal formulations	310
<b>62</b>	Pharmacokinetic profile of DOX (A) and VCR (B) in Sprague Dawley rats from various formulations.	314