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Development of biodegradable polymer based nanoparticles of doxorubicin for in silico and in-vitro evaluation of anticancer activity using MCF-7 cell line

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Abstract

The present study is aim at the development of PLGA based nanoparticles of Doxorubicin for anti breast cancer activity by in silico and in vitro cell line using MCF-7. Nano formulated Doxorubicin was standardized with various parameters such as drug excipient interaction, particle size, shape, surface morphology, polydispersity index, zeta potential, drug content, entrapment efficiency etc. Thereafter, tested as a pure compound, 99 percent of medications are released right away. Doxorubicin was prepared as PLGA nanoparticles with NP1 and NP2, an extended release was noted, indicating the drug release over a period of 2 days and NP3 and NP4 over a period of 4 days. Thereafter, molecular docking study was performed for Doxorubicin with PDB protein enzyme 3ERT and 4OAR. Finally, in vitro breast cancer activity was performed using MCF-7 cell line. Overall result concluded that doxorubicin showed binding energy of 8.5 with 3ERT protein and -8.8 with 4OAR protein complex. The best performing formulation was NPF3 which constituted doxorubicin nanoparticles of appropriate size and demonstrated benefits of controlled drug release over free doxorubicin and other formulations. Finally, in vitro MCF-7 cell line study for breast cancer was performed with best formulation which showed remarkable inhibition of cancer growth by measured dose dependent decreased in
Glutathione level and increased in lipid peroxidation level.

Key Word: Nanoparticle, Doxorubicin, PLGA, in silico, in vitro, MCF-7

Introduction

Nanoparticle can be used as the novel colloidal drug delivery system, where the particle size ranges from 10 to 1000 nanometer in diameter, that holds great promise for reaching the goal of control drug delivery as well as site specific drug delivery which result in reduction of dose and the reduction of the toxicity of the drugs too [1]. Doxorubicin is a red-orange, hygroscopic crystalline powder. It contains not less than 98% and not more than 102% of $C_{27}H_{29}NO_{11}$, HCl, calculated on the anhydrous, solvent free basis. It is soluble in water, in NaCl 0.9% and in methyl alcohol. It is practically insoluble in chloroform, in ether and in other organic solvents. It stored in air tight

container. A 0.5% solution in water has a pH 4.0 to 5.5. Doxorubicin is a chemotherapy medication used to treat cancer. This includes breast cancer, bladder cancer, Kaposi's sarcoma, lymphoma and acute lymphocytic leukemia [2]. But the Clinical value of this agent is limited by its cardio toxicity. The toxicity is essentially of two kinds: acute, usually reversible ECG changes, including a wide range of arrhythmias and a delayed, usually irreversible dose related cardio-myopathy, resulting in congestive heart failure. Also Blood count should be made routinely during treatment with doxorubicin. PLGA or poly (lactic-co-glycolic acid) is one of approved polymer by the US food and drug administration (FDA) for human use as surgical sutures, implantable device and drug delivery system, owing to its biodegradability and bio compatibility. Depending on the ratio of lactides to glycolides used for polymerization, different forms of PLGA are available viz. PLGA (85:15), PLGA (75:25), PLGA (65:35) and PLGA (50:50) and all are most widely used due to its extensive clinical background, advantageous breakdown properties, and potential for long-term medication delivery. According to recent research, PLGA breakdown can be used to implant drugs sustainably and at desired concentrations without the need for surgery and used in formulation of various control released studies [3-5]. Based on the earlier scientific evidences, the present study was investigated with PLGA based nanoparticles of doxorubicin for effective therapeutic efficacy as an anticancer agent by standardized various parameters to optimize the formulation. Further, in silico study for doxorubicin for anti breast cancer activity was not performed by earlier and hence it was worthwhile to perform molecular docking study for the preliminary confirmation of anticancer activity.

Materials & Methods Materials

Doxorubicin, was a gift sample from Sun Pharmaceuticals, Gujarat, India. PLGA ratio 65:35 was procured from Sigma-Aldrich India Pvt. Ltd, Bangaluru, India. Polyvinyl Alcohol (PVA) was purchased from SD Fine-Chemicals, Mumbai, India. Dichloromethane (DCM) was purchased from Merck life science Pvt. Ltd, Bengaluru, India. Pluronic F-127 were purchased from Sigma- Aldrich India Pvt. Ltd, Bangaluru, India.

Preparation of polymeric drug loaded nano-particles

The biodegradable polymer poly (D,L-Lactide-co-glycolide) based nanoparticle is prepared by multiple emulsification and solvent evaporation method [6]. Required amount of PLGA was taken in a beaker and dissolved in DCM. A specific percentage of PVA solution containing drug is prepared. Then aqueous phase containing PVA and drug was added drop wise into the oil phase and homogenized with a high speed homogenizer with a specific speed for 4 minutes.

The primary emulsion (w/o) was formed. The primary emulsion was then added drop wise into the aqueous phase containing PVA with specific concentration and homogenized in the same speed for 6 minutes and a multiple emulsion (w/o/w) was formed. The prepared emulsion was placed on a magnetic stirrer and stirred for 12 hours at room temperature for evaporation of organic solvent such as DCM. The nano particles were then washed thrice using double distilled water by cooling centrifugation at 4°C with 16000 rpm for 40 minutes. Particles were then freeze dried (by pre-freezing at – 20°C overnight and lyophilizing at – 40°C for 12 hours) in a lyophilizer and stored at 4°C [7].

Evaluation & characterization

Study of drug-excipient interaction using FTIR Spectroscopy

The interaction between drug and excipients was carried out by FTIR Spectroscopy (Magna-IR 750, series ll, Nicolet Instruments, Madison, Wiscosin, USA). To understand the interaction FTIR spectrogram of pure drug, PLGA, their physical mixture (1:1) and the formulation was carried out. Samples were mixed with IR grade Potasium bromide (1:100 ratio) and the pellet was prepared by compressing in a hydraulic press. The pellet was scanned over IR range of 4000-400 cm^{-1}.

Surface morphology of Nanoparticles

Surface Morphology of the prepared nanoparticles was analyzed by scanning electron microscope (SEM).

Assessment of particle size, Zeta potential and PDI of prepared nanoparticles

A small amount of lyophilized nanoparticles was taken with 2 ml of Milli-Q water and sonicated for 15 min followed by vortex for few min. Average particle size, zeta potential and polydispersity index (PDI) of the formulation were detected by using Zetasizer Nano ZS 90 (Malvern Instruments, Malvern, UK) with DTS software. For the determination of particle size, dynamic light scattering is used and the software collects and interprets data on particle size and zeta potential and calculates the average size and PDI by using the intensity, volume and number distribution.

