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POLY (D, L- LACTIDE)-GENTAMICIN COMPOSITE COATED ORTHOPAEDIC METALLIC IMPLANT

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ABSTRACT
Antibiotic prophylaxis is a routine procedure in orthopedic surgery. Various local antibiotic delivery techniques are used to reduce bone and soft tissue-related infection. The main objective of this study was to develop a prolonged gentamicin delivery system, which can sustain the drug for at least one month, using a biodegradable polymer, Poly (D,L- Lactide) coating on metallic implants. We investigated the release kinetics and antibacterial properties of gentamicin impregnated Poly (D, L- Lactide) coating. In the present study, two successive layers of drug-polymer composite were coated on SS 316 L strip using air suspension spray coating technique to achieve a drug loading of 25 μg /cm^2. In-vitro elution of gentamicin at regular intervals, for about 42 days, from strip was analyzed using high performance liquid chromatography (HPLC). It was observed that the drug was continuously released over a period of one month owing to swollen polymeric matrix and bulk erosion. Scanning electron microscopy (SEM) images indicate smooth and crack free coating on implant surface.

KEY WORDS: Gentamicin, Poly (D, L- Lactide), HPLC, SEM

INTRODUCTION

Post operative orthopedic infections greatly restrict the use of implants in humans (Andriole et al., 1974). Bones, which usually are well protected from infection, can become infected directly through open fractures, during bone surgery or from contaminated objects that pierce the bone. Long periods of antibiotic therapy or repeated surgical procedures are needed to treat such infections. Bacterial adhesion to implanted materials and the ability of many microorganisms to form a biofilm on the implant surface are some of the major contributors of post operative infection. The bacteria present in biofilms are protected from the body's immune system and exhibits increased resistance to antibiotics (Wu et al., 2006). Orthopedic implants can thus become a major .source of infection and sometimes requires removal and further implantation through surgery. This is a serious concern for the patients as well as surgeons. Conventional therapy with systemic antibiotics often becomes unsuccessful due to poor antimicrobial distribution at the site of infection and poor blood circulation to skeletal tissue. Systemic antibiotic therapy alone does not eradicate bacteria because of poor penetration into bone (Winkler et al., 2000). Thus, there is a need to achieve prolonged drug delivery that can persist at least over a month, to prevent post operative orthopedic infections. *Staphylococcus aureus* is the bacteria most commonly responsible for bone and tissue related infection (Ohtani et al., 1982). Objects thus represent an alternative to conventional systemic therapy (Russell et al., 2001). Gentamicin, the broad antimicrobial spectrum bactericidal drug of the amino glycoside antibiotic group, has good bone penetration (Eron, 1985) and can actively treat many different types of severe bacterial infections, particularly gram-negative infections (Panyam et al., 2003). This drug was approved by the FDA in 1966 (Gitelis et al., 2002). Gentamicin has general pattern of action which may be described in two main steps: (1) Transport of the Gentamicin through the bacterial cell wall and cytoplasmic membrane. (2) Binding to ribosomes resulting in inhibition of protein synthesis.

Several strategies have been developed for creating drug loaded implants that releases antimicrobial agents in a controlled manner. Drug impregnated PMMA bone cements/Beads have been commercially made available for clinical use. However, this polymer is not biodegradable and clinical failures can occur due to difficulty of bone regeneration. PMMA has poor adhesion to implant surface and residual MMA monomers can kill healthy cells around the implant device. This has led to the search for alternate biodegradable or biocompatible implant coatings. Several researchers have focused on use of drug impregnated ceramic materials such as tri-calcium phosphate or hydroxyapatite, since their chemical composition is similar to bone (Alt et al., 2006). Recently, peptide based implants, the so called RGD (arginine, glycine, aspartic acid)- peptide, have been reported to stimulate the adhesion of osteoblasts and, therefore, to improve the osteointegration of RGD-coated implants(Goliwitzer et al., 2003). The incorporated antibiotics showed a continuous release for a period of about 96 h with an initial peak of release in the first 6 h (Liang et al., 1999). That means the antimicrobial effect exists only for a few days, such high doses and/or lengthy

