Chapter-3

LITRATURE REVIEW
Abdallah Makhlof et al., (2011) formulated the chitosan nanoparticles by ionic cross-linking with hydroxypropyl methylcellulose phthalate (HPMCP) as a pH-sensitive polymer and evaluated for the oral delivery of insulin. After s.c. injection to rats, no significant difference in the hypoglycemic effect of insulin solution or insulin-loaded CS/HPMCP NPs was observed, confirming the physico-chemical stability and biological activity of the entrapped peptide. Following peroral administration, CS/HPMCP NPs increased the hypoglycemic effect of insulin by more than 9.8 and 2.8-folds as compared to oral insulin solution and insulin-loaded CS/tripolyphosphate (TPP) NPs, respectively.

Adnan Badwan et al., (2009) formulated an oral insulin delivery system by oily vehicle. Insulin in the preparations was stable for a period of one month at storage temperatures of 4 and 25°C. It was also biologically active and stable as demonstrated by the remarkable reduction of blood glucose level of the STZ-diabetic rats after oral administration of the preparation. Moreover, hypoglycemic effect of nanoparticles administered orally was sustained for a longer period of time compared to the subcutaneous injection. These results clearly evidenced the ability of the nanoparticles to enhance the pharmacological response of insulin when given orally and could be used to deliver other peptides.

Akbar Bayat et al., (2008) studied the Insulin (INS), has low therapeutic activity when administered orally due to degradation by proteolytic enzymes. He developed an INS nanoparticulate system by using chitosan (CS), triethylchitosan (TEC), and dimethyl-ethylchitosan (DMEC, a new quaternized derivative of CS). INS-polymer nanoparticles were prepared by the polyelectrolyte complexation method. The amount of INS loaded into the nanoparticles was determined by measuring the association
efficiency and also the content of INS in the nanoparticles. In vitro release studies showed a relatively small burst effect at the beginning and then a sustained release characteristic for 5 hours.

**Alok Dhawan et al., (2009)** prepared the small size of the nanoparticles (NPs) being used, there is a concern that they may interact directly with macromolecules such as DNA. ZnO NPs demonstrated a DNA damaging potential as evident from an increased Olive tail moment (OTM) of 2.13±0.12 (0.8 g/mL) compared to control 1.37±0.12 in the Comet assay after an exposure of 6 h. ZnO NPs were also found to induce oxidative stress in cells indicated by depletion of glutathione (59% and 51%); catalase (64% and 55%) and superoxide dismutase (72% and 75%) at 0.8 and 0.08 g/mL respectively. Our data demonstrates that ZnO NPs even at low concentrations possess a genotoxic potential in human epidermal cells which may be mediated through lipid peroxidation and oxidative stress.

**Amani Elsayed et al., (2009)** prepared the insulin nanoparticle and it has unimodal particle size distribution with a mean diameter of 108 ± 9 nm. Insulin was protected from gastric enzymes by incorporation into lipid-based formulation. The results of RP HPLC and ELISA indicated that insulin was able to withstand the preparation procedure. It was also biologically active and stable as demonstrated by the remarkable reduction of blood glucose levels of the STZ-diabetic rats after oral administration of the preparation. Moreover, hypoglycemic effect of nanoparticles administered orally was sustained for a longer period of time compared to the subcutaneous injection.

**Ammon C. Sintov et al., (2006)** demonstrated the application of a short-term iontophoresis on the topical delivery of lidocaine hydrochloride from a microemulsion-based system. In contrast, the application of aqueous solution-
iontophoresis resulted in a relatively lower drug accumulation (21.44±10.42 and 5.30±2.25 g cm$^{-2}$ in the epidermis and dermis, respectively, at $t = 30$) with more rapid clearance of the drug from the skin. Ten-minute application of a low-current electric field on a new topical microemulsion appears to make significant changes in skin permeability.

Amnon C. Sintov et al., (2010) the main purpose of this study was to investigate the nasal absorption of insulin from a new microemulsion spray preparation in rabbits. The bioavailability of insulin lispro via the nasal route using a W/O microemulsion was found to reach 21.5% relative to subcutaneous administration, whereas the use of an inverse microemulsion as well as a plain solution yielded less than 1% bioavailability. The profile of plasma glucose levels obtained after nasal spray application of the microemulsion (1 IU/kg lispro) was similar to the subcutaneous profile of 0.5 IU/kg at the first 90 min after application and resulted in a 30–40% drop in glucose levels. In view of the absorption differences of insulin between 20%, 50% water-containing microemulsions and an aqueous solution obtained in vitro and in vivo, it has been concluded that the acceleration in the intramucosal transport process is the result of encapsulating insulin within the nano-droplet clusters of a W/O microemulsion.

Amnon C. Sintov et al., (2007) reviewed the transdermal delivery of insulin is a non-invasive alternative to the subcutaneous injection of insulin in diabetic patients. It has been found that skin pretreatment with iodine followed by a dermal application of insulin results in reduced glucose and elevated hormone levels in the plasma. Topical iodine protects the dermally applied insulin presumably by inactivation of endogenous sulfhydryls such as glutathione and gamma glutamylcysteine which can reduce the disulfide bonds of the hormone. Thus, the effect of iodine is mediated by retaining the
potency of the hormone during its penetration via the skin into the circulation. The proposed procedure might be applicable for additional disulfide-containing peptides such as calcitonine, somatostatin, oxytocin/vasopressin and their analogs.

Andreas Bernkop-Schnürch et al., (2008) prepared the novel three-layered oral delivery system for insulin in vivo by the patch system consisted of a mucoadhesive layer, a water insoluble backing layer made of ethyl cellulose and an enteric coating made of Eudragit. The mucoadhesive layer, exhibiting a diameter of 2.5mm and a weight of 5 mg, comprised poly carbophil-cysteine conjugate (49%), bovine insulin (26%), glutathione (5%) and mannitol (20%). 74.8±4.8% of insulin was released from the delivery system over 6 h. Six hours after administration of the patch system mean maximum decrease of blood glucose level of 31.6% of the initial value could be observed. Maximum insulin concentration in blood was 11.3±6.2 ng/mL and was reached 6 h after administration. The relative bioavailability of orally administered patch system versus subcutaneous injection was 2.2%.

Anja Graf et al., (2009) optimised the insulin in poly(alklycyanoacrylate) nanoparticles prepared from microemulsions with different microstructure containing isopropyl myristate, caprylocaproyl macrogolglycerides, polyglyceryl oleate and insulin solution Insulin loading however, showed an opposite trend. In vitro release profiles of insulin from the nanoparticles dispersed in the microemulsion templates were controlled by the monomer concentration only. In vivo, a consistent and significant hypoglycemic effect over controls was found for up to 36h depending on the type of monomer. No significant serum insulin levels were detectable.

Anja Graf et al., (2009) prepared the insulin nanoparticles by poly (alklycyanoacrylate) microemulsions with different microstructure containing isopropyl myristate, caprylocaproyl macrogolglycerides, and polyglyceryl oleate.
Bioactivity of insulin was studied using a streptozotocin diabetic rat model. Nanoparticles were spherical with 200–400 nm in size without significant difference between different microemulsion templates, types and amounts of monomer. *In vivo*, a consistent and significant hypoglycemic effect over controls was found for up to 36 h depending on the type of monomer. No significant serum insulin levels were detectable. This study showed that the strategy of delivering insulin orally, entrapped in nanoparticles and dispersed in a biocompatible microemulsion.

Anja Graf et al., (2009) studied the optimise entrapment of insulin in poly(alkylcyanoacrylate) nanoparticles prepared from microemulsions with different microstructure containing isopropyl myristate, caprylocaproyl macrogolglycerides, polyglyceryl oleate. *In vitro* release profiles of insulin from the nanoparticles dispersed in the microemulsion templates were controlled by the monomer concentration only. *In vivo*, a consistent and significant hypoglycemic effect over controls was found for up to 36 h depending on the type of monomer. No significant serum insulin levels were detectable.

