ABSTRACT

Aim: Goserelin acetate is parenterally administered, a typical LHRH analogue for the treatment of prostate cancer. The objective of present work was to formulate and evaluate long acting in situ forming microparticles of goserelin using PLGA which would extend the drug release.

Materials and Methods: PLGA 50:50 were used as the biodegradable polymer, DMSO was as organic solvent and sesame oil was used as dispersion phase. HLB based system was used in present investigation for stabilizing non-aqueous emulsion prior to administration. Emulsifier mixtures of Span 80 and Tween 80 in different combination were prepared to stabilize microemulsion system with required HLB of external oil.

Result: Developed microsphere batch E06 was found to be more stable with external phase ratio 2:8 were visualized under an inverted biological microscope. In vitro drug release profile of goserelin acetate was investigated that ISMs were minimized burst effect and release up to 24 days and it was found to be stable after 1 month. The drug release was observed 95.97%, 94.02% and 97.77% after 24 days in the batches A, B and C respectively of emulsion E06.

Conclusion: ISMs of goserelin were successfully formulated and could be a potential substitute for a marketed formulation. The developed Biodegradable injectable in-situ microparticles (ISMs) were found to be optimized, formed stable, spherical and non-porous formulation.

Key words: Goserelin acetate, In-situ forming microparticles, PLGA, HLB, Burst effect.
added drop wise to oil phase II which composed of miglyol 812 and span 80 and homogenized to produce rubbery injectable dispersion of PLGA micro globules. The produced embryonic or pre-microparticles harden, shrink, were able to entrapping the drug and form true microparticles in situ within 17 min. Deadman et al. studied the effect of drug lipophilicity on its release profile from different controlled release vehicles such as, PLGA micro particles and in situ forming depots. They reported that, although there was minor effect of drug lipophilicity on the in vitro studies that effect was obvious in vivo which attributed to the interactions between the formulation and the biological tissue. Moreover, recent developments in this field such as the first Phase I trial of Risperidone-ISM™ suggest there is a possibility for prolonged release of bioactive molecules applied as intramuscular injection. In order to minimize burst release by decreased a viscosity of the system; a novel approach has been developed. The drug incorporating organic polymeric inner phase was emulsified with an oily external continuous phase containing oil soluble stabilizer. Micro droplets of an inner phase solidify after contact with physiological fluid to form solid in situ forming microparticles (ISM). An advantage of such formulation is that a lower viscosity as compare to ISI formulation, due to viscosity is assigned by the continuous phase. It has less painful and lower concentration of polymer can be used, additionally is showed reduced burst effect and lower mytoxicity as compared to ISI. Another advantages of it is the regular spherical shaped of microparticles, which is determined by the size of the previous droplets in emulsion and thereby minimize the morphological variations that provides more consistence and reproducible release pattern than ISI. Although for emulsion system, the malice use of emulsifier is main drawback could be lack knowledge of emulsion stability. In case of injection, ISM is prepared prior to administration does not make challenges itself. However, the coalescence of droplets could occur during their solidification. Burst reduction depending on the type of polymer and solvent used, increasing polymer concentration and decreasing drug loading. Higher viscosity of external oil phase, faster emulsification rate and internal: external phase ratio can also contribute to burst reduction.

MATERIALS AND METHODS

Materials

Goserelin Acetate was procured from Carbosynth Ltd, Berkshire, UK. PLGA (50:50) was obtained as a gift sample from Evonik, Mumbai, India. All other chemicals and regents used were of Research grade.

Methods

Drug characterization and Identification

Drug characterization was done by UV spectroscopy and Identification of Goserelin Acetate was carried out using FTIR study. For this the FTIR spectra of plain drug was recorded in FTIR- 8400 S Shimadzu spectrophotometer. The pure Goserelin Acetate drug was mixed with thoroughly with potassium bromide. Then the scans were obtained at a resolution of 4000- 400 cm⁻¹.

Drug polymer compatibility study

Chemical Compatibility of Goserelin Acetate with PLGA polymer was determined by FTIR study. Physical compatibility of Goserelin Acetate, polymer and physical mixture of drug and polymer were carried out using a Differential scanning calorimetry (DSC). The temperature ranges used for heating all the samples was 50- 300°C.

