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PLGA Based Nanoencapsulation For Controlled Release Of Doxorubicin For Cancer Therapy

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Abstract: Nanoparticle based drug delivery has been evolved to enhance the drug efficacy in recent times. Nanoparticles are used for drug delivery should be less toxic for normal cells and more effective drug delivery for tumor cells. In this study, the doxorubicin is encapsulated with Poly Lactic and Glycolic acid (PLGA) by desolvation technique. The doxorubicin loaded PLGA nanoparticles are prepared by desolvation technique. Ethyl alcohol was added intermittently into PLGA solution. Following the desolvation of the doxorubicin, the resulting nanoparticles were stabilized by the addition of glutaraldehyde. The morphology and characterization of nanoparticles were done by using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR). The drug release study was carried by in vitro setup. It was noted that 82% of drug has been released at the end of 72 hours. In addition, animal cell line study was done for anti-cancer efficiency. PLGA polymeric particles loaded with doxorubicin act as a promising vehicle for targeting tumor cells by killing them at drug concentration of as low as 50µg/ml.

Keywords: Poly Lactic and Glycolic acid (PLGA), desolvation method, SEM, FTIR, controlled drug release, Doxorubicin.

I. INTRODUCTION

One of the most challenging breakthroughs in drug delivery and therapeutics is the nanomedicine. As nanoparticles (NPs) possess the particle size in the range of 1-100 nm it can easily interact with body tissues and hence it finds applications in chemotherapy and analytics.

Polymeric nanoparticles are well engineered and efficient systems for drug release studies as these possess the property of biodegradability, biocompatibility and also biostable. Due to these properties, they can overcome various barriers and target the cancer cells without any harm to normal cells and tissues. Based on the mode of preparation, nanoparticles are broadly classified as ionic, non-ionic and based on method of preparation; nanoparticles are classified into nanospheres and nanocapsules. In nanospheres, the drug may be absorbed at the spherical surface and in nanocapsules; the drug is loaded *in situ* into polymeric particles. Poly Lactic and Glycolic acid (PLGA) is a synthetic polymer used to prepare nanoparticles for chemotherapy because of its biocompatibility and non-toxicity. Desolvation is preferred to prepare nanoparticles by dissolving doxorubicin completely in PLGA followed by purification. After this, the drug is entrapped by the polymeric surface.

In this study, PLGA is a preferred polymer as it is a type of copolymer and it shows a promising property of drug delivery to the target. It can also act as an antioxidant to protect the drug from free radicals and other chemicals agents. PLGA also finds applications in tumor detection and delivery of drug to cancer tissues. Doxorubicin is the selective anticancer drug for this delivery as doxorubicin is hydrophilic in nature. Doxorubicin is derived from bacterial species by chemical synthesis and is widely used for chemotherapy to cure cancers (breast, prostate) and various carcinomas. It is first investigated in mice to target HIV-1. Doxorubicin can lower WBC count if the dosage level is high; if it leaks out from vein it will damage tissues causing ulceration. So it should be carefully loaded into the polymer particles to target cancer cells. The PLGA-doxorubicin when spots the target, it binds with cancerous tissues and slowly releases the drug to the tissues in a controlled manner, where PLGA acts as a carrier vehicle or the delivery of drug.

II. MATERIALS

All chemicals and reagents used were of analytical grade and used without further purification. Lactic acid, Glycolic acid, ethyl alcohol (98 %) and glutaraldehyde were supplied by LOBA Chemicals, India. All aqueous solutions were prepared by double distilled water.

III. METHODS

I. PREPARATION OF NANOPARTICLES

PLGA-DOX NPs were prepared by the desolvation method. 100 mg PLGA powder was dissolved in 5.0 ml of 10 mM Nacl solution and Maintained the pH 7.0 with 0.1 N NaoH. 1.0 ml of doxorubicin drug was added to 4.0 ml of

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ethyl alcohol that ethanolic drug was continuously added drop wise to the PLGA samples under stirred (300 rpm) at room temperature, till the solution turbid. And these samples were transformed to NPs. The turbid solution was stirred at continuously 40 minutes for stabilize nanoparticles without adding of ethyl alcohol. After that, 150µl of 25% glutaraldehyde was added for cross linking purpose to generate nanoparticles.

II. Purification

The generated NPs were purified by 3 to 4 cycles of centrifugation (8000 rpm, 30 minutes). The pellet was redispersed by 10 mM Nacl (pH 7.0). Each redispersion step was performed shaker.

