Formulation and Optimization of Biodegradable Polylactic-co-glycolic acid Nanoparticles of Simvastatin using Factorial design

Anilkumar J. Shinde* and Harinath N. More

Department of Pharmaceutics, Bharati Vidyapeeth, College of Pharmacy, Kolhapur

ABSTRACT

The objective of the present work was to formulate nanoparticles for simvastatin drug. Simvastatin is a lipid lowering agent, undergoes extensive first pass extraction in the liver, the availability of the drug to the general circulation is low (< 5%). Nanoparticles were prepared by precipitation-solvent deposition method using $3^2$ full factorial design, Pluronic F-68 as polymeric stabilizer. From the preliminary trials, the constraints for independent variables $X_1$ (amount of PLGA) and $X_2$ (amount of Pleuronic F-68) have been fixed. The prepared formulations were further evaluated for % encapsulation efficiency, particle size, Polydispersity index, in vitro drug release pattern and drug excipient interactions. Drug: polymer ratio and concentration of stabilizer were found to influence the particle size and entrapment efficiency of simvastatin loaded PLGA nanoparticles. In vitro drug release study of selected factorial formulations (PS1, PS4, PS7) showed, 84.56%, 89.65% and 73.46 % release respectively in 24 hrs. The formulation batch PS3 having lowest particle size 122 nm. The release was found to follow first order release kinetics with fickian diffusion mechanism for all batches. These results indicate that simvastatin loaded PLGA nanoparticles could be effective in sustaining drug release for a prolonged period.

Keywords: Antilipidemic agent, PLGA, Pluronic, $3^2$ factorial design, Simvastatin, sustained release.

INTRODUCTION

Drug low solubility and stability in physiological environment constitutes a main hurdle in attaining the appropriate bioavailability. Several polymer-based nanotechnologies are being intended in order to optimize the technological (e.g., solubility, stability, bioavailability, etc.) aspects of drugs. Among them, polymeric nanoparticles, dendrimers, polymeric micelles and polymersomes appear as the most attractive and promising.[1,2] In the recent years, nanoparticle technology has emerged as a strategy to tackle such formulation problems associated with poorly water and lipid soluble drugs. The reduction of drug particle to the nano-scale, increases dissolution velocity and saturation solubility and have the potential power to improve drug
stability, increase the duration of the therapeutic effect and permit administration through enteral or parenteral administration, which may prevent or minimize the drug degradation.

Dyslipidemia, including hypercholesterolemia, hypertriglyceridemia, or their combination, is a major risk factor for cardiovascular disease. Generally, dyslipidemia is characterized by increased fasting concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), in conjunction with decreased concentrations of high-density lipoprotein cholesterol (HDL-C). At present, these lipid imbalances are most routinely treated with pharmacological therapy.

However, many cholesterol lowering agents, like, simvastatin, lovastatin, atorvastatin, cerivastatin, pravastatin are generally used. Simvastatin, is a crystalline compound, practically insoluble in water and hence poorly absorbed from the GI tract. It is a potent and specific inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase, which catalyzes the reduction of HMG CoA to mevalonate. Thus, simvastatin arrests a key step for cholesterol biosynthesis in liver, and is hence widely used in the treatment of hypercholesterolemia and dyslipidemia, as an adjunct to diet. After oral administration, simvastatin is metabolized to its β-dihydroxy acid form (simvastatin acid) by the cytochrome-3A system in liver, where it inhibits the rate-limiting step in cholesterol biosynthesis.[3] Being a Class II drug, it often shows dissolution rate limited oral absorption and high variability in pharmacological effects. Therefore, improvements in its solubility and/or dissolution rate may lead to enhancement in bioavailability.

Poly(lactic-co-glycolic acid) is a copolymer which is used in a wide array of FDA approved therapeutic devices. PLGA is a biodegradable, biocompatibility polymer that by hydrolysis of its ester linkages in the presence of water. It has been shown that the time required for degradation of PLGA is related to the monomers' ratio used in production: the higher the content of glycolide units, the lower the time required for degradation. Pluronic F68 is a difunctional block copolymer surfactant terminating in primary hydroxyl groups. A nonionic surfactant that is 100% active and relatively nontoxic.

