CHAPTER 5
Characterizations of Implant & In Vitro Study

5.1 Implant Preparation

Up to chapter four we have studied the composition selection, compatibility study in between selected drug and excipient(s) with each other. Along with that formulation strategy was identified and taken various trials to optimize it. Finally solvent casting method was optimized for casting of implant. Development of implant formulation consists of mainly selection and compatibility study between selected ingredients, composition optimization, formulation process, drug release optimization, impact of plasticizer on implant, qualification of implant in-process testing, analytical testing for quality and performance study etc. In this chapter we are summarizing the phases of implant formulation, qualification specially drug release and polymer degradation.

It was identified that there is a scope of targeted delivery of antibiotic in wound care. Targeted delivery of antibiotic drug at wound site will play important role to avoid or control the infections. This current work is about preparation and evaluation of PLGA based, Gentamicin sulphate (GE) loaded implant to get optimum drug at site for antibacterial action. Drug release, degradation behavior and implant tensile properties were studied up to 42 days. Drug release in IVRT was studied by UV absorbance of receptor solution. Physical characteristics such as molecular weight, tensile strength and change in morphology of biodegradable implant were studied as a function of time during IVRT studies. The biodegradable implant releases 3.4 µg/g/h drugs with a steady decrease in PLGA molecular weight as well as the implant tensile strength. In this manuscript it is demonstrate that the implant will release the drug with simultaneous degradation of bio-implant.

Wounds or surgical infections may result in patient discomfort by means of itching, swelling, pain or healing delay and sometimes leads to death (Reddy et al., 2012; Bagnall et al., 2009; Leaper, 2010). It is estimated that surgical wounds and therefore caused infections results in an increased length of hospital stay (Gaynes et al., 2001; Smyth et al., 2008). Thus, in wound care there is a major impact of prevention and management of wound infections on patient health and related health economics.
The term surgical site infection is associated with surgical wound and its infections in the body parts and tissues involved in the surgery or operation which procedure requires insertion of external devices or parts like implants or prosthetic devices (Leaper et al., 2008). Antibiotic prophylaxis is performed prior to surgery to control infections (Bernard et al., 1999; Kawy et al., 2013). For effective dose continuous dosing of antibiotics is mandatory cause of fast elimination properties of it. In most of the cases amino glycosides are used as antibiotic in prophylaxis for controlling infections during and after the surgery (Bratzler et al., 2004; Anderson et al., 2014). To attain medical effectiveness higher concentrations of such drugs are needed (Maher et al., 2002). However, there are reported problems associated with use of higher concentration of amino glycosides in critical operations or surgery or implantations or any other such type of treatments (Vargas et al., 2004; Roberts and Douglas, 1978; Gonzalez, 1998; Maurin, 2001). There are major side effects studied and reported such as nephrotoxicity and ototoxicity associated with use of amino glycoside administration (Prayle et al., 2010; Warchol, 2010; Black et al., 2004; Ahmed et al., 2012; Mingeot and Tulkens, 1999).

There is no exact therapy or dosage form available which can prevent the side effects associated with aminoglycoside administration. But care can be taken while giving broad spectrum antibiotic Gentamicin sulphate and/or a combination of amino glycosides intravenously by monitoring the creatinine levels of the patient under treatment (Radigan et al., 2010; Oliveria et al., 2009; Warner and Sanders, 1971; Nathens and Dellinger, 2000). But still this does not eliminate potential damage to susceptible patients and exact mechanism for this side effect is also unclear. Therefore, in order to overcome the aforementioned limitations there is a need to prepare a bio-degradable polymeric implant with superior physical strength, controlled drug release and durability with required properties.

There are some models are prepared and studied for their characteristics (Nicolas et al., 2013; Devrajan and Jain, 2015; Klemm, 2001, Spann et al., 2003; Nelson et al., 2002; Friberg et al., 2005; Gruessner et al., 2001; Iyer et al., 2006). But we have prepared such device which is having unique composition. The demo implant is prepared and studied for its drug delivery activity by using Gentamicin sulphate as model drug. In supporting to weight loss study, simultaneously degradation pattern was studied by monitoring molecular weight change using gel permeation chromatography.
(GPC) (Balke et al., 1969; Potthast et al., 2015; Sultana and Kadir, 2011). Also studied morphological changes by using Scanning Electron Microscope (SEM) (Ederle et al., 2015).

