Chapter-10

Research and Discussion
Identification of Pure Drugs

Determination of IR spectrum of Repaglinide

The FTIR studies showed that the significant peaks of Repaglinide where C-N stretching at 1444.02 cm\(^{-1}\), C=O cm\(^{-1}\) vibration at 1682.16 cm\(^{-1}\), C-O-C at 1085.35 cm\(^{-1}\), N-H cm\(^{-1}\) at 3301.77 cm\(^{-1}\), C=C group vibration at 1630.68 cm\(^{-1}\) and O-H vibration at 2799.45 cm\(^{-1}\) (fig 56). The functional groups were similar that of standard pure Repaglinide. Based on that FTIR spectrum of Repaglinide functional groups peak was coincided with standard Repaglinide pure drug. Based on this result the drug was confirmed as in its pure form without by-products.

Determination of IR spectrum of Insulin

The FTIR studies showed that the significant peaks of Insulin are C-N stretching at 1385.30 cm\(^{-1}\), C-H [CH\(_3\)] at 2926.56 cm\(^{-1}\), C=O cm\(^{-1}\) vibration at 1655.91 cm\(^{-1}\) and C-H [CH\(_2\)] bending at 1454.30 cm\(^{-1}\) and C-O-C at 1109.46 cm\(^{-1}\), N-H cm\(^{-1}\) at 3404.48 cm\(^{-1}\) (fig 49). Based on that FTIR spectrum of insulin functional groups peak was coincided with standard insulin pure drug. Based on this result the drug was confirmed as in its pure form without by-products.

Solubility studies

Solubility of Repaglinide was performed in various solvents like water, 0.1 N HCL, methanol, ethanol, Dichloro methane and phosphate buffer (pH 7.4). From the above solvent Repaglinide was freely soluble in Dichloro methane and phosphate buffer, whereas remaining solvents shows insoluble particle sediment in the bottom of test tube.

In the case of Insulin, it was polypeptide whereas degraded in acetic solutions like 0.1 N HCL, insoluble in water, methanol, and ethanol. Freely soluble in Tris buffer.
and phosphate buffer. From the above study the solubility of Repaglinide and insulin were found to be freely soluble in dichloromethane, Tris buffer respectively. The solubility study was found to be helpful in the development of analytical methods of drug and the selection of diffusion medium.

**Determination of \( \lambda_{\text{max}} \) of Repaglinide:**

On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. By scanning the drug in U.V spectrophotometer in 200-400 nm range, a sharp peak was observed at 275nm using distilled water as solvent. It was concluded that the drug has \( \lambda_{\text{max}} \) of 275nm.

**Determination of \( \lambda_{\text{max}} \) of Insulin:**

On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. By scanning the drug in U.V spectrophotometer in 200-400 nm range, a sharp peak was observed at 216nm using distilled water as solvent. It was concluded that the drug has \( \lambda_{\text{max}} \) of 216nm.

**The linear regression analysis:**

The linear regression was done on absorbance data points (table 10&11). The results are presented as follows.

**For standard curve pure Repaglinide:**

The slope \( = 0.014 \)

The intercept \( = 0 \)

The correlation coefficient \( = 0.996 \)

A straight-line equation \( (Y = mx + c) \) was generated to facilitate the calculation of amount of drug (fig 20). The equation is as follows:

\[
\text{Absorbance} = 0.014x \text{ Concentration} + 0
\]
For standard curve pure Insulin:

The slope = 0.050
The intercept = 0
The correlation coefficient = 0.993

A straight-line equation \( Y = mx + c \) was generated to facilitate the calculation of amount of drug (fig 21).

The equation is as follows:

\[
\text{Absorbance} = 0.050x \text{ Concentration} + 0
\]

Preparation of Repaglinide Solid Lipid Nanoparticles

SLN of Repaglinide was successfully produced by a double emulsification method based on w/o/w double emulsion. For the preparation of SLN, biodegradable lipids like lecithin, cephalin, and dynasan were employed (table 12). They are considered to be stable carriers for transdermal delivery. Poloxamer 407 was used as surfactant in aqueous phase while preparing the Repaglinide loaded SLN. In the case of RS1, RS2 formulation, lecithin in the concentration of 1%, 2% were used for the preparation of Repaglinide SLN. During the high speed homogenization of drug and polymer solution, white cloudy primary emulsion was formed. The formed primary emulsion was stabilized by poloxamer 407 as co-stabilizer to form stable multiple w/o/w emulsion.

In the case of RS3, RS4 formulations cephalin in the concentration of 1%, 2% were used. The primary emulsion was formed by homogenizing the drug and polymer solution. For the formation of w/o/w emulsion, additionally 30 minutes of homogenization is required.
In the case of RS5, RS6 formulations, dynasan is used as an oil phase. The primary emulsion was formed very quickly, but it was unstable. Later homogenization with co-stabilizer helped to form stable multiple emulsion.

Solid lipid nanoparticles of Repaglinide prepared by double emulsification method produced superior physical stability compared with liposomes. In the liposome and emulsion, the drug can diffuse and partition between aqueous and oily phase. These solid lipid shells minimize the partitioning of drug and prevent the drug leakage and degradation. Poloxmer 407 shows, increase surface area and adhesive properties of the skin and skin hydration, which leads to reduce the corneocyte packing and increases skin penetration properties.

**Morphological characters**

The morphology and surface characters of Repaglinide SLN were observed by SEM. The scanning election micrographs of SLN lecithin, cephalin, and dynasan were shown in figures 83-87 respectively, which revealed the formation of spherical shape with irregular surface. However, lecithin SLN (RS1, RS2) showed large population of small particles. Cephalin SLN (RS3, RS4) had relatively smaller with rough and irregular surface. On the other hand, RS5, RS6 SLN was somewhat spherical although they had imperfections on their surface. It is worth mentioning that during SEM samples preparation, both the water of bulk phase and particle were completely removed. In general, such drying apparently may lead to shrinking of surface of the particles. The morphology of Repaglinide SLN prepared by Lecithin showed, spherical shape with smooth surface compared with Cephalin, Dynasan. In aqueous solution, Lecithin phospholipids can forms lamellar structure, depending on hydration and temperature. The Lecithin had more emulsification and lubricant properties; it can totally metabolize by humans and excreted by kidney.
Particle size and polydispersibility index:

The particle size of prepared Repaglinide SLN was analyzed by Malvern particle size analyzer. All the formulations size range between 110± 0.8 to 180.6 ± 2.2nm. These sizes of SLN were only used to improve the transdermal delivery of Repaglinide. The effect of lipid type and concentration on the particle size of SLN [RS1-RS6] was investigated. In the table 13, fig 22 shows small significant increase in particle size as the concentration of lipid increased from 1% to 2%. The minor increase in particle size with increasing lipid concentration can be explained by the decrease in homogenization efficiency with increasing content of dispersed phase i.e., lipid phase. In the case of RS1, RS2, the observed particle size was of 116.2nm, 124.0nm respectively. In the case of RS3, RS4 the cephalin SLN particle size was 108.8nm and 110.6nm. But in case of RS5, RS6, the results showed huge variation of 110nm, 180.6nm respectively. Similarly (Souto, 2004) has reported that the particle size of SLN increased with increase in lipid concentration from 9.5 to 18%.

The Polydispersity index value of Repaglinide SLN formulation was 0.06 to 0.70 [table 13]. In case high value of RS3, RS4, and RS6 could be due to high viscosity of SLN dispersion, which could affect the homogenization efficiency and resulted in a monodispersed particle size distribution. The small particles of Repaglinide SLN formed by Cephalin containing preparations. The particle size of nanoparticles was the main factor for permeation through skin. Particles size of 20-200nm were easily transported via stratum corneum by passive diffusion (Vogt, 2006), whereas particles size of Repaglinide SLN formed were in the range of 108.8 nm to 124nm, which significantly increases the penetration through follicular and intracellular penetration into the inner layer of skin. PDI of SLN was showed
monodispersion, which influences the penetration and decrease accumulation of nanoparticle in the corneocyte or intercorneocyte space.