Various attempts to enhance the dissolution rate and bioavailability of simvastatin have been reported. Like that preparation of nanoparticles, various methods, currently used are precipitation, high pressure homogenization and pearl milling, either in water or in mixtures of water and water-miscible liquids or nonaqueous media.[4,5] Nanoprecipitation is a technique, where a 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 surfactants.[6]

The aim of present work was formulation of nanoparticles by nanoprecipitation–solvent diposition method and find out the effect of stabilizer on the formulation, when all parameters of operation are kept constant. Formulation of nanoparticles using Polylactide co-glycolide as lipophilic polymer, simvastatin as a model drug and Pleuronic F- 68 as surfactant stabilizer and evaluated with different parameters such as particle size, % encapsulation efficiency, zeta potential, in vitro drug release, fourier transform infra red study, scanning electron microscopy and differential scanning colorimetric study.[7,8]
MATERIALS AND METHODS

Simvastatin was obtained from a gift sample from Aurobindo Pharmaceutical Ltd., Hyderabad; Poly (D, L Lactide-co-Glycolide) (PLGA 50:50) was obtained as gift samples from Purac biochem ltd. Netherlands; Pluronic F 68 was purchased from sigma chemicals, Mumbai, dialysis bag (cellulose membrane, molecular weight cut off 10000-12000 Da, purchased from Hi-Media, Mumbai, India. All other reagents and chemicals used in this study were of analytical Grade.

Methods

Formulation of Nanoparticles

PLGA nanoparticles were prepared by the nanoprecipitation - solvent deposition method.[9] Simvastatin was dissolved in acetone. Hydrophilic stabilizer Pluronic F68 dissolved in 40 ml of water. PLGA was solubilized in acetone 20ml at various concentrations. The organic phase was poured into the aqueous solution drop wise, with syringe positioned with the needle directly into stabilizer containing water under magnetic stirring at 3000 RPM for 2 hrs, thus forming a milky colloidal suspension. The organic solvent was then evaporated by using a Rota evaporator. The resultant dispersion was dried using a freeze dryer.[10]

Experimental Design [11]

The formulations were fabricated according to a 3² full factorial design, allowing the simultaneous evaluation of two formulation variables and their interaction. The experimental designs with corresponding formulations are shown in table no.1 & 2. The dependent variables that were selected for study were particle size (Y1) and % drug entrapment (Y2).

Table 1: Experimental design and Parameters for 3² Full Factorial Design Batches

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Variables level in coded Form</th>
<th>Particle Size (nm)</th>
<th>% drug entrapment ± SD*</th>
<th>% Free Drug ± SD*</th>
<th>Polydispersity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS1</td>
<td>-1</td>
<td>140</td>
<td>78.23 ± 0.67</td>
<td>13.28 ± 0.31</td>
<td>0.3834</td>
</tr>
<tr>
<td>PS2</td>
<td>-1</td>
<td>137</td>
<td>72.25 ± 1.47</td>
<td>25.15 ± 1.21</td>
<td>0.5873</td>
</tr>
<tr>
<td>PS3</td>
<td>-1</td>
<td>122</td>
<td>61.58 ± 0.39</td>
<td>38.03 ± 1.22</td>
<td>0.7458</td>
</tr>
<tr>
<td>PS4</td>
<td>0</td>
<td>212</td>
<td>81.40 ± 0.38</td>
<td>18.60 ± 0.24</td>
<td>0.9663</td>
</tr>
<tr>
<td>PS5</td>
<td>0</td>
<td>189</td>
<td>76.57 ± 0.70</td>
<td>22.73 ± 0.37</td>
<td>0.9513</td>
</tr>
<tr>
<td>PS6</td>
<td>0</td>
<td>173</td>
<td>63.43 ± 0.25</td>
<td>36.32 ± 1.24</td>
<td>0.9130</td>
</tr>
<tr>
<td>PS7</td>
<td>+1</td>
<td>293</td>
<td>97.18 ± 0.4</td>
<td>02.82 ± 1.3</td>
<td>0.9425</td>
</tr>
<tr>
<td>PS8</td>
<td>+1</td>
<td>272</td>
<td>89.25 ± 0.36</td>
<td>10.75 ± 0.14</td>
<td>1.010</td>
</tr>
<tr>
<td>PS9</td>
<td>+1</td>
<td>205</td>
<td>87.03 ± 1.38</td>
<td>12.97 ± 0.23</td>
<td>0.8946</td>
</tr>
</tbody>
</table>

