ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Levofloxacin Loaded Polyhydroxybutyrate Nanodrug Conjugate for *In-Vitro* Controlled Drug Release.

C Valli Nachiyar[1]*, A Brinda Devi[1], S Karthick Raja Namasivayam[1], and Arul Maximus Rabel[2].

[1]Department of Biotechnology, Sathyabama University Chennai 600 119 Tamil Nadu, India.
[2]International Research Centre, Sathyabama University Chennai 600 119Tamil Nadu, India.

ABSTRACT

Biologically produced Polyhydroxy butyrate synthesized by *Bacillus cereus* has been modified into nanostructures which were in the size range of 250 – 300 nm. Loading of these nanoparticles with levofloxacin increased the size to 350-580 nm. *In vitro* drug release study indicated the controlled release of levofloxacin from PHB-levofloxacin nanoconjugate where 99% of the drug release was noticed after 22 h. This ability of PHB nanoparticles clearly indicates their potential for application as drug carrier for the controlled drug release.

Keywords: *Bacillus cereus*; Polyhydroxybutyrate; Nanoparticless; Levofloxacin; controlled drug delivery

Corresponding author

ISSN: 0975-8585

INTRODUTION

Drug delivery technology is emerging as an interdisciplinary science aimed at improving human health. Over the past few decades, there has been considerable interest in developing biodegradable drug carriers as effective drug delivery systems [1]. It has been shown that PHAs can be used to fabricate various items for medicine: surgery, transplantology, tissue engineering, and pharmacology [2].

Polyhydroxybutyrate (PHB) is a polyhydroxyalkanoates (PHA) produced by number of bacteria as granules using fatty acids, sugars and other carbon sources [3]. Among all bio-based plastics, they are unique by being entirely produced and degraded by living cells naturally and completely to CO_2 and H_2O under natural environment [4]. The biodegradable and biocompatible nature of PHB makes it suitable for drug delivery system [5].

In this study an attempt has been made to convert this microbially produced PHB into nanoparticless and check for their ability for controlled drug release using an antibiotic (Levofloxacin) as model compound.

Bacillus cereus showing appreciable PHA production was isolated from oil amended clothes (used to smear oil on 'dosa' pan). The bacterium was identified based on their biochemical and molecular characterization. The media used for the production of PHB, extraction and characterization of PHB produced by the organism were explained elsewhere [6, 7].

PHB nanoparticles were synthesized using this biogenic PHB by the triple emulsion method [8]. Briefly, 50 mg of hydrophobic polymers was added into 1 ml dichloromethane, and the mixture was stirred to ensure that all materials were dissolved. 5 ml of 1% PVA (w/v) was sonicated for 1 min and was slowly added with 1 ml of organic solution. Ultrasonication (Vibra cell V 501, Sonics, USA) was continued for 10 min. The mixture was gently stirred for 3–5 h at room temperature. The nanoparticles were collected by centrifugation at 1.5 × 104 × g for 10 min, followed by washing twice with deionized water.

0.1 mg of PHB nanoparticles was mixed with 0.001 mg of levofloxacin in 1 ml of chloroform, stirred under magnetic stirrer at room temperature for 3 hrs. After stirring, the homogenous slurry thus obtained was collected, lyophilized and used for further studies. The sample in chloroform was subjected to FTIR analysis after applying as a smear over the NaCl block and was analyzed using Perkin Elmer RX1 FTIR spectrophotometer. SEM analysis was carried out to understand the size and morphology of the PHB nanoparticless and levofloxacin loaded nanoparticless using Field emission Scanning electron microscope (Carl Zeiss, Germany).

The antibacterial activity of the PHB- Drug nanoconjugate was analyzed by agar diffusion method on the clinically isolated pathogenic strains of *E.coli*. 100 µl of PHB-drug conjugate was loaded onto the wells in the seeded agar plates and incubated for 24 hr at 30^0C and the antibacterial activity was recorded as zone of inhibition in mm [9].

In vitro release of levofloxacin was studied using dialysis bag and 1 x PBS. The Drug-PHB nanoconjugate in chloroform was taken in dialysis bag and dialyzed against physiological saline at 30^0C and at 75 rpm. An aliquot of dialysis medium was taken at regular intervals; filtered using a syringe filter and the amount of levofloxacin was quantified using spectrophotometric analysis at 291 nm [9].

