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RESEARCH ARTICLE

NANO ENCAPSULATION WITH INTERFACIAL DEPOSITION OF PHB (POLY-B-HYDROXYBUTYRATE) AS NANO PARTICLES WITH AMPICILLIN

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ABSTRACT

Different soil samples were collected for the isolation of PHB producing *bacillus sp* in and around Coimbatore area. As the preliminary analysis the screening, extraction and estimation of PHB, Physical optimization of PHB production, chemical characterization of extracted powder was done by FTIR analysis. Ampicillin is the drug of choice for per oral administration using nanoprecipitation technique. The production of Poly-β-hydroxybutyrate (PHB) as nanoparticle containing Ampicillin, increase the stability of loaded drug. The extracted PHB created as nanoparticles and the nano encapsulation was done by PCL immobilization method with presence and absence of drug. The resulting nanoparticle is characterized by scanning electron microscopy (SEM) analysis.

INTRODUCTION

Nanotechnology is an upcoming and fast developing field with potential application for human welfare. Nanomaterials have unique physicochemical properties, such as small size (10-1000 nm), large surface area to mass ratio, and high reactivity are different from bulk materials of the same composition. Recently many studies have been conducted to explore the synthesis of nanoparticle by the use of biodegradable polymers as a potential bio sources such as polyethylene glycol (PEG), polylactic glycolic acid (PLGA) and Poly-β-hydroxybutyrate (PHB) (Hans and Lowman, 2002). A nanoparticle is a sub microscopic solid material with the size ranging from 1-100nm. Materials used in the preparation of nanoparticles are sterilizable, non toxic and biodegradable like albumin, ethyl cellulose, gelatin polyesters etc. Pharmaceutical companies focused their research on creating nanoparticles formulations with high surface- to-volume ratios for personal administration of hydrophobic compounds. Various methods are used for the preparation of nanoparticles the salting-out (Bindschaedler *et al*., 1988), emulsification-diffusion and nano precipitation (Fessi *et al*., 1989) methods. One of the important methods for designing nanoparticle is the nanopreciptation. Ampicillin is the drug of choice for per oral administration using nanoprecipitation technique. The nano precipitation method is also called solvent displacement or interfacial deposition where the drug solution in a water miscible organic solvent is mixed with an aqueous solution containing a surfactant. Upon mixing, the supersaturated solution leads to nucleation and growth of drug particles, which may be stabilized by surfactant (Barichello *et al*., 1999). The production Poly-β-hydroxybutyrate (PHB) nanoparticles containing Ampicillin, increase the stability of loaded drug. The resulting nanoparticle is characterized by Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM), various physicochemical testing methods and the invitro release of drug is carried by dialysis method.

For the present study the PHB was selected for nanoparticle formation. Polymer sciences have been the backbone of pharmaceutics (Pillai and Panchagnula, 2001). Poly-β-hydroxybutyrate (PHB) has gained attention as a particulate

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carrier containing chemotherapeutic drugs (Allemann *et al*., 1993) due to their biodegradable, biocompatible and low toxicity properties, in which the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Ampicillin is considered to major drugs and since it is available in 1945 and it has saved thousands of human lives from fatal bacillary infections like plague. Ampicillin is prescribed for prevention and cure and is internationally accepted that Ampicillin was the best choice of treatment.

MATERIALS AND METHODS

The microbial isolates were screened for PHB production from soil samples collected from different geographical zones.

Isolation, Qualitative and Quantitative screening of PHB producing organisms from soil samples (Yilmaz *et al*, 2005).

The soil isolates were screened for PHB production. As a preliminary step, screening of PHB producers was carried out using viable colony staining technique. (Williamson and Wilkinson, 1958). The selected strains were grown on minimal broth and incubated at 37°C and extracted using chloroform extraction method.

Development of PHB Nano encapsulation by Emulsification, Solvent displacement and interfacial deposition Method

Emulsification-solvent evaporation involves two steps. The first step requires emulsification of the polymer solution in to the aqueous phase. During the second step polymer solvent is evaporated, inducing polymer precipitation as nanospheres.

Development of Nanoencapsulation with PHB

About 1gm of PHB powder was dissolved in 5 ml chloroform and mix thoroughly to that suspension about 0.1% PCL was added and the mixture was heated with magnetic stirrer. About 100ml of 1.2% sodium alginate solution was added to the above mixture and stirred with magnetic stirrer for about 15-30 minutes. The prepared solution was loaded in a syringe and poured on to the beaker/plate containing about 1 mol calcium chloride solution. The PHB nanoparticles are developed without drug.