Solubility and miscibility of polymer and drug in solvents and water

In order to determine the solubility, Goserelin Acetate 5 mg was added in 1 ml of water, 1 ml of solvent DMSO and 1 ml of polymer solution were incubated at 37°C overnight. Solubility was determined by visual inspection. Solubility of PLGA (50:50) in solvent; 200 mg of PLGA was added in 1 ml of solvent DMSO was stirred on magnetic stirrer for 1 h and then incubated at 37°C overnight. Solubility was determined by visual inspection. Miscibility of solvent and polymeric solution in water (dissolution medium), 1 ml of solvent DMSO and 1 ml of polymer solution containing 200 mg PLGA in DMSO were added in 1 ml of water and their miscibility was observed by visual inspection.

Preparation of ISM forming Non-aqueous injectable emulsion

First was prepared drug polymeric solution by dissolving 100mg PLGA in 0.2 ml DMSO on hot plate and allow cooling down at room temperature. Then add 10mg of drug in a polymeric solution and sterilized by membrane filtration. External oil phase was prepared by calculated amount of emulsifier mixture required to achieve desire HLB value of oil were added in a 0.8ml in oil phase, both phases were filled in syringe and homogenized by hand shaking prior to administration.  

Optimization of stable non-aqueous emulsion

Development and optimization of stable non-aqueous emulsion system based on HLB values of oils. Firstly selection of external oil phase based on HLB value. For
that we selected 3 different bio-edible oils had distinct HLB value such as castor oil, corn oil and sesame oil having HLB value 14, 10 and 7 respectively, but the castor oil have been rejected due to higher viscosity. Further was selected of suitable emulsifier mixture based on their solubility in water and bio-edible oil. The Span 80 with HLB 4.3 has more soluble in oil as compared to different span derivative and the Tween 80 with HLB 14 had chosen due to higher solubility in water. Allegation method was performed to determine percentage of emulsifier mixture required to stabilize emulsion with different HLB value of oils. Table 1 showed the percentages of emulsifier were required to stabilize.

Finally concentration of emulsifier mixture required to stabilize the emulsion were calculated. We prepared emulsion containing 10%, 15%, 20% and 30% emulsifier mixture produce less phase separated formulation descript in Table 2. There were 8 test tubes in which first 4 were corn oil emulsion and next 4 were sesame oil emulsions containing 10%, 15%, 20% and 30% emulsifier respectively. Further 30 min its showed that emulsion E06 is more stable than any other emulsion and no phase separation occurred, continuous observation till 6 hr remain stable emulsion. It’s was also indicate that the phase separation is increase with respect to the emulsifier concentration. Finally the conclusion was drawn that is sesame oil formed more stable emulsions as compared to corn oil. The emulsion E06 was selected as best formulation which has been fulfill the requirement of injectable emulsion.

**Evaluation of Non- Aqueous Injectable Emulsion**

**FT-IR studies**

Chemical compatibility of Goserelin Acetate with PLGA and DMSO were analyzed by FT-IR. Any changes in the chemical position or changes in functional groups characteristics peak of drug in IR spectra after combining with excipients were investigated.

**DSC analysis**

Compatibility of Goserelin Acetate and PLGA in physical mixture was analyzed by DSC thermo gram analysis. Any changes in thermo gram peaks of drug in physical mixture after combining with polymer were investigated.

**pH**

The pH of the polymeric droplet emulsion was tested using digital pH meter.

**Syringeability and Injectability**

Injectability in general means the injection into the desirable site of administration. Syringeability is defined as the ease of withdrawal of a product through a needle from the container. Sample (1 ml) was filled into 5 ml syringe with attached 18 gauge needle. The syringe was connected to rubber tube ended with air pump.

