A REVIEW ON INSULIN LIPOSOMES

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Accepted on: 24-08-2012; Finalized on: 29-09-2012.

ABSTRACT

The liposomal drug delivery has received a lot of attention during the past 30 years as pharmaceutical carriers of great potential. In recent times, many new developments have been seen in the area of liposomal drugs from clinically approved products to new experimental applications, with gene delivery and cancer therapy still being the principal areas of interest. For advance successful development of this field, promising trends must be identified and exploited, although with a clear understanding of the limitations of these approaches.

Keywords: Liposomes, Drug delivery, Insulin.

INTRODUCTION

The real breakthrough developments in the area during the past 15 years have resulted in the approval of several liposomal drugs, and the appearance of many unique biomedical products and technologies involving liposomes. The interest in the field remains high almost 3,000 papers and more than 250 reviews on various aspects of liposomology were published in 2008 alone. Clearly, within the frame of a single review paper it is impossible to address all of the pertinent issues, but I will attempt to identify the most important trends in liposomology, as well as the most significant achievements and challenges. Nowadays, the treatment of diabetes is healing predominantly by way of technical improvement of insulin injection. However, the classical procedure of insulin injection is in conflict with natural system for the maintenance of glucose homeostasys as on subcutaneous injecting the hormone does not arrive immediately at the liver. What is more, this procedure causes psychologic stress provoking the emission of contrainsulin hormones, which aggravate the pathological course of metabolism. Diabetes mellitus is a group of metabolic diseases characterized by increase in blood glucose levels (hyperglycemia). Type-1 diabetes where there is total loss of activity of β-cells of pancreas necessitates the administration of exogenous insulin to maintain the normal glucose levels. On the other hand, the glucose levels fluctuate throughout the day as per the meals and stress, making it compulsory the multiple injections of insulin. The challenging part may be taking care of postprandial glucose levels and the maintenances of the basal levels of insulin. Insulin, produced in the islets of langerhans in the pancreas, is an anabolic polypeptide hormone that regulates carbohydrate metabolism. Apart from being the primary agent in carbohydrate homeostasis, it has effects on fat metabolism and it changes the liver’s activity in storing or releasing glucose and in processing blood lipids. Regular human insulin is less than ideal for postprandial glucose control. The slow onset of action necessitates administration 20–40 minutes before meals, putting patients at risk of pre-meal hypoglycemia if the meal is delayed. The duration of action extends beyond the action of endogenous insulin; therefore, the risk of hypoglycemia is increased. However, their rapid waning of activity places greater dependence on sufficient inter-prandial basal insulin to maintain blood glucose control. Of the insulin formulations used traditionally for basal insulin replacement, NPH insulin and Lente are intermediate-acting, with durations of action considerably less than 24 hours, and pronounced peaks in activity within a few hours of administration.

LIPOSOMES

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule (Fig.1). A sustained release of encapsulated drugs, in combination with a long circulation time, makes the liposomes useful as a controlled drug delivery system. Liposomes can be utilized as a controlled release carrier for proteins and peptides, controlling the permeability of the lipidosome membrane, and thus avoiding drug release, minimizes the side effects.

Figure 1: Schematic diagram of a liposome
Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule. Liposomes were first described by Bangham in 1965, while studying the nature of cell membranes. It was found that liposomes are formed spontaneously when phospholipids are dispersed into water. Liposomes can simply be portrayed as spherical vesicles consisting of one or more phospholipid bilayers surrounding an aqueous cavity. The drug molecules can either be encapsulated in aqueous space or intercalated into the lipid bilayers. The exact location of the drug will depend upon its physico-chemical characteristics and the composition of the lipids.

### Classification of liposomes

**On the basis of composition**

Liposomes are composed of natural and/or synthetic lipids (phospho- and sphingo-lipids), and may also contain other bilayer constituents such as cholesterol and hydrophilic polymer conjugated lipids. The liposomes can be classified in listed in table 1.

