Chapter 2
Materials and Characterization
Applied
This chapter gives the informations regarding use of various materials and their specifications. It also acknowledges the places where the characterization test was conducted.

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**Gentamicin eluting orthopaedic implant**

- **Drug:** Gentamicin (Triomphe fine chemistry company Ltd, (China))
- **Polymer:** Poly(D,L-Lactide) having inherent viscosity 0.594 dl/g - (Bio-Invigor corporation, Taiwan)
- **Solvent:** Acetone GR, Acetone HPLC, Water HPLC, 2-Propanol were procured from Merck India Pvt Ltd.
- **Metal:** Corticle screw 45 mm long head Dia 4.5 mm and strip having size 1.5 cm x 3.0 cm x 0.25 cm supplied by Biomed Corporation were used for antibiotic coating.

Various tests were conducted for gentamicin eluting orthopaedic implant as follows.

1. **Drug loading**
   In this test, Gentamicin content was evaluated per cm$^2$ of the coated material.
   Test conducted at: Konark Research Foundation, Daman, Gujarat (INDIA)

2. **High performance liquid chromatography (HPLC)**
   Gentamicin coated strip was immersed in Phosphate buffer solution. The extracts of the material was analyzed using for various days.
   Test conducted at: Konark Research Foundation, Daman, Gujarat (INDIA)

3. **Scanning electron microscopy (SEM)**
   Coating morphology was studied by SEM at different magnification.
   Test conducted at: Electrical Research and Development Association, Vadodara, Gujarat (INDIA)

4. **Coating thickness measurement**
   The thickness of Drug-polymer coating in orthopedic implant was determined by Optical microscope
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Test conducted at: TCR advance engineering, Vadodara, Gujarat (INDIA)

5. **In-vitro antimicrobial activity test**
   The antimicrobial activity of gentamicin eluting orthopaedic system was assayed by a Kirby-Bauer technique.
   Test conducted at: Shree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram Kerala (India)

6. **Platelet adhesion**
   Haemocompatibility of the material was checked against human blood plasma.
   Test conducted at: Shree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, Kerala (India)

7. **Pyrogen test**
   Extracts of the material was examined in Rabbit to check pyrogenicity of the coated sample.
   Test conducted at: Bee Pharmo Labs Pvt Ltd, Mumbai (India)

8. **In-vitro cytotoxicity test**
   Toxicity of the material was checked against cultured L 929 mouse cell.
   Test Conducted at: Shree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram Kerala (India)

9. **Residual ethylene oxide**
   Residual ethylene oxide of the sterilized sample was determined using Gas Liquid Chromatography
   Test conducted at: Shree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram Kerala (India)

10. **Residual solvent**
    Residual solvent (acetone) of the material was determined using Gas Liquid Chromatography.
    Test conducted at: Shree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram Kerala (India)

11. **Sterility**
    Sterility of the sterilized material was checked in soyabean digest broth
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Test conducted at: Shree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, Kerala (India)

12. **Gentamicin/Poly(D,L-lactide) interaction study by Differential Scanning Calorimeter (DSC)**

Drug-polymer interaction was studied using change in Glass transition temperature (Tg) of the polymer.

Test conducted at: Bee Pharmo Labs Pvt Ltd, Mumbai (India)

13. **Gentamicin/Poly(D,L-lactide) interaction study by FTIR**

Possible interaction between drug/polymer was studied using FTIR.