---

**Table 1: Percentage of emulsifier mixture required for particular HLB.**

<table>
<thead>
<tr>
<th>Require HLB</th>
<th>% of Emulsifier required</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Span-80</td>
</tr>
<tr>
<td>5.4</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
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<td>10</td>
<td>45</td>
</tr>
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<td>11</td>
<td>37</td>
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<tr>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
</tr>
</tbody>
</table>

[* indicate the amount of tween 80 in 100%]

**Table 2: Optimization of concentration of emulsifier mixture.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Internal phase concentration (%/v/v)</th>
<th>External phase concentration (%/v/v)</th>
<th>Emulsifier concentration (%/v/v with respect to external phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Emulsifier mixture</td>
</tr>
<tr>
<td>Corn oil emulsions with HLB 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E01</td>
<td>20%</td>
<td>80%</td>
<td>10%</td>
</tr>
<tr>
<td>E02</td>
<td></td>
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<td>15%</td>
</tr>
<tr>
<td>E03</td>
<td></td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>E04</td>
<td></td>
<td></td>
<td>30%</td>
</tr>
<tr>
<td>Sesame oil emulsions with HLB 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E05</td>
<td>20%</td>
<td>80%</td>
<td>10%</td>
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<tr>
<td>E06</td>
<td></td>
<td></td>
<td>15%</td>
</tr>
<tr>
<td>E07</td>
<td></td>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>E08</td>
<td></td>
<td></td>
<td>30%</td>
</tr>
</tbody>
</table>
In vitro formation of microparticles

In order to investigate microparticles were formed in vitro from non-aqueous emulsion, final formulation was injected into 50 ml phosphate buffer pH 7.4 and 0.02% sodium azide, incubated for 24 hr at 37°C. Then microparticles were visualized with an inverted biological microscope until complete hardening of microparticles observed.[13]

Ex-vivo formation of microparticles

In order to confirm that the microparticles formed in the human tissues, ex-vivo study was performed. Isolated chicken leg muscles were used for this study. The formulation was mixed with methylene blue dye which locates the microparticle formation in the muscle. The formulation was injected to a depth of 0.5 ml using an 18 gauge marked needle. The muscle was immersed in 25 ml of phosphate buffer pH 7.4 and kept for 24 hr at 37°C. The confirmation of microparticles formation was observed under inverted biological microscope in the muscle after injection of formulation.[13]

In vitro drug release study

The novel polymeric emulsion was injected using syringe attached with 18 gauge needles followed by the addition of 1 ml of the release medium into pieces of dialysis tubing tied at one end. The other end of the sacs was tied with threads and the sacs were placed into a vial containing 10 ml of phosphate buffer pH 7.4 containing 0.02% w/v sodium azide. The vial was placed in a reciprocating incubator shaker maintained at 37°C. With an oscillation speed of 50 rpm. At different sampling points the release medium was removed from the vial and replace with fresh medium. The amount of drug release into the medium was determined by UV spectroscopy. The actual amount of the polymer-drug solution incorporated in the final formulation was taken as basis for the drug release and encapsulation efficiency.[14]

Stability studies

The internal polymeric phase was subjected to stability study for a period of 1 month at storage condition of 4°C. Appearance, pH, syringeability and drug content at different interval of one week during one month.[17]

RESULTS AND DISCUSSION

Drug characterization

FT-IR spectra of Goserelin Acetate was showed prominent peak at 1099.46 cm⁻¹ lying in the range characteristics for 1250-1020 cm⁻¹ for C-N bonds. It showed other peak at 1653.35 cm⁻¹ which indicate the C=O bonds. While peaks at 3417.96 cm⁻¹ and 2927.08 cm⁻¹ indicates hydroxyl and C-H stretching in Goserelin Acetate. Thus, from the obtained peak it can be concluded that the given sample of drug Goserelin Acetate. (Figure 1 (a) and Figure 1(b)). The UV spectroscopic analysis of Goserelin Acetate the Absorbance maxima wavelength was found at 278 nm which was reported value for major peptides. Hence, 278 nm was selected as a maximum wavelength. The calibration curve was taken in Phosphate buffer pH 7.4 within wide range of drug concentration about 20-50 µg/ml. The calibration curve showed a linear relationship with a regression coefficient (R²) of 0.994. The absorbance was found to lie within the range of Beer and Lambert law.