The bacterium used in this study was isolated from oil amended cloth and was identified as *Bacillus cereus* based on their biochemical and molecular characteristics. After optimizing the medium the organism was found to be capable of producing more than 50% PHB (w/w) [6]. The SEM graph of the PHB has shown the crystalline nature of the polymer [7].

The procedure to prepare PHB nanoparticles explained by Xiong [8] was followed which yielded structure quite larger than the expected size range. SEM graph clearly indicated the size to be in the range of 250 – 300 nm (Figure 1a). The nanoparticles were found to be attached to the PVA matrix unlike the nanoparticles obtained by other researchers [8, 10]. Size of these nanoparticles increased to 350-580 nm (Figure 1b) after loading with levofloxacin. The FTIR spectrum of PHB nanoparticles (Figure 2a) clearly indicated the presence of peaks at 3447 cm^{-1}, 2976 cm^{-1} & 2935 cm^{-1}, 1735 cm^{-1}, corresponding to OH

ISSN: 0975-8585

stretching of carboxylic acid, CH stretching of alkanes and C=O and C-O stretching of esters respectively. The FTIR spectrum of PHB-levofloxacin nanoparticles (Figure 2b) has also shown peaks at the same wavelengths and the peaks which were reported as characteristic peaks for levofloxacin (1294 cm^{-1} corresponding to C-N stretching of amine group and 1085 cm^{-1} of halogen group) could not be identified which may be due to the overlap of PHB peaks with that of levofloxacin [11, 12, 13]. PHB-levofloxacin nanoparticless exhibited a clear zone of inhibition with 14.2 mm diameter whereas the PHB alone did not show any zone of inhibition (Fig. 3) clearly indicating the presence of levofloxacin with PHB.

Figure 1: FESEM graph of (a) PHB nanoparticles, (b) PHB + levofloxacin nanoconjugate

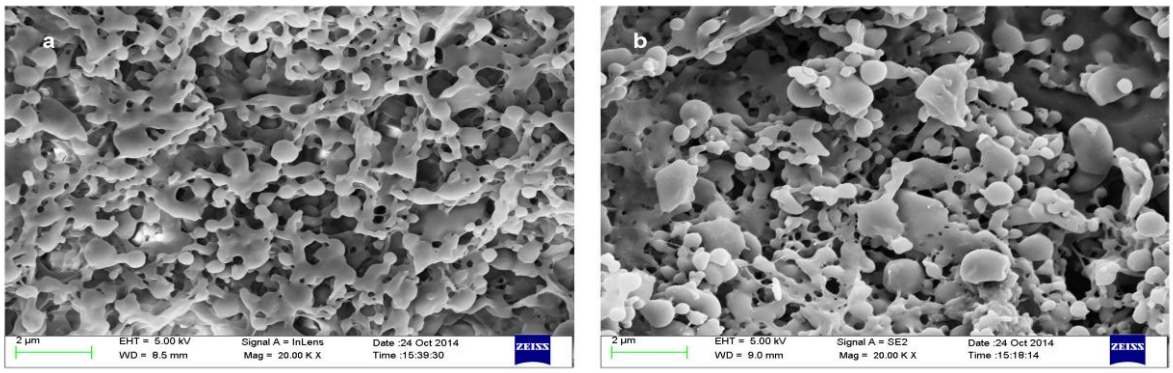


Figure 2: FTIR spectra of (a) PHB nanoparticles, (b) PHB + levofloxacin nanoconjugate