**Drug entrapment efficiency.**

The entrapment efficiency of Repaglinide SLN was determined by calculating the unentrapped drug present in centrifugation process. The entrapment efficiency of RS1-RS6 was in the range of 80.25 to 91.7%. The highest entrapment efficiency was observed in RS2 SLN. This could be explained on the basis of percentage lipid in the formula (table 13, fig 23) Concentration of lecithin was more in RS2, so entrapment was higher. Therefore, the structure of lecithin in the SLN was less ordered by arrangement compared with other lipids and this could be the reason for the highest drug entrapment in SLN. The entrapment efficiency is not affected based on type of lipid used (Trotta, 2005).

**Zeta potential:**

Zeta potential is a key factor to evaluate the stability of colloidal dispersion (Komatsu, 1995). In general, particle could be dispersed stabilized when value of zeta potential was in the range of -30 to + 30 mV which are due to electrical repulsion between particles (Muller, 2000). As shown in table 13, the average zeta potential obtained for formulations RS1 to RS6 SLN was about -14.50 ± 1.4mV to -30.84 ± 0.02Mv. It was concluded that the Repaglinide SLN obtained in this study was a dynamic stable system. Surface charge of nanoparticles influences their skin penetration. Kohli reported that only the negative charged particles were able to penetrate the SC to reach the inner epidermis. Whereas Repaglinide SLN shows -14 to – 30 mV with small particle size, which abundantly influence the penetration of nanoparticles through stratum corneum.

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FTIR spectra:
The FTIR studies showed that the significant peaks of Repaglinide are C-N stretching at 1444.02 cm$^{-1}$, C=O cm$^{-1}$ vibration at 1682.16 cm$^{-1}$, C-O-C at 1085.35 cm$^{-1}$, N-H cm$^{-1}$ at 3301.77 cm$^{-1}$, C=C group vibration at 1630.68 cm$^{-1}$ and O-H vibration at 2799.45 cm$^{-1}$ and mixture of Repaglinide and lecithin showed some significant peaks are N-H cm$^{-1}$ stretching at 3290.84 cm$^{-1}$, C=O stretching at 1732.47 cm$^{-1}$, C-O-C peak at 1047.76 cm$^{-1}$ and C-N stretching at 1376.13 cm$^{-1}$ and C-H [CH$_3$] at 2853.99 cm$^{-1}$, C-H [CH$_2$] bending at 1460.31 cm$^{-1}$ and O=P-[OR]$_3$ vibration showed at 1208.82 cm$^{-1}$. In the case of mixture of Repaglinide and cephalin showed five significant functional group peaks are N-H cm$^{-1}$ stretching at 2921 cm$^{-1}$, C=O stretching at 1679.45 cm$^{-1}$, C-N cm$^{-1}$ vibration at 1462.61 cm$^{-1}$ and CH$_3$ vibration at 1736.37 cm$^{-1}$ and O=P-[OR]$_3$ vibration showed at 1171 cm$^{-1}$. In the case of mixture of Repaglinide and dynasan showed some significant functional group peaks are N-H cm$^{-1}$ stretching at 3301.49 cm$^{-1}$, C=O stretching at 1631.03 cm$^{-1}$, C-N cm$^{-1}$ vibration at 2913.04 cm$^{-1}$ and C-O-C peak at 1087.78 cm$^{-1}$, C-H [CH$_3$] at 2848.29 cm$^{-1}$, C-H [CH$_2$] bending at 1469.79 cm$^{-1}$ Finally the FTIR studies of mixture of polymers and drug does not show any significant change. These results indicate that there is no interaction between drug and selected polymers. (table 14) (fig 56, 60-63 & 66-68).
The compatibility studies of polymer and drug reveals the entrapment efficiency of drug in the lipid matrix. If incompatibility occurs between drug and polymer, lead to cross liking in lipid matrix, which reduces the drug entrapment.

DSC spectra

The DSC spectrum of pure Repaglinide showed a sharp endothermic peak at 65.68°C and it was the melting point of drug. In the mixture of Repaglinide and
polymers shows, doesn’t changed the thermal behavior of sharp endothermic peak of drug at 65.68°C. the shoulder peaks were showed by polymers like Lecithin, Cephalin, Dynasan at 132.97°C, 23.6°C, 193.46°C respectively (fig 69-72). It indicated that there was no interaction between the drug and polymers.

**In-vitro release of drug from nanoparticles**

The *in vitro* release of Repaglinide from different biodegradable lipid nanoparticle is showing table 15, fig 24. The quantity of drug release in all the formulations (RS1-RS6) of polymeric nanoparticle was very low- in the range of 8.20 ± 1.2 % to 11.6 ± 0.6 % at initial period (2h). From this, it is obvious that the decreased percentage of drug release was due to the more compact wall around the drug by the biodegradable lipid and it signifies that they possess a sustained drug release for a prolonged period of time in all the formulations. At the end of 24 h, limited percentage of drug was released and was found to be 60.6 ± 1.2 to 89.1 ± 1.0 %. In the case of Repaglinide SLN prepared by Lecithin, shows low initial release (8.2%) at 2 h and the release rate was gradually maintain throughout period, at end of 24h only 60.6% of drug was released from SLN. The long chain ester of Lecithin, a strong effect of lipophilicity on their association with solid lipid matrix and thus on their release in the external medium was expected. Only the long chain fatty acid ester can release drug from SLN was more than 48 h. whereas shorter chain homologues still remaining partially associated with the lipid matrix, thereby initial release was more.

**Invitro release kinetic studies**

As shown in table 16 the *in vitro* release data was fitted into various kinetic equations i.e., zero order, first order, higuchi, korsemeyer. The release constant was calculated from the slope of appropriate plot and the regression [R²] was calculated.
In the Insulin polymeric nanoparticle preparations of all formulations [IP1-IP6], the *invitro* release kinetic was best fitted by zero order equation and the plots showed the high linearity $[R^2 = 0.979 \text{ to } 0.988]$ followed by first order $[R^2 = 0.816 \text{ to } 0.914]$ and higuchi equation $[R^2 = 0.907 \text{ to } 0.918]$. Hence the drug release kinetics demonstrated that the concentration was nearly independent of drug release. Korsemeyer- peppas equation has showed good linearity $[R^2 = 0.990]$. The release exponent $n= 0.70$, which appears to be showing anomalous diffusion. The release kinetics was often used employed for comparative purpose and relating the release parameters with important in bioavailability and used to study influences of formulations factors on the drug release for optimization as well as control of drug release from nanoparticles. The *invitro* release data was subjected to zero, first order, higuchi and korsemeyar peppas to establish the drug release mechanism and kinetics of drug release from SLN. When data was subjected to zero order and first order kinetic model, a linear relationship was observed with high $r^2$ values for zero order as compared to first order model; it suggested that the Repaglinide SLN were zero order controlled release of drug from lipid matrix. Higuchis model $r^2$ values suggested that the drug release from SLN followed diffusion mechanism as all the lipid were melting based matrix type. The exact release mechanism was analyzed by korsemeyar peppas model. The values of ‘n’ obtained for all the SLN formulations was $\geq 0.5 \text{ to } \leq 1.0$ suggested that the drug release followed non-fickian anomalous diffusion mechanism due to lipid matrix have less affinity with aqueous medium.

**Preparation of Insulin Solid Lipid Nanoparticles**
Insulin SLN were successfully prepared by a double emulsification-solvent evaporation method based on w/o/w double emulsion type. They are favored carrier for Insulin delivery via transdermal with diminished enzymatic degradation. The Insulin is a high proteneous substance which is more sensitive for heat, light, pH changes, enzymatic action. For this reasons, Insulin was coated with biodegradable natural lipids like lecithin, cephalin and dynasan. Poloxamer 407 was used as quasisurfactant in aqueous phase, while preparing the Insulin loaded SLN and to increase their stability (table 17). Lipid nanoparticles of Insulin may suffer aggregation during incubation in acidic medium, where as protective coating of Poloxamer 407 completely diminished these phenomena (Garcia-Fuentes, 2003).

In the case of IS3, IS4 lecithin is used in the concentration of [1%,2%] and it dissolved in dichloromethane and the Insulin was dissolved in 10 mM Tris buffer. Both the liquid mixtures are stirred at high homogenization speed i.e., 2000 rpm, but during the high agitation, Insulin may become unstable. To reveal the thermodynamic effect, homogenization was done in ice bath. The primary emulsion was thick viscous medium. It was then poured in to a Poloxamer 407 solution. After a short period of ultrasonication, a stable globule of Insulin nanoparticles of w/o/w type emulsion was produced.