Polymer characterization by FT-IR

FT-IR spectra of distinct functional groups of PLGA polymer exhibits molecular vibration as illustrated in Figure 2(a). The peak at 2931.90 cm⁻¹ falling within the range characteristic for 3010-2885 cm⁻¹ is attributed to C-H stretching. Intense band observed at 1749.49 cm⁻¹ in the range region between 1760-1750 cm⁻¹, are attributed to the stretching vibration of the C=O groups present in the monomers. Medium intensity bands at 1094.54 cm⁻¹ between 1300-1000 cm⁻¹ were attributed to asymmetric C-C(=O)-O stretches. A narrow peak at 1392.65 cm⁻¹

Terminal sterilization

The preparation of drug containing polymeric internal phase was carried out aseptically including sterilization. Terminal sterilization of polymeric solution was finalized by filter membrane with 2µ pore size.[15]

Drug Release kinetic models

The release kinetic of injectable in situ microparticle forming system was carried out. The release data were tested for the Zero order, First order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas equation.[16]
Chauhan, et al.: Goserelin loaded in-situ forming microparticles

in the region between 1450–850cm⁻¹ is attributed to C-H bonding. It was observed that the FT-IR peaks of PLGA are nearly equal to the peaks reported, therefore it can be concluded that polymer is PLGA.

**Drug Excipient compatibility study**

FT-IR spectra of the formulation in Figure 2 (b) depict all the entire prominent peak of present drug. There was no marked shift in the major peaks of drug in the physical mixture of drug and polymer. So it was concluded that drug was stable in the physical mixture. It also indicates that the drug did not interact chemically with the polymer.

**DSC analysis of formulation**

The DSC thermo gram of both Goserelin acetate and PLGA illustrated in Figure 3 (a) and 3 (b) It was showed no thermal peak, which indicates that drug and polymer both are in amorphous form. Figure 3(c) showed two thermal peaks which does not indicates the re- crystallization of either drug or polymer. Thus it can be concluded that drug and polymer were found to be compatible with each other in physical mixture.

**Solubility and miscibility of polymer and drug in solvent and water**

Goserelin acetate knowingly freely water soluble peptide drug. From the solubility studies it predicted that the drug was soluble in solvent as well as in polymer solution to form homogenous system with good syringe ability. The PLGA (50:50) was found to be soluble in DMSO and provides clear transparent polymeric solution. DMSO is polar aprotic solvent miscible with wide ranges of organic solvent and water. PLGA is synthetic biodegradable polymer, hydrolytically unstable and insoluble in water, but when added polymeric solution in water, liquid- liquid de-mixing lead to phase separation causes precipitation of PLGA form homogenous dispersion.

**Evaluation of ISM forming Non- Aqueous injectable emulsion**

**pH, appearance and syringe ability**

The pH of injectable emulsion was found to be 7.36 which suitable for subcutaneous injection, pH was found within the range of body fluid pH. Colour of emulsion comprising of sesame oil gave yellowish dark emulsion, after homogenization it has been easily withdrawal from vials, as it has no particulate that would hinder the syringe ability.

**In vitro formation of microparticles**

Emulsion was then injected in beaker containing 50 ml phosphate buffer pH 7.4 and 0.02 % w/v sodium azide. The microparticles were formed slowly in a buffer medium after leaching of solvent from oil globules. The hardening of microparticles in slow rate observed under inverted biological microscope. Figure 4 showed the microparticles were visualized with an inverted biological microscope until complete hardening of globules.
Slow diffusion phase inversion dynamics

After injection of emulsion upon contact with buffer media (in-vitro) and body fluid (in-vivo), the polymer system undergoes slow rate diffusion and phase separation due to presence of external oil phase. The rate of solvent diffusion is much more controlled thereby preventing sudden polymer precipitation at any point. Depot can be stay more or less viscous during time of prolonged solidification, produced less porous sponge like hardening structure are more homogenous. Drugs got entrapped within the matrix as it solidifies and was released by diffusion processes or as the implant biodegrades. Drug diffusivity was decreased as well as diffusion path length increased result a more gradual release pattern of drug. Figure 5 was showed the mechanism behind formation of ISM in water or body fluid by slow rate diffusion and phase separation of polymer.  

Ex- vivo formation microparticles

It was confirmed under inverted biological microscope that developed microparticles were formed slowly in buffer medium after leaching of solvent from oil globules (Figure 6).