5.2 Optimized Materials and Method for Preparation of Implant

Gentamicin sulphate was supplied by FDC Ltd, India. Poloxamer 188 was purchased from BASF Aktiengesellschaft, Germany, magnesium stearate from Ferro Corporation, Cleave Land, and PLGA (Mₙ=30,000) was purchased from Evonik Industries, Germany. Poly-Caprolactone (Mₙ=23,000) was purchased from Sigma Aldrich; Germany. All common solvents were purchased from Avantor Performance Materials and distilled prior to use.

According to IIG (Inactive Ingredient Guidance, USFDA) Poloxamer 188 having 0.1% to 5.5% is allowed limit as per to dosage form. Similarly allowed limit of magnesium stearate is 0.0008% to 1% according to dosage form. PLGA and Poly-Caprolactone are biodegradable polymers hence there is no specific limit for their use. According to ICH Q3 guidelines dichloromethane is a class two solvent having allowable limit of 600 ppm. Thus with these references study was planned. Following trials planned for optimization of formulation composition.

Drug loading was defined according to the therapeutic dose required to cure or to protect the wound from infection. Thus to treat bacterial infections in adults about 1.5 mg/kg to 2.0 mg/kg drug loading dose is required and followed to that about 1 mg/kg to 1.7 mg/kg by intravenous or intramuscular administration is required at every 8 hours. In another words for every 24 hours about 5mg/kg to 7 mg/kg intra venous drug is required. These limits are strongly given on the basis of brief toxicological data of Gentamicin sulphate by parenteral route of administration. Compared to that; we are directly targeting the site of where actual site is required. Drug loading is also depends on type of dosage form and route of administration. Along with that; duration of therapy is also one of the major factor which has to be consider when drug loading is there.

The duration of therapy is depending on severity of infection. But we know that Gentamicin sulphate eliminates rapidly from body and thus for its activity higher IV or IM doses are required. As per literature there are several reported methods available for implant blend preparation, but popular are by solvent casting method and hot melt extrusion method (Andjelic et al., 2016; James et al., 2010).
Thus drug loading was decided to put in implant in implant with respect to its weight and degradation time. On an average we considered for study purpose weight of implant is to be 1 gram and will degrade in about 200 to 250 days. Thus 5%, 10% and 20% of drug loading was kept for study.

Gentamicin sulphate loaded PLGA based implant is complete matrix system. We have not added any additive to control the drug release. Also polymer degradation and drug release are not interdependent processes here in this implant casting. As discussed; degradation of implant (PLGA) is completely different process; by hydrolysis or any other process. As implant is delivering drug at site of action, there are minimum chances of elimination with compare to IV or IM route. Thus drug loading was decided less with compare to IV and IM.

Other ingredients like Poloxamer 188 and magnesium stearate were selected according to IIG limits. Concentration of key ingredients like Poly-Caprolactone and PLGA were optimized according to physical properties of implant. At initial stage number of trials planned and taken to check the feasibility of blend and implant. Solvent casting method was selected for blend preparation as blend can be molded according to requirement and yield is also more. Dichloromethane (DCM) was used as a solvent. PLGA (79.1%), Poly-Caprolactone (10%) dissolved in dichloromethane. Homogenized the mixture for about 15 minutes at 2000 rpm. Followed to that added Poloxamer 188 (0.05 %) and Magnesium Stearate (0.05 %).

The mixture was homogenized overnight at 1000 rpm to obtain a homogeneous polymer solution. Slowly added Gentamicin sulphate (10.8 %) and homogenized it for about 60 minutes. In mixing process was monitored on microscope. Viscosity of slurry was measured and observed about 600 to 800 cps. Demo implant was prepared by carving the film prepared by using above slurry (Fig.5.1). The slurry was casted on to a glass made mold plate (to obtain film implant like structure) then dried in a vacuum oven at 50 °C for about 2 hours and then allowed to cool at room temperature.
5.3 Characterisation of Implant

5.3.1 Analytical Attributes for Casted Implant

Implant casting involves number of stages. Each stage was identified for quality improvement and used relevant analytical tool to assure the process of formulation and quality of material. Till chapter four; all relevant analytical methods and results are discussed. On that basis of satisfactory results only further formulation process was optimized. In this chapter performance verification, confirmation and characterization of implant is studied. Drug release study is briefly discussed and performed in this chapter. Along with this; hydrolysis or degradation of biodegradable implant is performed and discussed. All experiments are performed as per guidelines in medical and pharmaceutical field.