Lecithin was used in the case of IS1, IS2 formulations. After the formation of primary emulsion, the mixture of oily phase has been separated out. To avoid this effect, the homogenization speed was increased to 30000 rpm. After the formation of o/w type emulsion, it was further stabilized by double emulsification with Poloxamer 407 as a co-stabilizer. The Poloxamer 407 has reduced the interfacial tension between the liquid phases and helped to form w/o/w type stable emulsion of Insulin SLN. However in the case of IS5, IS6 dynasan was used for the preparation of Insulin SLN,
could produced quick primary emulsion. In later the globules were aggregated with each other. To prevent this aggregation, Poloxamer 407 was used to stabilize Insulin solid lipid nanoparticles. Solid lipid nanoparticles of Insulin prepared by double emulsification method produced superior physical stability compared with liposomes and emulsions. In the liposome and emulsion, the drug can diffuse and partition between aqueous and oily phase. These solid lipid shells minimize the partitioning of drug and prevent the drug leakage of Insulin from matrix and degradation. Poloxamer 407 shows, increase surface area and adhesive properties of the skin and skin hydration, which leads to reduce the corneocyte packing and increases skin penetration properties.

**Morphology**

Morphological study of Insulin SLN was observed under SEM [scanning electron microscopy]. The lyophilized Insulin SLN was uniformly coated with gold. In the case of IS1, IS2 SEM images showed in fig 90-94 which revealed the formation of spherical shape with irregular surface. However, IS4 showed larger population of small particles with spherical shape and a smooth surface. On the other hand IS5, IS6 had relatively smaller particle size with rough and irregular surface, due to improper solvent evaporation. The morphology of Insulin SLN prepared by Lecithin showed, spherical shape with smooth surface compared with Cephalin, Dynasan. In aqueous solution, Lecithin phospholipids can forms lamellar structure, depending on hydration and temperature. The Lecithin had more emulsification and lubricant properties; it can totally metabolize by humans and excreted by kidney.

**Particle size and Poly dispersity index**

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The lipid concentration and homogenization speed were the main factors in influencing the formation of uniform size distribution. The table 18 showed particle size has increased significantly with increase in concentration of lipid. The formulation of Insulin SLN showed the size range between 106.5±0.6 nm to 187.6±3.1 nm. In the case of IS3, IS4 SLN particle size of 106.5 nm & 119.0 nm, where there was no significance increase in particle size as the lipid concentration increased from 1%, 2% [table 18]. Where as, a little significance of particle size variation was seen in IS5, IS6 that is 144.6 nm, 186.2 nm respectively (fig 25).

Poly dispersity index values of Insulin SLN formulation were ≤ 0.3 [table 18] except IS5 containing dynasan SLN, this could be due to high viscosity of dynasan phase. All the formulation showed particle size distribution as monodispersed in nature. The small particles of Insulin SLN formed by Cephalin containing preparations. The particle size of nanoparticles was the main factor for permeation through skin. Particles size of 20-200 nm were easily transported via stratum corneum by passive diffusion (Vogt, 2006), whereas particles size of Insulin SLN formed were in the range of 106.5 nm to 187.6 nm, which significantly increases the penetration through follicular and intracellular penetration into the inner layer of skin. PDI of SLN was showed monodispersion, which influences the penetration and decrease accumulation of nanoparticle in the corneocyte or intercorneocyte space.

Zeta potential

The mean values of zeta potential of Insulin SLN were -12.4±7.6 mV to -24.1±3.1 mV. The mean diameter confirmed that the SLN produced submicron colloidal carriers which were suitable for enabling transdermal absorption. The zeta potential value was nearly close to the electrical neutrality, but it was possible to notice a slightly more negative value of Insulin loaded SLN, probably due to the
deposition of Insulin on the surface of the SLN. In fact, after production of SLN, the pH of Insulin SLN was found to be very less and it was lower than the isoelectric point of Insulin [IEP 5.3] which was responsible for the positive charge of Insulin. Surface charge of nanoparticles influences their skin penetration. Kohli reported that only the negative charged particles were able to penetrate the SC to reach the inner epidermis. Whereas Insulin SLN shows -12 to – 24 mV with small particle size, which abundantly influence the penetration of nanoparticles through stratum corneum.

**FTIR spectra:**

The FTIR studies showed that the significant peaks of Insulin are C-N stretching at 1385.30 cm\(^{-1}\), C-H [CH\(_3\)] at 2926.56 cm\(^{-1}\), C=O cm\(^{-1}\) vibration at 1655.91 cm\(^{-1}\) and C-H [CH\(_2\)] bending at 1454.30 cm\(^{-1}\) and C-O-C at 1109.46 cm\(^{-1}\), N-H cm\(^{-1}\) at 3404.48 cm\(^{-1}\) and mixture of Insulin and lecithin showed some significant peaks are N-H cm\(^{-1}\) stretching at 3309.47 cm\(^{-1}\) C=O stretching at 1644.42 cm\(^{-1}\), C-O-C peak at 1049.51 cm\(^{-1}\) and C-H [CH\(_3\)] at 2853.07 cm\(^{-1}\), C-H [CH\(_2\)] bending at 1463.21 cm\(^{-1}\) and O=P-[OR]\(_3\) vibration showed at 1209.03 cm\(^{-1}\) O-H peak at 2922.82 cm\(^{-1}\) and C-S peak at 718.61 cm\(^{-1}\). In the case of mixture of Repaglinide and cephalin showed five significant functional group peaks are N-H cm\(^{-1}\) stretching at 3646.07 cm\(^{-1}\) C=O stretching at 1735.84 cm\(^{-1}\) C-S cm\(^{-1}\) vibration at 720.58 cm\(^{-1}\) and CH\(_3\) vibration at 2851.04 cm\(^{-1}\) and O=P-[OR]\(_3\) vibration showed at 1248.27 cm\(^{-1}\) C-O-C stretching at 1010.35 cm\(^{-1}\). In the case of mixture of Repaglinide and dynasan showed some significant functional group peaks are N-H cm\(^{-1}\) stretching at 3666.14 cm\(^{-1}\) C=O stretching at 1731.41 cm\(^{-1}\), C-S cm\(^{-1}\) vibration at 714.58 cm\(^{-1}\) and C-O-C peak at 1056.73 cm\(^{-1}\) C-H [CH\(_3\)] at 2847.52 cm\(^{-1}\), C-H [CH\(_2\)] bending at 1414.61 cm\(^{-1}\) O-H peak at 2955 cm\(^{-1}\). Finally the FTIR studies of mixture of polymers and drug
does not show any significant change. This results in indicating that there is no interaction between drug and selected polymers. *(table 19) (fig 49, 53-56 & 66-68).*

**DSC spectra**

The DSC spectrum of pure Insulin showed a broad endothermic peak at 106.68°C and it was the melting point of drug. In the mixture of Insulin and polymers shows, doesn’t changed the thermal behavior of broad endothermic peak of drug at 106°C. the shoulder peaks were showed by polymers like Lecithin, Cephalin, Dynasan at 266.56°C, 274.2°C, 290.1°C respectively *(fig 76-79).* It indicated that there was no interaction between the drug and polymers.

**Entrapment efficiency**

The entrapment efficiency of Insulin SLN was calculated by unencapsuled Insulin in SLN. The entrapment efficiency of all formulation [IS1-IS6] was in the range of 80.6± 2.4 % to 90.3 ± 2.1% *(table 18) (fig 26).* These results indicated that about 10-20% of lipid is present in dispersed medium and it was solubilised by surfactant molecules. It is noteworthy that may be a hydrophilic molecules having low entrapment efficiency of Insulin within the lipid matrix of SLN was expected. Nonetheless, the w/o/w double emulsification method was shown to be a suitable production procedure to achieve relatively high entrapment of Insulin.