Test conducted at: Bee Pharmo Labs Pvt Ltd, Mumbai (India)

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Chapter 4

**Hydroxyapatite coating by various techniques**

4.1 Hydroxyapatite precipitation with Electrophoretically (EPD) deposited Calcium oxide on Metal surface

Material used:
- Calcium Oxide: Otto chemie, Mumbai
- Di-ammonium Hydrogen Phosphate: Finar chemicals, Mumbai
- Polyvinyl alcohol: Otto chemie, Mumbai
- Ethanol: Changshu Yangyuan Chemical, China
- Hydroflouric acid: Merck, India
- Nitric acid: Merck, India

4.2 Electrochemical Deposition of Hydroxyapatite on Titanium

Material used:
- Calcium Nitrate: Merck, Mumbai
- Tri-Potassium Phosphate: Otto Chemie, Mumbai
- Sodium Hydroxide: Fischer Chemicals, India
- 0.1 N Hydrochloric acid: RFCL, India

4.3 Hydroxyapatite coating on Stainless Steel by Plasma spray

Material used:
- Hydroxyapatite: Clarion pharmaceutical, India

Plasma coating was done by "Metalizing Equipment, Basani, Jodhpur, Rajasthan (INDIA)"
4.4 Hydroxyapatite coating on Titanium by Sol-gel method

Material used:
- Calcium Nitrate: Merck, Mumbai
- Triethyl Phosphite: Labort Fine Chem, India
- Ethanol: Changshu Yangyuan Chemical, China
- Sodium Hydroxide: Fischer Chemicals, India
- Nitrogen Gas: Marfatia Gas agency, Surat

4.5 Hydroxyapatite/Titania coating on Titanium strip by Brush coating

Material used:
- Hydroxyapatite: Plasma biotech, USA
- Tween 20: Merck, India

For all above coating techniques, metal was supplied by Matrix Meditech Pvt Ltd, Ahmedabad

Various tests were conducted for Hydroxyapatite powder and for coating.

1. **FTIR**
   Precipitated Hydroxyapatite powder was analyzed for functional group using FTIR
   Test Conducted at: Centre of Excellency, Vapi, Gujarat (India)

2. **Energy dispersive analysis by X-ray (EDAX)**
   Hydroxyapatite coated metals were analyzed for elemental analysis using EDAX.
   Test Conducted at: M S university, Baroda

3. **Scanning electron microscopy(SEM)**
   Coating morphology was studied by SEM
   Test Conducted at: Electrical Research and Development Association, Vadodara, Gujarat (India)

4. **X-ray diffraction(XRD)**
   To check phase purity, hydroxyapatite powder was analyzed using XRD
   Test Conducted at: Schulzan Shah research centre, Nadiad, Gujarat (INDIA)
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5. **Adhesion test**
   Test conducted at: Adhesion test was conducted by in-house developed system as per ASTM F-1147

Chapter 5

**Cefazolin eluting Hydroxyapatite coated Orthopaedic implant**

Material used:

- Hydroxyapatite: Clarion
- Cefazolin: Ranbaxy Laboratory, India
- Titanium strip: Matrix Meditech Pvt Ltd, Ahmedabad

Various tests were conducted for prepared samples

1. **Drug release by HPLC**
   Test conducted at: Shree Dhanvantary College of Pharmacy, Kim, Gujarat, India

2. **Bacterial endotoxin test**
   Test was conducted to check the Endotoxin present in the sample
   Test conducted at: Bio-division, Matrix Meditech, Ahmedawad

3. **Antimicrobial activity test**
   Released extracts from the material was tested for antimicrobial test using Cup-plate diffusion method
   Test conducted at: Bio-division, Matrix Meditech, Ahmedawad

4. **Coating thickness measurement by Scanning electron microscopy**
   Test conducted at: M S university, Baroda

5. **Cefazolin - Poly (D,L-Lactide) interaction study by FTIR**
   Possible interaction between drug/polymer was studied using FTIR.
   Test conducted at: Shree Dhanvantary College of Pharmacy, Kim, Gujarat, India

6. **Coating Morphology studied by SEM**
   Test conducted at: Electrical Research and Development Association (ERDA), Vadodara, Gujarat (INDIA)

7. **Degradation behavior in PBS**
   In-vitro degradation of product was analyzed using spectrophotometer in laboratory for released extracts of material for various days.