5.3.2 In Vitro Hydrolysis of Implant and Drug Release Study

*In vitro* drug release study was performed by placing above implant in pH 7.4 saline phosphate buffer solution at 37 °C ± 2 °C with mechanical shaking for defined period of time (Dave et al., 2015; Gasmi et al., 2015; Blanco et al., 2000). Study was performed in glass made bottles under covered condition having 20 mL of capacity. Collected the samples at specified time interval by complete decantation method and analyzed the same on UV spectrophotometer at 256 nm. Recorded the absorbance and calculated the cumulative drug release and controlled drug release. Following equation is used for the cumulative amount (Q) of drug released per unit area (Chandrasekaran et al., 2011; Davis, 1987),

$$Q = \left\{ C_nV + \sum_{i=1}^{n-1} C_iS \right\}/A \quad \text{(5.1)}$$
Here,
Q = Cumulative amount of drug released per unit area (μg/cm$^2$)
$C_n$ = Concentration of GE (μg/mL) determined at various time interval
V = Volume of individual Franz diffusion cell (mL)
$\sum_{i=1}^{n-1} C_i S_i$ = Sum of concentrations of GE (μg/mL) determined at sampling intervals; n-1
S = Sample volume withdrawal in (mL)
A = Surface area of sample and used area was 4.0 cm$^2$

5.3.3 Weight Loss of Implant

Implant was treated with thermal (37 °C), mechanical (Mechanical shaking at 100rpm) and chemically (saline buffered solution) in drug release (IVRT) study. Physical characterization of control and treated implant was performed (Martin, 1993). Control implant was termed to non-treated implant. After the completion of study, the treated samples were washed with de-mineralized water and dried at 40 °C in vacuum oven. These dried samples were used for further analysis and its characterization. The weight loss of these samples was studied. Prior to weight measurements, the initial non-treated samples were dried in vacuum. After the IVRT study samples were dried at 50°C in vacuum oven. Taken the weights of non-treated and treated samples and calculated difference between their weights. Then by taking difference of these weights calculated percent weight loss.

5.3.4 Molecular Weight Loss of Implant by GPC Analysis

Degradation of implant was studied by analyzing molecular weight change of treated and untreated sample on gel permeation chromatography (GPC) of make Waters510, and the data was processed by Waters Millennium 32 software. The GPC equipment consisted of ‘Waters 410 RI’ differential RI detector, ‘Shodex KF-800GPC’ column and ‘Waters 515’ HPLC-pump. Both treated and un-treated polymer samples were analyzed in chloroform solvent at a flow rate of 1.0 mL/min. The polystyrene standard was used as a relative standard for molecular weight determination.

5.3.5 Tensile Strength of Implant

Mechanical property was evaluated by testing the tensile strength of control sample and treated sample (Davis, 1987). Universal testing machine (UTM) make
Lloyd; USA, was used to measure tensile strength and analysis was performed under controlled room temperature and humidity. All the reported values are mean of three test trials. Tensile strength was calculated by using following formula (Chandrasekaran et al., 2011)

\[
\text{Tensile strength (mPa)} = \frac{(\text{load at break})}{(\text{original width}) (\text{original width})} \quad -------(5.2)
\]

5.3.6 Morphological Changes and Texture Analysis by SEM

The scanning electron microscope (SEM) of make Bruker was used to study the surface and texture morphologies of non-treated and IVRT treated samples at initial, five days, twenty first and forty-two days. Samples were dried at 40 °C and sputter coated with gold prior to SEM analysis.