Drug content and entrapment efficiency study

Prepared nanoparticles 2mg was dissolved in 2 ml of DMSO, sonicated for 30 min and centrifuged at 16000 rpm for 10 min. Absorbance of the supernatant was read against blank at 480 nm in the UV-Visible spectrophotometer [8]. The drug content and drug entrapment efficiencies were calculated by applying the following formulae:

$$\text{Drug loading (theoretical) (\%)} = \frac{\text{Amount of drug taken to prepare nanoparticles}}{\text{Amount of PLGA+ drug taken}} \times 100$$

$$\text{Drug loading(actual) (\%)} = \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of nanoparticles obtained}} \times 100$$

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Drug loading (actual) (\%)}}{\text{Drug loading (theoretical) (\%)}} \times 100$$

Drug release study

To measure the drug release of prepared nanoparticles at the different time points 5 mg of nanoparticles were suspended in 1 ml phosphate buffer saline (PBS) pH 7.4 in prelabeled microcentrifuge tubes and kept in an incubator shaker (Somex incubator Shaker) at 37°C with constant shaking at 72 rpm after short vortexing. Formulations were processed in triplicate and samples were kept for specific periods of time. At any particular time point only the sample for analysis was removed from the shaker, centrifuged at 15000 rpm for 30 min at 4°C and the drug from the supernatant was analyzed at a wavelength of 480 nm with a UV-Visible spectrophotometer (Beckman Instrument) [9]. The percentage of drug release was calculated as:

$$\text{Drug release (\%)} = \frac{\text{Amount of drug released}}{\text{Amount of drug loaded in 5 mg of nanoparticle}} \times 100$$

In silico docking study:

Preparation of target

The computational studies require target and ligand which are the important requirements of docking studies. The target is obtained as a protein (3ERT and 4OAR) from RCSB PDB (protein data bank) in pdb format. The obtained file is opened using MOE for energy minimization and the removal of water molecules, ligands, and heteroatoms. Further, it is saved in the form of pdb and converted to pdbqt by the addition of missing atoms, polar hydrogens, and charges (Kollman and gasteiger charges) using AutoDock 4.2.6.

Preparation of ligands

The ligands are those showing therapeutic activity and are selected from Doxorubicin and their 3D chemical structure is obtained using ChemDraw 3D in sdf format. The obtained sdf file is converted to a pdb file using Open Babel software. Further, the pdb file is converted to a pdbqt file by choosing the torsions and aromaticity criterion using AutoDock4.2.6.

Docking studies

The target and ligand which are prepared are used in docking studies. Auto grid is performed for target-ligand by adjusting the matrices of the grid in 60x60x60 on the 3D axis. The Auto

grid is done using the gpf file to get the glg file. The auto dock is performed by genetic algorithm in the Lamarckian form. The dpf file is converted to dlg file by running Autodock to get the output file that predicts the conformations along with the binding energy. Further, for visualization of binding sites, Discovery Studio is used.

Cell culture

Human Breast cancer cell line (MCF-7) was selected for the determination of cytotoxicity for all the four formulations (NPF1, NPF2, NPF3 and NP4). The cell line was procured from American Type Culture Collection (ATCC, Manassas, USA). The culture was facilitated by fetal bovine serum (FBS) (10%), $NaHCO_3$ (0.3%), and antibiotic solution (1 ml/100 ml of culture medium). The MCF-7 cells were cultivated at 37 °C in a CO_2 incubator. Moreover, trypan blue assay was performed to assess cell viability based on the cell viability was greater than 98% viability.

Cytotoxicity determination

The cytotoxicity for the formulations was determined by Neutral Red Uptake (NRU) assay method. The varying concentration (12.5 to 300 µg/ml) of the formulations was added to 96-well culture plate where cell density was 1×10^4 MCF-7 cells/well. After being incubated for 24 hours, Phosphate buffered saline (PBS) was used to wash the wells. Next, three more hours of incubation were spent in each well after adding 50 µg/mL of neutral red solution. A solution of 0.5% H.CHO and 1% CaCl2 was used to wash the cells. A solution of 1% acetic acid and 50% ethanol were added to each well contained various formulations.

Finally, absorbance was measured at 550 nm against blank [10].

Glutathione and lipid peroxidation analysis

Commercially available kit was used for the determination of the levels of lipid peroxidation and glutathione. MCF-7 cells were grown on 6-well plates and, over the course of 24 hours, exposed to 12.5, 25, 50, 100 and 150 µg/ml for all the formulations. Extra cells were removed by PBS washing and then homogenate was centrifuged for 25 min at 4000 rpm. After that, the supernatant was collected and analyzed [10].

Statistical analysis

The data for the result was replicated three times and stated as the mean ± SEm. Oneway ANOVA with Dunnett's post-hoc test was performed for data analysis. $p < 0.05$ was considered as statistically significant.

Results and Discussion

FTIR study for pure drug and pure drug with PLGA was carried out and result showed presence of Doxorubicin in PLGA (Figure-1a and 1b). The same way, FTIR study of Doxorubicin with PVA and Doxorubicin with PLGA and PVA was carried out (Figure-2a and 2b) and showed the range of Doxorubicin was 1770–1680 cm^{-1}.