**In-vitro drug release**

The *in-vitro* release behaviors of Insulin solid lipid nanoparticles with different lipids concentration have been performed in pH 7.4 phosphate buffer. In all the Insulin SLN formulations, there was slow initial release at 2 h and followed by sustained release at a constant rate. The initial release may be due to the loosely bounded surface present on the drug could be removed in the initial sink condition.
The observed amount might vary with the aggregation and disaggregation status of the particles. In the case of IS1, IS2 containing cephalin showed 65.4%, 61.3% of drug release at 24 h and followed by IS3, IS4 containing lecithin having low drug release pattern of 74.2%, 60% respectively at 24 h, more amount of drug release was observed in the formulation IS5, IS6 with a release of 76.1%, 81.6% respectively (table 20, fig 27). In the case of Insulin SLN prepared by Lecithin, shows low initial release (8.3%) at 2 h and the release rate was gradually maintain throughout period, at end of 24h only 60% of drug was released from SLN. The long chain ester of Lecithin, a strong effect of lipophilicity on their association with solid lipid matrix and thus on their release in the external medium was expected. Only the long chain fatty acid ester can release drug from SLN was more than 48 h. whereas shorter chain homologues still remaining partially associated with the lipid matrix, thereby initial release was more.

**Release kinetic studies**

As shown in table 21 the invitro release data was fitted in various kinetic equation i.e., zero order, first order, higuchi, korsemeyer. The release constant was calculated from the slope of appropriate plot and the regression coefficient \( R^2 \) was calculated. In the Insulin polymeric nanoparticle preparations of all the formulations [IP1-IP6], the *in vitro* release kinetic was best fitted by zero order equation, as the plots showed the high linearity \( R^2 = 0.966 \text{ to } 0.984 \) followed by first order \( R^2 = 0.810 \text{ to } 0.991 \) and higuchi equation \( R^2 = 0.966 \text{ to } 0.984 \). Hence the drug release kinetics demonstrates that the concentration was nearly independent of drug release. To explain the mechanism of drug release, korsemeyer- peppas equation has showed good linearity \( R^2 = 0.843 \text{ to } 0.961 \). The release exponent n= 0.5 to 0.62, which
appears to be anomalous diffusion mechanism. The release kinetics was often used employed for comparative purpose and relating the release parameters with important in bioavailability and used to study influences of formulations factors on the drug release for optimization as well as control of drug release from nanoparticles. The invitro release data was subjected to zero, first order, higuchi and korsemeyar peppas to establish the drug release mechanism and kinetics of drug release from SLN. When data was subjected to zero order and first order kinetic model, a linear relationship was observed with high $r^2$ values for zero order as compared to first order model; it suggested that the Insulin SLN were zero order controlled release of drug from lipid matrix. Higuchis model $r^2$ values suggested that the drug release from SLN followed diffusion mechanism as all the lipid were melting based matrix type. The exact release mechanism was analyzed by korsemeyar peppas model. The values of ‘n’ obtained for all the SLN formulations was ≥ 0.5 to ≤ 1.0 suggested that the drug release followed non-fickian anomalous diffusion mechanism due to lipid matrix have less affinity with aqueous medium.

**Preparation of Repaglinide Polymeric Nanoparticles**

Polymeric nanoparticles of Repaglinide were prepared by solvent evaporation method with three different biodegradable polymers (table 22). This method is comparatively easy to prepare than other methods. Suspension of polymer and drug in dichloromethane as organic phase. This organic solution was poured into an aqueous phase containing Poloxamer 407. The organic solvent used in these preparation rapidly partitioned into the external aqueous phase and the polymer precipitated around the drug particle. The subsequent evaporation of the entrapment solvent led to the formation of polymeric nanoparticles (Sunil, 2005).
In the case of RP1, RP2, poly (ε-caprolactone) was used as a polymer at different concentration. Poly (dl-lactic acid) was used in the formulation of RP3, RP4. On other hand RP5, RP6 formulation contain 1%, 2% of chitosan respectively. The polymeric nanoparticles prepared by solvent extraction method helps to vanished all organic residues in the formulations to avoid skin irritations. At the 20000 homogenized pressures, particles size was small compared with low homogenization pressure. When increasing pressure, particle size got reduced, which aggregated to form large size polymeric micelle. The quasi-emulsifier (Poloxamer 407) shows stabilized polymeric Repaglinide micelle formation and maintain uniformity of size and reduced aggregation properties of polymeric nanoparticles. The organic solvent was quickly removed by Rota evaporator at reduced pressure.

**Morphological examination**

The PCL –Repaglinide nanoparticles had spherical shape with rough surface, as the polymer did not completely dissolved in organic solvent and secondly due to faster evaporation of solvent. In the case of RP3, RP4 formulations were prepared with PLA polymer. It showed smooth surface of spherical shaped nanoparticles (fig 95-100). The surface morphology of formulated nanoparticles depends on (1) a saturated solution of polymer produced irregular and rod shaped nanoparticles: 2) the diffusion rate of solvent is varying fast and solvent may diffuse into the aqueous phase before stabilization of nanoparticles and caused to aggregation of nanoparticles. In this formulation, the polymer was fully saturated and the diffusion rate of solvent was minimal, leading to formation of smooth, spherical and homogeneously distributed particle, which have a smooth surface and complete removal of solvent from the formulated nanoparticle with good quality.
In the formulation RP5, RP6 chitosan was used as the polymer and it is dissolved in 0.25N acetic acid and drug was dissolved in organic solvent. The rate of evaporation was very slow and phase separation occurred due to the aqueous phase, which is more than that of organic phase that lead to phase separation. The separation was suppressed by increasing the homogenization time and that leads to precipitation of polymeric nanoparticles.

The PCL were produced smooth spherical shaped appearance due to saturated solution of polymer, produce smooth and high yield nanoparticles. The undissolved polymer produces irregular and projected shaped particles. The diffusion rate of solvent was too fast and the solvent may diffuse into aqueous phase before stable nanoparticle was developed. Smooth surface reveals complete removal of solvents from formulated nanoparticle and indication of good quality.

**Particle size and poly dispersity index**

The nanoparticles size was very important factor for drug permeation through the skin. The explanation for this difference in the polymers concentration has been given in the literature and can be employed for the charged co-polymer nanoparticles as well. Particle size is often used to characterize the nanoparticles facilitation via skin and understanding of aggregation (Duane, 2000). In the case of large surface area, the attractive force between the particles and chance for possible aggregation in smaller sized particles. To overcome such aggregation, addition of a surfactant in the preparation was necessary. Poloxamer 407 appeared to be the most suitable surfactant for reducing aggregation between nanoparticles, as it suspends quickly after formation (Duane, 2000). The formulations RP1-RP6 shows the particle size range between 108.6± 3.4 nm to 220.6± 1.2 nm. It is indicated that the particle size increases with increase in concentration of polymer (table 23, fig 28).
The particle size data showed that the nanoparticle produced submicron size and had low Polydispersity, which indicate relative narrow size distribution. The PDI of all formulations was found to be in the range of 0.06-0.44, which concluded that prepared nanoparticle was monodispersed in nature (table 23).

The particle size of Repaglinide nanoparticle used to characterize the polymeric nanoparticle, because it facilitates the penetration of nanoparticles via stratum corneum of skin. Very small size nanoparticle have large surface and attractive force between the particles, the chance of possible aggregation was high in small sized particles. To overcome such aggregation, addition of co surfactant (Poloxamer 407), this reduces the aggregation of nanoparticles lead to immediate formation of nanoparticles. The PDI of PCL Repaglinide nanoparticles were found to be 108.6 nm and 0.06 PDI. Insufficient polymer synthesis may form polymer with high PDI that degrade more rapidly. Particle size was critical factor in the variation of entrapment efficient, drug release, bioavailability, efficiency and penetration via stratum corneum.

**Entrapment efficiency:**

The entrapment efficiency is the functional characteristic of polymers, drug and surfactant etc. the entrapment efficiency was high in the case of RP1-RP4 formulations, due to high affinity of drug and the polymer in the same solvent. The low entrapment efficiency of remaining formulations was due to high affinity of drug and polymer in different solvents, i.e., drug in organic and polymer in aqueous phase. High entrapment efficiency has been shown by RP1, RP2, RP3, and RP4 and was in the range of 81.4± 1.8% to 92.7± 1.4%. However in the case of low entrapment efficiency was observed in RP5, RP6 i.e. 73.6, 75.1% respectively (table 23) (fig 29). The entrapment efficient depends on the polymer- drug concentration and the method.
used to prepare nanoparticles. The hydrophobic polymer s (PLA, PCL) encapsulate large amount of hydrophobic drugs, whereas hydrophilic polymer entrap greater amount of hydrophilic drugs. High entrapment observed in PCL, due to its poor aqueous solubility. The polymeric matrix decreases the drug leakage and drug release.