Ex- vivo study was carried out confirm formation of in situ microparticles in the muscles. The crystal violet dye added in the formulation differentiated the microparticles formation in the muscles. After injection of emulsion containing dye present in polymeric phase, the muscles sacs were appeared blue color which indicated formation of microparticles. Figure 7, the digital microscopy of transverse section of leg tissues after 24 hr was shows microparticles generated in tissues.

% In vitro drug release study

In vitro drug release study of emulsion E06 was carried out in three different batches A, B and C. Figure 8. Showed comparative drug release profile for batches A, B and C. The drug release was observed for all batches were shows controlled drug delivery from microparticles up to 24 h to obtained linear curve with negligible burst effect obeyed the zero order of release. The drug release was observed 95.97%, 94.02% and 97.77% after 24 days in the batches A, B and C respectively. From the % in vitro drug release profile of first 2 days showed in figure that no burst release of drug seen on different batches of formulations because of in ISM system the polymer phase was first emulsified with oil phase prior to administration, which formed a barrier between aqueous medium and polymer solution. The low solubility of drug in oil phase reason the drug to stay in the polymer phase as it were encapsulate within the precipitate polymer. The polymer hardening was depending upon the concentration of surrounded external phase. Globules were close to the surface of emulsion were rapidly solidify as compared to globules were present in depth in emulsion. Therefore, liquid-liquid demixing delayed and slow phase inversion in order to several days to weeks, thus result formed homogenous particle surface leads to control the drug release up to long time. Therefore, in situ forming
microparticles have less porous particle surface being another opportunity for the negligibly burst release of drug.

**Drug Release kinetic models**

From the results of mathematical model in Table 3 it appears that the formulation follows zero order models as it shows the highest $R^2$ value. The mechanism of diffusion was found to be non-fickian from the n value as shown in the table. Figure 9. Drug release kinetics, was indicates the integrity of the drug release from the in situ formulations when evaluated by different mathematical models.

### Stability Studies

The stability study was carried out at 4°C as PLGA is known to degrade fast to high temperature. The Table 4 showed different parameters were analyzed at different time interval of one week during one month. There was no difference showed in appearance of polymeric solution was able to pass through 18-gauge needle so there was no difference found with syringe ability. Drug content was found to stable during 1 month of stability study. The data from the Table 4 were analyzed by one way ANOVA. The $P$- value was found to be more than 0.05 for parameters shown Table 5. So it can be said that there was no significant changes in drug content and pH of polymer solution during 1 month of stability study. Hereby it can be said that polymeric solution of formulation was found to be stable at 4°C.

### CONCLUSION

This research study we concluded that formulation of in situ forming microparticles by adjusted the HLB value with selective emulsifier mixture of Span 80 and Tween 80 increased the physical stability of the non-aqueous emulsion. Microparticles were observed under inverted biological microscope until complete precipitation of polymer within the micro-globules, slow rate of diffusion and leaching of DMSO from globules formed spherical, non-porous and dense polymer matrix.

### ACKNOWLEDGEMENT

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---

**Table 3: $R^2$ value for drug release kinetic model.**

<table>
<thead>
<tr>
<th>Kinetic model</th>
<th>Formulation code</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>$R^2$</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>First order</td>
<td>$R^2$</td>
<td>0.847</td>
<td>0.870</td>
<td>0.786</td>
</tr>
<tr>
<td>Higuchi model</td>
<td>$R^2$</td>
<td>0.921</td>
<td>0.919</td>
<td>0.918</td>
</tr>
<tr>
<td>Hixson-Crowell model</td>
<td>$R^2$</td>
<td>0.939</td>
<td>0.946</td>
<td>0.915</td>
</tr>
<tr>
<td>Korsmeyer-Peppas model</td>
<td>$R^2$</td>
<td>0.897</td>
<td>0.890</td>
<td>0.911</td>
</tr>
</tbody>
</table>

|   |   | 0.772 | 0.793 | 0.796 |

---

**Table 4: Stability study data of 1 month of optimized formulation.**

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Drug content (%)</th>
<th>pH</th>
<th>Appearance</th>
<th>Syringe ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98.34</td>
<td>6.87</td>
<td>Clear solution</td>
<td>Pass</td>
</tr>
<tr>
<td>7</td>
<td>98.12</td>
<td>6.81</td>
<td>Clear solution</td>
<td>Pass</td>
</tr>
<tr>
<td>14</td>
<td>98.10</td>
<td>6.78</td>
<td>Clear solution</td>
<td>Pass</td>
</tr>
<tr>
<td>21</td>
<td>97.98</td>
<td>6.79</td>
<td>Clear solution</td>
<td>Pass</td>
</tr>
<tr>
<td>28</td>
<td>97.91</td>
<td>6.82</td>
<td>Clear solution</td>
<td>Pass</td>
</tr>
</tbody>
</table>