Anna Radomska-Soukharev et al., (2007) investigated the chemical stability of lipids used as excipients in the production of Solid Lipid Nanoparticles (SLN). Different lipids and amounts of surfactants were considered. The production process of SLN itself did not affect the chemical stability of lipid excipients forming the particle matrix. The formulations where lipids consisted of triglycerides showed a negligible decomposition of the structure during incubation at 25 °C. Dynasan 118 showed the highest chemical stability (loss < 4%) within two years. Basal values with a sustained hypoglycemic effect over 24 h. Pharmacodynamic and pharmacokinetic parameters were evaluated at a dose of 50 IU/kg nanoencapsulated insulin, and 13% oral bioavailability showed a threefold increase in comparison to free insulin.
Bruno Sarmento et al., (2006) prepared the Insulin-loaded nanoparticles by ionotropic pre-gelation of alginate with calcium chloride followed by complexation between alginate and chitosan. Individual and smaller sizing nanoparticles, around 800 nm, were obtained at pH 4.7 with an alginate:chitosan mass ratio of 6:1. Thermograms of insulin-loaded nanoparticles originated shifts on same unloaded nanoparticle peaks and suggested polyelectrolytes–protein interactions at pH around 4.5–5.0. FTIR spectra of insulin-loaded nanoparticles showed amide absorption bands characteristic of protein spectra and revealed the formation of new chemical entities.

Bruno Sarmento et al., (2007) worked to produce and characterize cetylpalmitate-based solid lipid nanoparticles (SLN) containing insulin, and to evaluate the potential of these colloidal carriers for oral administration. SLN were prepared by a modified solvent emulsification evaporation method based on a w/o/w double emulsion. The particle size, zeta potential and association efficiency of unloaded and insulin-loaded SLN were determined and were found to be around 350 nm, negatively charged and the insulin association efficiency was over 43%. After oral administration of insulin-loaded SLN to diabetic rats, a considerable hypoglycemic effect was observed during 24 hours.

Camile B. Woitiski et al., (2010) proved the Intestinal uptake of insulin and hypoglycemic effect of orally delivered insulin encapsulated in polyelectrolytically stable nanoparticles were evaluated in streptozotocin-induced Wistar diabetic rats. Nanoparticles with 396 nm mean diameter were formed by alginate and dextran sulfate nucleating around calcium and binding to poloxamer, stabilized by chitosan, and subsequently coated with albumin. The resulting negatively charged nanoparticles retained insulin bioactivity and enhanced pharmacological availability by shielding insulin from enzymatic degradation and through chemical and physical facilitation of
permeation through the intestinal membrane. Pharmacodynamic and pharmacokinetic parameters were evaluated at a dose of 50 IU/kg nanoencapsulated insulin, and 13% oral bioavailability showed a threefold increase in comparison to free insulin. Therefore the nanoformulation facilitated the oral delivery of insulin, and potentially that of other therapeutic proteins.

**Chander parkash dora et al., (2010)** formulated nanoparticles (NPs) containing glibenclamide (GB) prepared with Eudragit L100 to achieve a better release profile suitable for *per oral* administration with enhanced efficacy. The NPs were prepared by solvent displacement method. Addition of surfactants showed a promising result in decreasing particle size of NPs. Dissolution study revealed increased release of GB from NPs. Developed NPs revealed a decreased Tmin and enhanced bioavailability and hence superior activity as compared to plain GB in alloxan induced diabetic rabbit model. The developed NPs could reduce dose frequency, decrease side effects, and improve patient compliance.

**Chunhua Yin et al.,(2009)** Trimethyl chitosan-cysteine conjugate (TMC-Cys) was synthesized in an attempt to combine the mucoadhesion and the permeation enhancing effects of TMC and thiolated polymers related to different mechanisms for oral absorption. It have particle size of 100–200 nm, zeta potential of $\pm 12$ to $\pm 18$ mV, and high encapsulation efficiency. TMC-Cys/insulin nanoparticles (TMC-Cys NP) showed a 2.1–4.7-fold increase in mucoadhesion compared to TMC/insulin nanoparticles (TMC NP), which might be partly attributed to disulfide formation between TMC-Cys and mucin as evidenced by DSC measurement. Compared to insulin solution and TMC NP, TMC-Cys NP induced increased insulin transport through rat intestine by 3.3–11.7 and 1.7–2.6 folds, promoted Caco-2 cell
internalization by 7.5–12.7 and 1.7–3.0 folds, and augmented uptake in Peyer’s patches by 14.7–20.9 and 1.7–5.0 folds, respectively.

**Eitan Kimmel et al., (2000)** prepared the Electron-dense nano-particles in aqueous suspension were administered by immersion into the epidermis of fish using ultrasound in the therapeutic range. Enhanced permeability of the tissues to the particles was achieved by acoustic cavitation, which induced a controlled level of necrosis in the outer cell layers, and by non-cavitational exposures, which widened intercellular spaces of non-necrosed tissue in deeper regions of the epidermis. While cavitation-induced perforation was necessary for particles to penetrate into the tissues, non-cavitational exposures during immersions increased the particle flux towards the skin surface, as well as the diffusion rate of the particles within the epidermis and their depth of penetration.

**Frank Stracke1 et al., (2006)** introduced the Multiphoton microscopy of a dually fluorescence-labeled model system used to study the release, accumulation and penetration properties of drugs released from nanoscale carrier particles in dermal administration.

**Fude Cui et al., (2006)** Biodegradable nanoparticles loaded with insulin–phospholipid complex were prepared by a novel reverse micelle–solvent evaporation method, in which soybean phosphatidylcholine (SPC) was employed to improve the liposolubility of insulin, and biodegradable polymers as carrier materials to control drug release. Spherical particles of 200 nm mean diameter and a narrow size distribution were obtained under optimal conditions. The drug entrapment efficiency was up to 90%. Intra gastric administration of the 20 IU/kg nanoparticles reduced fasting plasma glucose levels to 57.4% within the first 8 h of administration and this
continued for 12 h. PK/PD analysis indicated that 7.7% of oral bioavailability relative to subcutaneous injection was obtained.

Gary Adams et al., (2005) investigated the normal physiological conditions; euglycaemia is maintained principally by the homeostatic balance of insulin and glucagon which are secreted from the pancreas. Clinical manifestations of diabetes, which arise from the metabolic disturbances vary between individuals but are often a serious threat to quality and length of life. Pancreas transplantation (Tx) and islet modifications are methods used to restore endogenous insulin secretion in insulin-dependent diabetic patients. In order for this to be achieved successfully, however, some of the problems such as hyperglycemia states (>150 mg/dl), which may harm pancreatic graft beta cells, immuno rejection, the effects of immunosuppression, This review will show the recent development in the use of pancreatic islets and their modification in a quest to halt the aberrations seen in diabetes mellitus.

Gregor Cevc et al., (2010) reviewed the essential to maintain this protective barrier even after breaching skin surface for purposes of transdermal drug delivery to cope with cutaneous microbiota. If properly designed and applied, such self-regulating, ultra-adaptable, and stable hetero-aggregates can open spontaneously and carry drugs through ≤109cm−2 cutaneous pores in the primary skin barrier and minimise cutaneous drug clearance; this permits deep/targeted deposition and prolonged action of the carrier-transported drugs. Particulates and delineating their molecular interactions within biological systems.

Guang-Hui Ma et al., (2008) prepared the uniform sized nanoparticles by a facile method combining emulsion-solvent removal and premix membrane emulsification for the first time. Several factors played key roles to obtain uniform-sized PLA nanoparticles, including type of organic solvent, the volume ratio of oil phase and
external water phase, pore size of the microporous membrane and transmembrane pressure. The coefficient of variation (CV) value of PLA nanoparticles could be controlled below 16.9% under an optimum condition. The novel method also has the advantages of high productivity, simplicity and easy scale-up.