*SD indicates standard deviation (n=3)

Table 2: Translation of coded levels to actual quantities

<table>
<thead>
<tr>
<th>Coded Levels</th>
<th>+1</th>
<th>0</th>
<th>-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug: Polymer ratios (X₁)*</td>
<td>1:3</td>
<td>1:2</td>
<td>1:1</td>
</tr>
<tr>
<td>Pleuronic F 68 (X₂)mg*</td>
<td>200</td>
<td>150</td>
<td>100</td>
</tr>
</tbody>
</table>

In Vitro Characterization of Nanoparticles

Determination of particle size

The particle size and size distribution of the simvastatin loaded PLGA (50:50) nanoparticles were characterized by photon correlation spectroscopy (PCS) using a Zetasizer 2000 Malvern Instruments, UK. Nanosuspension was diluted with filtered (0.22µm) ultra pure water and
analysed using Zeta sizer. This analysis yields the mean diameter (z-average, measuring range: 20–1000 nm), which allows sample measurement in the range of 0.020-2000.00 μm. [12]

**Determination of zeta potential**

The zeta potential of the drug-loaded PLGA nanoparticles was measured on a zetasizer (Malvern Instruments) by determining the electrophoretic mobility in a microelectrophoresis flow cell. All the samples were measured in water at 25 °C in triplicate.

**Determination of Encapsulation Efficiency**

The encapsulation efficiency of nanoparticles was determined by first separating the nanoparticles formed from the aqueous medium by centrifugation at 15000 rpm for 30 min. The amount of free simvastatin in the supernatant was measured by UV spectrophotometry at 238 nm (Shimadzu UV-1700,) after suitable dilution. The simvastatin entrapped in the nanoparticles was calculated as Eq 1.

\[
\text{Drug Entrapment (\%) = } \frac{T_p - T_f}{T_p} \times 100 \quad \text{...1}
\]

where \(T_p\) is the total simvastatin used to prepare the nanoparticles and \(T_f\) is the free simvastatin in the supernatant.

**Polydispersity index:** Polydispersity was determined according to the equation,

\[
\text{Polydispersity index} = \frac{D (0.9)-D (0.1)}{D (0.5)}
\]

Where, \(D (0.9)\) corresponds to particle size immediately above 90% of the sample. \(D (0.5)\) corresponds to particle size immediately above 50% of the sample. \(D (0.1)\) corresponds to particle size immediately above 10% of the sample.

**Statistical Analysis:** [15]

The results from factorial design were evaluated using PCP Disso 2000 V3 software. Step-wise backward linear regression analysis was used to develop polynomial equations for dependent variables particle size (Y1) and % drug entrapment (Y2) which form of equation-1:

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \epsilon \quad \text{...1}
\]

Where \(Y\) is estimate response of dependent variable, \(\beta_0\) arithmetic mean response of nine batches, and \(\beta_1\) estimated coefficient for factor \(X_1\). The main effects (\(X_1\) and \(X_2\)) represent average result of changing one factor at a time from its low to high value. The interaction term (\(X_1X_2\)) shows how the response changes, when two factors are simultaneously changed. The polynomial terms (\(X_1^2\) and \(X_2^2\)) are included to investigate non-linearity. \(\epsilon\) is the random error. The simplified models were then utilized to produce three dimensional response surface plots to analyze the influence of independent variables.

