Research Paper

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Development of Nano particles with Tetracycline with Interfacial Deposition of PHB (Poly-B-Hydroxybutyrate)

Microbiology

KEYWORDS : Extraction of PHB, Nano encapsulation, Nanoparticles, Interfacial deposition Tetracycline.

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ABSTRACT

Different soil samples were collected for the isolation of PHB producing bacillus sp present 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. Tetracycline is the drug of choice for per oral administration using nanoprecipitation technique. The production of Poly-β-hydroxybutyrate (PHB) as nanoparticle containing Tetracycline, 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. 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). 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. Tetracycline 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 tetracycline, 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 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. Tetracycline is prescribed for prevention and cure and is internationally accepted that Tetracycline was the best choice of treatment.

2. Materials and methods

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

2.1 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.

2.2 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.

2.3 Development of Nanoencapsulation with Tetracycline

About 1 gm of PHB was dissolved in 5 ml of chloroform about 0.1gm of Tetracycline 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 Tetracycline.

2.4 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 of PHB was taken for SEM image to observe the nanoparticles.

2.5 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.

2.6 Physical Characterization of Developed PHB Nanoparticles

Scanning electron microscopy (SEM) was used for the physical characterization of developed nanoparticles.

3. Results

3.1 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

3.2 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. Mass cultivation of PHB and extracted PHB was collected and stored.

3.3 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 (Poly Capro Lactone) and PHB nanospheres were produced by Emulsion polymerization were shown in plate 1.

3.4 Nanoencapsulation with Tetracycline

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

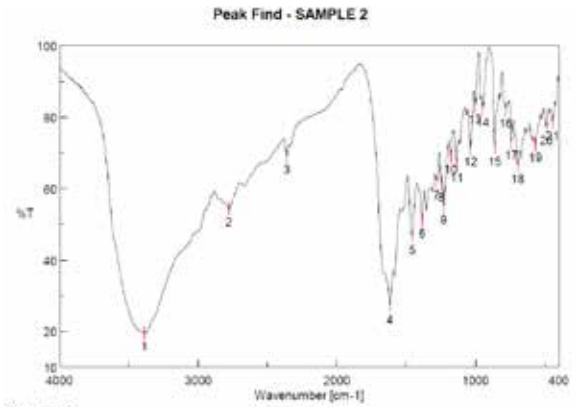
Plate 1 Nanoencapsulation with Tetracycline

3.5 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.

3.5.1 Chemical Characterization of Developed PHB Nanoparticles with Tetracycline-FTIR

3.5.1.1 Fig 1 FTIR Analysis of Tetracycline



[Comments]
Sample name SAMPLE 2
Comment FTIR SPECTRUM
User
Division
Company SRIPMS

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity
1	3386.39	19.5285	2	2777.96	54.3485
3	2360.44	89.0363	4	1617.02	26.9983
5	1456.99	46.5469	6	1383.68	51.1532
7	1280.5	61.9892	8	1246.75	61.1959
9	1227.47	54.8991	10	1177.33	69.0297
11	1135.87	66.7844	12	1036.55	71.1572
13	1001.84	83.1339	14	947.842	82.2821
15	861.06	71.2155	16	780.065	82.0084
17	741.496	72.9711	18	694.248	66.3151
19	568.898	72.3048	20	498.866	77.2299
21	443.547	76.5087			

Table 2: Comparative Analysis of FTIR Spectrum - PHB Nanoparticles with Tetracycline