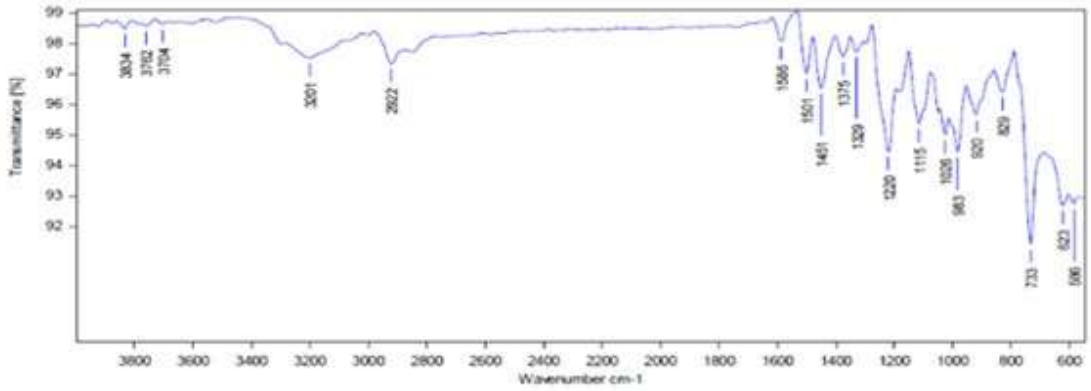


Figure-1a: FTIR data of pure Doxorubicin

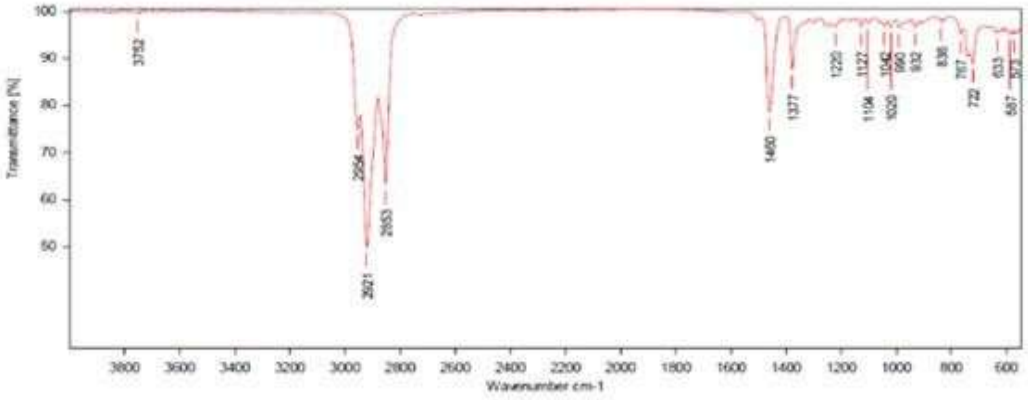


Figure-1b: FTIR study of Doxorubicin + PLGA

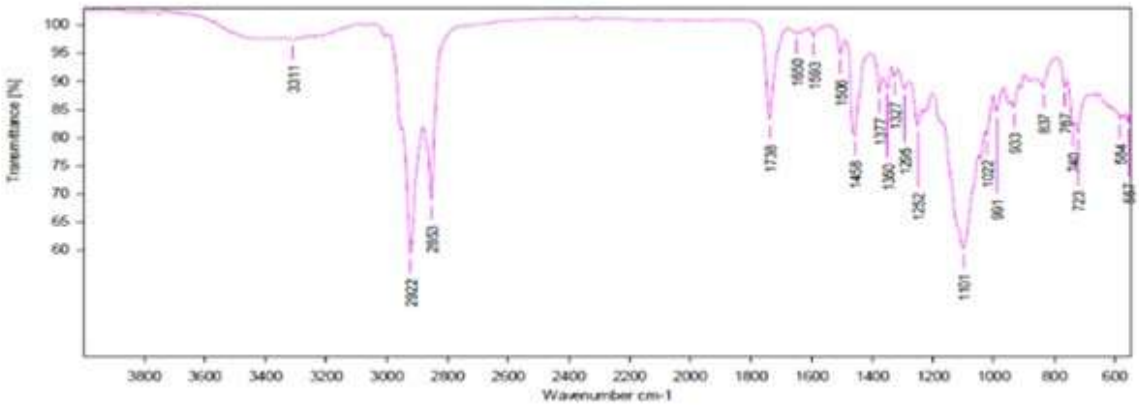


Figure-2a: FTIR Spectra of Pure Drug of Doxorubicin with PVA

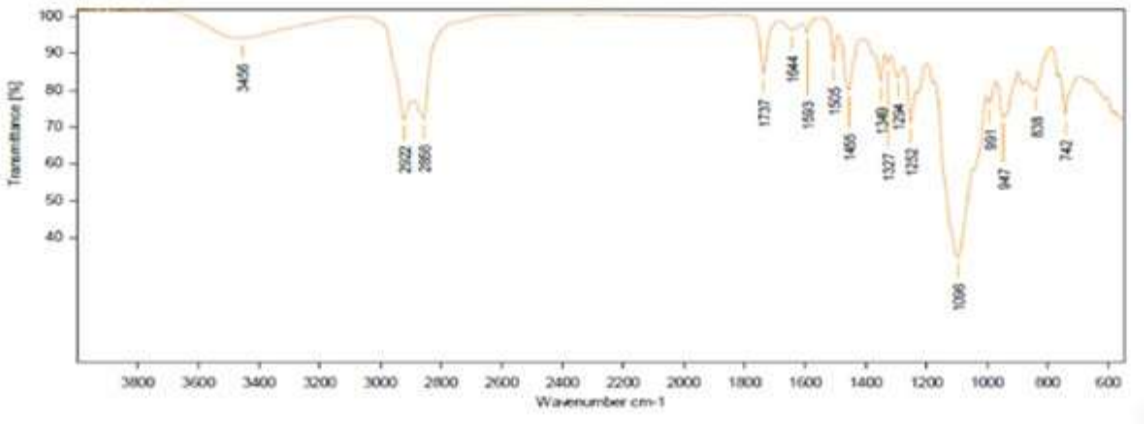


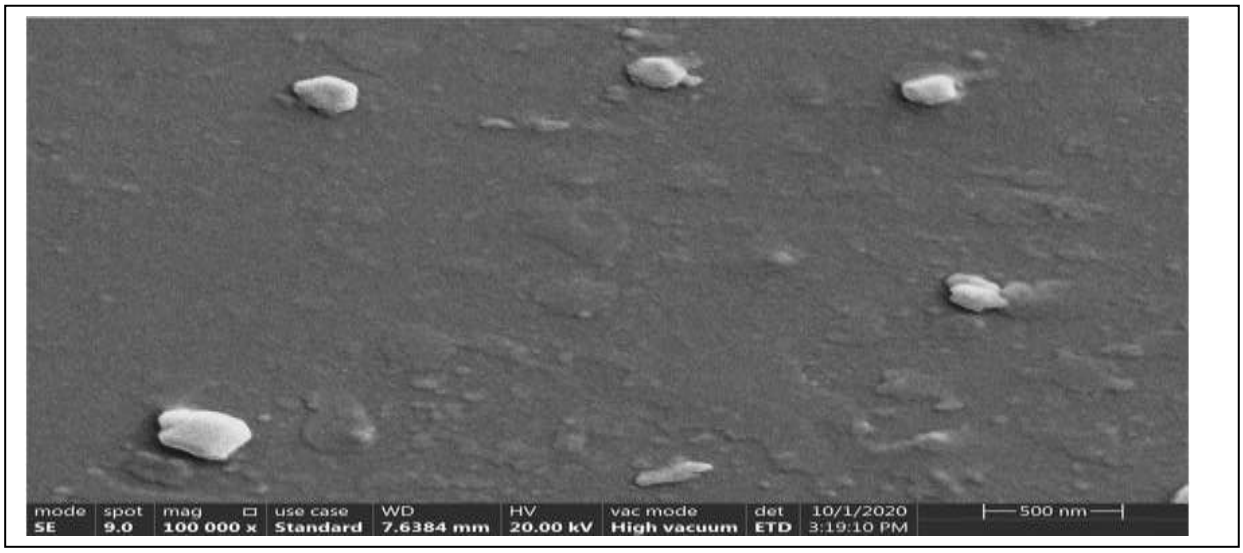
Figure-2b: FTIR Spectra of Pure Drug of Doxorubicin with PLGA and PVA

Further, four various formulations were prepared with two different ratios of PLGA and drug. The result showed percent drug loading and per cent entrapment efficient was higher with formulation 3 (NPF3) (Table-1). Per cent of drug loading for formulations NPF1 was 6.53±0.12, NPF2 was 6.84±0.02, NPF3 was 8.33±0.15 and NPF4 was 7.93±0.01. Same way per cent entrapment efficiency for NPF1 was 71.83±0.08, NPF2 was 75.24±0.17, NPF3 was 91.63±0.3 and NPF4 was 87.23±0.33. The speed of homoginization was changed with NPF112000, NPF2 14000, NPF3 16000 and NPF4 21000 rpm.