Development of Nanoencapsulation with Ampicillin

About 1 gm of PHB was dissolved in 5 ml of chloroform about 0.1gm of Ampicillin was added in to the mixture and mix thoroughly. About 0.1% PCL solution was added to the mixture and was mixed thoroughly using magnetic stirrer for about 15-30 minutes. About 100 ml of 1.2% sodium alginate solution was added to the mixture and stirred with magnetic stirrer for about 15-30 minutes. The prepared solution was loaded in a syringe and poured on to the beaker/plate containing about 1 mol calcium chloride solution. PHB nanoparticles are encapsulated with Ampicillin.

Development of PHB Nanoparticles

About 2 gm of PHB powder was mixed with 150 mg of propylene glycol and was dissolved in 5 ml chloroform and mixed separately. The dispersion was added to 10 ml of aqueous ethanol solution (70%). After 5 minutes the mixture of organic solvents were removed by evaporation at 35° C under normal pressure and centrifuged at 10000 rpm for 20 min. The supernatant were removed and pellet was washed with water and dried at room temperature. The dried powder were taken for SEM image to observe the nanoparticles.

Chemical Characterization of Developed PHB Nanoparticles

About 1 mg of extracted PHB powder was mixed with 5 ml chloroform in a screw cap tube. The samples were then subjected to FTIR analysis.

Physical Characterization of Developed PHB Nanoparticles

Scanning electron microscopy (SEM) was used for the physical characterization of developed nanoparticles. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nano meter. Specimens can be observed in high vacuum, in low vacuum, and (in environmental SEM) in wet conditions. For SEM, a specimen is normally required to be completely dry, since the specimen chamber is at high vacuum.

RESULTS AND DISCUSSION

Isolation of PHB Producing Organisms from Soil

All the eight isolates from soil samples were observed under the direct dilution and plating on minimal agar were observed on the basis of their colony morphology and sub cultured on to appropriate medium and subjected to further study. The results are presented in the table 1.

Table 1 Colony type of the isolates

Isolates	Colony Morphology
B1	Dry, Small, feathery, flat, creamy colonies
B2	Mucoid, large, circular, creamy colonies
B3	Dry, irregular, medium- larger, flat colonies
B4	Feathery, irregular, creamy-buff, flat colonies
B5	Mucoid, irregular, small, creamy, flat colonies
B6	Dull, Small, branchy, dry, flat colonies
B7	Dull, Creamy, branchy, large, flat colonies
B8	Dull, moist , small, flat colonies

Screening of PHB Producing Organisms Using Sudan Black Staining Technique

Different bacterial colonies appear on the Nutrient agar were subjected to screening of Poly-β- hydroxybutrate (PHB) producers using Sudan black B staining solution. The blue black coloured intracellular granules were observed within the cells by the uptake of Sudan black B stains.

Extraction of Poly hydroxy-butyrate (Chloroform Extraction method)

PHB produced from the selected isolates were extracted by the chloroform extraction method. Three phases are obtained. The upper phase consist of sodium hypochlorite solution was removed. The middle phase consist of chloroform with undisturbed cells was separated from the bottom phase (chloroform & PHB). The solution was then evaporated at 100^0C in water bath and the PHB powder was collected and stored.

Development of PHB Nano Encapsulation by Emulsification, Solvent displacement and interfacial deposition Method

Polymer deposition occurs at the interface between water and chloroform nanodroplets, forming nanocapsules with a shell-like wall. PCL and PHB nanospheres were produced by Emulsion polymerization were shown in plate 1. As one of the first methods for production of nanoparticles, surfactants or protective soluble polymers were used to prevent aggregation in the early stages of polymerization reported by Exman and Sjfholm (1978).

Nanoencapsulation of PHB Nanoparticles

Preparation of Microspheres

Porous microspheres were prepared by the emulsion solvent diffusion method.

The smallest particles produced had a minimum size of 1 to 5 µm, obtained by air atomization. The preparation of alginate nanoparticles was first achieved in a diluted aqueous sodium alginate solution in which gelation was induced by the addition of a low concentration of calcium and leads to the formation of invisible clusters of calcium alginate gels, alginate particles have been produced by using a modified emulsification/internal gelation method by Reis *et al.*,(2005).