**Zeta potential**

Zeta potential of Repaglinide polymeric nanoparticles is presented in the table 23. Zeta potential is an essential factor to evaluate the stability of nano dispersion. Zeta potential values mainly reflect the electrical repulsion between the particles (Muller, 2000). The average zeta potential value of Repaglinide polymeric nanoparticle was in the range of \(-16.4 \pm 2.0 \text{ mV}\) to \(-30.5 \pm 3.2 \text{ mV}\). Even though a high zeta potential could provide an electric repulsion between the particles. Poloxamer 407 also provides the stearic stability for maintaining stability in polymeric nanoparticles (Lim & Klin, 2002).

Surface charge of nanoparticles influences their skin penetration. Kohli reported that only the negative charged particles were able to penetrate the SC to reach the inner epidermis. Whereas Repaglinide polymeric nanoparticles shows \(-16\) to \(-30 \text{ mV}\) with small particle size, which abundantly influence the penetration of nanoparticles through stratum corneum.

**FTIR spectra:**

The FTIR studies showed that the significant peaks of Repaglinide are C-N stretching at 1444.02 cm\(^{-1}\), C=O cm\(^{-1}\) vibration at 1682.16 cm\(^{-1}\), C-O-C at 1085.35 cm\(^{-1}\), N-H cm\(^{-1}\) at 3301.77 cm\(^{-1}\), C=C group vibration at 1630.68 cm\(^{-1}\) and O-H vibration at 2799.45 cm\(^{-1}\) and mixture of Repaglinide and chitosan showed some significant peaks are N-H cm\(^{-1}\) stretching at 3301.59 cm\(^{-1}\), C=O stretching at 1631.06
cm$^{-1}$ O-H cm$^{-1}$ at 2918.50 cm$^{-1}$ C-O-C peak at 1031.78 cm$^{-1}$ and C-N stretching at 1084.40 cm$^{-1}$ . In the case of mixture of Repaglinide and poly (ε-caprolactone) showed five significant functional group peaks are N-H cm$^{-1}$ stretching at 1683.85 cm$^{-1}$ C=O stretching at 1633.51 cm$^{-1}$ C-N cm$^{-1}$ vibration at 1086.50 cm$^{-1}$ and CH-CH$_2$ vibration at 1446.87 cm$^{-1}$ and C-O-C peak at 1033.80 cm$^{-1}$ In the case of mixture of Repaglinide and poly (dl-lactic acid) showed some significant functional group peaks are N-H cm$^{-1}$ stretching at 3301.49 cm$^{-1}$ C=O stretching at 1631.03 cm$^{-1}$ ,C-N cm$^{-1}$ vibration at 1425.04 cm$^{-1}$ and C-O-C peak at 1086.32 cm$^{-1}$ Finally the FTIR studies of mixture of polymers and drug does not show any significant change. This results in indicating that there is no interaction between drug and selected polymers (table 24) (fig 56, 57-60 & 63-65).

**DSC spectra**

The DSC spectrum of pure Repaglinide showed a sharp endothermic peak at 65.68°C and it was the melting point of drug. In the mixture of Repaglinide and polymers shows, doesn’t changed the thermal behavior of sharp endothermic peak of drug at 65.68°C.the shoulder peaks were showed by polymers like Chitosan, PLA, PCL at 193.46°C, 193.10°C, 95.16°C respectively (fig 69, 73-75). It indicated that there was no interaction between the drug and polymers.

**In-vitro release**

The *invitro* release of Repaglinide from different biodegradable nanoparticles is shown in table 25. The quantity of drug release in the all formulations (RP1-RP6) of polymeric nanoparticle was as very low and in the range of 7.6 ± 1.2 % to 11.4 ± 0.6 % in the initial period (2h). From this, it is obvious that the decreased percentage of drug release was due to the formulation of more compact wall around the drug by the biodegradable polymer and it significantly possess the sustained drug release for a
prolonged period of time. At the end of 24 h limited percentage of drug was released in the range of 73.6±0.6 to 86.9±1.2% (table 25, fig 30).

The drug release was attributed to the physical and chemical properties particularly on the $\text{p}K_a$ and solubility profile of drug. For the polymer like PCL that possess plastic and hydrophobic properties, drug particles present in the surface of matrix is initially release into medium generating many pores and cracks which facilitate further drug release. The fact can be substantiated by the fact that release profile of drug molecules, irrespective of their chemical nature was almost linear with time. Invitro medium mimics the pH and salt concentration in the body particularly for hydrophobic drugs, it is critical during dissolution testing that sink condition were maintain and pH and salt concentration of biological fluid were appromatially.

**Release kinetic studies**

In order to determine the release patten, the *invitro* release data were substituted in zero, first order, higuchi model, korsemeyer peppas. The release constant was calculated from the slope of appropriate plots and the regression coefficient ($R^2$) was determined and the result was tabulated in table 26. In the Repaglinide polymeric nanoparticle, the *in-vitro* release kinetics of all formulations [RP1-RP6] was fitted by zero order equation, as the plots showed the high linearity [$R^2=0.971$ to $0.989$] followed by first order [$r^2=0.808$ to $0.910$] and higuchi equation [$R^2=0.910$ to $0.943$]. Hence the drug release kinetics demonstrates that the concentration was nearly independent of drug release. On explaining the mechanism of drug release, korsemeyer- peppas equation has showed good linearity [$R^2=0.987$]. The release exponent n= 0.80, which appears to be the coupling of diffusion and erosion mechanism i.e. anomalous diffusion.
The release kinetics was often used employed for comparative purpose and relating the release parameters with important in bioavailability and used to study influences of formulations factors on the drug release for optimization as well as control of drug release from nanoparticles. The invitro release data was subjected to zero, first order, higuchi and korsemeyar peppas to establish the drug release mechanism and kinetics of drug release from polymeric nanoparticles. When data was subjected to zero order and first order kinetic model, a linear relationship was observed with high \( r^2 \) values for zero order as compared to first order model; it suggested that the Repaglinide polymeric nanoparticle were zero order controlled release of drug from polymeric matrix. Higuchi's model \( r^2 \) values suggested that the drug release from polymeric nanoparticle followed diffusion mechanism as all the polymer was gelated based matrix type. The exact release mechanism was analyzed by korsemeyar peppas model. The values of ‘n’ obtained for all the Repaglinide nanoparticles formulations was \( \geq 0.5 \) to \( \leq 1.0 \) suggested that the drug release followed non-fickian anomalous diffusion mechanism due to polymeric matrix have less affinity with aqueous medium.

**Preparations of Insulin Polymeric Nanoparticles**

Polymeric nanoparticles of Insulin were prepared with different biodegradable polymers. In IP1, IP2, formulations poly (dl lactic acid) was used and poly (ε-caprolactone) was used in IP5, IP6. The above polymeric nanoparticle was prepared by using solvent evaporation method. Polymeric suspension was prepared by dissolving polymer in dichloromethane as organic solvent, whereas Insulin was dissolved in 10 mM Tris buffer (table 27). The organic solvent used in these preparations rapidly partitioned into external aqueous phase containing poloxamer
and the polymer precipitated around the drug particle. The subsequent evaporation, the entrapped solvent led to the formation of Insulin polymeric nanoparticle.