Table-1: Percent drug loading and entrapment efficiency of various formulations

Formulations	**PLGA : DRUG**	**Speed of Homoginization**	**Stabilizer Used**	**% Drug Loading (mean ± SEm) (n=3)**	**% Entrapment Efficiency (mean ± SEm) (n=3)**
NPF1	30 : 2	12000	Pluronic – 127 (0.5% w/v)	6.53 ± 0.12	71.83 ± 0.08
NPF2	30 : 2	14000	Pluronic – 127 (0.5% w/v)	6.84 ± 0.02	75.24 ± 0.17
NPF3	20 : 2	16000	Tween-80	8.33 ± 0.15	91.63 ± 0.3
NPF4	20 : 2	21000	Tween-80	7.93 ± 0.01	87.23 ± 0.33

In the present study, the bounded drug with polymer showed particles at 500 nm (Figure-3).



For the formulations, further zeta potential, average particle diameter and Polydispersity index were determined. It was seen that zeta potential value was maximum less in formulation 3 (NPF3) followed by NPF4 (Table-2 and Figure-4). The ZP of nanoliposomes directly relates to their stability; a value of +30 mV or less than −30 mV is regarded as appropriate for the vesicle's stability. The more the nanoliposomes oppose and resist the formation of aggregates, the higher the absolute value of ZP.

Table-2: Determination of various parameters with the formulations

Formulations	**Average particle diameter (Z-average) (nm)**	**Polydispersity index**	**Zeta potential (mV)**	**Formulations**
NPF1	291.6±24	0.591±23.8	-7.26± 0.004	NPF1
NPF2	280.2±22.57	0.554±46.6	-7.71±0.012	NPF2
NPF3	120±9.8	0.310±14.3	-10.8±0.008	NPF3
NPF4	122.56±9.1	0.330±15.7	-10.23±0.003	NPF4

Mean ± SEm (n =3).

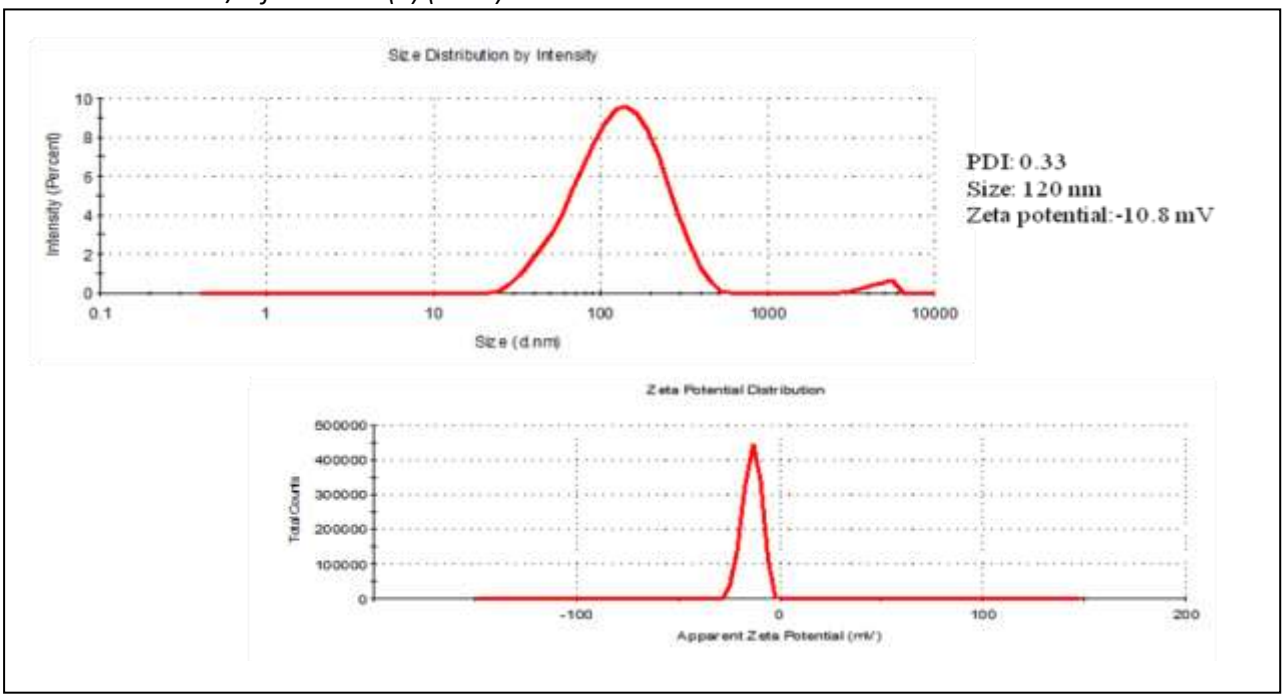


Figure-4: Zeta potential determination for the formulation NPF3

Drug release study:

The comparative release of doxorubicin as a pure compound and when formulated into PLGA nanoparticles are given in Figure-5 and Table-3. The results demonstrated that more than 99% of drugs get released immediately after administration when tested as a pure compound. However, an extended release was observed when doxorubicin was formulated as PLGA nanoparticles with NPF1 and NPF2, showing the drug release over a period of 2 days and NPF3 and NPF4 over the period of 4 days.