treatment (orally or parentally in high dose) with antibiotics often lead to adverse effects like ear and kidney damage (Baro et al., 2002). Controlled release of antimicrobial drugs from the implanted objects thus represents an alternative to conventional systemic therapy (Hascicek et al., 2003). The objective of the present investigation is to develop and examine drug loaded biodegradable polymer films that can bind to the orthopedic implants and prevent bacterial infection through controlled release of the drug. The release kinetics should be such that the drug is delivered at least for a period of one month so that no complications arise during the osteointegration period. In present research work, coating of anti-bacterial drug on metallic implants using biodegradable polymer Poly (D, L- Lactide) as a carrier in multi layers control release is investigated. Several clinical studies support the fact the Poly (D, L Lactide) is osteo-conductive, i.e. it facilitates bone growth. Moreover, since the soft tissues are not in direct contact with the coated implant due to the polymeric coating, it prevents corrosion up to some extent.

MATERIALS AND METHODS

The 1.5 cm x 3.0 cm x 0.25 cm SS 316L (ASTM-F 138) strips and cortical screws supplied from Biomed Corporation, India were used in the study. Gentamicin drug was obtained from Triomphe Fine Chemistry Company Ltd., China, and used without further purification. Polymer; Poly (D, L- lactide) (Bio Invigor Corporation; Taiwan) having inherent viscosity 0.594 dl/g was used as a carrier for the drug. The solvent acetone, water and other chemicals used in the current investigation were of HPLC grade procured from Merck, India. Polymer/antibiotic complex preparation and coating on Medial grade 316 L SS. Biodegradable polymer; Poly (D, L- Lactide) was dissolved in HPLC grade acetone (solution A) and fixed quantity of Gentamicin was dissolved in water (solution B). Coating solution was formulated by mixing of both solution A and solution B at the proportion of 20% Gentamicin and 80% Poly (D, L-Lactide). Strips were stored in amber colored glass vials after washing with de-ionized water followed by acetone and followed by vacuum drying for solvent evaporation. Before coating, the strips and cortical screws was weighed using analytical balance (Citizen Model CX-220) having 0.01 mg accuracy. Base layer of Strips and Cortical screws were coated by aerosol spray technique to achieve a loading of 30 $\mu g/cm^2$ gentamicin. Further only polymeric top layer of Poly (D, L- Lactide) was coated for protection against moisture on base layer and to prevent premature drug release. Coated materials were followed by vacuum drying for solvent evaporation. All coated samples were sterilized by Ethylene Oxide.

The HPLC system used for gentamicin drug analysis was LC-2010CHT pump (Shimadzu, Japan) equipped with UV-VIS detector: SPD- 10AVP (Shimadzu, Japan), Rheodyne 1303 integrator (Rheodyne, USA). The column used was ODS PR C-18 (10µ pore size) 12.5 cm x 4.6 m (Phenomenex). Drug content was analyzed using mobile phase solution containing 0.55 %w/w of sodium heptane sulphonate monohydrate in a mixture of methanol: water: acetic acid (70: 25:5 v/v) at flow rate of 1.5 ml/min. Detector wavelength was set at 330 nm. Three strips were evaluated for gentamicin content and three strips for in-vitro gentamicin drug dissolution kinetics from biodegradable polymer matrix for a period of 28- 30 days under simulated biological conditions. Standard solutions were prepared by dissolving 10 mg of Gentamicin drug in 10 ml of mobile phase. This mobile phase was then diluted up to 100 ml. Diluted 20 µl standard was injected in the HPLC column and standard chromatogram for this standardized solution was obtained. The strip was immersed in phosphate buffered saline (mobile phase). This mobile phase (20 µl) containing Gentamicin was injected in the HPLC column and chromatogram was obtained. Gentamicin content on the strip was found by comparing the area under the peak with that of the standard curve. For in-vitro Gentamicin kinetics study, strips were incubated in 20 ml of phosphate buffered saline (PBS) solution at 37^0 C with constant agitation at 60 rpm. Strips were evaluated for Gentamicin release by withdrawing aliquots at 1, 2, 3, 4, 7, 14, 21, 28, 35 and 42 days and analyzed for amount of gentamicin release in PBS. Coating thickness was measured microscopically. In this study acetone was used for coating solution preparation. Coated samples were vacuum dried for solvent removal. According to european medicine agency acetone is less toxic and covered by class 3. So, solvent should be less toxic and less than 0.5%. Residual solvent was determined gas liquid chromatography using Head space method. The column used was 30M x 0.53 mm id Capillary BP 624 (G43). To evaluate the possible interaction between the compounds, the thermal analysis was performed by differential scanning calorimetry (DSC 2920, TA instruments Inc). The nature and extent of interaction between drug and polymer carrier in the solid state are probe by FTIR studies. In polymer blends, mixing of the components at the molecular level will cause changes in the oscillating dipole of the molecules. This will manifest itself of changes in the frequency and band width of stretching groups in spectrum, if the drug and PDLLA interact, the functional group in FTIR show band shift and broadening compared to the spectra of pure drug and PDLLA.