**In vitro drug Release Study:**

The simvastatin loaded PLGA nanoparticles, after separation by centrifugation, were re-dispersed in 5mL phosphate buffer solution pH 6.8, placed in a dialysis membrane bag, tied and immersed in 150mL of PBS in a 250ml beaker. The entire system was stirred continuously at 37 °C with a magnetic stirrer at 100 rpm. Required quantity 5ml of the medium was withdrawn at specific time periods (0.5, 1, 2, 3 ,4, 6 , 8,10, 12, 24 hours) and same volume of dissolution
medium was replaced in the flask to maintain a constant volume. The withdrawn samples were filtered through a filter paper (0.22 µm, Whatman Inc., USA) and 5 ml filtrate was made up to volume with 100 ml of Phosphate buffer pH 6.8. The samples were analyzed for drug release by measuring the absorbance at 238 nm using UV-visible spectrophotometer and calculate percent cumulative release of simvastatin. [16]

**Fourier Transform Infrared Spectroscopy Study:**
Infrared spectrum of simvastatin, nanoparticle formulation was determined by using Fourier Transform Infrared Spectrophotometer (FTIR-4100, Shimadzu) using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run. [17]

**Differential Scanning Calorimetry Study:**
Differential scanning calorimetry (DSC) is one of the most powerful analytical techniques, which offers the possibility of detecting chemical interaction of Simvastatin, PLGA and simvastatin nanoparticles. DSC measurements were carried out on a modulated DSC Instrument: SDT Q600 V20.9 Build 20 equipped with a thermal analysis data system (TA instrument). Samples of 2–10 mg were placed in aluminium pans and sealed. The probes were heated from 25 to 250ºC at a rate of 10 K/min under nitrogen atmosphere. [18,19]

**X-ray Diffraction Study:**
X-ray diffraction analysis was employed to detect the crystallinity of the pure drug and the nanoparticle formulation, which was conducted using a Philips PW 3710 x-ray diffractometer (XRD) with a copper target and nickel filter (Philips Electronic Inst, Holland). Powders were mounted on aluminium stages with glass bottoms and smoothed to a level surface. The XRD pattern of each sample was measured from 10 to 50 degrees 2-theta using a step increment of 0.1 2-theta degrees and a dwell time of 1 second at each step. [20]

**Scanning Electron Microscopy Study:**
The morphology of nanoparticles was examined by using scanning electron microscopy (SEM, JSM-6360LV scanning microscope Tokyo, Japan). The nanoparticles were mounted on metal stubs using double-sided tape and coated with a 150 Å layer of gold under vacuum. Stubs were visualized under scanning electron microscope. SEM has been used to determine particle size distribution, surface topography, texture and examine the morphology of fractured or sectioned surface. The same generally used for generating three dimensional surface relief images derived from secondary electrons.

**RESULTS AND DISCUSSION**
All the factorial formulations developed by the nanoprecipitation-solvent disposition method, formulations, were found to be free flowing and white, powdery in appearance.

**Particle Size and Entrapment Efficiency:**
A graphical representation of the particle size of PLGA nanoparticles obtained is shown in fig. no.1&2. Particle size is an important parameter because it has a direct relevance to the stability of the formulation. The amount of stabilizer used also has an effect on the properties of nanoparticles. If the concentration of stabilizer is too low, aggregation of the polymer will take place, whereas, if too much stabilizer is used, drug incorporation could be reduced as a result of the interaction between the drug and stabilizer. The effect of the concentration of the polymer, increasing the concentration causes the emulsion to have larger droplets, hence leading to larger
particles. From Fig.1 and Fig. 3 and Table-1, it is revealed that as Simvastatin:PLGA ratio increased from 1:1 to 1:3, particle size increased significantly and drug entrapment also increased.

This can be explained by observing drug entrapment efficiency of factorial formulations PS1, PS4, PS7, where drug: polymer ratio increased from 1:1, 1:2 and 1:3 respectively with constant concentration of Pluronic F68 of 100 mg. Drug entrapment efficiency increased from 78.23%, 81.40% and 97.18%. It is also observed that as percentage of stabilizer increased from 100 mg to 200 mg, entrapment efficiency and particle size decrease significantly. The same can be explained with respective to factorial formulation PS1, PS4, PS7 and PS2, PS3, PS5, PS6, PS8, where it is observed that as drug: polymer ratio increases, entrapment efficiency increased significantly. For factorial formulation PS7, PS8, PS9, where drug: polymer ratio is constant i.e.1:3 and concentration of stabilizer increased from 100 mg to 200 mg, drug entrapment efficiency deceased from 97.18 to 87.03% and particle size deceased from 293 nm to 205 nm. Thus it can be concluded that the stabilizer had greater influence on both dependent parameters particle size and drug entrapment as compared to drug: polymer ratio.