On the other hand, IP3, IP4 formulation, the polymeric Insulin nanoparticle were prepared by polyelectro complex formation. The complex formation in between polyelectrolyte polymer of chitosan and Insulin is by cumbic interactions. The pH of the Insulin solution will influences the properties of polymeric nanoparticles. As reported previously, at pH 7.4, the human Insulin has two negative charges per molecules. It has been observed that desirable Insulin nanoparticle could be formed when pH of Insulin solution was maintained between 8.0 to 8.5 (Mao, 2006) and the pH of chitosan solution was adjusted to 5.5, because at this pH more than 90% of all amino group were protonated (Mao, 2006). This method has several characteristics favorable for cellular uptake and colloidal stability, including suitable particle diameter, zeta potential and spherical morphology and low PDI. A major advantage of this method is high entrapment efficiency and absence of organic chemical for production of Insulin nanoparticles. The insulin polymeric nanoparticles prepared by PEC method showed, mechanical strength and permeability barrier of nanoparticle can be increase by addition of oppositely charged polymeric to Insulin, which formed gelated Insulin nanoparticles. Strong electrostatic interaction of amino groups of chitosan with carboxyl groups of insulin lead to formulation of chitosan insulin complex, due to protination of amino group on chitosan and the ionization of carboxylic acid group on insulin containing amino acids, the stability of chitosan influenced by ionic strength. The formed nanoparticle was more stable.
**Morphology**

The morphological characters of Insulin polymeric nanoparticle were observed under SEM. In formulations of IP1, IP2, spherical shaped particles were observed, unfortunately the particle surface were rough. Even though IP5, IP6, poly (dl-lactic acid) formulations had small spherical shaped particles some with projected pits, which is due to quick evaporation of organic solvent during homogenization. In the case of IP3, IP4, chitosan Insulin nanoparticles showed smooth surface and spherical shaped particles (fig 101-106). Fortunately they produced narrow size distribution; whereas, the chitosan nanoparticle prepared by PEC method has diminished the organic solvent. At particular pH condition, the nanoparticles precipitated. The insulin chitosan nanoparticles were smooth and spherical shaped particles formed compared with other polymers. The chitosan polymer has efficiently protonated on the surface of insulin molecules to form aggregation protonated charges on dense surface to produce smooth surface. The smooth surface particle can easily penetrate through SC compared to rough surface nanoparticles.

**Particle size and poly dispersity index**

The particle size of IP1 to IP6 was in the range of 110.2 – 341.6 nm respectively. It is shown in table 28, fig 31. In the case of IP3, IP4 particles formed are very small in size, depends on method of preparation. In this case of PEC method, nanoparticles were produced without homogenization, temperature induction and with the absence of organic solvent.

Polydispersity index of IP3, IP4 was very less compared to other formulations. In these formulations PDI was found to be 0.09, 0.08 respectively. The produced particles were monodispersed and these particles would easily cross the stratum
corneum. Although, other formulations, PDI was in the range of 0.10 to 0.62. It is assumed that they are monodispersed. Unfortunately, those particles find it some what difficult to penetrate through stratum corneum layer (table 28). The particle size of Insulin nanoparticle used to characterize the polymeric nanoparticle, because it facilitates the penetration of nanoparticles via stratum corneum of skin. Very small size nanoparticle have large surface and attractive force between the particles, the chance of possible aggregation was high in small sized particles. The PDI of chitosan Insulin nanoparticles were found to be 110.2 nm and 0.08 PDI. Insufficient polymer synthesis may form polymer with high PDI that degrade more rapidly. Particle size was critical factor in the variation of entrapment efficient, drug release, bioavailability, efficiency and penetration via stratum corneum.

**Entrapment efficiency**

The entrapment efficiency of Insulin polymeric nanoparticle was shown in table 28, fig 32. Ideally, a successful nanoparticulate system should have high entrapment efficiency. As several studies has shown, the electrostatic interaction between the acidic group of Insulin and amino group of chitosan play a dominant role in the entrapment efficiency of Insulin in nanoparticles (Calvo, 2007). In the chitosan nanoparticle formulations, IP3, IP4, they have more entrapment efficiency of 87.8, 91.3 % respectively. In case of PCL nanoparticle [IP1, IP2] they showed less entrapment efficiency of 81.8, 84.2 % respectively. On other hand PLA Insulin nanoparticles [IP5, IP6] have less entrapment of 80.6, 84.8 % respectively. It concludes that all formulations, the entrapment efficiency increased with increase in polymer concentration. In the case of Insulin chitosan nanoparticles, entrapment efficiency was more compared with other polymers. The entrapment efficiency was
altering depending on type of polymer and method of preparation used from preparation off insulin. The chitosan cross linking with Insulin was more and form association of charges on the surface. It can minimize the leakage of peptide from polymeric matrix.

**Zeta potential**

Zeta potential is a key factor to evaluate the stability of colloidal dispersion (Komatsu, 1995). In general, particle could be dispersed in stable form when value of zeta potential was in the range of -30 to +30 mV due to electrical repulsion between particles (Muller, 2000). As shown in **table 28**, the average zeta potential obtained in formulations RS1 to RS6 was in the range of -12.6 ± 1.4mV to -27.8 ± 3.2mV. It was concluded that the Insulin nanoparticles showed a dynamic stable colloidal system. Surface charge of nanoparticles influences their skin penetration. Kohli reported that only the negative charged particles were able to penetrate the SC to reach the inner epidermis. Whereas Insulin polymeric nanoparticles shows -12 to –27 mV with small particle size, which abundantly influence the penetration of nanoparticles through stratum corneum.

**FTIR spectra:**

The FTIR studies showed that the significant peaks of Insulin are C-N stretching at 1385.30 cm⁻¹, C-H [CH₃] at 2926.56 cm⁻¹, C=O cm⁻¹ vibration at 1655.91 cm⁻¹ and C-H [CH₂] bending at 1454.30 cm⁻¹ and C-O-C at 1109.46 cm⁻¹, N-H cm⁻¹ at 3404.48 cm⁻¹ and mixture of Insulin and chitosan showed seven significant peaks are N-H cm⁻¹ stretching at 3665.11 cm⁻¹ C=O stretching at 1641.64 cm⁻¹ O-H cm⁻¹ at 2917.57 cm⁻¹ C=S cm⁻¹ vibration at 571.58 cm⁻¹ and CH-CH₃ vibration at 2850.18 cm⁻¹ CH-CH₂ vibration at 1415.78 cm⁻¹ C-O-C peak at 1022.72
cm$^{-1}$. In the case of mixture of Insulin and poly (ε-caprolactone) showed some significant functional group peaks are N-H cm$^{-1}$ stretching at 3665.33 cm$^{-1}$ C=O stretching at 1650.03 cm$^{-1}$ O-H cm$^{-1}$ at 3608.86 cm$^{-1}$ C-S cm$^{-1}$ vibration at 572.47 cm$^{-1}$ and CH-CH$_3$ vibration at 2380.39 cm$^{-1}$ CH-CH$_2$ vibration at 1235.13 cm$^{-1}$ and C-O-C peak at 1022.72 cm$^{-1}$ In the case of mixture of Insulin and poly (dl-lactic acid) showed some significant functional group peaks are N-H cm$^{-1}$ stretching at 3665.93 cm$^{-1}$ C=O stretching at 1691.02 cm$^{-1}$ O-H cm$^{-1}$ at 2954.50 cm$^{-1}$ C-S cm$^{-1}$ vibration at 683.96 cm$^{-1}$ and CH-CH$_2$ vibration at 1427.76 cm$^{-1}$ and C-O-C peak at 1096.71 cm$^{-1}$ Finally the FTIR studies of mixture of polymers and drug does not show any significant change. These results indicate that there is no interaction between drug and selected polymers (table 29) (fig 49, 50-53 & 62-65).

**DSC spectra**

The DSC spectrum of pure Insulin showed a broad endothermic peak at 106.68°C and it was the melting point of drug. In the mixture of Insulin and polymers shows, doesn’t changed the thermal behavior of broad endothermic peak of drug at 106°C the shoulder peaks were showed by polymers like Chitosan, PLA, PCL at 294.6°C, 214.4°C, 221.6°C respectively (fig 76, 80-82). It indicated that there was no interaction between the drug and polymers.

**Invitro release**

The invitro release of Insulin nanoparticle was done in pH 7.4 phosphate buffer and it is shown in table 30, fig 33. The amount of drug release from polymeric nanoparticle was very less in all the formulations [IP1-IP6]. The release rate was a minimum of 8.1 ± 0.1 % and maximum of 12.5 ± 0.6 %. In the formulations of IP1, IP2, IP5, IP6 initial burst release was very less compared to IP3, IP4 formulations,
due to more affinity between the drug and polymers. But in the case of IP3, IP4, the initial burst release of 12.6± 1.4%, 10.2 ± 0.2 % has been observed respectively. It has good burst release and maintained a controlled release of 69.0 ± 1.2%, 51.6 ± 1.4 % respectively over 24h. After the initial release the polymeric matrix swelled and slowly eroded the drug from the nanoparticle. The drug release was attributed to the physical and chemical properties particularly on the $P_{\text{ka}}$ and solubility profile of drug. For the polymer like chitosan that possess plastic and hydrophilic properties, drug particles present in the surface of matrix is initially release into medium generating many pores and cracks which facilitate further drug release. The fact can be substantiated by the fact that release profile of drug molecules, irrespective of their chemical nature was almost linear with time. It was critical during dissolution testing that sink condition were maintain and pH and salt concentration of biological fluid were appromatially.