5.3.7 Microbiological Studies

Objective of this study is to verify the activity of Gentamicin sulphate which is bounded in PLGA based biodegradable implant system. Thus activity of Gentamicin sulphate in matrix form was studied. Minimum concentration of Gentamicin sulphate required to heal or to avoid the bacterial infection is reported according to bacteria. Here by taking two bacteria which are mainly associated with wound infection; efficacy test was performed. Minimum inhibitory concentration (MIC) is a concentration of antibiotic or drug or any chemical entity required to inhibit visible planktonic bacterial growth of microorganism. Most of the antibiotics are having reported MIC and according to that dose optimization can be done. We have studied MIC of free GE, GE loaded implant, placebo implant and blank. Broth micro dilution method in 96 well micro plates was used to determine MIC. Turbidity analysis was performed for all the above samples to measure optical density at 600 nm in order to examine the visible bacterial growth. In this study, optical density (OD) 600<0.1 was observed which is a clear indication for zero bacterial growth (Davis, 1987).

Selection of bacterial strain and preparation of inoculum: For our studies we have selected Escherichia coli ATCC 25922 and P. Aeruginosa ATCC 27853 as bacterial strains. These are mostly associated with wound infection. Nephelometric turbidity method (Nephelometer, Phoenix and Becton Dickinson, USA) was used to prepare bacterial suspension equivalent to 108 CFU/ml (turbidity of 0.5 McFarland standard) from fresh subculture of both the strains. This suspension was diluted to 106
CFU/ml using Trypticase soy broth (TSB). Gentamicin sulphate as antimicrobial agent: Gentamicin sulphate a broad spectrum antibiotic was a key active ingredient of implant. Healing of bacterial infections associated with wound by Gentamicin sulphate is one of the objectives of the study. Efficacy of Gentamicin sulphate when realizing from implant is studied. Gentamicin sulphate solutions were prepared according to percent drug loading in concentrations ranging from 0.1 μg/mL to 50 μg/mL.

Materials and Experimental Methods: MIC study was performed by 96 well plate methods. Inoculated different concentrations of GE in plates. Examination of visible growth was performed by measuring an optical density (OD) of that at 600nm. The observation where OD 600 < 0.1 indicated zero bacterial growth. Trypticase soya broth (TSB) was used to grow the both the strains (P. Aeruginosa ATCC 27853 and Escherichia coli ATCC 25922) at 37 °C to obtain an optical density of one at 600nm.

Further this bacterial cell suspension was diluted hundred folds. Solutions of a range between 50 μg/ml to 0.1 μg/ml were prepared from as such active GE and GE-loaded implant released (in water at pH 6.9 and at 37 °C temperature) and were serially diluted in 100μL TSB in such way that to obtain in a total volume of 200μL. Then, 100μl of diluted P. Aeruginosa and Escherichia coli ATCC 25922 inoculums were added to each well containing free GE and GE-loaded implant in 100μL TSB. These samples were incubated for 16 hours at 37 °C. After incubation, these plate wells were visually examined and optical density of these samples recorded at 600nm. Minimum inhibitory concentration was determined as the lowest concentration which was yielded OD≤0.1.

5.4 Results and Discussion

GE is well known broad spectrum antibiotic used widely in wound care. But there are adverse side effects of it when given by traditional routes like intravenous and intra muscular etc. In order to fulfill the objective of local action, antibiotic drug Gentamicin sulphate loaded biodegradable implant is prepared by solvent casting method. PLGA having composition 75:25 is selected as major biodegradable component. The other excipients of the biodegradable implants are Poly-Caprolactone (acts as plasticizer and avoid cracks), Poloxamer 188 (show synergistic activity as lubricant and anti-inflammatory action), and Magnesium stearate (lubricant). Here the utilized excipients were approved for human and animal consumption. Our
compatibility study (using powder XRD, TGA and IR analysis) revealed that drug Gentamicin sulphate is compatible with all excipients.

5.4.1 In-Vitro Drug Release Study

IVRT is one of the most important tools to evaluate the drug release through the designed dosage form. It is a blue print of in-vivo study data if all parameters are selected to mimic the bio-system. During development stage it was observed that about 10% of drug loading is appropriate as per duration of therapy and amount required to heal the infection. Drug release kinetics was studied with different mathematical models which are associated with drug release. In this formulation, there is no any enhancer used to increase or decrease the drug release. Drug release is completely controlled by drug loading. Thus drug release study at different concentrations showed that drug loading of 10% in implant releases the appropriate amount of drug. Here in this chapter; we are demonstrating the optimized formulation of implant and drug release study associated with it. Experimental parameters are followed as mentioned in analytical methods.