IV. CHARACTERIZATION

III. SEM (Scanning Electron Microscope)

SEM was performed using ZEISS SEM. PLGA is nonconductive. Signals are generated when the electron beam hits the sample and these signals are used for the analysis of structural details and compositional information in the sample. Composition of the nanoparticles was can be identified by performing EDS analysis.

IV. FTIR (Fourier Transform Infrared Spectroscopy)

FTIR is the special method of spectroscopy suitable to check the quality or purity of the nanoparticle. The samples were kept in the field of IR radiation and the absorbance spectrum was checked to produce the spectrum of the nanoparticle sample. It helps to find the amount of sample in a mixture and thus finds application in qualitative analysis of PLGA nanoparticles.

V. *IN VITRO* RELEASE

To ensure the release of drug in aqueous solution *in vitro* drug release was carried out with dialysis membrane of molecular weight between 12000 to 14000. In this, the dialysis bag was pretreated with phosphate buffer for about 15 minutes. About 50mg of the prepared NPs was added to dialysis bag followed by addition of 1ml phosphate buffer. This membrane was kept immersed in a 50ml phosphate buffer saline solution of pH 7.4. Then the setup was kept at magnetic stirrer at 100 rpm. After 1:30 hrs drug release study was carried out by withdrawing 2ml of sample solution from the medium and checking for absorbance at 266nm. This reveals the release of drug from the dialysis membrane and 2ml of fresh buffer was added in to the medium. This entire setup was kept at 37ºC to match the body temperature. The sample was withdrawn from the medium at regular time intervals (1:30, 3, 4:30, 6, 48, 72 hrs) and checked for absorbance.

VI. CELL LINE STUDY

I. Cell line

Cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin 20 µl (100 U), and streptomycin (1000 µg/ml) and amphotericin B (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37 °C.

II. Reagents

MEM, Fetal bovine serum (FBS), Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), Dimethyl sulfoxide (DMSO) and antibiotics were purchased from Hi media & Sigma Aldrich. **Inverted Microscope** (Lobomed) Magnefication 40X.

III. *IN VITRO* ASSAY FOR CYTOTOXICITY ACTIVITY (MTT ASSAY)

The anticancer activity of samples on **MCF7 cells** was determined by the MTT assay (*Mosmann et al*., 1983). Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates and incubated at 5 % CO_2 incubator for 72 hours. Then, various concentrations of the samples were added in 0.1% DMSO for 48hrs at 5 % CO_2 incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of **MCF7 cells** were expressed as the % cell viability using the following formula:

% Cell viability = A540 of treated cells / A540 of control cells × 100

VII. RESULTS AND DISSCUSION

I. SEM (Scanning Electron Microscopy)

The nanoparticles were coated in a carbon sheet for microscopic examination. This Doxorubicin loaded PLGA nanoparticles were then examined for size, topology, structure of nanoparticles, which then reveals that this nanoparticles ranges between 100-150nm in diameter with a smooth and spherical surface. The image of doxorubicin loaded PLGA nanoparticles were shown in the figure 1.

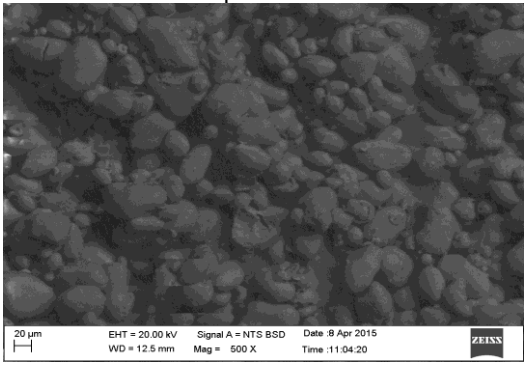

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Fig. 1: Microscopic image of PLGA-Dox under SEM

II. FTIR

The graph (Fig. 2) represents the absorption spectrum of the Doxorubicin loaded PLGA nanoparticles to check for the purity of the nanoparticles with the help of infrared rays. This shows that the nanoparticles were pure at 2167cm^{-1}, 2124cm^{-1} and had impurity peaks at 3342cm^{-1}. The nanoparticles were checked for purity by before and after purification and the resulting spectrum shows that the nanoparticles have had purity peaks and no impurity peaks after purification. This graph proves that the resulting nanoparticles are pure.