**Table 1:** Classification of liposomes based on their composition

<table>
<thead>
<tr>
<th>Type</th>
<th>Composition</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional liposomes (CL)</td>
<td>Neutral phospholipids and cholesterol</td>
<td>Subject to coated-pit endocytosis; contents ultimately delivered to lysosomes, if they do not diffuse from endosome; useful for RES targeting; short circulation half life; dose dependent PK</td>
</tr>
<tr>
<td>pH-sensitive liposomes</td>
<td>PE or DOPE with either CHEMS or OA</td>
<td>Subject to coated-pit endocytosis; at low pH, fuse with cell or endosome membranes and release their contents in cytoplasm; suitable for intracellular delivery of weak bases and macromolecules.</td>
</tr>
<tr>
<td>Charged liposomes</td>
<td>Generally SA/ODA for positive charge; DCP for negative charge</td>
<td>Possibly fuse with cell or endosome membranes; suitable for delivery of DNA, RNA, etc; structurally unstable; toxic at high doses</td>
</tr>
<tr>
<td>Long-Circulating Liposomes (LCL)</td>
<td>neutral high T, lipids, Chol, plus 5-10% of PEG-DSPE, GM1 or HPI</td>
<td>Hydrophilic surface coating; low opsonization and thus low rate of uptake by RES; long circulation half life (~ 40 hr); dose independent PK</td>
</tr>
<tr>
<td>Immuno-liposomes</td>
<td>CL or LCL with attached antibody or recognition sequence</td>
<td>Subject to receptor-mediated endocytosis; cell specific binding (targeting); can release contents extracellularly near the target tissue and drugs may diffuse through plasma membranes to produce their effects</td>
</tr>
</tbody>
</table>

**Liposome on the basis of size**

On the basis of their size and number of bilayers, liposomes can also be classified into one of three categories: The size and characteristics of these types of liposomes are listed in table 2.

**Table 2:** Classification of liposomes based on size

<table>
<thead>
<tr>
<th>Type</th>
<th>Usual size</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLV (Multilamellar vesicles)</td>
<td>&gt;0.1 µm</td>
<td>More than one bilayer; greater encapsulation of lipophilic drugs; mechanically stable on long term storage; rapidly cleared by RES</td>
</tr>
<tr>
<td>LUV (Large unilamellar vesicles)</td>
<td>&gt;0.1 µm</td>
<td>Single bilayer; useful for hydrophilic drugs; high capture of macromolecules; rapidly cleared by RES</td>
</tr>
<tr>
<td>SUV (Small unilamellar vesicles)</td>
<td>&lt;0.1 µm</td>
<td>Single bilayer; homogenous in size; thermodynamically unstable; long circulation half life</td>
</tr>
</tbody>
</table>

**Factors affecting the performance of liposomes in-vivo data**

**Surface Charge**

Based on the head group composition of the lipid and pH, liposomes may bear a negative, neutral or positive charge on their surface. The nature and density of the charge on the surface of the liposomes influences stability, kinetics, and extent of biodistribution, as well as interaction with and uptake of liposomes by target cells. Liposomes with neutral surface charge have a lower tendency to be cleared by cells of the reticuloendothelial system (RES) after systemic administration and the highest tendency to aggregate. Although negatively charged liposomes reduce aggregation and have increased stability in suspension, their non-specific cellular uptake is increased in vivo. Negatively charge liposomes containing phosphatidylserine (PS) or phosphatidylylycerol (PG) were observed to be endocytosed at a faster rate and to a greater extent than neutral liposomes. Negative surface charge is recognized by receptors found on a variety of cells, including macrophages.

**Surface Hydration or steric effect**

The surface of the liposome membrane can be modified to reduce aggregation and avoid recognition by RES using hydrophilic polymers. This strategy is often referred to as surface hydration or steric modification. Surface modification is often done by incorporating gangliosides, such as GM-1 or lipids that are chemically conjugated to hygroscopic or hydrophilic polymers, usually poly(ethylene glycol) (PEG). This technology involves the...

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**Figure 2:** Types of liposomes according to their size and number
conjugation of PEG to the terminal amine of phosphatidylethanolamine. This added presence of hydrophilic polymers on the liposome membrane surface provides an additional surface hydration layer. 9,10 Depending on the length of the PEG polymer, PEG on the liposome membrane occupies an additional 5 nm of surface hydration thickness without significantly modifying the overall charge property of liposome membranes. One of the key advantages of using PEG-conjugated lipid is the long standing human safety data on the use of PEG as excipient for parenteral preparations. On the other hand, heterogeneity of long-chain PEG polymers, purified from petroleum products, and the slow renal clearance of extremely large PEG polymers may be concerns.

