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# FORMULATION DEVELOPMENT OF NOVEL PLGA NANOPARTICLE FOR CO-DELIVERY OF DOCETAXEL AND PREDNISONE FOR THE TREATMENT OF PROSTATE CANCER

**Suparna Ghosh (Ray)[1] and Sayantan Ray[2]***

[1]Department of Radiotherapy, Calcutta National Medical College, Kolkata.

[2]Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

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***Corresponding Author**
**Sayantan Ray**
Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

## ABSTRACT

In the current study PLGA [poly (D, L-lactide-co-glycolide)] polymer encapsulated dual drug loaded (Prednisone and Docetaxel) nanoparticle has been reported for treatment of prostate cancer. Two different drugs have been intercalated within polymeric carrier by simple single emulsion solvent evaporation technique. After intercalation of the drug within polymeric matrix system the resultant nanoformulation known as PLGA-prednisone –docetaxel was characterized by using laser light scattering to determine average particle size and size distribution, further dissolution study was carried out in phosphate buffer saline (PBS) at pH 7.4, which exhibited sustained release profile of both the drug prednisone and docetaxel for a prolonged period of 240 h. The *in vitro* cell cytotoxicity study of PLGA-prednisone-docetaxel nanoparticle was carried out using human prostate cancer cell line, as per standard protocol, that exhibited significant inhibition of the cancer cell proliferation approximately two times more compared to the drug alone prednisone and docetaxel. To the best of our knowledge, this is the first ever report of the use of PLGA polymer encapsulated prednisone and docetaxel dual drug delivery for treatment of prostate cancer.

**KEYWORDS:** Prednisone; cell viability; *in-vitro*; sustained release**.**

## INTRODUCTION

Prostate cancer (PC) is the second most common cancer in men (333,000 cases, 10.0% of the total cancers) in UK and the second in (570,000 cases, 9.4% of the total cases) worldwide after lung cancer.[1] It is a major concern that incidence rates of PC in Indian immigrants to

the United Kingdom and USA are much higher, suggesting that life styles and dietary habits (e.g., high intake of fat, alcohol, red meat, obesity, sexually transmitted disease, smoking and lack of physical exercise) are the reasons behind PC.[2,3] Combination of medical or surgical castration and hormonal therapy is the preferred mode of treatment for carcinoma prostate, but in hormone resistant cases and in advanced stages chemotherapy with docetaxel and prednisone is the most preferred treatment.[4] Radiotherapy is considered for treatment of early carcinoma prostate as an alternative treatment of radical prostatectomy.[5] Prostate is relatively radio-resistant due to low alpha-beta ratio. Combination drug therapies are being explored as the synergistic action of multiple drugs could potentially lead to better therapeutic efficacy and reduced possibility of drug resistance development by the cancer cells. Docetaxel and prednisone are some of the drug combinations in phase III clinical trials have already been established for first-line chemotherapy treatment of hormone resistant carcinoma prostate to enhance the improvement both the survival and quality of life.[6] However, these conventional treatments are limited by rapid relapse, systemic toxicity, and non-specific delivery of the drug combinations used, leading to low therapeutic efficacy. Therefore, it is crucial to develop a system that can overcome the above limitations and provide targeted and controlled releases of these therapeutic reagents for effective anti-cancer therapy to treat these advanced prostate cancer patients.

In this scenario, the role of nanoparticles is well documented, due to increased site-specific targeting, reduced systemic toxicity, improved drug solubility and bioavailability, and incorporation of multiple components that impart multi-functionality for diagnosis and therapy. Although a myriad of efforts on enhancement of efficacy of the anticancer drug have been reported in various polymeric systems, but the area of combination chemotherapy by using polymeric nanocarrier for delivery of docetaxel and prednisone is not been explored for treatment of prostate cancer.

In the present work, our aim to develop a dual drug loaded PLGA nanoparticulate formulation for controlled release of anticancerous drugs docetaxel and prednisone, to explore the possibility of its application in treatment of prostate cancer with minimal or no side effects. The PLGA encapsulated docetaxel and prednisone nanopaticle was developed by single emulsion technique and particle size and surface charge was analyzed. The *in vitro* drug release study, and comparative *in-vitro* cell viability assay was carried out in (human prostate cancer cells). We believe that such nanoparticulate novel formulation could have

several advantages over conventional dosage forms: reduction in wastage of costly drugs, dosage frequency and several toxic side effects on account of high plasma concentration and thereby improving the overall patient compliance to a great extent.