Drug release (in µg per gram of implant) was calculated and presented in Fig. 5.2 and 5.3. Samples were collected and analyzed in time intervals like initial, one day, three days, five days, seven days, twenty-one days and forty-two days respectively. Cumulative drug release is as shown in Fig. 5.2, where it is demonstrated that the cumulatively in forty-two days about 3.4 mg of drug was released at receptor site per gram of sample. Fig. 5.3 is representing a flux; microgram of drug released per unit per hour (µg/unit/hr).

As per the published MIC data (Baxter, 2012) of Gentamicin sulphate for gram positive and negative bacteria is discussed here. MIC data for E. Coli ATCC 25922 is 0.25 to 1 mcg/ml and zone diameter in mm is 19-26. Similarly for Pseudomonas A. ATCC 27853 MIC is reported as 0.50 to 2.0 mcg/ml and zone diameter in mm was 16-21 mm.
5.4.2 Sample Weight Loss After IVRT Study

When implant is treated for drug release study, along with drug release there is a hydrolysis of PLGA and Poly-Caprolactone occurred. To identify this sample weight loss, control and treated sample (treatment to pH 7.4 saline phosphate buffer at 37 °C) were studied. Triplicate study was performed to monitor the weight loss. Samples were
weighed at initial and at the end of degradation study (about 6 weeks) by proper vacuum drying process. As shown in Table 5.1 there was weight loss observed (Fig.5.4).

This weight loss is not only because of drug release but also due to polymer degradation. It is confirmed in molecular weight loss study and morphological study. It demonstrated that drug was released through the implant along with polymer degradation.

**Table 5.1 Weight Loss Study Data for Implant after 42 Days’ Buffer Treatment**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Initial Wt</th>
<th>Wt after 42 days</th>
<th>Wt loss</th>
<th>% wt loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>937.5</td>
<td>786.0</td>
<td>151.5</td>
<td>16.2</td>
</tr>
<tr>
<td>2</td>
<td>961.0</td>
<td>809.0</td>
<td>152.0</td>
<td>15.8</td>
</tr>
<tr>
<td>3</td>
<td>1001.3</td>
<td>867.0</td>
<td>134.3</td>
<td>13.4</td>
</tr>
</tbody>
</table>

![Fig. 5.4 Weight Loss of IVRT Exposed Sample after 42 Days](image)

5.4.3 Molecular Weight Change and GPC Analysis

From weight loss study, it was proven that there is a weight loss of implant when treated in saline phosphate buffer solution of pH 7.2 at 37°C. This may be due to drug release but values from weight loss study data are representing polymer weight loss is also there. Molecular weight of the PLGA of biodegradable implant was analyzed
during the degradation studies. It was observed that at the initial stage the control sample was having $M_n2.05\times10^4$ g/mole with a PDI of 2.48. But when it was treated for degradation in pH 7.4 saline phosphate buffer solution at 37 °C decrease in molecular weight was observed with time. For instance, the samples that were drawn on 21st and 42nd days showed $M_n$ of $1.47 \times 10^3$ with PDI of 1.76 and $1.15 \times 10^3$ with PDI of 1.34 respectively (Table 5.2 and Fig.5.5). This controlled GPC analysis clearly indicating that there was a degradation of implant with respect to time during the in vitro studies.

**Table 5.2 GPC Data for Control and IVRT Treated Sample**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Day</th>
<th>$M_n$</th>
<th>$M_w$</th>
<th>$M_w/M_n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zero day</td>
<td>$2.05 \times 10^4$</td>
<td>$5.08 \times 10^4$</td>
<td>2.48</td>
</tr>
<tr>
<td>2</td>
<td>Twenty first day</td>
<td>$1.47 \times 10^3$</td>
<td>$2.60 \times 10^3$</td>
<td>1.76</td>
</tr>
<tr>
<td>3</td>
<td>Forty second day</td>
<td>$1.15 \times 10^3$</td>
<td>$1.55 \times 10^3$</td>
<td>1.34</td>
</tr>
</tbody>
</table>