**Table 5: Comparison of drug content, pH by ANOVA.**

<table>
<thead>
<tr>
<th>Time in days</th>
<th>Drug content (%)</th>
<th>pH</th>
<th>Appearance</th>
<th>Syringe ability</th>
<th>$P$- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98.34</td>
<td>6.87</td>
<td>Clear solution</td>
<td>Pass</td>
<td>1.4607</td>
</tr>
<tr>
<td>7</td>
<td>98.12</td>
<td>6.81</td>
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<tr>
<td>14</td>
<td>98.10</td>
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<tr>
<td>28</td>
<td>97.91</td>
<td>6.82</td>
<td>Clear solution</td>
<td>Pass</td>
<td></td>
</tr>
</tbody>
</table>
support to carry out this research work. I sincerely express my deep gratitude to Evonik, Mumbai for providing PLGA as a gift sample.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ISM: In situ Microparticle; PLGA: Poly Lactic-Co-Glycolic Acid; DMSO: Dimethyl Sulfoxide; HLB: Hydrophile Lipophile Balance.

REFERENCES


PICTORIAL ABSTRACT

SUMMARY

- The current study has been made to develop biodegradable injectable in-situ microparticles (ISMs) of goserelin acetate for the treatment of prostate cancer. The research work was carried out in which PLGA 50:50 as biodegradable polymer, DMSO as organic solvent and sesame oil as dispersion phase. The developed formulation was evaluated for Preformulation study including FTIR, DSC and in vitro drug release study, ex vivo study on isolated chicken leg muscles, stability study.
- It was observed and concluded from in vitro and ex vivo study that developed biodegradable Injectable in-situ microparticles (ISMs) of goserelin acetate was providing new dimension to the treatment of prostate cancer. It was found and confirmed that developed and optimized batch was successfully stable as per mentioned stability study.

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ABOUT AUTHORS

Dr. Bhavesh Akbari, Currently working as Professor and Head, Department of Pharmaceutics, Shree Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India. He has 12 years of Teaching and Research experience. He has published more than 35 research and Review papers in reputed peer review National and International journals. He has filed 03 Indian Patents at IPO, Mumbai. He has published 01 International Book as a main Author. He has guided 33 PG and 20 UG students for their research Dissertation. He is reviewer in various National and International peer review journals. He has received more than 7 lakhs research grant from GUJCOST(DST),Gujarat. He has attended 22 National and 06 International Conferences/Seminars. He has attended 03 Faculty Development Program sponsored by AICTE/PCI. He has presented more than 25 research paper at Various Conferences/Seminars. He is life member of APTI, IPA and GSPC.

Mr. Rohan Barse, Currently working as Assistant Professor and Head, Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India. He has 6 years of Teaching & Research experience. He has published 5 research articles in reputed peer review National & International journals. He has filed 03 Indian Patents at IPO, Mumbai. He has published 01 book as a main Author. He has guided 3 PG students for their research Dissertation. He has attended 06 National & International Conferences/Seminars. He has attended 01 Faculty Development Program sponsored by PCI. He has presented more than 25 research paper at Various Conferences/Seminars.

Mrs. Velenti Chauhan, Currently working as an Assistant Professor of Department of Pharmaceutics, Shree Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India. She has 1.5 years of Teaching and Research experience. She has Co-guided 1 PG student for their research Dissertation. She has attended 02 National and 02 International Conferences/Seminars. She has presented 02 research paper at Various Conferences/Seminars.

Mr. Vimal Patel, has completed M. Pharm in Pharmaceutics from Shree Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India. He has attended various National and International Conferences Seminars.