Other amphiphilic polymers with similar properties, such as poly(acryloylmorpholine) (PacM), poly(acrylamide) (PAA), and poly(vinylpyrrolidone) (PVP), have also been conjugated to phospholipids and used as liposome steric protectors with varying degrees of success 11,12.

**Fluidity of Lipid Bilayer**

Lipid bilayers and liposome membranes exhibit a well-ordered or gel phase below the lipid phase transition temperature ($T_c$) and a disordered or fluid phase above the $T_c$. The lipid phase transition is measured and expressed as $T_c$, the temperature at which equal proportions of the two phase coexist. At a temperature corresponding to $T_c$, maximum liposome leakiness is observed. Because the $T_c$ varies depending on the length and nature (saturated or unsaturated) of the fatty acid chain, the fluidity of bilayers can be controlled by selection and combination of the lipids. Encapsulated drugs can be released into the target tissue by modulating local tissue temperature by external heating using various sources of energy, such as infrared, microwave, or laser light. However, drugs bound to lipid membranes or protein-bound lipid membranes may shift the transition temperature or abrogate the phase transition behaviour all together 13.

**Liposome size**

Size of the liposome affects vesicle distribution and clearance after systemic administration. The general trend for liposomes of similar composition is that increasing size results in rapid uptake by RES. Whereas RES uptake in-vivo can be saturated at high doses of liposomes or by predosing with large quantities of control liposomes, this strategy may not be practical for human use because of the adverse effects related to the impairment of RES physiological functions. Complement activation by liposomes and opsonization depend on the size of the liposomes 14. Even with the inclusion of PEG in the liposome compositions to reduce serum protein binding to liposomes, the upper size limit of long circulation PEG-PE liposomes is ~150-200 nm.

### Preparation of Peptide and protein liposomes

A wide range of different methods for the preparation of liposomes are available leading to a variety of vesicle types and sizes. Table 3 shows each vesicle type has advantages and shortcomings that should be considered with respect to the physico-chemical properties of the drug and the individual application. From the peptide drug point of view those processes which avoid using organic solvents are preferred. This eliminates a number of difficult variables in the manipulation of proteins, including precipitation effects and deactivation.

<table>
<thead>
<tr>
<th>Method</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic-film MLV Method</td>
<td>The process involves the formation of a thin lipid film on a round-bottom flask by roteovaporation of a solution of phospholipid, usually in chloroform followed by hydration of the film by an aqueous solution of drug.</td>
</tr>
<tr>
<td>Freeze-Thaw method</td>
<td>Method is based on repetitive freezing-thawing of MLVs which causes breakage and reformation of the membranes and enhances loading of the drug from aqueous solution. Drug loading can be accomplished either by drying a mixture of drug and lipid solution or by rehydrating freeze-dried vesicles in a solution of the drug.</td>
</tr>
<tr>
<td>Osmotically-Derived Vesicles</td>
<td>Method is based on deformation reformation mechanism derived by an internal osmotic pressure rather than lipid-phase transitions.</td>
</tr>
<tr>
<td>Solvent requiring Methods</td>
<td>Methods have also been employed to prepare peptide liposomes. Many preparation techniques of liposomes such as injection methods (ethanol injection, ether injection), demulsification methods (reverse phase evaporation and double emulsion), stable plurilamellar vesicles (SPLV) and the monophasic vesicle process (MPV) require the use of solvent.</td>
</tr>
</tbody>
</table>