## MATERIALS

Prednisone (molecular weight, 358.428 g/mol) and Docetaxel (molecular weight, 807.879 g/mol) were obtained as a gift sample from M/s Sigma Aldrich, USA. The polymer used in this work, poly (D, L-lactide-co-glycolide) (PLGA), with a copolymer ratio of D, L-lactide to glycolide of 50: 50 (molecular weight in the range, 24 000–38 000 g/mol) (M/s Boehringer Ingelheim Pharma Gmbh & Co., Germany). The non-ionic surfactant poly (vinyl alcohol), PVA (water soluble, 87–90% hydrolysed, molecular weight 30 000–70 000) were purchased from M/s Merck Specialties Pvt Ltd, Mumbai, India. The solvent dichloromethane (DCM), chloroform, acetone and other chemicals of AR grade were purchased from Sigma Aldrich, St. Louis, MO, USA. Deionised and decarbonated ultrapure water (Millipore, specific resistivity 18.2MΩ) was used for all the syntheses and the chemicals utilized in this study were used as received without further purification.

## METHODS

Nanoparticles were prepared by single emulsion (O/W) cum solvent evaporation method as described by Sayantan et al.[7] Briefly, 500 mg of PLGA, 125 mg of docetaxel and 125 mg of prednisone (2: 1:1) were dissolved in 10 ml of acetone and chloroform mixture (1:1), and then added drop wise through a 16 gauge needle syringe to 10 ml of a 2% (w/v) poly (vinyl alcohol) aqueous solution. The emulsion thus formed was homogenized (@ 18,000 rpm) using a high speed homogenizer for 8 min and was stirred for a period of 8 h for complete evaporation of the solvent, to precipitate the PLGA encapsulated prednisone and docetaxel nanoparticles. The resulting sample was collected by centrifugation at 8519g for 15 min and was washed several times with decarbonated water to remove the excess non-ionic surfactant, PVA. Finally, the particles were re-suspended in a cryoprotectant (1% w/v mannitol solution) and freeze dried at-82º C at a vacuum pressure of 20 Pa to receive PLGA-prednisone-docetaxel nanoparticle (Sample A).

### Determination of drug content in Sample A (PLGA-prednisone-docetaxel nanoparticle)

A known quantity of (100 mg) sample A (PLGA-prednisone-docetaxel nanoparticle) was dispersed in 5 ml of solvent mixture (acetone: dichloromethane; 1:1) and shaken vigorously for 30 min to dissolve the PLGA coating.[8] A 10 ml solution of phosphate buffer saline

(PBS) was added to the above solution and sonicated further for 10 min to extract the drug in the same. The extract was filtered, diluted with PBS and drug concentration was measured using UV spectrophotometer (Perkin Elmer, Lambda 45, USA). The analytical methods were validated according to linearity, precision, accuracy, and specificity.

Drug content (%) = Amount of drug (prednisone and docetaxel) per mg of sample (A) × Total weight of sample A × 100 / Amount of drug (prednisone and docetaxel) taken to develop a batch.

***In vitro* drug release study of prednisone and docetaxel from (PLGA-prednisone and docetaxel nanoparticle/samples A)**

*In vitro* drug release of prednisone and docetaxel from samples A was carried out using type-II USP dissolution test apparatus (Electrolab TDT-146 08L, Mumbai, India). In this, a known quantity of samples A (comprising prednisone-docetaxel) equivalent to 50 mg of active drug were placed in a dialysis tubing cellulose membrane bag (cut off molecular weight 14 KD, M/s Sigma-Aldrich, St. Louis, MO 63178 USA) separately and immersed in 900 ml of PBS (pH 7.4) maintained at 37.2 °C with constant stirring @ 50 rpm.[9] At specific time intervals, 10 ml of aliquot was withdrawn and replenished immediately. The aliquots were filtered using a 0.2 mm, 13 mm diameter Nylon 66 filter paper (Pall Corporation, USA). The absorbance of filtered solution mentioned as above was measured by using UV-VIS sptrophotometer (Lambda1010 Bischoff, Switzerland, wavelength range 190-800 nm).