RESULTS & DISCUSSION

Coating characteristics

The thickness of polymer coating in orthopedic implant was determined by Optical microscopic observation done by Embed Resin Matrix Technique. Samples were prepared for microscopic imaging, by resin embedding, sectioning, grinding & polishing. Due to difference in reflectivity of the metal, Drug-polymer coating and resin, the interfaces were clearly identified with optical microscope, with optical microscope. Drug-polymer matrix coating thickness was measured from the microscopic images (Carl Zeiss, Germany) at 1200X magnification (Fig 1). Thickness measured from different parts of sample is summarized in Table.1. The mean thickness for Gentamicin coated orthopedic strip was 9.56 µm.

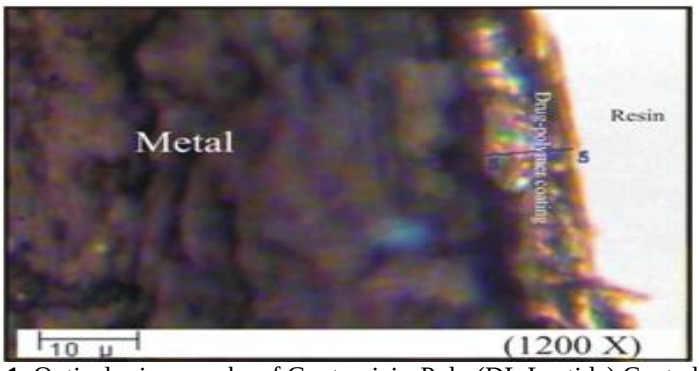


FIGURE 1. Optical micrographs of Gentamicin-Poly (DL Lactide) Coated SS Strip

TABLE 1. Coating thickness measurement from different parts of the Drug –Polymer Coated Strip

Frames	Drug-Polymer Coating Thickness Measurement (Microns)
1	7.9
2	8.8
3	12.8
4	9.0
5	9.3
Average of 5 frames	9.6

Drug content determination per cm^2 by HPLC

The amount of gentamicin loaded per unit area of polymer coating was estimated from HPLC analysis. The concentration of Gentamicin in the polymer matrix, film thickness etc are important parameters to control the release of drug. It is desirable that the elution should be controlled and programmed. Drug- polymer ratio is one of the factor that controls the release kinetics e. To evaluate the drug content, drug-polymer coated strip having a surface area of 11.75 cm^2 (size 1.5 cm x 3.0 cm x 0.25 cm) was dissolved in tetrahydrofuran. The gentamicin content on strip was found to be 212 µg by HPLC which corresponds to a drug content of 18 µg/cm^2.

In-vitro release profile by HPLC method

Similarly; the release kinetics of strips was evaluated using HPLC technique. Table 2 represents the in-vitro gentamicin dissolution kinetics for 28-30 days at regular intervals. For coated strip, $t_{1/2}$ (i.e. time taken for a cumulative release of 50% of the drug) is found to be about 7 days. The release profile depicted table -2 shows that the release rate is time dependent. The slow and controlled release of the drug arises from the fact that the drug embedded deep inside the polymer matrix has to diffuse over long distance. This is further facilitated from the degradation of the polymer with time, leading to controlled release.

