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Efficacy of Polymer Nanoparticles for Controlled *in vitro* release of Doxorubicin for Cancer treatment

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Abstract — **Nanoparticles have been evolved as a promising tool for controlled drug delivery to enhance the drug efficacy in recent times. Polymer nanoparticles are used as drug carriers and are less toxic for normal cells and more effective drug delivery for tumor cells. In this study, the doxorubicin is loaded on to various types of natural polymers like starch, bovine serum albumin and poly hydroxyl butyric acid (PHB) by various techniques. The morphology and characterization of these nanoparticles were determined by using Scanning Electron Microscopy (SEM). The drug release study was carried by *in vitro* membrane setup. It was noted that there is controlled release of drug during the entire period and a maximum (82%) of drug which has been released at the end of 72 hours.**

Index Terms — **PLGA, PHB, controlled drug release, Doxorubicin.**

I. INTRODUCTION

The most important breakthroughs in drug delivery and therapeutics is the nanomedicine. Nanomedicine is an emerging field dealing with the formation and exploitation of materials at a nano scale for the diagnosis, treatment and imaging of diseases. Nanoparticles (NPs) have the particle size in the range of 1-100 nm and they can easily interact with body tissues thus finding applications in diagnosis and treatment. In cancer treatment, the nanoparticles are used as drug delivery systems since it has the advantage of bioavailability, *in vivo* stability, intestinal absorption, solubility, sustained and targeted delivery, and therapeutic effectiveness of several anticancer drugs.

Polymeric nanoparticles may be synthetic or natural which may act as 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.

Bovine serum albumin is a polymer as it is a type of protein (albumin) obtained from Bovine Serum 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. It finds applications in tumor detection and delivery of drug to cancer tissues. Starch nanoparticles produced from gelatinized starch. Poly hydroxyl butyric acid (PHB) has also been used as a suitable nanoparticle for its biodegradability and biocompatibility in biomedical applications.

Doxorubicin is derived from bacterial species by chemical synthesis and is widely used for chemotherapy to cure cancers (breast, prostate) and various carcinomas.

II. MATERIALS

All chemicals and reagents used were of analytical grade and used without further purification. All aqueous solutions were prepared by double distilled water.

III. METHODS

I. PREPARATION OF NANOPARTICLES

Biodegradable nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic biodegradable polymers. The selection of the base

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polymer depends on various designs and end application criteria. It depends on factors like size of the desired nanoparticles, properties of the drug (solubility, stability, etc.), surface characteristics and functionality, nature and degree of biodegradability and biocompatibility and drug release efficiency. The methods of preparation of nanoparticles can be classified as Solvent evaporation, Spontaneous emulsification/solvent diffusion, Nanoprecipitation, Salting out etc.

(A) SOLVENT EVAPORATION METHOD - In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate. The drug is dissolved or dispersed in the preformed polymer solution followed by emulsification of the mixture to form an oil/water (o/w) emulsion using an appropriate surfactant/emulsifying agents. Most commonly used surfactant/emulsifying agents for this purpose are gelatin and polyvinyl alcohol. After formation of a stable emulsion the organic solvent is evaporated by increasing the temperature or pressure along with continuous stirring of the solution.

(B) SPONTANEOUS EMULSIFICATION / SOLVENT DIFFUSION METHOD - This is a modified solvent diffusion method where a water-miscible solvent such as acetone or methanol along with a water-insoluble organic solvent such as dichloromethane or chloroform are used as an oil phase. Due to the spontaneous diffusion of solvents, an interfacial turbulence is created between the two phases leading to the formation of smaller particles. As the concentration of water-soluble solvent increases, smaller particle sizes of NPs can be achieved.

(C) NANOPRECIPITATION METHOD - This method is used for hydrophobic drug and hydrophilic drugs. Polymers and drugs are dissolved in a polar, water-miscible solvent such as acetone, acetonitrile, ethanol, or methanol. The solution is then poured in a controlled manner (drop-by-drop addition) into an aqueous solution with surfactant. Nanoparticles are formed instantaneously by rapid solvent diffusion. Finally, the solvent is removed under reduced pressure.

(D) SALTING OUT METHOD - In this method, the polymer is dissolved in the organic phase, which should be water-miscible, like acetone or tetrahydrofuran (THF). The organic phase is emulsified in an aqueous phase, under strong mechanical shear stress. The aqueous phase contains the emulsifier and a high concentration of salts which are not

soluble in the organic phase. Typically, the salts used are 60% w/w of magnesium chloride hexahydrate or magnesium acetate tetrahydrate in 1:3 polymer to salt ratio. The fast addition of pure water to the o/w emulsion under mild stirring reduces the ionic strength and leads to the migration of the water-soluble organic solvent to the aqueous phase inducing nanosphere formation. The final step is purification of nanoparticles by cross flow filtration or centrifugation to remove the salting out agent.

II. PURIFICATION OF NANOPARTICLES

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 by using shaker.