***In vitro* Bioassay study of sample A (PLGA-prednisone-docetaxel nanoparticle)**

**Cell Culture**

The human prostate cancer cells (DU 145) were obtained from ATCC (Rockville, MD, USA). All the cell lines were routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Invitrogen, UK), 2 mg/ml sodium bicarbonate, 1 µg/ml penicillin G and 1 mg/ml streptomycin, in 10 cm diameter plastic petri dishes at 37 °C in a humidified atmosphere with 5% $CO_2$ in an incubator (HF90 Heal Force, China). The cells were sub-cultured using trypsin–EDTA when they were 90–95% confluent. All experiments were done with the cells when they were within six passages after revival from cryopreservation.[10]

***In-vitro* cell inhibitory assay of samples A on DU-145 cell line**

Briefly, $2\times10^4$ cells/well were taken in a 96 well plate, after 24 h incubation at 37 °C in humidified atmosphere containing 5% $CO_2$, the cells as above were treated with drug encapsulated nanoparticles PLGA-prednisone-docetaxel (sample A), having drug concentration ~50 µg/ml (calculated from % drug entrapment efficiency) along with bare drug, for 24, 48, 72h in the triplicate manner whereas cells which received 200 µl culture medium containing 5% DMSO served as control. Next, the cell viability was measured by MTT assay, as mentioned above and the absorbance of each well was read at 550 nm using ELISA reader (iMark™ Microplate Absorbance Reader Bio-Rad, USA), to determine the efficacy of PLGA encapsulated prednisone-docetaxel nanoparticle or samples A.

**Characterization**

Particle size of both bare PLGA nanoparticle and its drug intercalated part samples A were measured by dynamic light scattering technique using a Microtrac Zetatrac, PA, (USA) on respective aqueous suspensions.

**RESULTS AND DISCUSSION**

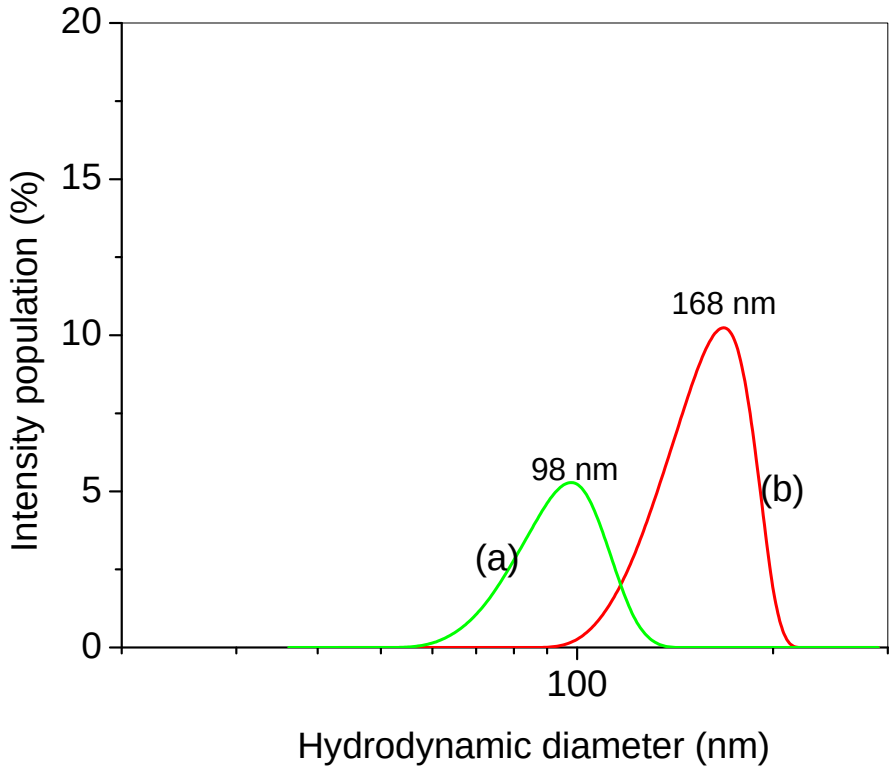


**Fig. 1: Particle size distribution (intensity) of (a) PLGA nanoparticle without drug and (b) PLGA-prednisone -docetaxel nanoparticle (Sample A).**

**Particle size analysis of sample A**

Figure 1 exhibits the particle size distribution (intensity) of drug intercalated PLGA-prednisone-docetaxel (Sample A) nanoparticle in aqueous suspension and bare PLGA nanoparticle without drug, measured by dynamic light scattering technique. This is already well established that an optimum particle size play a major role for reaching the target cells are in the range in between 100–200 nm. This leads to easy uptake by the diseased cells via 'endocytosis' mechanism, coupled with a longer retention time, on account of slow and controlled drug release process. Majority of the drug intercalated particles in aqueous suspension exhibit an unimodal distribution in the size range 50-200 nm with $D_{50}$ value of 168 nm in sample A, compared to bare or PLGA nanoparticle (~98 nm) and hence, is well within the limit. The polydispersity index (PDI) for all the nanoparticle formulations are below 0.5 (0.143, 0.212 for bare PLGA nanoparticle and sample A respectively).