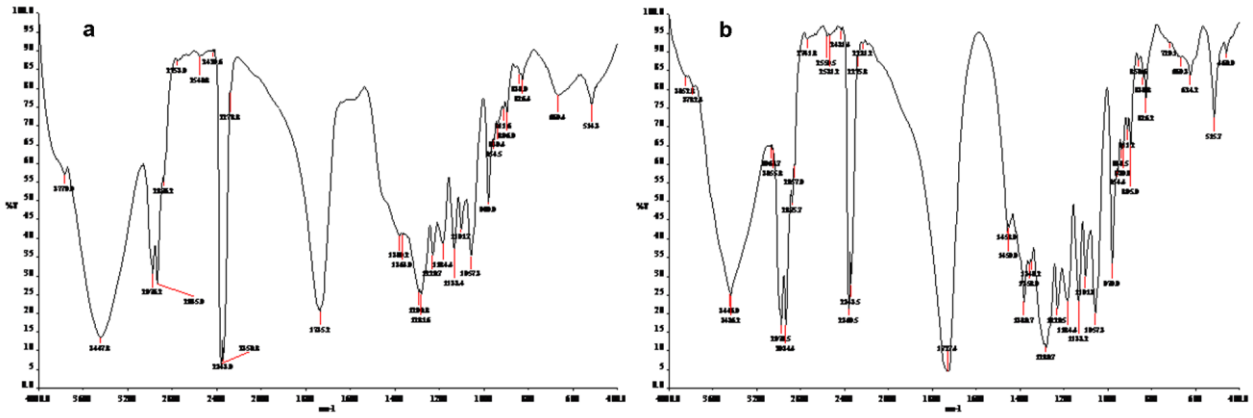
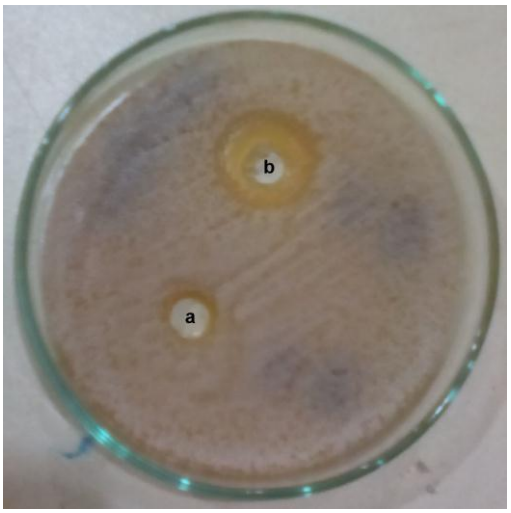
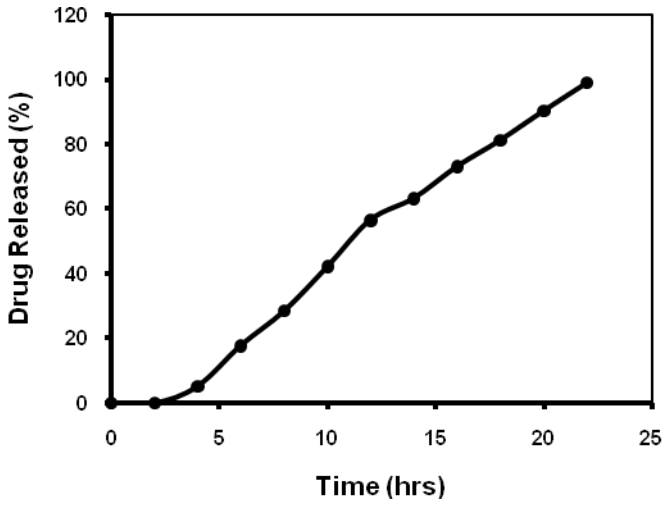


Figure 3: Antibacterial activity of (a) PHB nanoparticles, (b) PHB + levofloxacin nanoconjugate



In vitro drug release study indicated that the significant release of drug was noticed after 4 hrs of observation (Figure 4). The rate in which the drug was released increased up to 0.045 µg / h by 12 hrs of observation and remained constant after this, reaching a maximum of 99% by 22 hrs. The controlled release profile of levofloxacin indicates the potential application of PHB nanoparticless as carrier molecules for drugs [9]. The sustained release of levofloxacin from chitosan nanoparticles has been reported by Gevariya et al. [14] where the drug was released over a period of 20 h making this conjugate a promising agent for the effective management of ocular infection.

Figure 4: *In vitro* release of levofloxacin from PHB-levofloxacing nanoconjugate



The biodegradable, biocompatible nature of the PHB produced by the *Bacillus cereus* and the controlled release of drug from its nanoparticles qualify this polymer as carriers for drugs.

ACKNOWLEDGMENT

The authors would like to thank the management of Sathyabama University for providing the equipments and facilities to carry out this work.

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