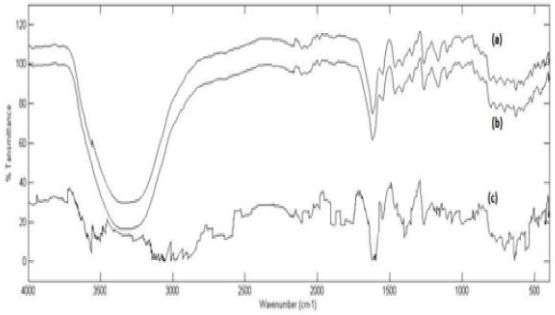

Fig. 2: FTIR result of PLGA -Dox Nanoparticles: (a) Native PLGA, (b) PLGA -Dox NPs with purification, (c) PLGA-Dox NPs without purification.

III. *IN VITRO* DRUG RELEASE

Drug release studies was done using dialysis bag kept in PBS (Phosphate Buffer Solution, pH 7.4) solution at 37^{o}c. The figure (Fig. 3) represents absorbance of the sample at various time intervals. The absorbance is low at initial periods; this indicates that the drug release is sustained and gets increased at greater periods. The cumulative absorbance is of the range between 0.438 and 1.638 and the results are tabulated in Table 1.

Table 1: *IN VITRO* release of PLGA -Dox Nanoparticles

Time (hrs)	Absorbance at 266nm
1 ½	0.438
3	0.080
4 ½	0.085
6	0.094
48	1.331
72	1.682

The figure (Fig. 3) represents the plot between absorption and transmittance. The release of drug was estimated to be about 82% at the end of 72 hours.

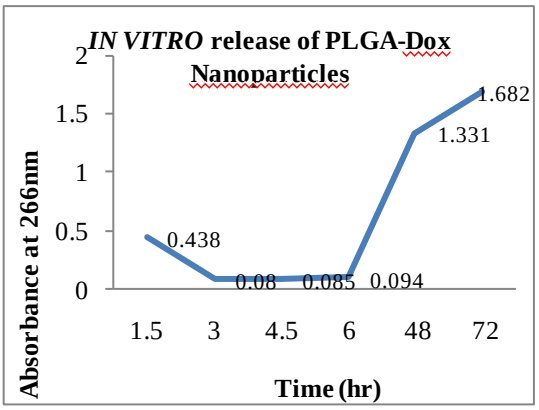

Fig. 3: *IN VITRO* release of PLGA-Dox Nanoparticles

IV. MCF7 CELL LINE STUDY

Cell line studies was carried out using MCF-7 (Michigan Cancer Foundation-7) a breast cancer cell line culture which is a breast ductal carcinoma. Initially without the incubation of the drug, absorbance is checked in this case the cells are 100% viable. The prepared nanoparticles were injected in to the cell line at suitable times and check or absorbance and also check or viability. The table given below entails the details about the drug efficiency to kill cells at various concentrations. At initial concentrations cells have the capacity to withstand drug effects at 50µg/ml cells were completely destroyed.

Table 2: Results of animal cell line studies

S. No.	Concentration µg/ml	Dilution	Absorbance 540nm	% cell Viability
1	Control	-	0.81	100
2	3.12	1:32	0.45	55.5
3	6.25	1:16	0.38	46.9
4	12.5	1:8	0.25	30.8
5	25	1:4	0.16	19.7
6	50	1:2	0.10	12.3
7	100	1:1	0.00	0.0
8	200	Neat	0.00	0.0

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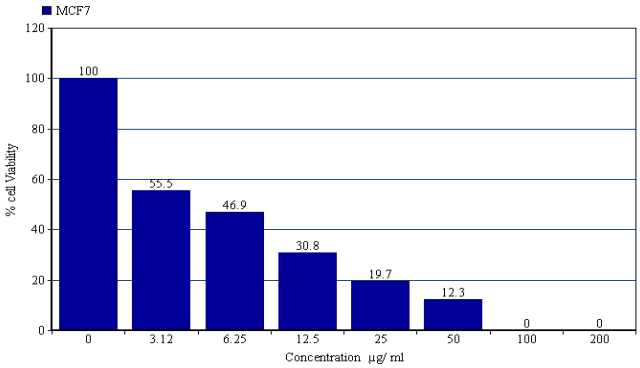
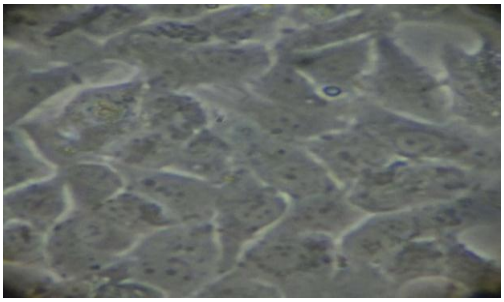
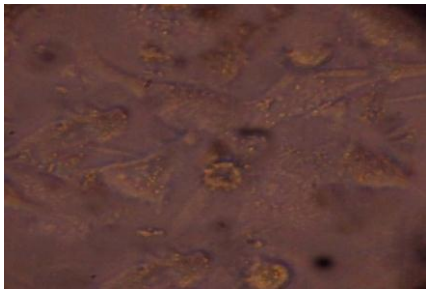