**Fig. 5.5 GPC Data for IVRT Treated Sample; Initial, Twenty First and Forty Second Day**

5.4.4 Mechanical Property Change Evaluation Study

We have evaluated the implant for its tensile strength. Mechanical properties of the sample were studied after treating them in saline phosphate buffer having pH 7.4 at 37 °C with mechanical shaking. At defined time intervals samples were withdrawing and analyzed. With time the tensile strength of the implant gradually dropped down. For instance, the un-treated sample showed tensile strength of 357.0 mPa, whereas the
sample treated for 42 days showed 267.4 mPa. From this we can conclude that the implant is degrading with respect to time when treated in *in-vitro* studies.

5.4.5 Morphological Changes

SEM analysis was performed to study and understand the micro level changes related to fracture, cracks, gaps and porosity of control and IVRT treated samples. It clearly demonstrated detail information on degradation of sample. Microscopic images taken on SEM are shown in Fig. 5.5. From these images, it is clear that after seven days’ texture change along with cracks was observed in all samples (Fig. 5.5 and Fig.5.6). As per literature, when there is a breaking of weak intermolecular forces i.e. vander Waals force and weak hydrogen bonding; texture change will be observed like cracks etc. (Nguyen and Alsberg, 2014; Hirenkumar and Steven, 2011). Simultaneously there was a release of drug and other excipients. These texture changes during *in vitro* study were clearly observed in Fig. 5 and 6. But it was not observed any cracks up to five days’ study. (Fig. 5.5(b))

It means that there is a release of surface drug and excipients initially along with degradation of polymer matrix. After this buffer enters inside the polymer matrix and releases the inside drug along with polymer degradation. It was observed that after forty-two days, in addition to the breaks and cracks some micro holes having diameter of about $1.3 - 2.6 \mu m$ (Fig.5.5.D) were observed. The micro holes are generated after drug release. For easy understanding, this entire phenomenon is represented in Fig. 5.7.
Fig. 5.6a SEM Images of Bio-Implant During IVRT Studies (a) Control Sample (b) Fifth Day
Fig. 5.6b SEM Images of Bio-Implant During IVRT Studies (c) Twenty First Day (d) Forty-Two Day
Fig. 5.7 Increase in Cracks Development in Bio-Implant during IVRT Studies as a Function of Time as Seen in its SEM Images during 7th Day (a), 21st Day (b) and 42nd Day (c) Respectively

Similarly control implant was evaluated for possible antibacterial activity against P. Aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 bacteria. Minimum inhibitory concentrations were determined by 96 well plate methods by separately cultivating both the strains and measured its optical density at 600nm. Samples were prepared by inoculating bacterial suspensions to placebo and GE loaded implant solution in the range of 0.1 to 50 µg / mL. These plates were incubated at 37 °C for about 24 hours. Agar plate method was used to measure colonies and expressed in the Colony Forming Units (CFU). MIC of GE from implant was observed as 2.5 µg for P. Aeruginosa and about 3 µg for Escherichia Coli (Fig. 5.8 and Fig. 5.9).
A : Drug loaded sample  
B : Placebo sample  

Fig. 5.8 Efficacy test of GE loaded PLGA based biodegradable implant at MIC
Fig. 5.9 Antibacterial Activity and Effect of GE Loading on it

(a) MIC Plot of GE for P. Aeruginosa  (b) MIC Plot of GE for E. Coli
Fig. 5.9 Antibacterial Activity and Effect of GE Loading on it

(c) Antibacterial Activity on P. Aeruginosa (d) Antibacterial Activity of GE on E. Coli
5.4.6 Susceptibility Testing of Implant

Gentamicin sulphate loaded PLGA based biodegradable implant was studied for its antibacterial activity. As a part of study and confirmation of the antibacterial activity of implant prepared is only due to Gentamicin sulphate was performed. Here in this study control sample which is an implant without Gentamicin sulphate was compared with major ingredient of implant PLGA and blank. Purpose of this study was only to confirm the claim that antibiotic drug loading is responsible for inhibition of microbial growth in wound. Similar procedure followed like inoculation of strains in to these four prepared samples and compared the CFU count data with blank in it.

