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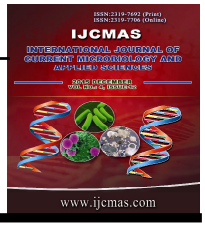

Original Research Article

Design and Characterization of Poly-Hydroxy Butyric Acid (PHB) Based Polymeric Nanoparticles for Controlled Release of Doxorubicin for Cancer Treatment

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Keywords

PHB, Doxorubicin, Nano-precipitation, Nanocapsules, Surfactant, Phosphate buffer

A B S T R A C T

Nanoparticles based on polymeric compounds provide enhanced solubility of Doxorubicin in the aqueous solution. Various parameters like presence of organic solvent, surfactant concentration, loading efficiency, doxorubicin concentration affect the size of doxorubicin-polymeric nanoparticle. Under controlled conditions, doxorubicin loaded *in situ* on to the polymer like polyhydroxy butyric acid with particle size ranging between 10nm to 100nm was achieved by nanoprecipitation method. These nanoparticles were characterized under Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) to find the surface topology, particle size, drug loaded inside the polymer, structure of DOX-PHB nanoparticles. The doxorubicin loaded PHB nanoparticle was observed for *in vitro* drug release study in a buffer solution mainly phosphate buffer solution for 5-7 days. This reveals that the PHB polymer acts as an efficient carrier vehicle for the anticancer agents.

Introduction

Nanoparticles (NPs) are ultrafine particles ranging between 1 and 100 nm in size. The chemical nature of nanoparticles may be metallic or polymeric. Polymeric nanoparticles may be synthetic or natural and exhibit nanosized colloidal structures. Based on the preparation of nanoparticles, drugs can be loaded in to, on to the surface. In nanocapsules, the drug is entrapped by the polymeric surface surrounding the molecule. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) display the structure of these finest nanoparticles. Techniques like desolvation, emulsification and evaporation are the recent techniques used for the preparation of nanoparticles. Among these, nanoprecipitation process is widely used process for the preparation of PHB-doxorubicin nanoparticles. The nanoparticles must be prepared in such a way that it should be biodegradable and biocompatible to body tissues (1,2,3).

Natural polymers like polyhydroxy butyric acid (PHB) exhibit biodegradability and also is a new and well known polymer which has a good drug delivery application with cells and tissues. These polyhydroxy butyric acid nanoparticles range between 10-100nm in size(4,5). Since these are hydrophilic, easily degradable and compatible with body tissues, polyhydroxy butyric acid is a well preferred polymer for this work. For instance, PHB nanoparticles can be loaded with different drugs like testosterone, nifedipine etc(6,7).

PHB is a biological polymer produced by bacteria like *Alkaigenes eutrophus* in which it acts as a storage material. PHB is mainly composed of polyhydroxyalkanoate. Many investigations used polymeric nanoparticles as a transdermal drug delivery system. These nanoparticles are mainly prepared by nanoprecipitation, emulsification and homogenitization. Due to its simplicity and reproducibility nanoprecipitation is preferred for preparation of these nanoparticles under controlled particle size by controlled use of surfactants and ethanol for purification (8, 9).

In this study, PHB nanoparticles were prepared by nanoprecipitation method. The loading of Doxorubicin *in situ* on to PHB to form Doxorubicin loaded PHB nanoparticles was done by precipitation of the preformed polymer particles and by water oil emulsion method. This provides a major advantage that there is no need of any organic solvent and no rising temperature for preparation of nanoparticles (10,11). It requires only small amount of organic solvent like ethanol to remove oily residues and also surfactants during precipitation process. The type of surfactant used plays a major role in the loading efficiency of doxorubicin-PHB nanoparticles as these surfactants should be non-ionic and nontoxic in nature(12,13). Also surfactant concentration determines the particle diameter. The anticancer drug loaded *in situ* in to PHB is doxorubicin which is a widely used drug for chemotherapy in treating leukaemia, lymphomas, soft tissue carcinomas, bladder, and breast cancers. Recent investigations in anticancer research have coupled doxorubicin with monoclonal antibody to eliminate HIV-1 in mice(14,15,16). Doxorubicin is a vesicant (i.e.) it is a chemical that causes tissue damage if it blisters out from vein. Doxorubicin should be carefully encapsulated in to PHB so that when it targets cancer tissue it does not cause damage to normal cells (17,18,19).