Fig. 4: Representation of cell viability with different concentrations MCF7 Control Cell line

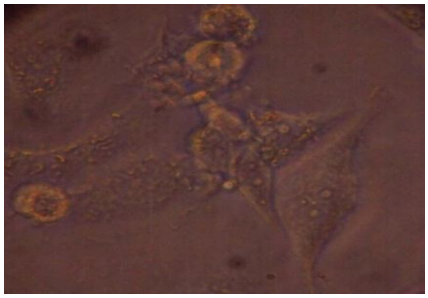


Before loading with **PLGA-Dox Nanoparticles**

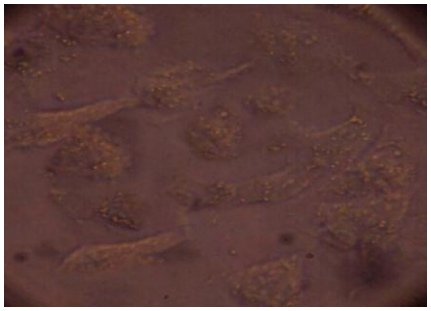


At concentration of 3.12

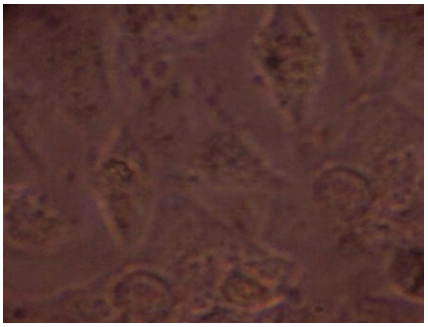
At concentration of 6.25µg



At concentration of 12.5 µg

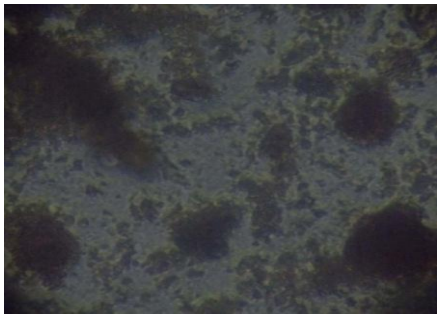


At concentration of 25 µg



At concentration of 50µg

At concentration of 100µg



At concentration of 200 µg

Fig. 5: Representation of cell morphology at different concentration of Nanoparticles

Table 3: Results of animal cell inhibition study

Concentration of drug (µg)	Description
Nil (before loading)	Cells with 100% viability
3.12	Change in structure was observed

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6.25	Morphology of cells gets changed
12.5	Internal cell components destroyed
25 and 50	Cells destroyed completely

A. CONCLUSION

The anti-cancer drug, Doxorubicin, has successfully loaded into PLGA polymeric nanoparticle using desolvation technique. The loading efficiency can be increased by type of reaction medium, surface concentration, doxorubicin concentration and pH of the medium. Loading of 2.5×10^{-5} M doxorubicin to polymeric particles under suitable condition for each preparation was found to have a high loading efficiency of doxorubicin onto each polymeric surface. The *in vitro* release observed for these nanoparticles was performed in a controlled manner in a dialysis membrane and the absorbance is taken at regular intervals. Cell line studies were performed using MCF-7 cancer cell line and the cancer cells were destroyed completely at 50 µg/ml concentration of the released drug. In conclusion, this system serves as an efficient tool for delivery of anticancer agents as about 82% of drug has been released at the end of 72 hours and 100% cancer cells were destroyed at a minimum concentration of drug as low as 25µg.

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