TABLE 2 Quantitative detection of gentamicin for 1, 2, 3, 4, 7, 14, 21, 21, 28, 35 & 42 days by HPLC

Day	Drug Released	Cumulative	
		(µg)	%
1	24.88	24.88	11.96
2	59.17	84.05	40.41
3	12.71	96.76	46.53
4	8.75	105.51	50.73
7	49.3	154.81	74.44
14	4.9	159.71	76.79
21	12.45	172.16	82.78
28	35.81	207.97	100.00
35	not detected		0.00
42	not detected		0.00

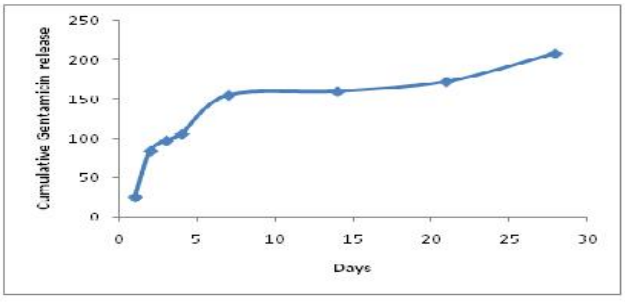


FIGURE 2. Cumulative gentamicin release profile of strip in PBS (pH 7.4) for 28 days at 37 ºC

Gentamicin is liberated with an initial burst of 30-40% for first 3-4 days where the risk of infections is maximum. This initial burst is followed by moderate and sustained release for prolong period of time of 28-30 days maintaining drug levels high enough for bactericidal activity. The total drug released from the polymer coating is found to be about 97.6% of the drug loaded. i.e. From a strip containing 212 µg of drug, about 207 µg gentamicin was released within 28 days.

Coating surface characteristics before and after immersion in PBS solution

Representative SEM images of the surface of coated strip containing 20% gentamicin drug and 80% polymer matrix are shown in Figure 3. Morphology of the drug-polymer coated surface reveals a discrete particulate phase in contrast to the uncoated strip surface that reveals a smooth & homogeneous appearance. The surface of the coated strip was found to be free from any irregularities such as cracking, flacking and delamination (figure 3).

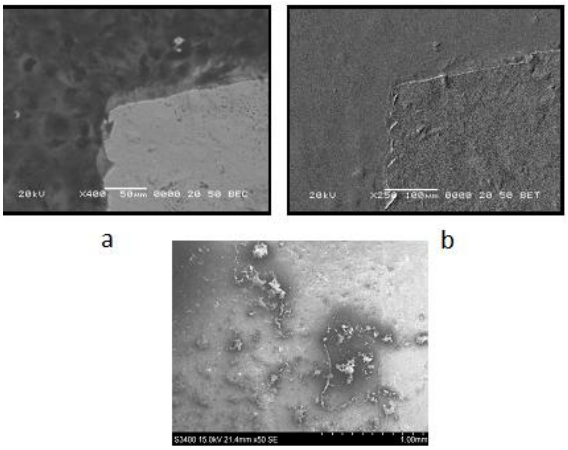


FIGURE 3. a. Back Scattering Image, Topographical Mode (250X) also confirms (Left) coated surface is smoother than Bare (Right) Metallic Surface

b. Back Scattering image, Composition Mode indicates that the Dark portion (Left) of the image is more homogenous and having smooth surface than the Light portion (Right) at 400X Magnification.

c. Back scattering image of Drug-Polymer surface after dissolution in PBS for 42 days. Erode surface was found and Pores were seen

Residual solvent determination by gas liquid chromatography

Two chromatograms were evaluated for standard and sample. The oven temperature was increased from room temperature to 55°C in 6 minutes then increased to 230°C with the rate 30°C/min. For standard 0.1027 mg/ml in water of acetone and for sample one drug-polymer coated screw + 1.0 ml water were taken in HSS vial, volatilized and injected individually. From the fig. 4a & b it can be concluded that no peak was found to be reported. Indicated the absence of residual solvent acetone.