Table-3: Percentage Cumulative drug release of free doxorubicin and different

Time (Days)	NPF 1		NPF 2		NPF 3		NPF 4		Free drug	
	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.57	0.02	0.67	0.02	14.57	0.02	14.57	0.02	45.76	0.43
0.5	0.75	0.05	0.75	0.05	18.75	0.05	18.75	0.05	65.17	0.34
1	10.93	0.02	11.93	0.02	20.93	0.02	20.93	0.02	76.55	0.76
2	23.56	0.11	26.56	0.11	21.00	0.11	21.00	0.11	99.97	0.63
4	31.33	0.08	29.48	0.08	25.33	0.08	25.33	0.08	112.47	0.83
8	38.39	0.29	38.39	0.29	29.39	0.29	29.39	0.29		
12	50.25	0.42	55.25	0.42	48.25	0.42	48.25	0.42		
24	70.91	0.89	78.91	0.89	55.91	0.89	55.91	0.89		
48	92.34	1.41	94.00	1.41	69.32	1.41	69.32	1.41		
72					84.32	1.52	84.32	1.52		
96					93.16	1.21	96.12	0.83		

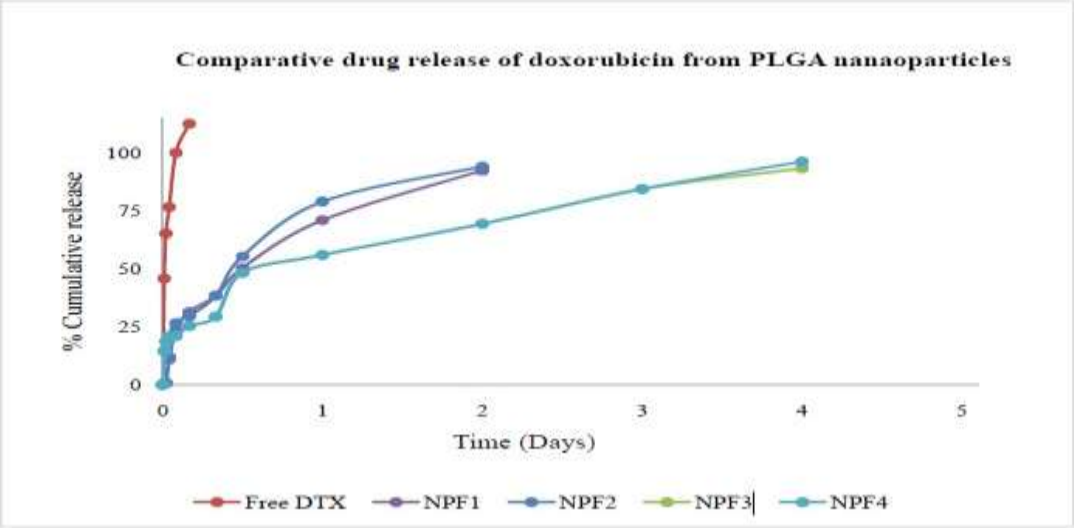


Figure-5: Comparative drug release of Doxorubicin from different formulations

In silico docking study

Docking study was performed using AutoDock 4.2.6 and result found that docking scores were -8.5 kcal/mol for protein 3ERT and -8.8 kcal/mol for protein 4OAR. Both protein shows good affinity for ligand Doxorubicin and the amino acids to which protein 3ERT binds by conventional hydrogen bond with LEU:536 and LEU:539, pi-alkyl bond with PRO:535, and for protein 4OAR,conventional hydrogen bond with GLU:695, pi-alkyl bond with VAL:698 (Figure-6 and 7).

Thereafter, Protox-III was used for the determination of toxicity level of Doxorubicin and the result was tabulated in figure-8 and 9 where inactive and active data showed respectively.

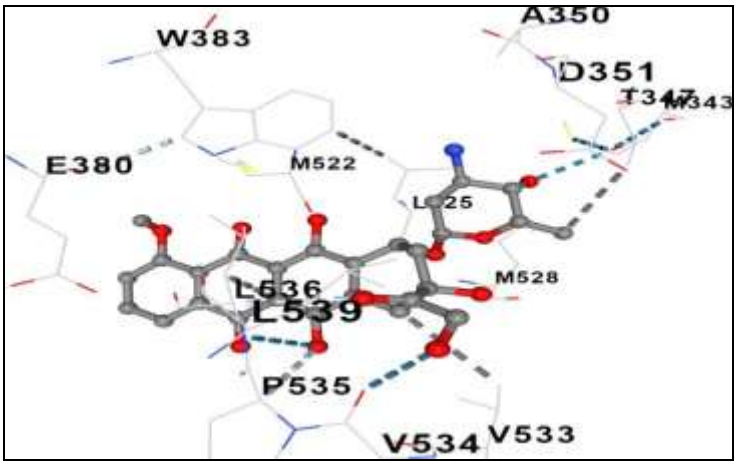


Figure-6: Doxorubicin-3ERT Complex (Binding Energy: -8.5 Kcal/mol)

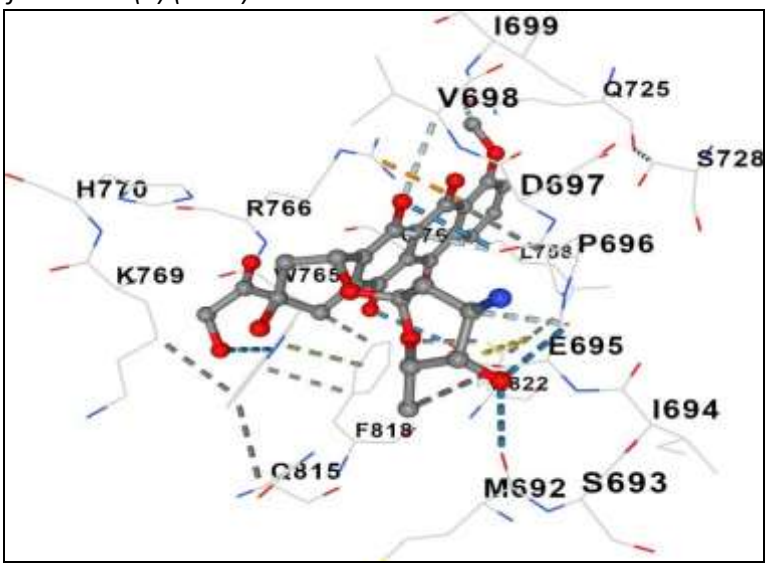


Figure-7: Doxorubicin-4OAR Complex (Binding Energy: -8.8 Kcal/mol)