### Why Need For Insulin Liposomes ?

The conventional formulations available for sc administration have the drawback of hypoglycemic shock after administration. One way of improving the compliance and minimizing discomfort arising from frequent injections is to couple protein and peptide drugs with sophisticated parenteral delivery systems that reduce the frequency of injection by providing a sustained release of the drug over time. The release of insulin can be controlled by incorporating it into liposomal matrix which can avoid the same and can sustain the insulin effect for longer period of time. Biodistribution and pharmacokinetics of the drug changes in carrier system compared to free drug. Type of the liposome (charged, PEG etc.) and the release of the drug from it govern the overall pharmacokinetics as well as distribution of the encapsulated drug. Different types of available lipids and using their different ratios provide an ample number of options to optimize the liposomes for the desired purpose. This necessitates the study of biodistribution and pharmacokinetics to determine the in vivo fate of the drug after administration in the carrier system. Pharmacokinetic study of exogenously administered insulin is complexed by the presence of the endogenous levels of the insulin in the species under study. Most of
the research work assumes the endogenous level of insulin to be constant during the pharmacokinetic study. However the levels of insulin changes in response to stress during the study (e.g. blood withdrawal). Quantitation of Insulin in serum is widely done by RIA (Radioimmunoassay) which cannot distinguish between the endogenous levels of insulin and exogenously administered insulin. This fact highlights the need of the method which can allow the quantitation of the administered insulin in the presence of endogenous insulin.

**Diabetes Mellitus**

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is a multisystem disease; insulin replacement does not completely prevent the onset of complications. Type 1 diabetes has been called IDDM, "brittle diabetes", and juvenile onset diabetes. It is characterized by beta-cell destruction, usually leading to absolute insulin deficiency, which makes insulin injections compulsory for the treatment. Type 2 diabetes has been called NIDDM and adult-onset diabetes. It is characterized by insulin resistance, so exogenous insulin is not always required. The insulin resistance is primarily exhibited by the liver and skeletal muscle, and to a lesser extent, by adipose tissue.

**Insulin**

Insulin is a hormone secreted from β-pancreatic cell in response to glucose and other stimulants (e.g., amino acids, free fatty acids, gastric hormones, parasympathetic stimulation, β-adrenergic stimulation). It is used as replacement therapy in the management of diabetes mellitus. It supplements deficient levels of endogenous insulin and temporarily restores the ability of the body to properly utilize carbohydrate, fats, and proteins. Insulin therapy is indicated in all cases of insulin-dependent (type 1) diabetes mellitus and is mandatory in the treatment of diabetic ketoacidosis. Insulin is also indicated in patients with non-insulin dependent (type II) diabetes mellitus when weight reduction and/or proper dietary regulation have failed to maintain satisfactory concentrations of blood glucose in both the fasting and postprandial state.

**Pharmacokinetics**

After sc injection, insulin is absorbed directly into the blood stream, bypassing the lymphatic system. The rate limiting step of insulin activity after sc administration is absorption of insulin from the injection site, which depends on the type of insulin administered. Fig 3 shows the approximate duration and onset of action of different insulins. Exogenous insulin is degraded at both renal and extra-renal (liver and muscle) sites. Degradation also takes place at the cellular level after internalization of the insulin-receptor complex. Approximately 30-80% of insulin is cleared from the systemic circulation by the kidneys, which have larger role in clearing exogenously administered insulin. Endogenous insulin is secreted directly into the portal circulation and is primarily cleared by liver in non-diabetic individuals (60%). Insulin is filtered by the glomerular capillaries, but > 99% is reabsorbed by the proximal tubules. The insulin is then degraded in glomerular capillary cells and post-glomerular peritubular cells.

![Figure 3: Schematic diagram showing the approximate onset and duration of action of insulin preparations.](image)

**Pharmacodynamics**

Clinically, the most important differences between the insulin products relate to their onset and duration of activities. The onset of action, peak effect and durations of action of each insulin category is given in table 4.

### Table 4: Comparison of onset of action, peak effect and durations of action of different insulins

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Onset (hr)</th>
<th>Peak (hr)</th>
<th>Duration (hr)</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin lispro</td>
<td>½</td>
<td>½-1½/2</td>
<td>4-5</td>
<td>Clear</td>
</tr>
<tr>
<td>Insulin aspart</td>
<td>5-10 min</td>
<td>1-3</td>
<td>3-5</td>
<td>Clear</td>
</tr>
<tr>
<td>Regular</td>
<td>½-1</td>
<td>2-4</td>
<td>5-7</td>
<td>Clear</td>
</tr>
<tr>
<td>NPH</td>
<td>1-2</td>
<td>6-14</td>
<td>24%</td>
<td>Cloudy</td>
</tr>
<tr>
<td>Lente</td>
<td>1-3</td>
<td>6-14</td>
<td>24%</td>
<td>Cloudy</td>
</tr>
<tr>
<td>Ultralente</td>
<td>6</td>
<td>18-24</td>
<td>36%</td>
<td>Cloudy</td>
</tr>
<tr>
<td>Insulin glargine</td>
<td>1.5</td>
<td>Flat</td>
<td>24</td>
<td>Clear</td>
</tr>
<tr>
<td>Exubera*</td>
<td>10-20 min</td>
<td>1-3</td>
<td>6-8</td>
<td>Aerosol</td>
</tr>
</tbody>
</table>