**Henkin et al., (2010)** reviewed the intrapulmonary administration of insulin was made to understand the physiological basis for its use, its efficacy in controlling hyperglycemia, its side effects and a comparison of its efficacy with other delivery methods. Many studies were performed to optimize each of these factors using several delivery systems to enhance pulmonary absorption. Availability was about 80% of subcutaneous administration with peak activity within 40– 60 min of administration. Intranasal insulin delivery faces a smaller surface area (w180 cm2) with quite different absorption characteristics in nasal epithelium and its associated vasculature.

**Huabing Chen et al., (2008)** worked on hydrogel-thickened nanoemulsion system (HTN) for topical delivery of active molecules. HTN was prepared to deliver an oily mixture of 5% camphor, 5% menthol and 5% methyl salicylate for topical therapy of arthritis, minor joint and muscle pain using soybean oil as the oil phase, soybean lecithin, Tween 80 and poloxamer 407 as the surfactants, propylene glycol as the cosurfactant, carbomer 940 as a thickening agent. The permeation rates of camphor, menthol and methyl salicylate from the optimal HTN formulation were 138.0±6.5, 63.6±3.3, 53.8±3.2 g cm−2 h−1 and showed the significant advantages over the control gel.

**Huabing Chen, et, al., (2009)** proved the transdermal delivery of insulin remains a significant challenge due to low permeation rates at therapeutically useful rates. They report unilamellar nanovesicles with membrane thickness of 3–5 nm and entrapment efficiency of 89.05±0.91%, which can be driven by iontophoresis for enhancing
Transdermal delivery of insulin through microneedle-induced skin microchannels. The permeation rates of insulin from positive nanovesicles driven by iontophoresis through skins with microneedle-induced microchannels were 713.3 times higher than that of its passive diffusion. The in vivo studies show that the blood glucose levels of diabetic rats induced by the positive nanovesicles driven by iontophoresis through skins with microneedle induced microchannels are 33.3% and 28.3% of the initial levels at 4 and 6 h, which are comparable to those induced by subcutaneous injection of insulin.

Jia-You Fang et al., (2006) developed and evaluate liposomal formulations encapsulating tea catechins, which possess antioxidant and chemo preventive activities. Incorporation of anionic surfactants such as deoxycholic acid (DA) and dicetyl phosphate (DP) in the liposomes in the presence of 15% ethanol increased the (+)-catechin permeation by five to seven-fold as compared to the control. Intercellular spaces within the stratum corneum but not shunt routes are the major pathways for catechin delivery from liposomes. (+)-Catechin and (−)-epicatechin are isomers which showed similar encapsulation efficiencies and skin permeation in liposomes. (−)-Epigallocatechin-3-gallate showed the highest encapsulation rate and in vivo skin deposition level in liposomes among all catechins tested.

Jia-You Fang et al., (2008) developed the Solid lipid nanoparticles (SLN) by using Precirol ATO 5 as the solid core of the particles for topical psoralen delivery. Nanostructured lipid carriers (NLC) consisting of Precirol and squalene, a liquid lipid, were also prepared for comparison. SLN and NLC showed respective mean particle sizes of 300 and 200 nm, respectively. The in vitro permeation results showed that NLC-Tw increased the 8-MOP flux 2.8 times over that of a conventional emulsion.
Hyperproliferative or psoriasis-like skin produced by repeated strippings in the dorsal skin of nude mouse was also used as a permeation barrier.

**Jie Liu et al., (2007)** prepared the isotretinoin-loaded SLN by using PRECIROL ATO 5 as the lipid Tween 80 and soybean lecithin were used as the surfactants to stabilize SLN. The hot homogenization method was performed to prepare the drug-loaded SLN. All the formulations had high entrapment efficiency ranging from 80% to 100. The in vitro permeation data showed that all the IT-SLN formulations can avoid the systemic uptake of isotretinoin in skins; however the control tincture had a permeation rate of $0.76\pm0.30\; g\; cm^{-2}\; h^{-1}$ through skins. The IT-SLN consisting of 3.0% PRECIROL ATO 5, 4.0% soybean lecithin and 4.5% Tween 80 could significantly increased the accumulative uptake of isotretinoin in skin and showed a significantly enhanced skin targeting effect.

**Jie Liu et al., (2008)** developed an alternative for non-invasive systemic delivery of therapeutic agents. A novel nebulizer-compatible solid lipid nanoparticles (SLNs) for pulmonary drug delivery of insulin were developed by reverse micelle-double emulsion method. The influences of the amount of sodium cholate (SC) and soybean phosphatidylcholine (SPC) on the deposition properties of the nanoparticles were investigated. Fasting plasma glucose level was reduced to 39.41% and insulin level was increased to approximately 170 _IU/mL 4 h after pulmonary administration of 20 IU/kg Ins–SLNs. A pharmacological bioavailability of 24.33% and a relative bioavailability of 22.33% were obtained using subcutaneous injection as a reference. Incorporating fluorescent-labelled insulin into SLNs, we found that the SLNs were effectively and homogeneously distributed in the lung alveoli.

**Joke A. Bouwstra et al., (2005)** concluded the transdermal and dermal drug delivery is problematic, Because the skin, as a natural barrier, has a very low permeation rate.
Therefore several methods have been assessed to increase this rate locally and temporarily. One approach is the use of vesicle formulations. In this effectiveness of conventional and deformable vesicles as drug delivery systems as well as their possible mode of action as permeation enhancers or transdermal drug carriers was discussed.

**Juergen Lademann et al., (2007)** investigated the penetration and storage behavior of dye-containing nanoparticles (diameter 320 nm) into the hair follicles. The results were compared to the findings obtained with the same amount of dye in the non-particle form. In the first part of the experiments, the penetration of the dye into the hair follicles was investigated in vitro on porcine skin, which is an appropriate model for human tissue. It was found that the nanoparticles penetrate much deeper into the hair follicles than the dye in the non-particle form, if a massage had been applied. Without massage, similar results were obtained for both formulations. Subsequently, the storage behavior of both formulations in the hair follicles was analyzed in vivo on human skin by differential stripping. Using the same application protocol, the nanoparticles were stored in the hair follicles up to 10 days, while the non-particle form could be detected only up to 4 days. Taking into consideration the surface structure of the hair follicles, it was assumed that the movement of the hairs may act as a pumping mechanism pushing the nanoparticles deep into the hair follicles.

**Kai Shi et al., (2006)** prepared insulin solid lipid nanoparticles by anhydrous co-solvent lyophilization method. In addition, the hypoglycemic effects were evaluated in normal rats to determine if the production process for the phospholipids complex might destroy or change the bioactivity of insulin. The liposolubility in the hydrophobic organic solvent, dichloromethane, demonstrated the improved lipophilicity of insulin in the phospholipid complex. The low ionic strength and pH
close to the isoelectric point of insulin (pI 5.3) will increase the binding stability of the complex. Moreover, the bioactivity was maintained when insulin was complexed with phospholipid, and the production process of the complex did not change or destroy the molecular structure of the insulin inside the complex.

**Karsten Mader et al., (2001)** reviewed the Solid lipid nanoparticles (SLN) have attracted increasing attention during recent years. His overview about the selection of the ingredients, different ways of SLN production and SLN applications. Aspects of SLN stability and possibilities of SLN stabilization by lyophilization and spray drying are discussed. Special attention is paid to the relation between drug incorporation and the complexity of SLN dispersions, which includes the presence of alternative colloidal structures (liposomes, micelles, drug nanosuspensions, mixed micelles, liquid crystals) and the physical state of the lipid (supercooled melts, different lipid modifications).