Nanoencapsulation with Ampicillin

Porous microspheres were prepared by the emulsion solvent diffusion method by ampicillin (0.1mg/ml). The resulting solution was poured into an aqueous solution of PCL. The encapsulated particles are round in calcium chloride solution shown in Plate no1.

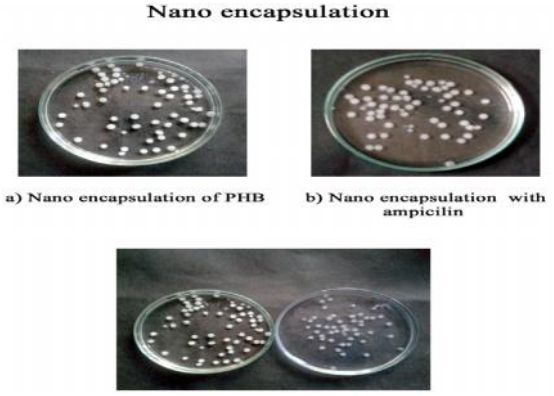

Plate 1 Nanoencapsulation with Ampicillin

Characterization of Developed PHB Nanoparticles

The physical characterization of developed PHB nanoparticles were done by SEM (Scanning Electron microscopy) and chemical characteristics of developed PHB nanoparticles were investigated by FTIR analysis.

Chemical Characterization of Developed PHB Nanoparticles with Ampicillin-FTIR

The chemical characterization of PHB, Ampicillin and developed PHB with Ampicillin were studied.

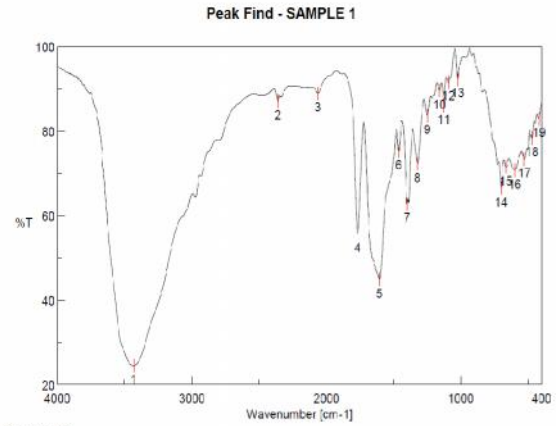

Fig 2 FTIR Analysis of Ampicillin

The FTIR spectra of PHB revealed the presence of PHB and Ampicillin

Peak 1 revealed the presence of functional group associated with hydroxyl absorption band, the similar peaks were observed from the FTIR analysis of drug with PHB (3432.67 cm^{-1}). On comparison with the absorption spectra of peak 2 revealed the presence of CH, CH3 group the similar peak were observed from the FTIR analysis of drug with PHB (2360.44 cm^{-1}). On comparison with the absorption spectra of peak 4 and 5 revealed the presence of Carbonyl absorption of peptide group, the similar peak were observed from the FTIR analysis of drug (1774 cm^{-1} , 1100.1cm^{-1}) and drug with PHB (1766.48 cm^{-1} , 1604.48 cm^{-1}) respectively. Peak 6 obtained revealed the presence of CH2.NH group and similar peak obtained from the FTIR analysis of drug (1373 cm^{-1}) and drug with PHB (1399.1 cm^{-1}). Peak 7, 8 and 9 obtained revealed the presence of C-O, the similar peak were obtained from the FTIR analysis of drug and drug with PHB.