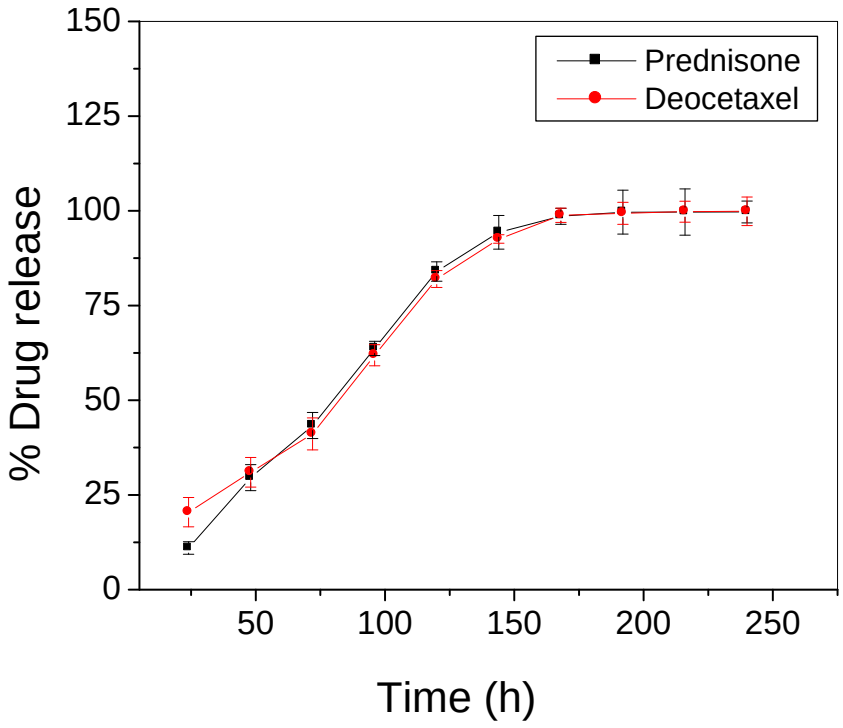


**Fig. 2: Cumulative drug release profile of prednisone and docetaxel from sample A (PLGA-prednisone-docetaxel nanoparticle).**

***In-vitro* dissolution study of PLGA-prednisone-docetaxel nanoparticle (sample A)**

The release profile of prednisone and docetaxel from sample A in phosphate buffer saline (pH 7.4) are shown in Figure 2. As observed, the release behaviour for both the drug prednisone and docetaxel from sample A or PLGA-docetaxel-prednisone exhibited a biphasic pattern,

characterized by an initial burst release of docetaxcel and prednisone within a period of 150 h followed by a slow and continuous release up to a period of 240 h. Around 75% of drug release within 120 h may be attributed to the loosely bound drug present at the PLGA surface/exposed area. Further a slower rate of remaining ~25% of the drug release in a period of next 120 h, which is due to slower diffusion of the intercalated drug molecule present in the polymeric matrix.