**Release kinetic studies**

As shown in table 31, the invitro release data was fitted in various kinetic equation i.e., zero order, first order, higuchi, korsemeyer. The release constant was calculated from the slope of appropriate plot and the reggration [$R^2$] was noted. In the Insulin polymeric nanoparticle preparation of all the formulations [IP1-IP6], the invitro release kinetic was best fitted by zero order equation, as the plots showed the high linearity [$R^2$= 0.983 to 0.996] followed by first order [$R^2$= 0.782 to 0.930] and higuchi equation [$R^2$= 0.967 to 0.989]. Hence the drug release kinetics demonstrates that the concentration was nearly independent of drug release, for further explanation of the mechanism of drug release, the korsemeyer- peppas equation showed good
linearity \( R^2 = 0.969 \). The release exponent (n) was 0.67, which appears to be anomalous diffusion.

The release kinetics was often used employed for comparative purpose and relating the release parameters with important in bioavailability and used to study influences of formulations factors on the drug release for optimization as well as control of drug release from nanoparticles. The invitro release data was subjected to zero, first order, higuchi and korsemeyar peppas to establish the drug release mechanism and kinetics of drug release from polymeric nanoparticles. When data was subjected to zero order and first order kinetic model, a linear relationship was observed with high \( r^2 \) values for zero order as compared to first order model; it suggested that the Insulin polymeric nanoparticle were zero order controlled release of drug from polymeric matrix. Higuchis model \( r^2 \) values suggested that the drug release from polymeric nanoparticle followed diffusion mechanism as all the polymer was gelated based matrix type. The exact release mechanism was analyzed by korsemeyar peppas model. The values of ‘n’ obtained for all the Insulin nanoparticles formulations was ≥ 0.5 to ≤ 1.0 suggested that the drug release followed non-fickian anomalous diffusion mechanism due to polymeric matrix have less affinity with aqueous medium.

**Stability studies of nanoparticles**

The stability studies were performed on optimized four formulations of nanoparticles at \( 25^0 \text{C} \pm 2^0 \text{C} / 60\% \pm 5\% \ \text{RH} \), \( 5^0 \text{C} \pm 0.2^0 \text{C} / 60\% \pm 5\% \ \text{RH} \) and \( 45^0 \text{C} \pm 2^0 \text{C} / 75\% \pm 5\% \ \text{RH} \) for 3 months. After 3 months particle size, zeta potential and drug content were analyzed. There was no significance difference observed for above parameters and the optimized formulation shows good stability over 3 months.
A successful gene delivery system requires efficiency and stability during storage.

This is why stability studies are imperative for new pharmaceutical products. Targeted nanomedicines are mostly incorporated into biotechnological products such as targeting Peptides or loaded with proteins and genes.

**Preparation of transdermal patch containing nanoparticles**

The selected formulations of nanoparticles [RS2, RP4, IS4, and IP4] are incorporated in transdermal patch prepared by Methocel K100M polymer. The nanoparticles were prepared by hydrophobic biodegradable polymers like lecithin, poly (dl-lactic acid) and Chitosan. The hydrophobic nanoparticles were casted on hydrophilic Methocel K100M transdermal patch. In these preparations there was no interaction between transdermal patch and nanoparticles. These nanoparticles did not dissolve in hydrophilic portion. Thereby the stability of nanoparticles has not been interrupted in transdermal patch. The transdermal patch containing permeation enhancer (DMSO) could significantly influence the permeability of nanoparticles by enlarging skin pores; thereby nanoparticles were passively diffused through the skin.

**Evaluation of nanoparticulated transdermal patches**

The SLN and polymeric nanoparticles of Repaglinide and Insulin was incorporated in transdermal patches. It was evaluated by thickness and drug content of each patch measures by triplicatelly. The Repaglinide SLN and polymeric nanoparticles (RS2, RP4) containing transdermal patches thickness was 20.6, 20.1µg/patch respectively. In case of Insulin SLN and polymeric nanoparticles (IS4, IP4) containing transdermal patches thickness was 21.0, 21.4 µg/patch respectively.
In case of RS2, RP4 nanoparticles containing transdermal patches drug content was 2.04 mg, 2.10 mg per patch respectively. In the case of IS4, IP4 nanoparticles containing transdermal patches were 1.94 IU, 2.09 IU per patch respectively.

**Ex-vivo permeation studies**

The percentage of drug permeated through the rat skin placed in Franz diffusion cell was measured for permeation. The percentage of drug transported from nanoparticles was at pH 7.4 dissolution medium. It has been shown in table 33 the percentage of drug permeated across the rat skin was calculated for RS2, RP4, IS4, IP4 nanoparticles formulations at 24h.

In the initial hours of drug release of RS2, RP4, IS4, IP4 transdermal formulations were 7.1%, 6.9%, 7.4%, 7.0% respectively. The initial release due to nanoparticle contains surface free drug molecules. After that, the drug release was consequently maintained gradual. At end of the experiment (60h) all the formulations (RS2, RP4, IS4, IP4) were released around half of the quantities of drug, i.e. 54.6%, 52.7%, 57.4%, 51.3% respectively. Thereby, it was concluded that all the four formulations of transdermal patch containing nanoparticles were maintained in steady state concentration over the prolonged periods.

**Histopathological Studies**

The differences in morphological changes of blank transdermal patch and nanoparticulated transdermal patch is shown in Fig 36. This fig. shows the blank transdermal patch with clearly defined subcutaneous layer having well swollen structure and the inflammatory cell infiltration. It is observed due to response of HPMC. And the loaded with nanoparticles in transdermal patch, the cell structure loosened causing more cell infiltration and resulting in permeation of nanoparticles in
dermis region (degeneration appendages were also seen (Fig 37-40) with nanoparticulated transdermal patch containing chemical permeation enhancer (DMSO) treated. Destruction of subcutaneous layer and sub epidermal region occurred because of which there was easy penetration of nanoparticles in subcutaneous and dermis layer. This order was confirmed by the histopathological changes in the rat skin.

In-Vivo Evaluation Studies

Repaglinide PLA nanoparticle containing transdermal patch

Hypoglycemic effects:

The result of reduction in plasma glucose levels of transdermal patch containing Repaglinide nanoparticle in comparison with oral administration of Repaglinide (2 mg) in diabetes rats are shown in table 43. The hypoglycemic effect that was observed was significant (P< 0.001; compared to control) in oral and transdermal patch treated animals upto 60 h. The Repaglinide (oral) produced a decrease in blood glucose level upto 85.12 ± 1.2 mg/dl (P<0.05) for 4 h. In case of transdermal patches containing nanoparticles (2.10 mg), the hypoglycemic response was gradual. A maximum hypoglycemic response was observed after 36 h and remained stable upto 60h (fig 47).

The result of Insulin level in hypoglycemic activity of Repaglinide in diabetes rats are shown in table 44 the plasma Insulin level was elevated to the maximum in oral and transdermal nanoparticle system treated group upto 4, 36 h respectively, compared to control group (P< 0.01). Repaglinide (oral) produced an increase in plasma Insulin level upto 40.12 at 4 h. In case of transdermal patch containing nanoparticle, the elevation in the plasma Insulin level was gradual. A maximum
increase in Insulin level was observed at 36 h and remained almost constant upto 60 h (fig 48).

**Bioavailability parameters:**

The plasma concentration of Insulin and time was plotted in trapezoidal method and pharmacokinetic parameters were calculated. The AUC, AUMC, Cmax, Tmax, MRT and $T_{1/2}$ of oral administration of Repaglinide were 1059.88 µIU/mL/h, 372061 µIU/mL/h, 40.12 µIU/mL, 4 h, 116.75h, 85.18h respectively. In case of transdermal patch, The AUC, AUMC, Cmax, Tmax, MRT were 2218.88 µIU/mL/h, 381630.3 µIU/mL/h, 41.88 µIU/mL, 36 h, 83.24h, and 52.79h respectively [table 45]. The values were significant compared to oral administration. The measurable concentrations of Insulin were obtained within an hour, after application of the patch relatively maintains steady plasma Insulin concentration of Insulin over 60 h. The relative bioavailability of transdermal patch containing Repaglinide nanoparticle was 75.06 as compared with oral administration. Therefore transdermal patch containing Repaglinide nanoparticle maintained the bioavailability by 75 fold, compared with oral Repaglinide administration. (table 46).