![Fig. 1. Particle size of the formulations PS1-PS9](image1)

![Fig. 2. Particle size of the formulations PS3 (122 nm)](image2)
Polydispersity Index:
The Polydispersity index of the data revealed that the particle size distributions of all the formulations are uniform, the results shown in table 1.

Zeta Potential Study:
The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. Particles with zeta potentials more negative than -30mV are normally considered stable. All the formulation of nanoparticle zeta potentials observed in the range of -25 mv to -31 mv.

In-Vitro Drug Release Study:
In vitro drug release study conducted according to highest % drug entrapment and lowest particle size below 293 nm batches PS1, PS4, and PS7 were selected, shown in table 3 & fig. 4. The release rate of nanoparticles by diffusion and biodegradation process. It is generally anticipated from a bulk eroding polymer such as 50:50 PLGA to give an initial burst release followed by a controlled release, in contrast to the release pattern observed in other controlled release systems.

Table 3. Cumulative % release of simvastatin formulation

<table>
<thead>
<tr>
<th>Time in hr</th>
<th>Cumulative % release (mean ± SD, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PS1</td>
</tr>
<tr>
<td>0.5</td>
<td>21.40 ± 1.02</td>
</tr>
<tr>
<td>1</td>
<td>38.17 ± 0.26</td>
</tr>
<tr>
<td>2</td>
<td>42.26 ± 1.32</td>
</tr>
<tr>
<td>3</td>
<td>51.27± 1.02</td>
</tr>
<tr>
<td>4</td>
<td>53.29 ± 0.29</td>
</tr>
<tr>
<td>6</td>
<td>58.34 ± 0.23</td>
</tr>
<tr>
<td>8</td>
<td>62.10 ± 1.28</td>
</tr>
<tr>
<td>10</td>
<td>65.79 ± 1.25</td>
</tr>
<tr>
<td>12</td>
<td>76.82 ± 1.20</td>
</tr>
<tr>
<td>24</td>
<td>84.56 ± 0.10</td>
</tr>
</tbody>
</table>

*SD indicates standard deviation
The drug release follows first order release kinetics with fickian diffusion mechanism. Drug release for selected factorial formulations PS1, PS4, PS7 are 38.17 %, 39.39 % and 26.19 % respectively, after 1 hr. These formulation show initial burst release followed by a controlled release. Kinetic exponent ‘n’ for these formulations indicate diffusion through the nanoparticle matrix as well matrix erosion. Finally, it can be concluded that the different drug release rates may be attributed to different sizes of the nanoparticles. It is expected as the particle size of nanoparticle is smaller, their surface area will be more and the drug release is faster.

Development of Polynomial Equations:
The experimental design and Parameters in table no.1 & 2 for factorial formulations PS1 to PS9, polynomial equations for two dependent variables, particle size and % drug entrapment have been derived using PCP Disso 2000V3 software.

The equation derived for particle size is:
\[ Y_1 = 200.01 + 64.095X_1 - 24.166X_2 + 5.765X_1^2 - 13.025X_2^2 - 13.575X_1X_2 \ldots \]

The equation derived for % drug entrapment is:
\[ Y_2 = 74.420 + 10.0913X_1 - 7.4617X_2 + 6.978X_1^2 - 0.9310X_2^2 + 0.8520X_1X_2 \ldots \]

In equations no. 2 negative sign for coefficient of X2 indicates that the particle size of nanoparticles deceases, when concentration of stabilizer Pluronic F68 is increased and positive sign for coefficient of X1 indicate positive effect of concentration PLGA on particle size. In equation no.3, positive sign for coefficient of X1 indicates that the % drug entrapment increases, when concentration of PLGA increases and negative sign for coefficient of X2 indicates that % drug entrapment of nanoparticles decreases, when concentration of stabilizer Pluronic F 68 increases. The closeness of predicted and observed values for particle size and % drug entrapment indicates validity of derived equations for dependent variables.