*Inhaled human insulin*
Role of Insulin in Carbohydrate Metabolism

Glucose metabolism and the metabolic effects of insulin differ in diabetic and non-diabetic subjects during the fed (postprandial) and fasting (postabsorptive) states. Haemostatic mechanisms maintain plasma glucose concentrations between 55 and 140 mg/dL. A minimum concentration of 40–60 mg/dL is required to provide adequate fuel for the central nervous system (CNS), which use glucose as its primary energy source and is independent of insulin for glucose utilization. When blood glucose concentrations exceed the re- absorptive capacity of the kidneys (approx 180 mg/dl), glucose spills into the urine resulting in loss of calories and water.

The actions of insulin on the global human metabolism level include:

- Control of cellular intake of certain substances, most prominently glucose in muscle and adipose tissue (about ⅔ of body cells).
- Increase of DNA replication and protein synthesis via control of amino acid uptake.
- Modification of the activity of numerous enzymes (allostERIC effect).

The actions of insulin on cells include:

- Increased glycogen synthesis – insulin forces storage of glucose in liver (and muscle) cells in the form of glycogen; lowered levels of insulin cause liver cells to convert glycogen to glucose and excrete it into the blood. This is the clinical action of insulin which is directly useful in reducing high blood glucose levels as in diabetes.
- Increased fatty acid synthesis – insulin forces fat cells to take in blood lipids which are converted to triglycerides; lack of insulin forces reduction in conversion to triglycerides; lack of insulin causes the reverse.
- Increased esterification of fatty acids – forces adipose tissue to make fats (i.e., triglycerides) from fatty acid esters; lack of insulin causes the reverse.
- Decreased proteinolysis – forces reduction of protein degradation; lack of insulin increases protein degradation.
- Decreased lipolysis – forces reduction in conversion of fat cell lipid stores into blood fatty acids; lack of insulin causes the reverse.
- Decreased gluconeogenesis – decreases production of glucose from various substrates in liver; lack of insulin causes glucose production from assorted substrates in the liver and elsewhere.
- Increased amino acid uptake – forces cells to absorb circulating amino acids; lack of insulin inhibits absorption.
- Increased potassium uptake – forces cells to absorb serum potassium; lack of insulin inhibits absorption.
- Arterial muscle tone – forces arterial wall muscle to relax, increasing blood flow, especially in micro arteries; lack of insulin reduces flow by allowing these muscles to contract.

Pharmacokinetics of Liposomes

The association of drugs with carriers like liposomes can result in dramatic change in pharmacokinetics and biodistribution of drugs. A drug, when associated with a liposome, is sequestered away from the interaction with its normal site of action until it is released from the liposome. Measuring the pharmacokinetics of the liposomal drugs is combination of two separate pharmacokinetic processes: the pharmacokinetics of the liposomal carrier, which include any drug remain associated with the carrier and the pharmacokinetics of that drug which is released from the carrier. The rate of release of the drug from its carrier will therefore influence the overall pharmacokinetic parameters.

Table 5: Effect of drug properties on their association and retention by liposomes

<table>
<thead>
<tr>
<th>Solubility</th>
<th>log P&lt;sub&gt;oct&lt;/sub&gt;</th>
<th>Liposomal association</th>
<th>Approximate degree of retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophilic</td>
<td>&gt; 1.7</td>
<td>Readily retained in liposome aqueous interior</td>
<td>Slowly released from liposomes over several hours to several days.</td>
</tr>
<tr>
<td>Hydrophobic</td>
<td>&lt; 5.0</td>
<td>Readily inserted into the hydrophobic interior of the liposome bialayer.</td>
<td>Excellent retention in liposomes</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1.7-5.0</td>
<td>Rapidly partition between the liposome bilayer and the aqueous phase</td>
<td>Rapid release from liposomes but pH manipulation or formation of molecular complexes can result in good retention</td>
</tr>
</tbody>
</table>

Increased fatty acid synthesis – insulin forces fat cells to take in blood lipids which are converted to triglycerides; lack of insulin forces reduction in conversion to triglycerides; lack of insulin causes the reverse.