Materials and Methods

Ethanol (98%), Tween 80 (polysorbate80), were procured from LOBA Chemicals, India. Oil used was sunflower oil. All chemicals and reagents were used without further purification and were of analytical grade. The aqueous solutions were prepared by double distilled deionized water. PHB was obtained from bacteria.

Preparations by Nanoprecipitation

PHB-Doxorubicin nanoparticles were prepared by nanoprecipitation technique. The surfactant (Tween 80- an emulsifying agent) was initially added to ethanol to form ethanolic surfactant. Then 1 ml of doxorubicin was added with 15 ml of ethanolic surfactant solution, followed by the addition of 5 ml of oil and the mixture was stirred for 2 hrs at room temperature. Then 1% PHB solution was prepared by using double distilled deionized water. From that 1 ml of PHB solution was added drop wise to the mixer and stirred continuously for 2 hrs, doxorubicin was loaded *insitu* with PHB nanoparticles. The medium containing nanoparticles as fine aggregates was centrifugated at 15000rpm in a research centrifuge so that the doxorubicin loaded

PHB nanoparticles were formed as pellet leaving the supernatant above which has to be discarded. The pellet was taken out for purification followed by air dried in an incubator at 37ºC for 3 hours.

Purification of the Prepared Nanoparticles

The resultant pellet (unpurified) was taken out and then washed with ethanol for 5 times to remove the excess drug that gets loaded on the polymeric surface. After purification the pellet is air dried to form powdered nanoparticles.

Characterization

SEM

SEM was performed using ZEISS SEM. This helps to focus the sample to extract structural and chemical information of the nanoparticles and also helps to image the PHB-Doxorubicin combination. It helps to ensure the loading of drug in to the polymer. In this study the sample is coated with carbon coating cressington units. EDS analysis can be used to find the comparison of different materials in the PHB – doxorubicin nanoparticle combination.

FTIR

FTIR spectroscopy incorporates the use of infra-red radiation to find the structure of the resulting nanoparticle in the range of 50-4000cm^{-1}. This helps to find the structure of molecules and also its absorbance spectrum. By using FTIR the amount of drug present in the nanoparticles can be investigated and the resulting spectrum reveals the characteristics of the PHB-doxorubicin nanoparticles.

***In Vitro* Drug Release**

The drug release studies were carried out in phosphate buffer solution using dialysis membrane under controlled conditions. The dialysis membrane of molecular weight ranges between 12000 and 14000 was used. Initially the dialysis bag was treated with phosphate buffer for above 15 minutes. Then 50mg of the formulated NPs were added to the dialysis bag followed by addition of 1ml buffer solution and the setup is immersed in a 50ml phosphate buffer saline solution and the pH of the phosphate buffer is 7.4. The entire setup kept at a magnetic stirrer at 100 rpm at 37ºC. After 1:30 hrs 2 ml of sample solution was withdrawn and the absorbance is measured using UV Spectrophotometer at 266 nm. After the sample is withdrawn from the setup 2ml of fresh buffer was added to the medium. The absorbance is measured at different time intervals to ensure the release of drug from the dialysis bag (20,21,22).

Results and Discussion

SEM (Scanning Electron Microscopy)

Analysis of the prepared nanoparticles reveals the structure and size of nanoparticles and also loading of drug in to the polymeric surface. The nanoparticles were first coated in a carbon sheet in a crystalline form for examination under SEM. This images the loading of drug in to the polymer as shown in the figure given below with size range between 100-150nm with a smooth surface having spherical space inside.

FTIR

FTIR (Fourier Transform Infrared Spectroscopy) is done to check the purity of the prepared nanoparticles by using infrared light. The absorption spectrum of the nanoparticle sample was obtained, from that the purity was checked by plotting a graph

between the wave number and transmittance. The graph shows the sample remains impure at 3013.11cm^{-1} and it is pure after the purification process at 2190.74cm^{-1}, 2049.06cm^{-1}, 1550.49cm^{-1}.