**Kesavan Bhaskar et al., (2009)** prepared the aqueous dispersions of flurbiprofen solid lipid nanoparticles (FLUSLN) and flurbiprofen nanostructured lipid carriers (FLUNLC) by hot homogenization followed by sonication technique and then incorporated into the freshly prepared hydrogels for transdermal delivery. They are characterized for particle size, for all the formulations, more than 50% of the particles were below 300 nm. The bioavailability of flurbiprofen with reference to oral administration was found to increase by 4.4 times when gel formulations were applied. The SLN and NLC dispersions and gels enriched with SLN and NLC possessed a sustained drug release over a period of 24 h but the sustained effect was more pronounced with the SLN and NLC gel.

**Khemariya et al.,(2010)** Designed the Solid lipid nanoparticles for Nateglinide by modified solvent injection method. Spherical SLNPs with an average particle size of
~173 nm were formulated. Delivery of Nateglinide by SLNPs led to a significantly higher accumulation by the endothelial cell monolayer as compared to the drug in aqueous solution. In vitro release kinetics based on a dialysis method demonstrated that Nateglinide was released in a prolonged fashion for 24 h. Both free and encapsulated drug reduced the time spent on the blocks in the bar test, although the action of encapsulated Nateglinide was more rapid in onset and prolonged than free drug.

**Lehr et al., (2010)** formulated the nanoparticles by using two different propyl-starch derivatives PS-1 and PS1.45 with high degrees of substitution: 1.05 and 1.45 respectively. A simple o/w emulsion diffusion technique, avoiding the use of hazardous solvents such as dichloromethane or dimethyl sulfoxide, was chosen to formulate nanoparticles with both polymers, producing the PS-1 and PS-1.45 nanoparticles. Finally, the potential use of these nanoparticles as transdermal drug delivery systems was also tested, displaying a clear enhancer effect for flufenamic acid.

**Lifeng Qi et al., (2004)** evaluated the in vitro antibacterial activity of chitosan nanoparticles and copper-loaded nanoparticles against various microorganisms. Chitosan nanoparticles were prepared based on the ionic gelation of chitosan with tripolyphosphate anions. Copper ions were adsorbed onto the chitosan nanoparticles mainly by ion-exchange resins and surface chelation to form copper-loaded nanoparticles. The MIC values were less than 0.25 lg/mL, and the MBC values of nanoparticles reached 1 lg/mL. AFM revealed that the exposure of S. choleraesuis to the Chitosan nanoparticles led to the disruption of cell membranes and the leakage of cytoplasm.
Ma et al., (2006) developed the insulin nano-aggregates with sizes of 100–230 nm were prepared by the salting out method with NaCl and encapsulated via the layer-by-layer (LbL) adsorption to provide the insulin nanoparticles shelled with two oppositely charged polyelectrolytes. Poly(l-malic acid) (PMA) and water-soluble chitosan (WSC) as the weak polyelectrolytes with good biodegradability and biocompatibility in vivo were chosen to be the encapsulating materials of the LbL adsorption. After eight adsorption cycles of the polyelectrolytes on the insulin nano-aggregates, the insulin–polyelectrolyte nanoparticles with the sizes of 100–250 nm were obtained with about 20% insulin loss. The insulin release from the nanoparticles was mostly pH-dependent owing to sensitivity of the weak polyelectrolytes to pH. Insulin was hardly released from the nanoparticles in a medium at pH 4–5 while it could be released at pH 7.4, corresponding to the pH of the human blood and the body fluid.

Manish Mishra et al., (2008) About 150 million people suffer from diabetes in the world and it has been predicted that this number will be doubled within 15 years. Type 2 diabetes accounts for about 85% of all cases with diabetes. Type 2 diabetes is considered a paradigm for a multi factorial polygenic disease where common variations in several genes interact to cause the disease when exposed to the affluent environment of too much food and too little exercise. Recently many scientists focused their research on nanomedicine and nanodiagnostics for many diseases, like diabetes, cancer, spinal card injury etc. For the scientists to synthesize the nanomedicine for diabetics is on the top priority to reduce the cost and pain of the patients. This article was attempted to illustrate the diabetes and the use of nanomaterials for benefit of diabetic patients.
María J. Alonso et al., (2001) studied the potential of chitosan (CS) nanoparticles as a new vehicle for the improvement of the delivery of drugs to the ocular mucosa was investigated. Cyclosporin A (CyA) was chosen as a model compound because of its potential usefulness for the treatment of these local diseases. These nanoparticles had a mean size of 293 nm, a zeta potential of +37 mV and high CyA association efficiency and loading (73 and 9%, respectively). In vitro release studies, performed under sink conditions, revealed a fast release during the first hour followed by a more gradual drug release during a 24-h period. In vivo experiments showed that, following topical instillation of CyA-loaded CS nanoparticles to rabbits, it was possible to achieve therapeutic concentrations in external ocular tissues during at least 48 h while maintaining negligible or undetectable CyA levels in inner ocular structures, blood and plasma.

Martin J. King et al., (2002) developed a novel transdermal lipid-based system (Biphasix) suitable for macromolecule delivery across the skin. A decrease in blood glucose of 43.7 from 63.8% was observed compared with initial blood glucose levels. The duration of the response was 51.5 from 63.7 h. Serum insulin after application of the transdermal Biphasix-insulin patch was 20.08 from 65.44 mIU/mL during the steady state, which was not statistically different from the insulin levels obtained 2 h after subcutaneous injection of 1 mg of recombinant human insulin solution. Insulin bioavailability from the transdermal Biphasix-insulin patches was 21.5 from 66.9% based on serum insulin and 39.5 from 68.5% based on the pharmacodynamic blood glucose-lowering effects. The Biphasix system successfully delivered insulin transdermally, as evidenced by a significant sustained decrease in blood glucose in diabetic rats, with a corresponding increase in serum insulin. These results support the feasibility of developing a transdermal insulin patch for human applications.
Megumu Higaki et al., (2006) evaluated the pharmacokinetic and pharmacodynamic effects of a transdermally delivered insulin using novel CaCO3-nanoparticles in normal mice and those with diabetes. CaCO3-nanoparticles encapsulating insulin (nanoinsulin) were transdermally applied to the back skin of normal ddY mice and dB/db and kkAy mice with diabetes after fasting for 1 h. Maximum serum insulin was 67.1 from 25.9 IU/mL at 4 h with 200 mg of transdermal nanoinsulin in ddY mice, whereas that after subcutaneous injection of 3 mg of monomer insulin was 46.2 from 20.9 IU/mL at 20 min. Insulin bioavailability until 6 h with transdermal nano insulin in ddY mice was 0.9% based on serum insulin level and 2.0% on pharmacodynamic blood glucose-lowering effects.

Michael S. Roberts et al., (2011) reviewed that, Skin is a widely used route of delivery for local and systemic drugs and is potentially a route for their delivery as nanoparticles. The skin provides a natural physical barrier against particle penetration, but there are opportunities to deliver therapeutic nanoparticles, especially in diseased skin and to the openings of hair follicles. Nanoparticles in 100 nm in diameter are unlikely to penetrate through the stratum corneum into viable human skin but will accumulate in the hair follicle openings, especially after massage. However, significant uptake does occur after damage and in certain diseased skin. Current chemistry limits both atom by atom construction of complex

Ming-Guang Li et al., (2007) investigated the distribution, transition, bioadhesion and release behaviors of insulin loaded pH-sensitive nanoparticles in the gut of rats, as well as the effects of viscosity agent on them. The release profiles in the gut were plotted by the percentages of FITC-insulin released versus time. FITC-insulin nanoparticle aqueous dispersion showed similar stomach but lower intestine empty rates, and enhanced intestinal mucosa adhesion in comparison with FITC insulin
solution. Addition of the HPMC reduced the stomach and intestine empty rates, enhanced the adhesion of FITC-insulin to the intestine mucosa. Addition of HPMC was prolonged the release half-life from 0.77 to 1.51 h.