It was clearly observed that CFU count of placebo (without Gentamicin sulphate) is near to similar to blank and PLGA preparations. Which clearly demonstrates that, the blank implant do not possess any antibacterial activity. The antibacterial effect is mainly due to the Gentamicin sulphate loading in to the implant.

![Fig. 5.8 CFU Count from Blank, PLGA and Control without Drug Susceptibility Testing](image)

Fig. 5.8 CFU Count from Blank, PLGA and Control without Drug Susceptibility Testing
Fig. 5.9. Schematic Representation of Drug Delivery and Polymer Degradation

Initial GPC

GPC after 21 days

GPC after 42 days

Initial SEM image

SEM image after 42 days

Cavities after drug release

21 days

42 days

= drug

= polymer chain
5.5 Conclusions

Here we have prepared PLGA based biodegradable Gentamicin sulphate (GE) drug loaded implant by solvent casting method. We have evaluated its physical properties during and after in-vitro drug release study. Drug release (in µg per gram of implant) from implant was calculated by collecting the samples in regular interval of times for a period of 42 days. In forty-two days about 3.4 mg of drug was released (cumulative) at receptor site per gram of sample. Average 3.5 µg/unit/hr drug was released which was sufficient to control infection and same was confirmed by MIC study of GE loaded implant.

During in-vitro studies, with time the tensile strength as well as the weight of the implant was dropped down gradually. This weight loss is not only because of drug release but also due to polymer degradation. It was confirmed in molecular weight loss study and morphological study. After 42 days of in vitro analysis the molecular weight of PLGA reduced from Mn 2.05x104 g/mole (PDI of 2.48) to 1.15 X 103 (PDI of 1.34). This decrease in molecular weight of PLGA in biodegradable implant represents its biodegradation nature.

The morphological study on biodegradable implant by SEM during the in vitro analysis revealed that the device gets degraded with expanded breaks and cracks with a change in texture. In SEM images of biodegradable implants in addition to the breaks and cracks some micro holes having diameter of about 1.3 – 2.6 µm were observed, which were generated after drug release. Thus we demonstrated that PLGA based biodegradable Gentamicin sulphate (GE) drug loaded implant as a potential material for controlled drug delivery system in wound care.
Summary & Conclusions

- A composition for Gentamicin sulphate delivery system is designed. Successfully studied the drug (Gentamicin sulphate) and excipients (Poloxamer 188, Magnesium stearate, Poly-caprolactone and PLGA) interaction by using various techniques and established compatibility data between them and found compatible with each other.

- Developed, verified and validated simple analytical tools for monitoring the process and quality of products. With reference to analytical data, processing parameters like composition, mixing time & drying time were optimized.

- PLGA based biodegradable Gentamicin sulphate (GE) drug loaded implant by solvent casting method was prepared. We have evaluated its physical properties during and after in-vitro drug release study. During in-vitro studies, with time the tensile strength as well as the weight of the implant was dropped down gradually.

- The samples that were drawn on 21st and 42nd days showed $M_n$ of $1.47 \times 10^3$ with PDI of 1.76 and $1.15 \times 10^3$ with PDI of 1.34 respectively. This decrease in molecular weight of PLGA in bio implant represents its biodegradation nature.

- The morphological study on bio implant by SEM during the in vitro analysis revealed that the device gets degraded with expanded breaks and cracks with a change in texture. In SEM images of bio implants in addition to the breaks and cracks some micro holes having diameter of about $1.3 - 2.6 \mu m$ were observed, which were generated after drug release. This weight loss is not only because of drug release but also due to polymer degradation. It was confirmed in molecular weight loss study and morphological study.

- Drug release (in $\mu g$ per gram of implant) from implant was calculated by collecting the samples in regular interval of times for a period of 42 days. In 42 days about 3.4 mg of drug was released (cumulative) at receptor site per gram of sample. Average 3.5 $\mu g$/unit/hr drug was released which was sufficient to control infection and same was confirmed by MIC study of GE loaded implant.

- Thus we demonstrated that PLGA based biodegradable Gentamicin sulphate drug loaded implant as a controlled drug delivery system in wound care.