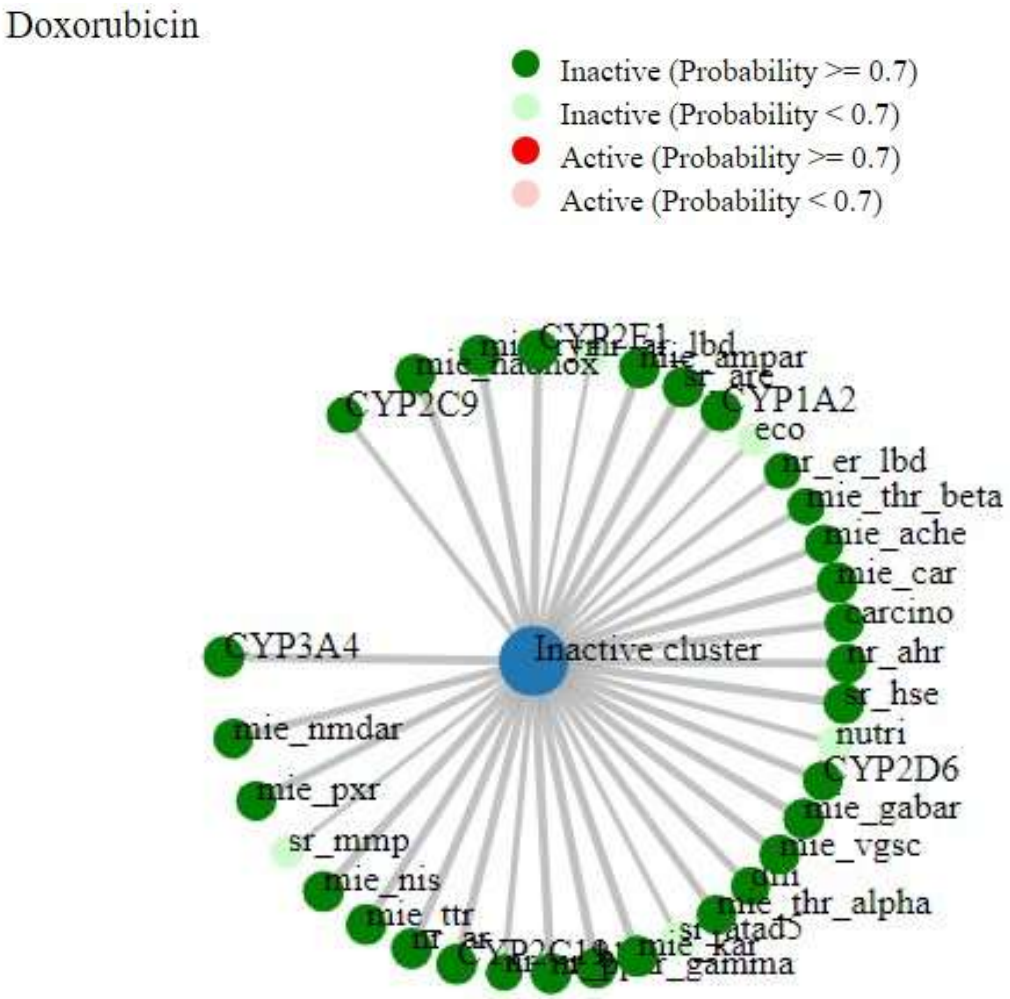


Figure-8: Inactive parameters of Doxorubicin

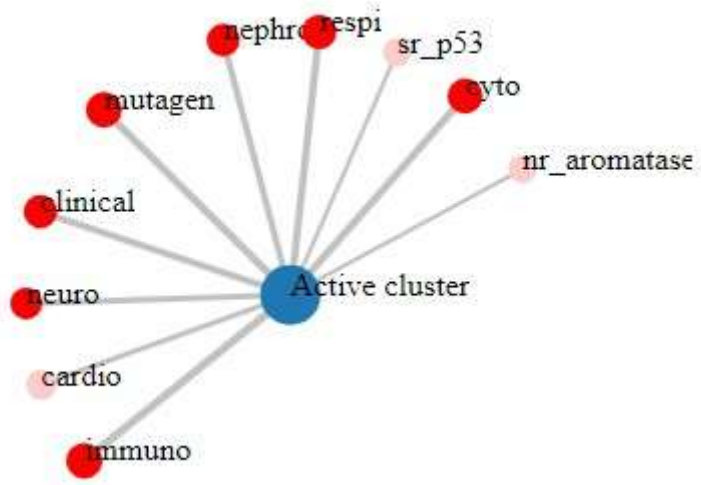
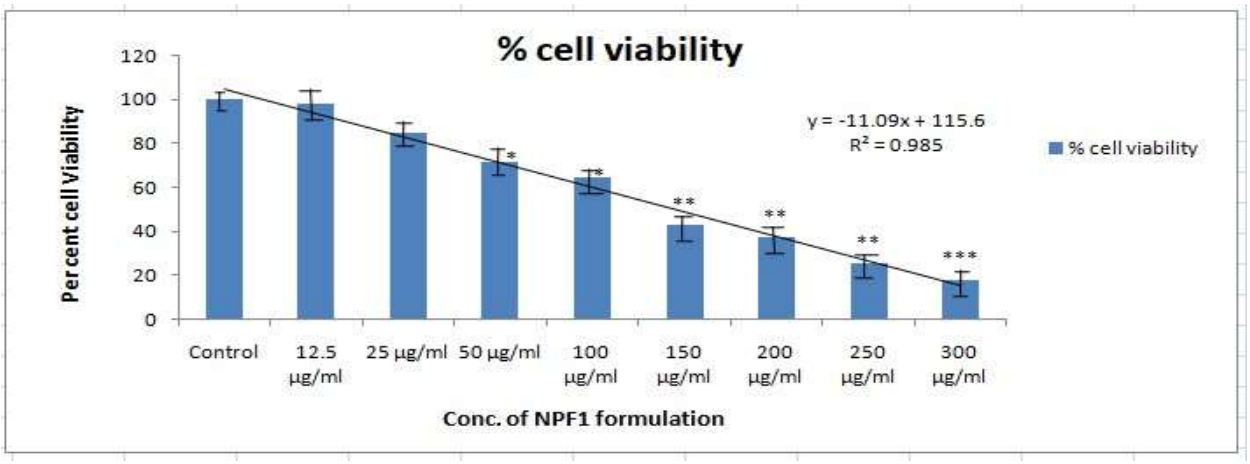


Figure-9: Active parameters of Doxorubicin

Cytotoxicity determination:

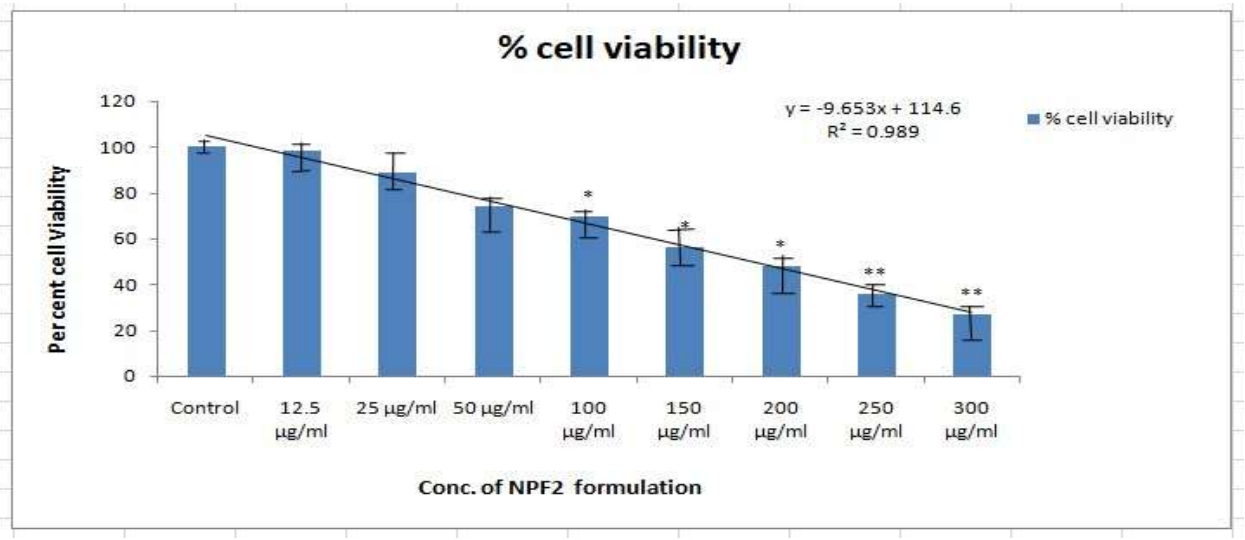
Cytotoxicity for all the four formulations was carried out by NRU method. Figure-10 to 13 showed changes in MCF-7 cells by NRU assay after the treatment of different concentrations of various formulations. It was observed that the cell viability decreased with increased concentration. NPF3 showed better inhibition than other formulations (Figure-10 to 13). It resulted 8.4 % in conc. 300 µg/ml and 94.3% in conc. 12.5 µg/ml with formulation 3 i.e NPF3.

Figure-10: Per cent cell viability for NPF1 formulation at different concentration



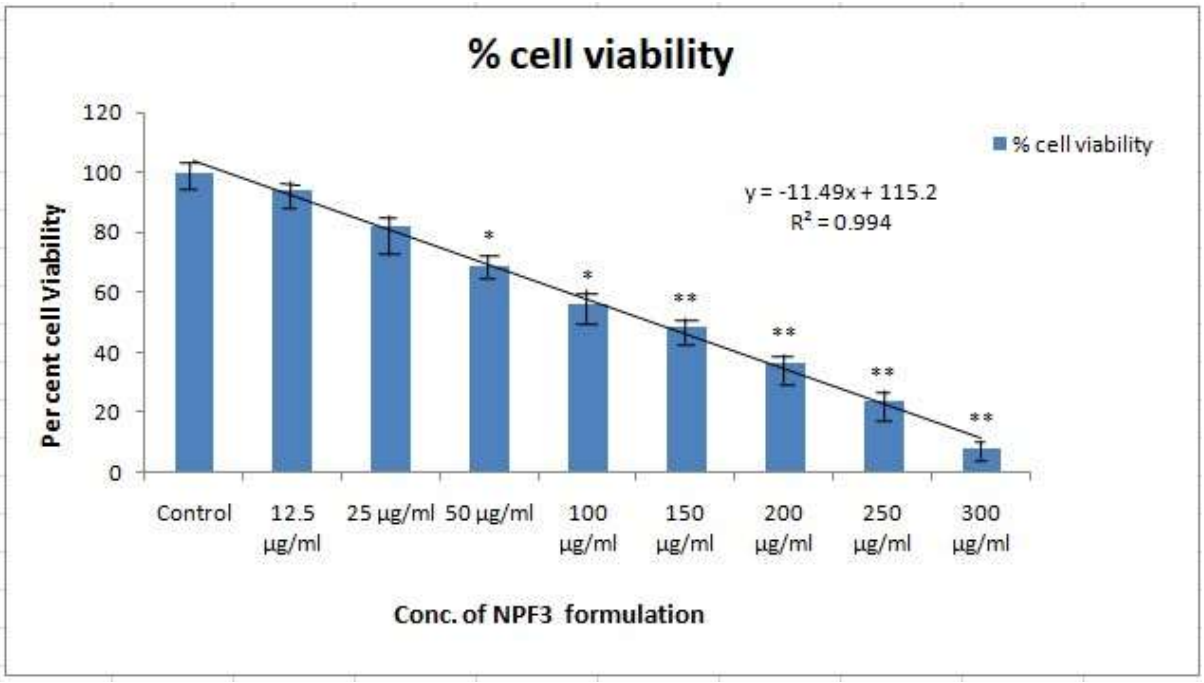
- Results are expressed as the mean ± SEm of three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 Vs Control.

Figure-11: Per cent cell viability for NPF2 formulation at different concentration



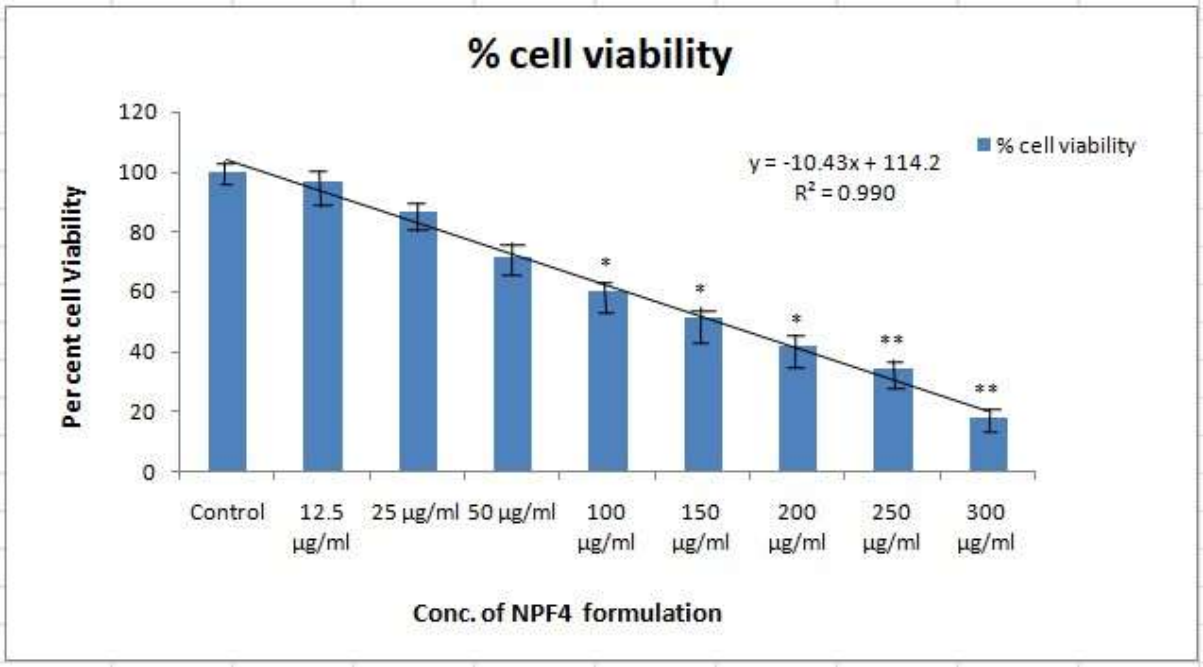
- Results are expressed as the mean ± SEm of three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 Vs Control.