Table.1 *In vitro* Drug Release of PHB-Dox Nanoparticles

Time (hrs)	Absorbance at 266nm
1 ½	0.352
3	0.269
4 ½	0.396
6	0.674
48	1.218
72	1.693

Fig.1 Microscopic Image of PHB-Dox under SEM

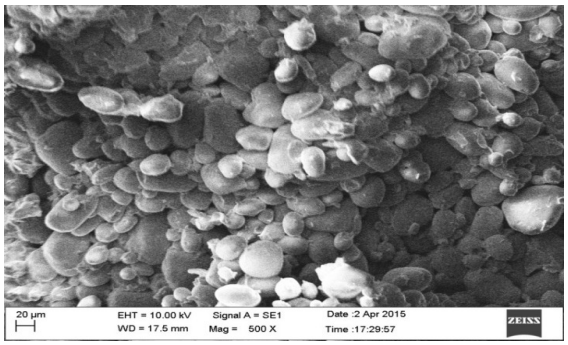


Fig.2 FTIR Result of PHB-Dox Nanoparticles :(a) Native PHB, (b) PHB-Dox NPs with Purification, (c) PHB-Dox NPs without Purification

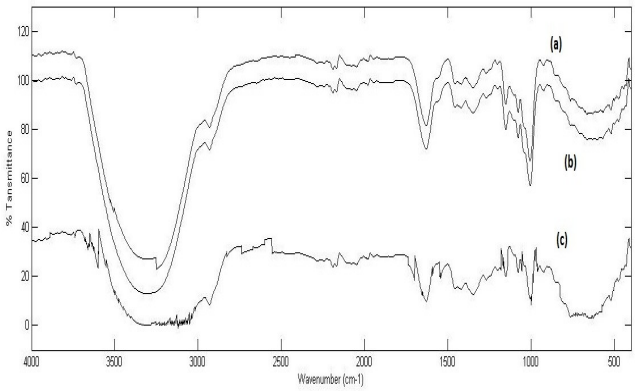
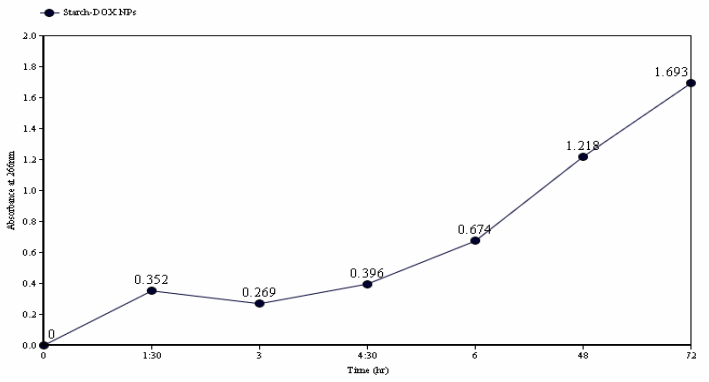


Fig.3 *In vitro* Drug Release of PHB-Dox Nanoparticles



In Vitro Drug Release

Drug release measurement of the sample for the period of 72 hours was in the table 2. The nanoparticle is put in a dialysis bag and then kept in PBS (Phosphate Buffer Solution) of pH 7.4 at 37^{o}c and then stirred in a magnetic stirrer. The absorbance is low (0.352) at the initial period and it increases over the period of time; this indicates that the drug releases in a controlled fashion.

In this study, the drug, doxorubicin was successfully loaded on to the PHB polymeric nanoparticles using nanoprecipitation method and its size range between 250 to 300 nm with smooth surface having spherical cavity inside which contains the anticancer drug (Doxorubicin). Various parameters like loading efficiency, molecular weight of the drug as well as the concentration of the drug affects release of nanoparticles from the dialysis bag. The absorbance was checked periodically after 1hr of treating with the phosphate buffer solution of pH 74 or the drug release studies. FTIR was carried out to check the purity of the prepared nanoparticle sample. At the last, Hence, it is concluded that PHB nanoparticle serves as an excellent carrier for drug delivery systems.

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