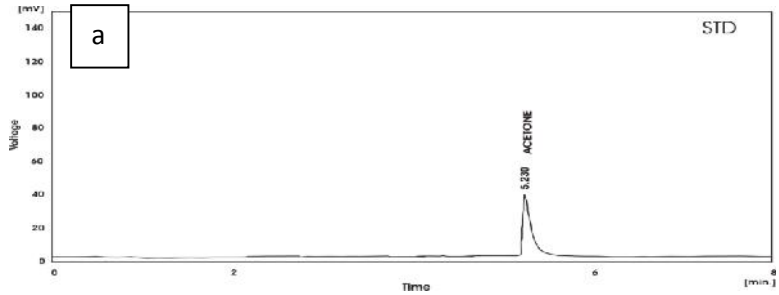
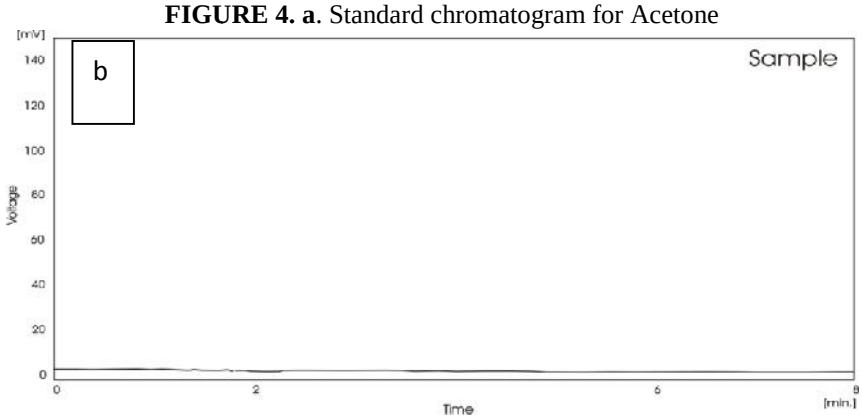


FIGURE 4. a. Standard chromatogram for Acetone



b. Chromatogram for sample

Drug-polymer interaction by differential scanning calorimeter (DSC)

The Tg of material can be assigned by DSC. The samples Poly (D,L-Lactide) and Gentamicin- Poly (D,L.-Lactide) mixture was heated in sealed aluminum pans and the first scan was measured at a heating rate of 10°C.min^{-1} from -50 to 150°C individually.