Figure-12: Per cent cell viability for NPF3 formulation at different concentration



- Results are expressed as the mean ± SEm of three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 Vs Control.

Figure-13: Per cent cell viability for NPF4 formulation at different concentration



- Results are expressed as the mean ± SEm of three independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001 Vs Control

Glutathione and lipid peroxidation analysis

Similarly, glutathione and lipid peroxidation level for all the formulations were analyzed in varied concentrations. Result was tabulated in table-4, which revealed the similar effect as above i.e formulation 3 (NPF3) showed better significant results than others. It showed that significant decreased in glutathione content of 21.27 % followed by 28.73 % at 150 µg/ml for formulations NPF3 and NPF4 respectively (Table-4).

Table-4: Glutathione level for all the formulations

Conc.	NPF1 (%)	NP2 (%)	NPF3 (%)	NPF4 (%)
Control	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
12.5 µg/ml	97.73 ± 0.52^{ns}	97.83 ± 0.20^{ns}	87.47 ± 0.46***	90.53 ± 0.56***
25 µg/ml	87.73 ± 0.75***	84.67 ± 0.27***	68.73 ± 0.34***	72.73 ± 0.54***
50 µg/ml	74.83 ± 0.75***	68.70 ± 0.25***	50.60 ± 0.47***	58.17 ± 0.32***
100 µg/ml	57.53 ± 0.47***	51.83 ± 0.48***	37.80 ± 0.23***	41.63 ± 0.38***
150 µg/ml	42.03 ± 0.73***	44.63 ± 0.38***	21.27 ± 0.26***	28.73 ± 0.44***

- Values are presented as mean ± SEm; one-way ANOVA followed by Dunnett's test. *p < 0.05, **p < 0.01, and ***p < 0.001 were considered as significant compared with the control. ns = Non significant.

In contrast, lipid peroxidation level was increased significantly with all the formulations than control but significant increased with NPF3 followed by NPF4 with increased concentration. It

was showed that the value was 150.9 % followed by 147.50 % at conc. 150 µg/ml for formulations NPF3 and NPF4 respectively (Table-5).

Table-5: Lipid peroxidation level for all the formulations

Conc.	NPF1	NP2	NPF3	NPF4
Control	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
12.5 µg/ml	103.04 ± 0.55*	103.30 ± 0.55 *	115.60 ± 0.62***	104.9 ± 0.68**
25 µg/ml	115.20 ± 0.26***	107.6 ± 0.53***	125.50 ± 0.69***	112.80 ± 0.84***
50 µg/ml	124.40 ± 0.44***	119.7 ± 0.43***	134.60 ± 1.04***	126.80 ± 0.87***
100 µg/ml	131.40 ± 0.52***	130.7 ± 0.58***	144.2 ± 0.75***	139.4 ± 0.55***
150 µg/ml	137.90 ± 0.26***	144.6 ± 1.17***	150.9 ± 0.79***	147.50 ± 0.78***

- Values are presented as mean ± SEm; one-way ANOVA followed by Dunnett's test. *p < 0.05, **p < 0.01, and ***p < 0.001 were considered as significant compared with the control.

From the FTIR analysis we found there is no such significant chemical interaction between drug and polymer. After trying number of formulations we finalize four different formulations by varying the PLGA and Drug ratio. It was reported that Pharmaceutical product production process supervision, quality control, and quantitative analysis have all benefited greatly from the extensive application of FTIR [11, 12]. Various pharmaceutical preparations such as liquids, solutions, pastes, powders, films, fibers, gasses, and surfaces can all be analyzed by carefully selecting this sample technique. Therefore, in this investigation FTIR study was carried out for the pure drug and also mixed with PLGA and PVA.

Pluronic-127 as surfactant for first two formulations and Tween-80 for next two formulations were used after the method standardization. Percentage loading increases due to the co-surfactant Tween-80 and Pluronic -127. The present work was similar with the earlier researches revealed in their researches [13, 14].

From the particle size, PDI and Zeta potential (ZP) data, it was found that NPF3 and NPF4 were proceeding for the further development. Although, comparing NPF3 and NPF4, there is no significance change observed in case of particle size and PDI in both formulation and the overall, data represented that NPF3 showed to be good nano carrier for potent therapeutic activities. Thereafter, particle size was determined by SEM. One of the most popular methods for characterizing nanomaterials and nanostructures is the SEM. The signals that result from electron-sample interactions provide details about the sample, such as its chemical makeup and surface morphology (texture) [15, 16].

Furthermore, the surface charge of nanoliposomes is directly reflected in the value of ZP [17, 18]. ZP for formulation 3 showed higher negative value which was directly proportional with the Polydispersity index and the result was similar with the earlier reports [19, 20]. It was observed that a PDI of 0.3 or below is regarded as acceptable in drug delivery applications employing lipid-based carriers, such as liposome and nanoliposome formulations, and denotes a homogeneous population of phospholipid vesicles. The same result of PDI observed in formulation-3 [21].

Thereafter, in silico study was performed by selected protein for breast cancer with the mother compound doxorubicin and showed very good binding effect which confirmed preliminary as potent anti breast cancer drug. Earlier many study was revealed molecular docking study as preliminary confirmation as anticancer activity [22, 23].

Thereafter, Neutral Red Uptake method was adopted for determination of cytotoxicity for all the formulations and revealed decreased in cell viability with increased in concentration which was similar result as per the earlier study [10].

Not only that, in case of per cent of glutathione content was depleted with increased concentration where as reversed effect observed for per cent content of lipid. Lipid content was increased with increased in concentration. The similar effects were also reported by earlier scientific reports [10, 24, 25].

Conclusion

Overall the result concluded that formulations NPF3 and NPF4 are good formulations among the four formulations but there is no significance change observed in case of particle size and PDI. Overall data represented that NPF3 showed to be good nano carrier for potent therapeutic activities. Further, the study will provide us a nano-particulate delivery system of doxorubicine which will reduce the dose and therapeutic toxicity of the drug in the patient and provide more patient compliance. Based on that further molecular docking study and other in vitro tests were performed and resulted nano formulated doxorubicin was a potent anti breast cancer drug.

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Conflict of Interest

No conflict of interest. **References**

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