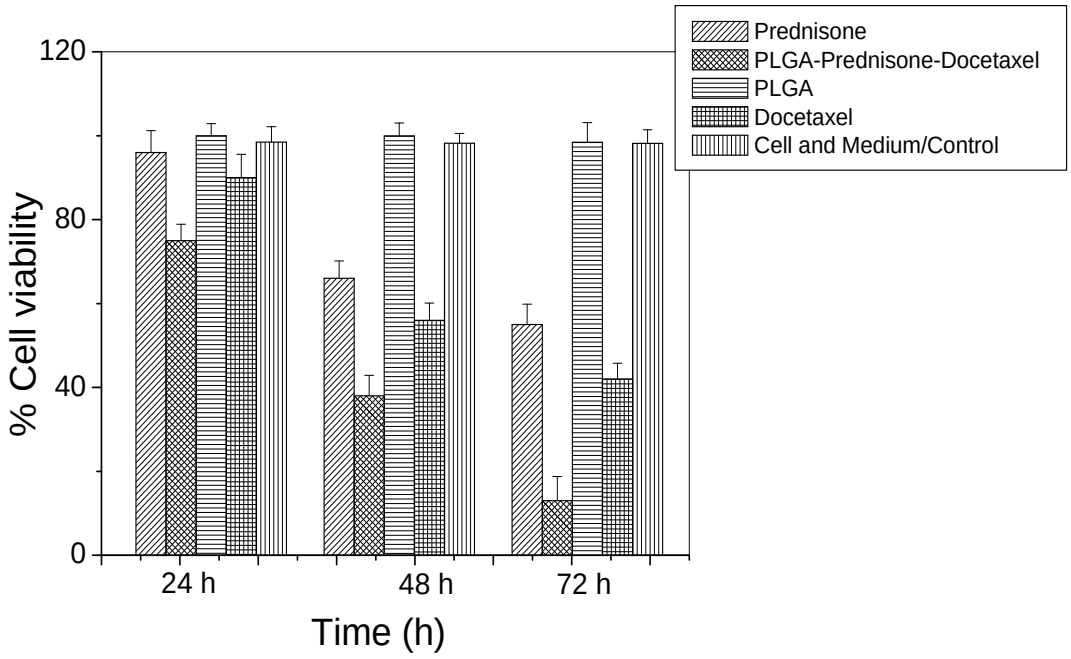


**Fig. 3: *In-vitro* cell viability assay of sample A (PLGA-prednisone-docetaxel nanoparticle), prednisone bare drug, docetaxel bare drug and PLGA nanoparticle without drug.**

***In vitro* cell viability assay of PLGA-prednisone-docetaxel nanoparticle (sample A)**

The efficacy of PLGA-docetaxel-prednisone nanoparticle to inhibit the growth of human prostate cancer cells (DU-145) at different incubation times has been shown in Figure 3. Interestingly, dual drug loaded PLGA nanoparticle (Sample A) shows significant effect to inhibit the cancer cells compare to bare drug e.g. prednisone and docetaxel drug at all the time points at 24 h, 48 h and 72 h respectively, but no significant effect was notified by bare PLGA nanocarrier. After 24 h of administration, the effect of the PLGA-prednisone-docetaxel nanoparticle formulation starts and it exhibits ~25% inhibition (~75% cell viability) of cell growth in comparison to the bare drug docetaxel (~12%) and prednisone (~6%) at 24h. After 48 h, the efficacy of the above dual drug loaded nanoformulationis enhanced to more than two times than bare docetaxel and prednisone. A much higher efficacy

of PLGA-prednisone-docetaxel nanoparticle, compared to bare docetaxel and prednisone monitored is distinctly noticeable for a period of 72 h. The PLGA-docetaxel-prednisone nanoparticle formulation exhibits cell inhibition to the extent of~98%, whereas in case of the bare drug, it is ~40 to 55%. This confirms the higher efficacy of the nanoformulation in comparison to the bare drug.

## CONCLUSION

In the present communication, an attempt was made to encapsulate two anticancerous drug within PLGA polymeric matrix by simple laboratory based single emulsion technique to form PLGA-prednisone docetaxel nanoparticle that was in turn confirmed by particle size analysis. Further a substantial drug loading (~62 wt %) in the PLGA-prednisone-docetaxel nanoparticle formulation was obtained, which was in good agreement to the drug entrapment efficiency analysis. Cumulative release for PLGA-docetaxel-prednisone nanoparticle was approximately 55% in a period of 120 h at 37°C, while, the entire drug (99%) was released by a period of 240 h. *In vitro* cell viability assay shows the better efficacy of PLGA-prednisone docetaxel nanoparticle in comparison to the bare prednisone and docetaxel when tested over DU-145 human prostate cancer cell line for a period of 72 h. Hence, the cellular response of the drug loaded nanoparticle may be correlated to the higher drug uptake into the target cells, being encapsulated within the polymeric nanocarriers. Finally, this work demonstrates the potential of the PLGA-prednisone docetaxel nanoparticle as a delivery vehicle in the significant area of cancer drug delivery that can limit the side effects of the highly toxic cancer drug like prednisone and docetaxel.

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### Disclosure statement

The authors [S. Ghosh (Ray), S. Ray] declare that they have no conflicts of interest.

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