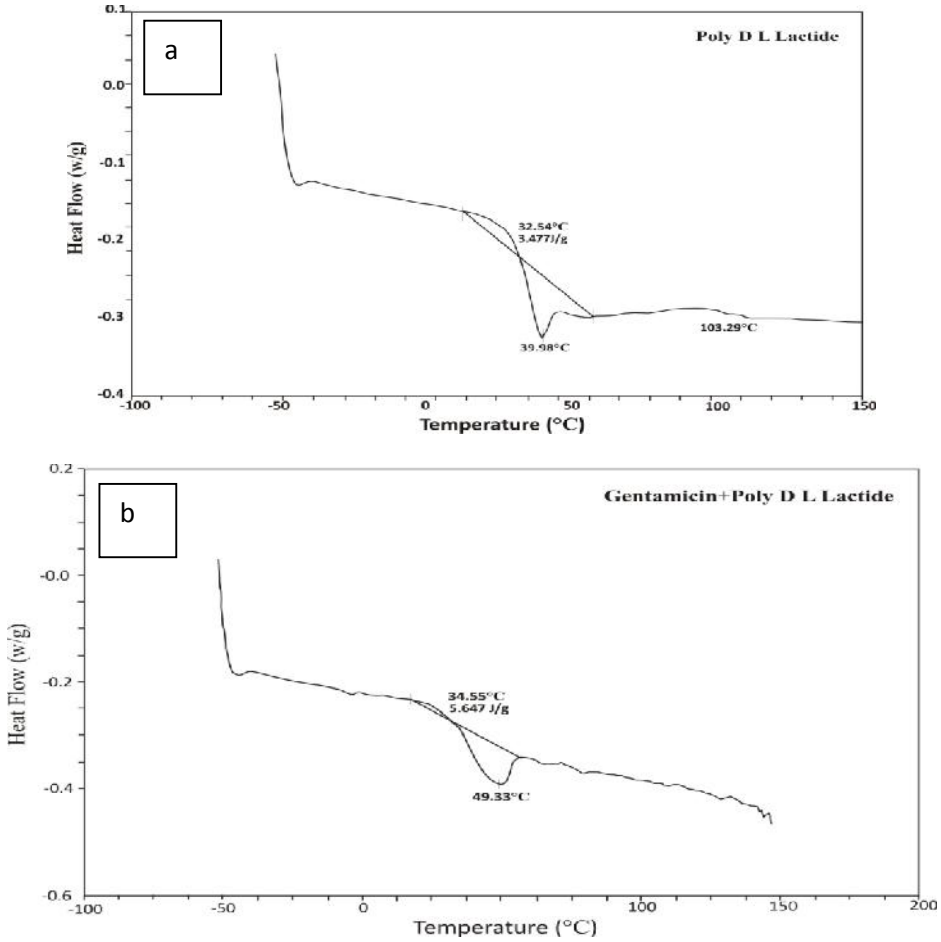
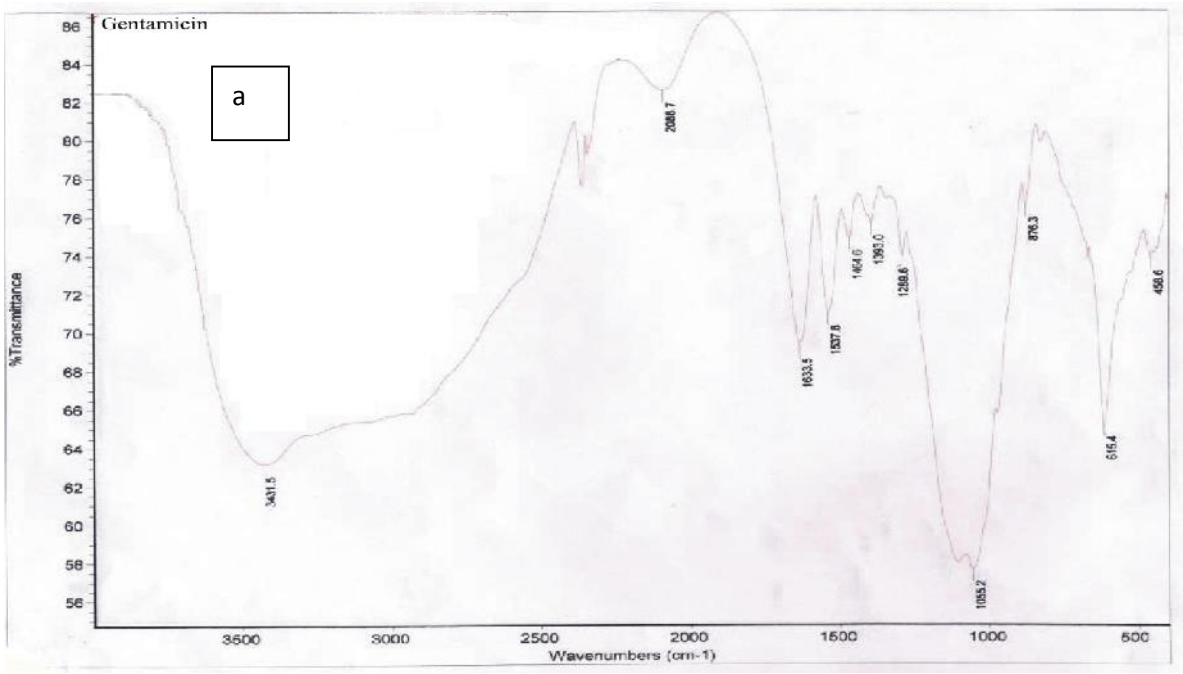