Peaks	Absorption spectra of PHB	Absorption spectra of Drug	Absorption spectra of PHB nano particle With Drug-Tetracycline	Functional Group
Peak 1	2924 cm^{-1} -2854 cm^{-1}	3383.39 cm^{-1}	3386.39 cm^{-1}	Associated hydroxyl absorption
Peak 2	2900 4 cm^{-1} 2426.0 cm^{-1}	2776 cm^{-1}	2777.96 cm^{-1}	CH,CH,.CH3
Peak 3	2429.8 cm^{-1} 2358.5 cm^{-1}	-	2360.44 cm^{-1}	=COOH
Peak 4	1596.7 cm^{-1} 1148.4 cm^{-1}	1618 cm^{-1}	1617.02 cm^{-1}	Carbonyl absorption
Peak 5	911.2 cm^{-1} 1383.6 cm^{-1}	1452.1cm^{-1}	1455.99 cm^{-1}	Carbonyl absorption of peptide
Peak 6	1111.7 cm^{-1}	1358 cm^{-1}	1383.68 cm^{-1}	CH2.NH
Peak 7	1067.4 cm^{-1}	1234.4 cm^{-1}	1280.5 cm^{-1}	-C=O
Peak 8	838.5 cm^{-1}	1234.4 cm^{-1}	1246.75 cm^{-1}	C-O
Peak 9	417.5cm^{-1}	1234.4 cm^{-1}	1227.47 cm^{-1}	C-O
Peak 10	-	1178 cm^{-1}	1177.33 cm^{-1}	C-N/C-H
Peak 11	-	1137 cm^{-1}	1135.87 cm^{-1}	C-C
Peak 12	-	1037 cm^{-1}	1036.55 cm^{-1}	C-C
Peak 13	-	1002 cm^{-1}	1001.84 cm^{-1}	C-O
Peak 14	-	950 cm^{-1}	947.842 cm^{-1}	C-H
Peak 15	-	-	861.06 cm^{-1}	N-H
Peak 16	-	771 cm^{-1}	780.065 cm^{-1}	C-C
Peak 17	-	744 cm^{-1}	741.496cm^{-1}	C-C Out of plane bending

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3.5.1.4 The FTIR spectra of PHB revealed the presence of PHB and Tetracycline

Peak 1 revealed the presence of functional group associated with hydroxyl absorption band, the similar peak were observed from the FTIR spectra of drug (3383.39 cm^{-1}) and drug with PHB (3386.39 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 (2776 cm^{-1}) and drug with PHB (2777.96 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 spectra of drug (1618 cm^{-1}, 1452.1cm^{-1}) and drug with PHB (1617.02 cm^{-1} , 1455.99 cm^{-1}) respectively. Peak 6 obtained revealed the presence of CH2.NH group and similar peak obtained from the FTIR spectra of drug (1358 cm^{-1}) and drug with PHB (1383.68 cm^{-1}). Peak 7, 8 and 9 obtained revealed the presence of C-O, the similar peak were obtained from the FTIR spectra of drug and drug with PHB.

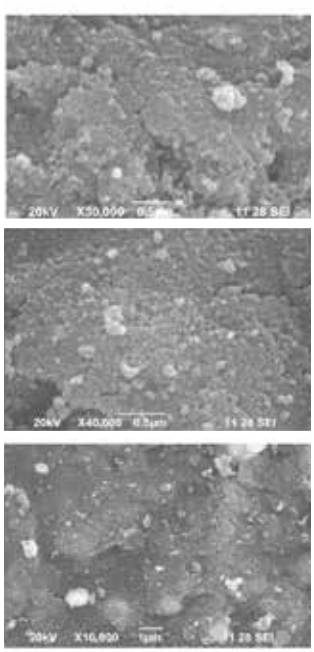
On Comparison of the absorption spectra obtained from the peak 10 to peak 17 revealed the presence of C-O, C-H, N-H functional groups are considered to be the weak bands and such type of weak bands were observed from the FTIR analysis of drug and drug with PHB.

3.6 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.

Plate no 2

PHB - Nano particles



Discussion

New methods have recently developed in the controlled release of drug. PHB as biomaterial from natural sources play an important role for control release, supported by Yu-Cui-Xiong *et al.,* (2010). PHBs are totally biosynthetic, biodegradability, biocompatibility and good thermo mechanical properties. The discussion of the Research work describes the eight effective PHB producing soil isolates. The developed Nano capsules with shell like spherical porous microspheres structure formed between water and chloroform and nanodroplets. 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). Comparative FTIR analysis of developed nanospheres with Tetracycline (fig 2) revealed two anti-symmetric stretching vibrations of C-H are observed at 2969 cm^{-1} and 2916 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} (fig 2). Hence, the similar band was 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-) (fig 2), the similar absorption spectrum was obtained in fig 1. In the synthetic heterocyclic compound, Tetracycline, 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). On comparison of FTIR analysis of developed nanospheres revealed the presence of functional groups of PHB and tetracycline.

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 Tetracycline). PHB with drug can be used more effectively to achieve longer intercellular controlled drug release.

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