IV. *IN VITRO* DRUG RELEASE STUDY

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.

V. RESULTS AND DISSCUSION

I. *IN VITRO* DRUG RELEASE

Drug release studies was done using dialysis bag kept in PBS (Phosphate Buffer Solution, pH 7.4) solution at 37°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 0.080 and 1.693 and the results are tabulated in Table 1.

Table 1: *In vitro* drug release using various Nanoparticles

Time (hrs)	Absorbance at 266nm		
	Starch	BSA	PHB
1 ½	0.362	0.438	0.352
3	0.222	0.080	0.269
4 ½	0.401	0.085	0.396
6	0.644	0.094	0.674
48	1.192	1.331	1.218
72	1.561	1.682	1.693

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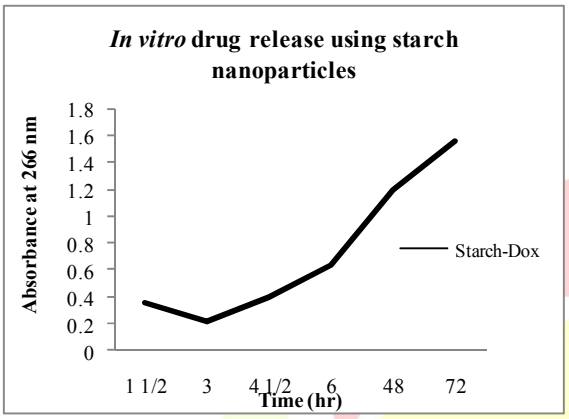

Fig 1. ***In vitro*** **drug release using starch nanoparticles**

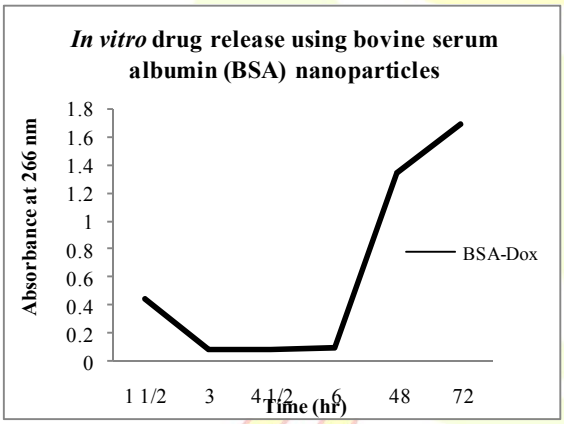

Fig 2. ***In vitro*** **drug release using BSA nanoparticles**

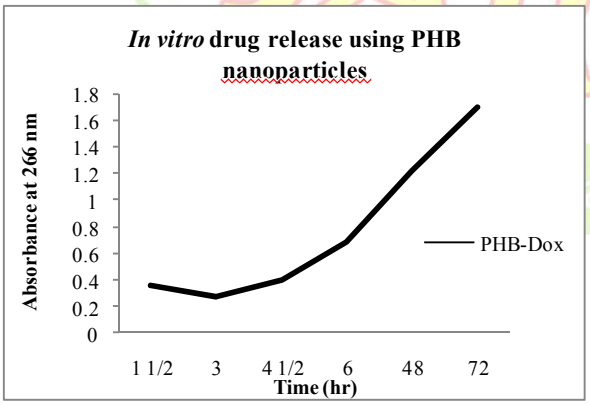

Fig 3. ***In vitro*** **drug release using PHB nanoparticles**

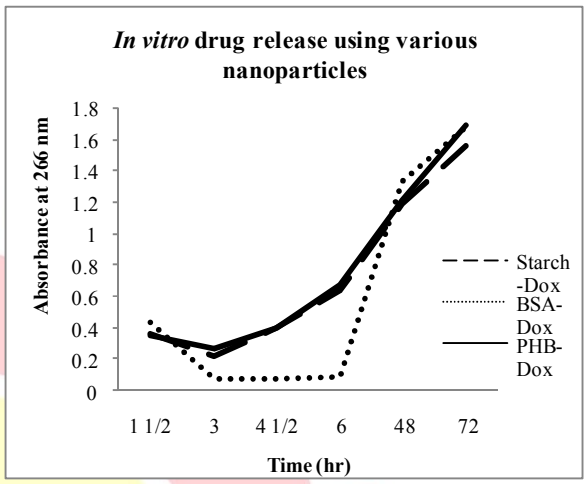

Fig 4. Comparison of *in vitro* drug release using various Nanoparticles

The figures (Fig. 1,2,3,4) represent the plot between absorbance and time in hours. Both starch and PHB showed similar kind of drug release whereas BSA showed lower release in the first few hours (up to 6 hours) and a sudden increase in the release of doxorubicin. The release of drug was estimated to be about 82% at the end of 72 hours.

VI. CONCLUSION

The anti-cancer drug, Doxorubicin, has successfully loaded into polymeric nanoparticles using various techniques. 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.

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