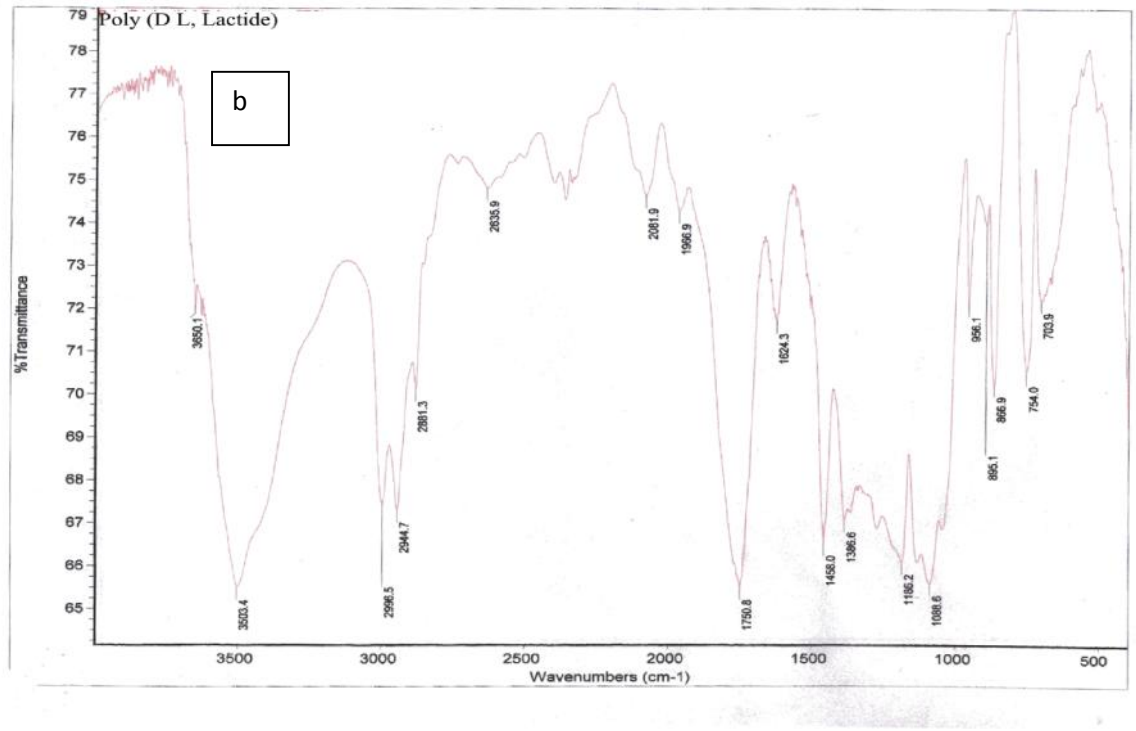
FIGURE 5. a. DSC curve for PDLLA
b. DSC curve for 20% gentamicin and 80% PDLLA

The evaluations of the Tg of the PDLLA and Gentamicin-PDLLA in the systems were obtained from the DSC curves. 39.98°C point in DSC curve of PDLLA was glass transition temperature (fig.5a). The Tg was seen at 49.88°C for DSC curve of mixture of Drug-polymer (fig. 5b). The increasing of Tg for PDLLA may be due to ionic interaction between amino groups of the gentamicin and the terminal carboxylic anions of the PDLLA. In general, factors increasing the stiffness of the polymeric molecular segments will tend to increase Tg. As the polymeric molecular rotations become more difficult or hindered, the Tg will increase. Here gentamicin was act as an anti-plasticizing agent because it increases the Tg of PDLLA showed the possibility for chemical and physical interaction.

FTIR studies of Drug- polymer and their of mixing

The FTIR spectra of pure gentamicin showed peaks at 1628 cm^{-1} and 1532 cm^{-1} correspond to amide band.(from fig. 6a, b & c) The peak observed at 1128 cm^{-1} was due to HSO_4^{-1} group. The peak observed at 617.23 cm^{-1} was due to SO_2 band. The FTIR spectra of without PDLLA showed peaks at 1751 cm^{-1} due to ester carbonyl group. The region 1300-1047 cm^{-1} was due to –CO-O- sequence. The peak observed between 2900- 3500 cm^{-1} was due to –OH end groups at polymer chain. In this study, the FTIR spectra of gentamicin-PDLLA mixture showed all peaks which were observed in spectra of pure gentamicin and Poly (D,L-Lactide). Neither appreciable shifts nor disappearance or appearance of new bands were observed, the gentamicin + PDLLA spectra being the result of superimposing those of the pure PDLLA and gentamicin, which indicated the absence of significant drug/polymer chemical interactions in the solid state.So, the increased in Tg of PDLLA with the gentamicin additive was due to physical interaction.