Mohammad Reza Avadi et al., (2010) developed the nanoparticulate system based on ionic gelation between chitosan and Arabic gum for loading of insulin. Increased solubility of chitosan in acidic medium and better swelling of Arabic gum chains at pH N6.5 resulted in lower insulin release of nanoparticles at pH 6.5 in comparison with that of the other pH mediums. The values of the exponent n were 0.49 and 0.82 for formulations F8 and F5, respectively, indicating a non-Fickian transport. This suggests that release is possibly controlled by diffusion or relaxation of the polymer chains.

Monika Schäfer-Korting et al., (2007) studied the Particulate carrier systems may mean an option to improve dermal penetration. Since epidermal lipids are found in high amounts within the penetration barrier, lipid carriers attaching themselves to the skin surface and allowing lipid exchange between the outermost layers of the stratum corneum and the carrier appear promising. He described the potential of these carrier systems and compare the dermal uptake from SLN and NLC to the one of alternative vehicle systems. A special focus is upon the interactions of active ingredients and the lipid matrix as well as the quantification of dermal penetration.

Morteza Rafiee-Tehrani et al., (2008) investigated the insulin nanoparticles by using chitosan (CS), triethylchitosan (TEC), and dimethyl-ethylchitosan (DMEC, a new quaternized derivative of CS). In vitro release studies showed a relatively small burst effect at the beginning and then a sustained release characteristic for 5 hours.

Mounir S. Mesiha et al., (2005) Dispersed of insulin poly (isobutylcyanoacrylate) nanoparticles were obtained by anionic in situ polymerization using aqueous pluronic
acid solution. Results showed a decrease in particle size diameter by increasing the pluronic acid concentration. Nanoparticles prepared in the presence of 2.5% pluronic acid resulted in particles of 85 nm average diameter and 59% intraparticular insulin load without the use of the oily. In vivo testing was performed on streptozocin induced diabetic rats. The subcutaneous injection of insulin nanoparticles was able to prolong its duration of hypoglycemic effect from 6 to 72 h. Effective oral absorption of the entrapped insulin was significantly better \((p < 0.01)\) when compared with non-encapsulated insulin or the control experiments.

**Mustafa M.A. Elsayed et al., (2007)** developed the lipid vesicles as carriers for skin delivery of drugs. Despite this long history of intensive research, lipid vesicles are still considered as a controversial class of dermal and transdermal carriers. Accordingly, this article provides an overview of the development of lipid vesicles for skin delivery of drugs, with special emphasis on recent advances in this field, including the development of deformable liposomes and ethosomes.

**Nathalie Ubrich et al.,(2007)** prepared the Nanoparticles by biodegradable polyester (poly(ε-caprolactone)) and a polycationic non-biodegradable acrylic polymer have been used as a drug carrier for oral administration of insulin. When administered orally by force-feeding to diabetic rats, insulin nanoparticles decreased fasted glycemia in a dose dependant manner with a maximal effect observed with 100 IU/kg. These insulin nanoparticles also increased serum insulin levels and improved the glycemic response to an oral glucose challenge for a prolonged period of time. FITC-Insulin-loaded nanoparticles strongly adhered to the intestinal mucosa and labeled insulin; either released and/or still inside nanoparticles, was mainly taken up by the Peyer's patches.
Nicholas A. Peppas et al., (2006) investigated the intelligent therapeutics or “smart drug delivery” calls for the design of the newest generation of sensitive materials based on molecular recognition. Synthetic networks that can be designed to recognize and bind biologically significant molecules are of great importance and influence a number of emerging technologies. These synthetic materials can be used as unique systems or incorporated into existing drug delivery technologies that can aid in the removal or delivery of biomolecules and restore the natural profiles of compounds in Niccolosi et al., (2008) compared the transdermal application of a nano-sized emulsion versus a micron-sized emulsion preparation of delta tocopherol as it relates to particle size and bioavailability. The particle size of the micron-sized emulsion preparation was 2788 nm compared to 65 nm for the nano-sized emulsion formulation. Two hours post-application, hamsters that were applied the nano-sized emulsion had a 36-fold significant increase of plasma delta tocopherol, whereas hamsters that were applied the micron-sized emulsion only had a 9-fold significant increase, compared to baseline, respectively.

Omathanu Pillai et al., (2003) studied the gel formulation of insulin which was formulated by using poloxamer 407 and was evaluated by ex vivo and in vivo skin permeation studies in rat with chemical enhancer and/or iontophoresis. Menthone pre-treatment resulted in rapid attainment of peak PIC, but the reduction in PGL was less than other treatment groups. There was no direct relation between PIC and PGL and is attributed to the fact that the action of insulin in mediated by a cascade of cellular mechanisms, before a reduction in PGL is observed. However, iontophoresis either alone or in combination with linoleic acid produced a reduction in PGL to the extent of 36–40%.
Patel et al., (2009) reviewed the nanoparticles have gained considerable attention in recent years as one of the most promising drug delivery systems owing to their unique potentials via combining the different characteristics of hydrophilicity and hydrophobicity with a nanoparticle (e.g., very small size). Several polymeric nanoparticulate systems have been prepared and characterized in recent years, based on both natural and synthetic polymers, each with its own advantages and drawbacks. Among the natural polymers, chitosan has been studied extensively for preparation of nanoparticles. Chitosan nanoparticles have been reported with different characteristics with respect to drug delivery. His review presented various types of chitosan based nanoparticles in drug delivery.

Pavan Kumar et al., (2010) reviewed the transdermal drug delivery system is a type of convenient drug delivery system where drug goes to the systemic circulation through the protective barrier i.e. Skin. Ethosomes have been found to be much more efficient in delivering drug to the skin; Ethosomes are the non invasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. For optimal skin delivery, drug should be efficiently entrapped within ethosomal vesicles. Ethosomal drug delivery system is a new state of the art technique and easier to prepare in addition to safety and efficacy. Ethosomes have become a area of research interest, because of its enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency.

Priscilla Vanessa Finotelli et al., (2010) investigate the potential of alginate/chitosan beads containing magnetite nanoparticles as a drug delivery system. The insulin beads were prepared by dripping a solution of sodium alginate containing insulin into a CaCl$_2$ solution. Magnetite nanoparticles of 5 nm mean size were synthesized inside the alginate egg-box structure by co-precipitation of Fe(III) and Fe(II) in the presence
of NH₄OH. Quantitative analysis revealed that insulin encapsulation depends on the initial protein content and 35% of insulin was entrapped by alginate beads for a protein concentration of 10 wt%. It was verified that approximately 50% of the insulin was released to Milli-Q water in 800 h release experiments. The application of oscillating magnetic field increased threefold the insulin release.

Qiang Zhang et al., (2004) demonstrated the increased concentrations of chitosan up to 1.5% (w/v) caused an increase in the permeability of insulin across the nasal mucosa. Insulin given intranasally in hypo- or hyperosmotic formulation showed a higher hypoglycemic effect than insulin delivered in isoosmotic formulation. A formulation containing both 1% chitosan and 0.1% ethylene diamine tetra acetic acid (EDTA), 5% polysorbate 80 (Tween 80) or 1.2% β-cyclodextrin (βCD) did not lead to a higher Fr than insulin formulated with 1% chitosan alone. The formulation containing both 5% hydroxypropyl-β-cyclodextrin (HPβ-CD) and 1% chitosan was more effective at reducing blood glucose levels than the formulation containing 5% HP-β-CD or 1% chitosan alone.