Response Surface Plots:
The response surface plots of particle size and % drug entrapment are shown in fig. 5 & 6 respectively. The response surface plots illustrated that as concentration of PLGA increases, the value of dependent variable, particle size increases and as concentration of Pluronic F 68
increases the value of dependent variable, particle size decreases. Similarly the response surface plots for % drug entrapment shows positive effects of independent variable, PLGA concentration and negative effect of other independent variable, concentration of Pluronic F 68.

![Response surface plot showing effect of factorial variables on particle size.](image)

**Fig. 5: Response surface plot showing effect of factorial variables on particle size.**

![Response surface plot showing effect of factorial variables on % drug entrapment](image)

**Fig. 6. Response surface plot showing effect of factorial variables on % drug entrapment**

**Fourier Transform Infrared Spectroscopy Study:**
F.T.I.R. study was carried out to confirm the compatibility between the selected polymer PLGA, drug simvastatin and nanoparticles are presented in fig.7. The spectra obtained from the I.R. studies are from 3600cm−1 to 400cm−1. It was confirmed that there are no major shifting as well as no loss of functional peaks between the spectra of drug, polymer and drug loaded nanoparticles (1265cm−1, 1380cm−1, 1458cm−1, 1765cm−1, 2968cm−1, 3652cm−1.)
Differential Scanning Calorimetry Study
Differential Scanning Calorimetry study gives information regarding the physical properties like crystalline or amorphous nature of the samples. The DSC thermogram of simvastatin (Fig. 8-A), shows an exothermic peak at 143.99 °C corresponding to its melting temperature. However, no sharp endotherm was seen at 147.99°C for the DSC curves of the simvastatin nanoparticles in Fig. 8-C. This shows the crystallinity of the drug has been reduced significantly in the nanoparticles. Hence it could be concluded that in both the prepared PLGA nanoparticles, the drug was present in the amorphous phase and may have been homogeneously dispersed in the PLGA matrix.

XRD study:
XRD pattern of the simvastatin, PLGA and selected nanoparticle formulation are shown in Fig 9. Characteristic diffraction peaks were observed for commercial simvastatin. On the other hand, the nanoparticles prepared with PLGA was characterized by less intensity of the diffraction peak when compared to that of simvastatin. This clearly indicates the reduction in the crystallinity of the precipitated simvastatin nanoparticles.
Scanning Electron Microscopy Study:
The morphology of nanoparticles was examined by scanning electron microscopy (SEM, JSM-5310LV scanning microscope Tokyo, Japan. The external morphological study using SEM revealed that all nanoparticles were spherical in shape shown in fig.10 & 11.

CONCLUSION
This study confirms that the nanoprecipitation- solvent deposition technique is suitable for the preparation of simvastatin nanoparticles with high encapsulation efficiency and low particle size. Drug: polymer ratio and concentration of stabilizer were found to influence the particle size and
entrapment efficiency of simvastatin loaded PLGA nanoparticles but the concentration of stabilizer had greater influence on both dependent variables, particle size and % drug entrapment as compared to drug : polymer ratio. In vitro drug release study of selected factorial formulations PS1, PS4, PS7 showed, 84.56%, 89.65 %, and 73.46 % release respectively in 24 hrs. The release was found to follow first order release kinetics with fickian diffusion mechanism for all batches. So, we can conclude that simvastatin loaded PLGA nanoparticles could be effective in sustaining drug release.

Acknowledgements
Authors are wishing to acknowledge Aurobindo Pharmaceutical Ltd. (Hyderabad, India), for providing simvastatin as gift sample. We also grateful to Bharati vidyapeeth, Poona College of Pharmacy, Pune for providing Malvern Mastersizer facility, Shivaji university Kolhapur for SEM, DSC & XRD facilities, Bombay College of Pharmacy, Mumbai for providing Malvern Mastersizer Zeta potential and Purac biochem, Netherland for providing, Polylactic co- glycolic acid as gift samples. Author also thank to Principal Dr. H. N. More Bharati Vidyapeeth, College of Pharmacy, Kolhapur for providing excellent facility to carry out this work.

REFERENCES