**Insulin chitosan nanoparticle containing transdermal patch**

**In vivo hypoglycemic effects:**

Wister rats exposed to Streptozocin and the plasma glucose level was increased from 84.17 ± 0.12 to 331.67± 0.02 mg/dL. In this experiment, the Streptozocin induced diabetic condition was stabilized over for three days. Once stabilized, plasma glucose level in diabetic induced rats stopped fluctuation during experiment. After application of a single transdermal patch containing Insulin nanoparticle (2.09IU Insulin per patch) to diabetic induced rats, showed the decrement of plasma glucose 90.27 ± 0.02 from 334.17±0.1 mg/dL. During this
experiment, plasma glucose level was normally maintained from 80.34±0.06 to 92.18± 0.62 mg/dL up to 60 h (fig 41) (table 34).

Plasma Insulin level of transdermal nanoinsulin patch treated rats showed drastic increase in Insulin level from 15.2± 0.44 to 30.82± 0.62µIU/mL after 2 h, and then slowly fluctuating in plasma level, after which normal Insulin level maintained up to 60 h. The control of subcutaneous Insulin injection showed an immediate rise in Insulin level 46.68 ±0. 32 µIU/mL within 2 h, later it showed a drastic decrease and reached the initial level. The transdermal Insulin nanoparticle was suggested for safe and viable drug delivery of Insulin. Hence the plasma glucose level decreased slowly in transdermal nanoinsulin when compared with subcutaneous injection, reflecting the differences in Insulin adsorption rates. The absorbed Insulin nanoparticles might remain in the dermis and slowly degrade the chitosan polymer and release free Insulin into blood. (fig 42, table 35).

**Bioavailability parameters:**

The bioavailability parameters like AUC, AUMC, Cmax, Tmax, MRT, T½ . Relative bioavailability of transdermal nanoinsulin patch was determined by Trapezoid method, which showed in table 46. Where as AUC of plasma Insulin level of transdermal nanoinsulin was 2106.28 µIU/mL/h as compared with subcutaneous Insulin level 1006.08 µIU/mL/h and AUMC, Cmax, Tmax of transdermal nanoinsulin showed 58994.96µIU/mL/h, 45.80 µIU/mL, 8 h, 144.9h, 28.23h respectively [table 36]. This experiment proved that the transdermal nanoinsulin was able to deliver a significant proportion of Insulin from the transdermal patch over a prolonged period of 60 h.

The relative bioavailability of transdermal patch containing Insulin nanoparticle was 84.40 as compared with subcutaneous administration [table 46]. Therefore
transdermal patch containing Insulin nanoparticle maintained the bioavailability of 84 fold, compared with subcutaneous Insulin administration.

**Repaglinide lecithin nanoparticle containing transdermal patch**

**Hypoglycemic effects:**

The result of reduction in plasma glucose levels of transdermal patch containing Repaglinide nanoparticle (2.04mg/patch) compared with oral administration of Repaglinide (2 mg) in diabetes rats are shown in **table 40**. The hypoglycemic effect was significant (P< 0.01: compared to control) in oral and transdermal patch treated animals upto 60 h. Repaglinide (oral) produced a decrease in plasma glucose level upto 84.60 ± 3.0 mg/dL (P <0.05) at 2 h. in case of transdermal patches containing nanoparticles, the hypoglycemic response was gradual. A maximum hypoglycemic response was observed after 36 h and remained stable upto 60 h (**fig 45**).

The result of plasma Insulin level in hypoglycemic activity of Repaglinide in diabetes rats were shown in **table 41**. The plasma Insulin level has been elevated maximum in oral and transdermal nanoparticle system treated group upto 4, 8 h respectively, when compared to control group (P< 0.05). Repaglinide (oral) produced an increase in plasma Insulin level upto 36.42 µIU/mL at 4 h. In case of transdermal patch containing nanoparticle (2.04mg/patch), the elevation in the plasma Insulin level was gradual. A maximum Insulin level increase was observed at 8 h and remained almost constant upto 60 h. (**fig 46**)

**Bioavailability parameters:**

The plasma concentration of Insulin and time was plotted in trapezoidal method and pharmacokinetic parameters were calculated. The AUG,AUMC Cmax,
Tmax, MRT, T\textsubscript{1/2} values of oral administered Repaglinide were 1108.96 µIU/mL/h, 353576.9 µIU/mL/h, 49.14 µIU/ML, 4 h, 119.12h, 79.15h respectively. In the case of transdermal patch, AUG, AUMC, Cmax, Tmax, MRT, T\textsubscript{1/2} was 2019.66 µIU/mL/h, 610037.9 µIU/mL/h, 36.42 µIU/mL, 8 h, 116.75h, 77.57 h respectively [table42]. The values were significantly compared with oral Repaglinide administration. Measurable concentration of Insulin was observed immediately after application of the patch and relatively steady plasma Insulin concentration over 60 h. The relative bioavailability of transdermal patch containing Repaglinide nanoparticle was 76.75, when compared with oral administration. Therefore transdermal patch containing Repaglinide nanoparticle maintained 76 fold bioavailability, compared with oral Repaglinide administration (table 46).

**Insulin solid lipid nanoparticle containing transdermal patch**

**In vivo hypoglycemic effects:**

Wister rats were exposed to Streptozocin and the plasma glucose level was increased from 84.10 ± 0.4 to 331.62± 0.2 mg/dL. In this experiment, the Streptozocin induced diabetic rats condition was stabilized over three days. During this period, the plasma glucose sensitive subcutaneous Insulin gets diminished. Once stabilized, plasma glucose level in diabetic induced rats stopped fluctuation during experiments. After application of a single transdermal patch containing Insulin nanoparticle (1.94 IU Insulin per patch) in diabetic induced rats, which decrease plasma glucose level from 89.10 ± 0.2 to 331.62±0.2 mg/dL. During this experiment, plasma glucose level has been normally maintained from 80.64±1.6 to 89.10± 0.2 mg/dL up to 60h. (fig 43) (table 37)

Plasma Insulin level of transdermal Insulin SLN patch treated rats showed drastic increase from 15.02± 0.6 to 32.76± 0.2µIU/ mL after 2 h, followed by small
fluctuations of plasma level was observed. There by normal Insulin level up to 60 h is maintained. In control (subcutaneous Insulin injection) showed an immediate rise in Insulin level 40.42 ±3.2 µIU/mL within 2 h. After that, it shows a drastic decrease to reach the initial level. The transdermal Insulin nanoparticle can be suggested for safe and viable drug delivery of Insulin. Hence the plasma glucose level decreased slowly after transdermal nanoinsulin in comparison with subcutaneous injection, reflecting the differences in Insulin absorption rates. The absorbed Insulin nanoparticles might remain in the dermis and slowly degrade from lipid and release free Insulin in situ. (fig 44, table 38)

Bioavailability parameters:
The bioavailability parameters like AUC,AUMC, Cmax, Tmax, MRT, T1/2. Relative bioavailability of transdermal nanoinsulin patch was determined by Trapezoid method which showed in table no 39 whereas relative bioavailability was determined by AUC of standard S.C. Injection and AUC of transdermal nanoinsulin. The AUC of plasma Insulin level of transdermal nanoinsulin was 2248.28 µIU/mL/h compared with subcutaneous Insulin level showed 1004.52 µIU/mL/h and AUMC,Cmax, Tmax, MRT, T1/2 of transdermal nanoinsulin were, 65965.44 µIU/mL/h, 41.18 µIU/mL, 36 h, 303.2h, 53.30h respectively [table 39]. Compare with subcutaneous Insulin injection (table 46). This experiment proved that the transdermal nanoinsulin was able to deliver a significant proportion of Insulin from the transdermal patch over a prolonged period of 60 h. The relative bioavailability of transdermal patch containing Insulin nanoparticle was 87.81 as compared with subcutaneous administration. Therefore transdermal patch containing Insulin nanoparticle maintained 87 fold bioavailability, compared with subcutaneous Insulin administration.