Qiang Zhang et al.,(2007) investigated the distribution, transition, bioadhesion and release behaviors of insulin loaded pH-sensitive nanoparticles in the gut of rats, as well as the effects of viscosity agent on them. Insulin was labeled with fluorescein isothiocyanate (FITC). The FITC-insulin solution and FITC-insulin nanoparticle aqueous dispersions with or without hydro propyl methylcellulose (HPMC, 0.2%, 0.4%, or 0.8% (w/v)) were orally administered to rats, respectively. The release of FITC-insulin from nanoparticles in the gut showed an S-shape profile, and addition of HPMC prolonged the release half-life from 0.77 to 1.51 h.

Rabinarayan Parhi et al., (2010) reviewed the Solid lipid nanoparticles (SLNs) for an alternative carrier system to traditional colloidal carriers, such as emulsions,
liposomes and polymeric micro and nanoparticles. A number of administration routes such as topical, oral, parenteral, nasal and pulmonary have been proposed for the delivery of SLNs. He reviewed various production techniques for SLNs including their advantages and disadvantages, drug incorporation, loading capacity with the factors affecting drug incorporation and loading capacity and drug release, especially emphasizing on mechanism drug release.

Rachna Rastogi et al., (2009) prepared the self-assembled vesicles of poly(caprolactone)–poly(ethylene glycol)–poly(caprolactone) co-polymer of 122±20nm were prepared by solvent evaporation method. Permeation studies of fluorescently labelled vesicles in cadaver epidermis confirmed their presence across the stratum corneum within 2 hours of application and accumulation in the deeper layers thereafter. The flexible nature of these nanosystems makes them an efficient alternative to liposomes for targeting melanomas and basal cell carcinomas.

Rafael A. Shimkunas et al.,(2009) Enhanced the specificity in drug delivery aims to improve upon systemic elution methods by locally concentrating therapeutic agents and reducing negative side effects. In this work, bovine insulin was non-covalently bound to detonated nano diamonds via physical adsorption in an aqueous solution and demonstrated pH-dependent desorption in alkaline environments of sodium hydroxide. Nanodiamonds combined with insulin at a 4:1 ratio showed 79.8 to 4.3% adsorption and 31.3 to 1.6% desorption in pH-neutral and alkaline solutions, respectively. Additionally, a 5-day desorption assay in NaOH (pH 10.5) and neutral solution resulted in 45.8 _ 3.8% and 2.2 _ 1.2% desorption, respectively. For applications in sustained drug delivery and therapy developed a therapeutic protein–ND complex with demonstrated tunable release and preserved activity.
Ravi Kumar et al., (2010) developed the Insulin loaded microemulsions by adopting a low shear reverse micellar approach using didoeyl dimethyl ammonium bromide (DMAB) as the surfactant, propylene glycol (PG) as the co-surfactant, triacetin (TA) as the oil phase and insulin solution as the aqueous phase. The microemulsions displayed a 10-fold enhancement in bioavailability compared with plain insulin solution administered per oral in healthy rats. The short-term in vivo efficacy in STZ induced diabetic rats provided the proof of concept by a modest glucose reduction at a dose of 20 IU/kg. Together this preliminary data indicate the promise of microemulsions for oral delivery of insulin.

Ronald J. Neufeld et al., (2010) developed a topical delivery vehicle that is capable of releasing therapeutic levels of bioactive insulin for several weeks with the potential to stimulate and sustain healing. By encapsulating the crystalline form of insulin within poly(d,l-lactide-co-glycolide) microspheres, we succeeded in stabilizing and then releasing bioactive insulin for up to 25 days. To determine whether the slow release insulin could stimulate keratinocyte migration, wounding was simulated by scratching confluent cultures of human keratinocytes (HaCaT). Coverage of the scratch “wounds” was significantly faster in the presence of insulin released from microspheres than in the insulin-free control. Extended and sustained topical delivery of active insulin from a stable protein crystal-based reservoir shows promise in promoting tissue healing.

Sadeghi et al., (2008) studied the different quaternized derivatives with degree of substitution of approximately 50 ± 5% were synthesized and their effect on the permeability of insulin across intestinal Caco-2 monolayers was studied and compared with chitosan both in free-soluble form and in nanoparticulate systems. In accordance with these results, the insulin loaded nanoparticles showed much less
permeation across the Caco-2 cell monolayer in comparison to the free-soluble polymers. Mass balance transport studies revealed that a substantial amount of the nanoparticles has been entrapped into the Caco-2 monolayer or attached to the cell surface. It can thus be stated that while free-soluble polymers can reversibly open the tight junctions and increase the permeation of insulin, the nanoparticles had basically only a low effect on the opening of the tight junction and the paracellular transport of insulin across the Caco-2 cell monolayer.

Sanjay K. Motwani et al., (2008) studied the mucoadhesive chitosan (CS)-sodium alginate (ALG) nanoparticles were investigated as a new vehicle for the prolonged topical ophthalmic delivery of antibiotic, gatifloxacin. He designed nanoparticles have average particle size from 205 to 572 nm (Polydispersity from 0.325 to 0.489) and zeta potential from 17.6 to 47.8 mV. Nanoparticles revealed a fast release during the first hour followed by a more gradual drug release during a 24-h period following a non-Fickian diffusion process.

Shaoyun Yu et al., (2004) investigated the nasal insulin delivery by in vitro and in vivo. The penetration of insulin through the mucosa of rabbit nasal septum was investigated by measuring the transmucosal flux in vitro, while the nasal absorption of insulin in vivo was assessed by the efficiency in lowering the blood glucose levels in normal rats. It was demonstrated that increasing concentrations of chitosan up to 1.5% (w/v) caused an increase in the permeability of insulin across the nasal mucosa. The studies indicated that chitosan concentrations, osmolarity, medium and absorption enhancers in chitosan solution have significant effect on the insulin nasal delivery.

Sharma et al., (2010) Insulin loaded microemulsions were developed adopting a low shear reverse micellar approach using didodecyl dimethyl ammonium bromide (DMAB) as the surfactant, propylene glycol (PG) as the co-surfactant, triacetin (TA)
as the oil phase and insulin solution as the aqueous phase. The droplet sizes of the microemulsions were 161.7 to 24.7 nm with PDI of 0.447 to 0.076 and insulin entrapment of 85%. The short-term in vivo efficacy in STZ induced diabetic rats provided the proof of concept by a modest glucose reduction at a dose of 20 IU/kg.  

Sheree E. Cross et al., (2007) designed the topically applied solid nanoparticles can penetrate the stratum corneum and access the underlying viable epidermis and the rest of the body is a great potential safety concern. Therefore, human epidermal penetration of a novel, transparent, nanoparticulate zinc oxide sunscreen formulation was determined using Franz-type diffusion cells, 24-hour exposure and an electron microscopy to verify the location of nanoparticles in exposed membranes. Less than 0.03% of the applied zinc content penetrated the epidermis (not significantly more than the zinc detected in receptor phase following application of a placebo formulation). No particles could be detected in the lower stratum corneum or viable epidermis by electron microscopy, suggesting that minimal nanoparticle penetration occurs through the human epidermis.  

Simeonova et al., (2003) designed the Poly(butylcyanoacrylate) nanoparticles (PBCN) as a drug carrier of 5-fluorouracil (5FU) intended for topical treatment of skin lesions. The presence of 5FU (as saline solution, pH 10–11) in the polymerization medium affected the polymerization as well as the nanoparticle formation by influencing the initiation of the polymerization reaction. 5FU acted as an initiator in the anionic polymerization of n-butylcyanoacrylate monomer through its nucleophilic nitrogen centers. The results obtained by GPC, 1H NMR, and X-ray diffraction allude to a possible mechanism of cytostatic immobilization in the polymer matrix, with evidence for both free and bound forms of the drug.
Sion A. Coulman et al., (2009) investigated the assesses of physicochemical factors to the rate and extent of nanoparticle delivery through microchannels created in a biological tissue, the skin, by novel delivery technologies such as the microneedle array. Wet-etch microneedle array devices can be used to significantly enhance the intra/transdermal delivery of nanoparticle formulations.