Gentamicin
a
%Transmittance
Wavenumbers (cm-1)
3431.5
2088.7
1633.5
1537.8
1464.6
1393.0
1288.6
1055.2
876.3
616.4
456.6


Poly (D L, Lactide)
b
%Transmittance
Wavenumbers (cm-1)
3650.1
3503.4
2996.5
2944.7
2881.3
2835.9
2081.9
1996.9
1750.8
1624.3
1458.0
1386.6
1186.2
1088.6
956.1
895.1
866.9
754.0
703.9

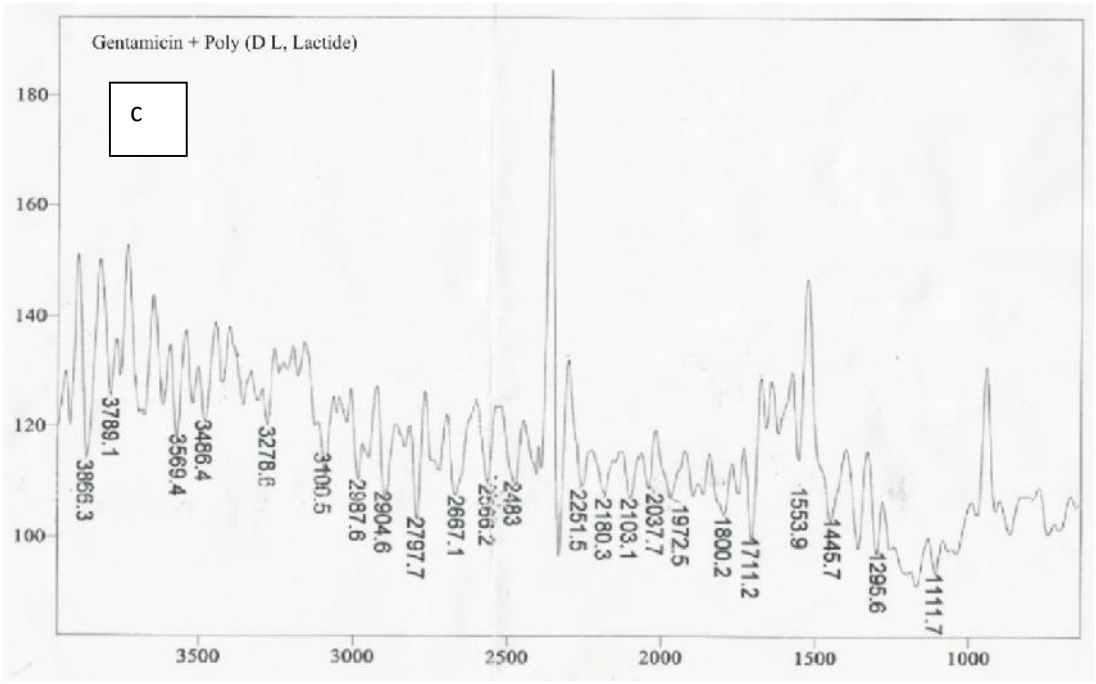


FIGURE 6. a. FTIR spectra gentamicin
b. FTIR spectra of PDLLA
c. FTIR spectra of gentamicin and PDLLA

CONCLUSION

In conclusion, we have developed a novel methodology for coating of biodegradable polymer-drug composites on orthopedic implants, using aerosol spray technique. The drug-polymer composite was coated with a second layer of poly lactide polymer. The in-vitro drug release studies indicate slow release of the drug over a period of 28 days. Drug coated specimens indicate zone of inhibition comparable to free drug for antimicrobial activity against gram-positive and gram-negative bacteria. This indicates that multilayered biodegradable PDLLA- gentamicin delivery system has potential in the controlled delivery of drug in orthopedic implant for the treatment and prophylaxis of postoperative infection.

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