Peaks	Absorption spectra of PHB	Absorption spectra of Drug	Absorption spectra of PHB nano particle With Drug- Ampicillin	Functional Group
Peak 1	2924 cm^{-1} -2854 cm-1	-	3432.39 cm^{-1}	-COOH,NH3,
Peak 2	2900 4 cm -1 2426.0 cm^{-1}	-	2360.96 cm^{-1}	CH,CH,.CH3
Peak 3	2429.8 cm^{-1} 2358.5 cm^{-1}	1311.3cm^{-1}	2062.5 cm^{-1}	=COOH
Peak 4	1596.7 cm^{-1} 1148.4 cm-1	1774 cm^{-1}	1766.48 cm^{-1}	Carbonyl absorption
Peak 5	911.2 cm-1383.6 cm^{-1}	1100.1cm^{-1}	1604.48 cm^{-1}	Carbonyl absorption of peptide
Peak 6	1111.7 cm^{-1}	1373 cm^{-1}	1399.1 cm^{-1}	CH2.NH
Peak 7	1067.4 cm^{-1}	687.4 cm^{-1}	1467.5 cm^{-1}	-C=O
Peak 8	838.5 cm^{-1}	1389 cm^{-1}	1321.6 cm^{-1}	C-O
Peak 9	417.5cm^{-1}	1263 cm^{-1}	1249.65 cm^{-1}	C-O
Peak 10	-	1169 cm^{-1}	1158.04 cm^{-1}	C-N/C-H
Peak 11	-	1118 cm^{-1}	1127.19 cm^{-1}	C-C
Peak 12	-	1078 cm^{-1}	1093 cm^{-1}	C-C
Peak 14	-	736 cm^{-1}	700.03 cm^{-1}	C-C
Peak 15	-	646 cm^{-1}	665.32 cm^{-1}	N-H
Peak 17	-	500 cm^{-1}	528.4 cm^{-1}	C-C Out of plane bending

On Comparison of the absorption spectra obtained from the peak 10 to peak 17 revealed the presence of C-O, C-H, N-H functional groups are considered to be the weak bands and such type of weak bands were observed from the FTIR analysis of drug and drug with PHB.

In the spectrum of Ampicillin two anti-symmetric stretching vibrations of C-H are observed at 2969 cm^{-1} and 2969 cm^{-1} is due symmetric stretching of methyl group. The carbonyl group in the four membered ring have a strong absorption at around 1775 cm^{-1}. Hence, a band observed 1774 cm^{-1} assigned to carbonyl vibrations by Johan (1978). Two strong bands at 1688 cm^{-1} and 1607 cm^{-1} are assigned to carbonyl vibrations of carboxylic group (COO-). In the synthetic heterocyclic compound, Ampicillin, S-C stretching vibrations are observed in the region of 650-750 cm^{-1} .The bands of medium to weak intensity at 646, 697, 711 and 736 cm^{-1} are assigned to heterocyclic S-C streching vibration supported by Hill and Rendell (1975).

Physical Characterization of Developed PHB Nanoparticles

SEM used to investigate the morphology of developed PHB nanoparticles as a discreate spherical structure with aggregation on 30,000 X and 40,000 X magnification revealed the property as smooth, moderate uniformity in shape. The size and increased number of spherical structure influences the impact strength of the developed PHB nanoparticle as shown plate 2.

Catarina *et al*., (2006) reported scanning electron micro photograph of polymetacrylic acid nanoparticles containing lamivudine, the nanoparticles appear as a discreate spherical structure with aggregation in different magnification. The PLGA nanoparticles containing drugs appears to be homogeneous, smooth, moderate uniformity, spherical in shape and did not cause aggregation of particles after lyophilization and these particles were readly re dispersible which is confirmed by surface morphology studies.

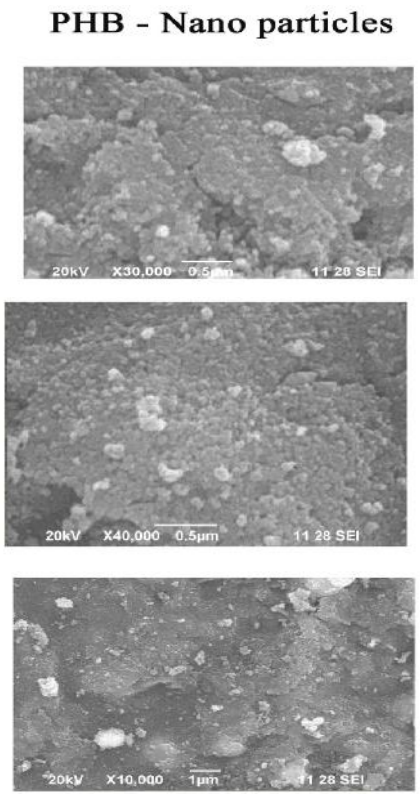

Plate no 2

CONCLUSION

PHB and PHB with drug were prepared and the studies showed that the nano encapsulation of PHB. The FTIR Spectrum of PHB and tetracycline of encapsulated nanoparticle revealed the presence of similar peaks as in extracted dry powder (PHB and Ampicillin). PHB with drug can be used more effectively to achieve longer intercellular controlled drug release.

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