Snow Stolnik et al., (2010) investigated the potential of chitosan nanoparticles by the ionic gelation with triplyphosphate (TPP), to open the cellular tight junctions and in doing so, improve the permeability of model macromolecules. Subsequently, a concentration of chitosan nanoparticles and solution exhibiting minimal toxicity was used to investigate the effect on TEER and macromolecular permeability across filter-cultured Calu-3 monolayer. Chitosan nanoparticles and solution were also tested for their effect on the distribution of the tight junction protein, zonula occludens-1 (ZO-1). Chitosan nanoparticles produced a sharp and reversible decrease in TEER and increased the permeability of two FITC-dextrans (FDs), FD4 (MW 4 kDa) and FD10 (MW 10 kDa), with effects of a similar magnitude to chitosan solution. Chitosan nanoparticles produced changes in ZO-1 distribution similar to chitosan solution, indicating a tight junction effect.

Stuart A. Jones et al., (2010) developed the colloidal carriers to administer therapeutic agents from semi-solid preparations adds an extra dimension to the already complex process of topical drug delivery. The mobility of nanoparticles influenced the delivery of a model drug when the carriers were suspended in a hyaluronic acid (HA) vehicle. TA was delivered into porcine skin regardless of the vehicle characteristics and this suggested that drug release from the LN was the rate limiting step in the delivery process and not the nanoparticle–vehicle–skin interactions.
Swarnlata Saraf et al., (2009) developed the glipizide (GPZ) loaded biodegradable nanoparticles by using a biodegradable polymer, poly (D,L-lactic-co-glycolic acid) (PLGA) as a sustained release carrier by modified emulsification solvent evaporation technique. The drug release pattern consisted of two phases releasing about 40% (within first 24 h) followed by a slow releasing phase (up to 90%) within next 48 h.

Tarl W. Prow et al., (2011) reviewed the potential delivery of microparticles and nanoparticles via skin. Most drug delivery particles were based a lipid carriers like SLN, nanoemulsion of around 300nm in diameter and it have more penetration. Less than 10nm range particles are unlikely penetrated through the stratum corneum into viable human skin but it was accumulate in the hair follicles opening, especially after massage.

Tejraj M. Aminabhavi et al., (2004) reviewed the considerable research efforts have been directed towards developing safe and efficient chitosan-based particulate drug delivery systems. Methods of their preparation, drug loading, release characteristics, and applications are covered. Chemically modified chitosan or its derivatives used in drug delivery research are discussed critically to evaluate the usefulness of these systems in delivering the bioactive molecules. From a literature survey, it is realized that research activities on chitosan micro/nanoparticulate systems containing various drugs for different therapeutic applications have increased at the rapid rate.

Tin W. Wong et al., (2009) reviewed the Chitosan and its derivatives or salts have been widely investigated as functional excipients of delivering insulin via oral, nasal and transdermal routes. The overview of various recent patented strategies on non-injection insulin delivery denotes the significance of chitosan for its mucoadhesive and able to protect the insulin from enzymatic degradation, prolong the retention time.
of insulin, as well as, open the inter-epithelial tight junction to facilitate systemic insulin transport. The chitosan is modifiable chemically to produce water-soluble low molecular weight polymer which renders insulin able to be processed under mild conditions, and sulphated chitosan which markedly opens the paracellular channels for insulin transport. Combination of chitosan and fatty acid as hydrophobic nanoparticles promotes the insulin absorption via lymphoid tissue.

Trotta et al., (2005) designed the solid lipid insulin-loaded micro-particles by the solvent-in-water emulsion–diffusion technique, using isobutyric acid as solvent phase, glyceryl monostearate or cetyl palmitate as lipid, soya lecithin and taurodeoxycholate as emulsifiers. Isobutyric acid, a partially water-miscible solvent with low toxicity, was used due to its high insulin-solubilization capacity.

Varsha B. Pokharkar et al., (2006) studied the Vitamin E acetate was encapsulated into liposome for improving its topical delivery. Amount of phospholipid (PL) and cholesterol (CH) were taken at three different levels and liposomes were prepared using ethanol injection method. Prepared liposomal dispersion (50 mg PL: 6 mg CH) showed seven-fold increase in drug deposition compared to control (plain drug dispersion). Gel formulation demonstrated six-fold and four-fold increase in drug deposition compared to control gel and marketed cream, respectively. Varsha B. Pokharkar et al.,(2006) studied the Vitamin E acetate was encapsulated into liposome for improving its topical delivery. However preparation of liposomes is very difficult due to number of formulation variables involved therein. In this work systematic statistical study for the formulation of liposomes for topical delivery of Vitamin E using the factorial design approach was undertaken. Gel formulation demonstrated six-fold and four-fold increase in drug deposition compared to control gel and marketed cream, respectively. Liposome dispersion and gel formulation were
found to be stable for 3 months. Factorial design was found to be well suited to identify the key variables affecting drug deposition. Improved drug deposition from liposomal preparations demonstrates its potential for dermal delivery.

**Vivek Kumar Gupta et al., (2010)** reviewed the Nanoparticle formulations have many advantages over traditional dosage forms, such as enhanced dissolution properties and the potential for intracellular drug delivery. Specifically, pure drug nanoparticles, polymeric nano-particles and polyelectrolyte complexes offer some encouraging results for delivering drugs to various organs and through various routes. Traditional techniques such as spray drying and grinding, and more recent advances in supercritical fluid extraction, precipitation, and double solvent evaporation have been employed to produce nanoparticle formulations for delivery of hydrophilic & hydrophobic drugs here, the benefits of nanoparticle formulations and current progress are compared in light of the practical encumbrances of producing formulations, and possible toxicological effects of these materials.

**Vjera Grabovac et al., (2008)** prepared a patch by mucoadhesive layer with water insoluble backing layer made of ethylcellulose and Eudragit. Drug release studies were performed in media mimicking stomach and intestinal fluids. For *in vivo* studies patch systems were administered orally to conscious non-diabetic rats. Compared with insulin aqueous solution. After the oral administration of the patch systems a decrease of glucose and increase of insulin blood levels were measured. The mucoadhesive layer, exhibiting a diameter of 2.5mm and a weight of 5 mg, comprised polycarbophil-cysteine conjugate (49%), bovine insulin (26%), glutathione (5%) and mannitol (20%). 74.8±4.8% of insulin was released from the delivery system over 6 h. Six hours after administration of the patch system mean maximum decrease of blood
glucose level of 31.6% of the initial value could be observed. Maximum insulin concentration in blood was 11.3±6.2 ng/mL and was reached 6 h after administration.

Wang Chun et al., (2007) studied the potential utility of water-soluble chitosan (WSC) as vehicles to load and deliver proteins. WSC nanoparticles (WSC NP) with various formations were prepared based on ionic gelation of WSC with penta sodium tripolyphosphate (TPP) anions. Blank and BSA-loaded WSC nanoparticles were examined and determined to have a spherical shape with diameters between 35—190 nm, and zeta potential between 35—42 mV. Introduction of poly ethylene glycol (PEG), BSA release was accelerated. Nanoparticle preparation from WSC with various deacetylation degrees (DDs) from 72.6% to 90% and MWs ranging from 3.5 to 15.8 kDa promoted loading efficiency and decreased the release rate. These results indicate that WSC nanoparticles are promising carriers for protein delivery.

Waree Tiyaboonchai et al., (2003) reviewed the Chitosan nanoparticles as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method, and providing versatile routes of administration. Their sub-micron size not only suitable for parenterally application, but also applicable for mucosal routes of administration, i.e., oral, nasal, and ocular mucosa, which are non-invasive route. The application for mucosal delivery also facilitated by chitosan absorption enhancing effect. Furthermore, chitosan nanoparticles also showed to be a good adjuvant for vaccines. Therefore, the objectives of this review are to summarize the available preparation techniques involved chitosan nanoparticles, the application of explored chitosan nanoparticles, and the mechanism of cell entry.

Waree Tiyaboonchai et al., (2003) developed an aqueous nanoparticle using oppositely charged polymers polyethyleneimine (PEI) and dextran sulfate (DS) with zinc as a stabilizer. Spherical particles of 250 nm mean diameter were produced under
optimal conditions which have a zeta potential of approximately +30mV. Up to 90% drug entrapment efficiency was observed when insulin was used as a model protein drug. Biological activity in steptozotocin-induced diabetic rats, however, exhibited a prolonged hypoglycemic effect. This system offers the following advantages: (1) ease of manufacturing under mild preparation conditions; (2) employment of completely aqueous processing conditions; (3) use of biocompatible polymers which can be prepared aseptically; (4) ability to control particle size; (5) a high level of drug entrapment and (6) an ability to preserve protein secondary structure and biological activity.

Xiang Yuan Xiong et al., (2007) studied the feasibility of using PLA-F127-PLA vesicles as oral delivery carrier for insulin. Both in vitro and in vivo release behavior of insulin loaded in PLA-F127-PLA vesicles were studied. A biphasic release behavior was observed for the in vitro release of insulin from PLAF127-29 vesicles. More importantly, it was found in the diabetic mice tests that the blood glucose concentration of oral insulin-loaded PLAF127-29 vesicles decreased from 18.5 to 5.3 mmol/L within 4.5 h and the minimum blood glucose concentration (about 4.5 mmol/L) was achieved after about 5 h. Furthermore, the blood glucose concentration was maintained at this level for at least an additional 18.5 h. These results proved that PLA-F127-PLA vesicles could be promising polymeric carriers for oral insulin delivery application due to their prolonged hypoglycemic effect.

Xiangliang Yang et al., (2006) constructed microemulsion-base hydrogel formulation for topical delivery of ibuprofen. Ethyl oleate (EO) was screened as the oil phase of microemulsions, due to a good solubilizing capacity of the microemulsion systems and excellent skin permeation rate of ibuprofen,. Various microemulsion formulations were prepared and the abilities of various microemulsions to deliver
ibuprofen through the skin were evaluated in vitro using Franz diffusion cells fitted with porcine skins. The in vitro permeation data showed that microemulsions increased the permeation rate of ibuprofen 5.72–30.0 times over the saturated solution.

**Xiangliang Yang et al., (2006)** studied the solid lipid nanoparticles as the topical carrier for epidermal targeting of podophyllotoxin (POD). The high pressure homogenization was employed to prepare drug-loaded solid lipid nanoparticles. P-SLN showed an average diameter of 73.4 nm and a zeta potential of -48.36 mV. The in vitro permeation study showed that P-SLN increased the accumulative amount of POD in porcine skin 3.48 times over 0.15% tincture. The penetration of POD from P-SLN seemed to follow two pathways along the stratum corneum and hair follicle route. The imaging revealed that P-SLN had a strong localization of POD within epidermis. The penetration of P-SLN with low particle size into stratum corneum along the skin surface and the consequent controlled release of POD might lead to the epidermal targeting. P-SLN provides a good epidermal targeting effect and may be a promising carrier for topical delivery of POD.

**Xuan Huang et.al., (2009)** Prepared low-molecular-weight chitosan (LMWC) by enzymatic degradation and ultrafiltration separation. LMWC nanoparticles with LMWC having 20 kDa weight average molecular weight (Mw) were then prepared by solvent evaporation method. The resultant nanoparticles were spherical with a narrow particle size distribution. LMWC nanoparticles loaded with insulin as a model drug were prepared. The average entrapment efficiency of insulin could reach up to 95.54%. The in vitro drug release profiles from the nanoparticles showed an initial burst of release in the first 2 h, followed by zero order release kinetics. In vivo pharmacodynamics of chitosan nanoparticles containing insulin showed that the
nanoparticles showed some hypoglycemic activity. Compared with an insulin solution, a relative bioavailability of

**Y. Chen et al., (2008)** aimed to prepare biodegradable hydrophobic particles by using poly (D,L-lactide-co-glycolide) (PLGA) by both solvent evaporation and solvent diffusion methods. Bovine insulin was chosen as a model peptide for formulation development and evaluation. By forming a complex between insulin and protamine, 50% incorporation of the model peptide in PLGA particles was achieved and a sustained release of insulin was observed over one week with improved stability of insulin in the PLGA matrix.

**Yan Chen et al., (2007)** examined the effect of charge ratio on the formation and properties of the chitosan (CS) dextran sulfate (DS) nanoparticles developed for the delivery of water-soluble small and large molecules, including proteins. Depending on the concentration and charge ratio of DS and CS, nanoparticles with varied size (≥244 nm) and zeta potential (−47.1-60 mV) were obtained. High entrapment efficiency (98%) was achieved for both R6G and BSA when the charge ratio of the 2 ionic polymers was greater than 1.12. The release of both R6G and BSA from nanoparticles was based on the ion-exchange mechanism. BSA showed much slower continuous release for up to 7 days while still maintaining its integrity for an extended period.

**Ying-zhe LI et al., (2008)** investigated the trypsin, used to enhance the transdermal delivery of insulin by applying its specific biochemical properties to react with the stratum corneum (SC) of skin. The *in vivo* hypoglycemic effects of bovine insulin with or without the trypsin pretreatment. Trypsin significantly increased the transdermal permeability of bovine insulin in pH 3.0 solution, but no effect was observed in pH 6.0 solution. The enhancement of trypsin was dependent on the concentration in the range of 0.5—2.5%. Furthermore, with trypsin pretreatment, the
plasma glucose level was reduced to less than 60% of the initial value after 8 h, but did not return to the initial value during an 8-h experiment. He concluded that trypsin would be effective as a biochemical enhancer for the transdermal delivery of peptide and protein drugs such as insulin.

**Zengshuan Ma et al.,(2005)** Studied the effects of formulation parameters on the in vivo pharmacological activity of the chitosan–insulin nanoparticles. Chitosan–insulin nanoparticles were prepared by ionotropic gelation at different pH and it was administered orally at insulin doses of 50 U/kg and/or 100 U/kg were effective at lowering the serum glucose level of streptozotocin-induced diabetic rats. The 100 U/kg-dose F5.3np sustained the serum glucose at pre-diabetic levels for at least 11 h. In comparison, F6.1np had a faster onset of action (2 h versus 10 h) but lower efficiency. The effectiveness of peroral F5.3np and F6.1np in lowering the serum glucose level of streptozotocin-induced diabetic rats was ascribed to the local effect of insulin in intestine. Confocal micrographs showed strong interaction between rat intestinal epithelium and chitosan nanoparticles 3 h post-oral administration.

**Zhirong Zhang et al., (2007)** developed the Solid lipid nanoparticles (SLNs) loaded with insulin-mixed micelles (Ins-MMs) by a novel reverse micelle-double emulsion method, in which sodium cholate (SC) and soybean phosphatidylcholine (SPC) were employed to improve the liposolubility of insulin, and the mixture of stearic acid and palmitic acid were employed to prepare insulin loaded solid lipid nanoparticles (Ins-MM-SLNs). Differential scanning calorimetry (DSC) of Ins-MM-SLN indicated no tendency of recrystallisation. The drug release behavior was studied by in situ and externally sink method and the release pattern of drug was found to follow Weibull and Higuchi equations. Results of stability evaluation showed a relatively long-term stability after storage at 4 °C for 6 months.