Increased esterification of fatty acids – forces adipose tissue to make fats (i.e., triglycerides) from fatty acid esters; lack of insulin forces reduction of protein degradation; lack of insulin increases protein degradation.

Decreased proteinolysis – forces reduction of protein degradation; lack of insulin increases protein degradation.

Decreased lipolysis – forces reduction in conversion of fat cell lipid stores into blood fatty acids; lack of insulin forces reduction in conversion of fat cell lipid stores into blood fatty acids; lack of insulin causes the reverse.

Decreased gluconeogenesis – decreases production of glucose from various substrates in liver; lack of insulin causes glucose production from assorted substrates in the liver and elsewhere.

Increased amino acid uptake – forces cells to absorb circulating amino acids; lack of insulin inhibits absorption.

Increased potassium uptake – forces cells to absorb serum potassium; lack of insulin inhibits absorption.

Arterial muscle tone – forces arterial wall muscle to relax, increasing blood flow, especially in micro arteries; lack of insulin reduces flow by allowing these muscles to contract.

Not all drugs can be easily and stably associated with liposomes. The solubility of drugs, which affects their association with and retention by liposomes, defines the pharmacokinetics of the liposomal drugs. Table 5 summarizes the effect of drug properties on their association and retention by liposomes.
Proteins differ from conventional small-molecule drugs in several respects, including size, but one of the most important differences affecting delivery and biological effectiveness is the complexity of the protein structure. The full biological activity is dependent on preserving the integrity of this complex structure. Because of the close relation between protein efficacy and the molecular-three dimensional structure, it is essential to maintain the structural integrity throughout all the formulation steps of the delivery system and while the drug is released from the dosage form at the site of action. Another problem relates to the physical and chemical stability of the peptide. Instability of liquid protein formulations and their stabilization had been reviewed extensively.

**Insulin Controlled Drug Delivery**

In past few years a number of attempts have been made to either stabilize the insulin in gastric environment or to sustain its effect by its incorporation to various matrices. A biocompatible polymer system had been developed that permitted the release of biologically active insulin in a controlled manner. The effect of release from sodium-insulin matrix to that of zinc insulin matrix was compared. Effect of geometry of the release device on in-vitro release profile was studied. They observed that the large concentration of the insulin within the matrix provided the driving force for the release. Release kinetics from the polymeric devices was enhanced by increasing the insulin solubility, the insulin powder particle size, the loading of insulin within the matrix and the porosity of the matrix.16,17

A diabetic hairless rat model was developed where they studied the effect of iontophoresis and the role of pH on the transdermal permeation of insulin. The iontophoresis was found to occur at pH values higher and lower than the isoelectric pH of 5.3. The blood glucose reduction data indicated that insulin was more effective at pH 3.68 than 7.10 or 8.0. Comparison of the results in hairless and normal model revealed no significant difference.

The developed a new formulation for prolonged pulmonary insulin delivery based on the encapsulation of an insulin: dimethyl-cyclodextrin (INS:DM-CD) complex into PLGA microspheres. The hypoglycemic effect of the complex-loaded microspheres, after intratracheal administration to diabetic rats, was investigated and compared with that obtained from administration of insulin solution sc. Time to reach maximum glucose reduction was 8 hr compared to 2 hr for insulin solution given sc.

The use of stabilizing excipients has been shown to diminish protein denaturation/aggregation and enhance protein encapsulation examined the effect of alpha, beta and gama-CDs on: (1) the thermal denaturation of insulin in solution, and (2) encapsulation and release kinetics of bovine insulin from ethyl-cellulose microcapsules. All CDs improved thermal stability of insulin by lowering the enthalpy of unfolding by 16–52%. Alpha- and gama-CDs also increased the encapsulation efficiency of insulin and improved uniformity of the microcapsule formulations. Two mathematical models were proposed to account for insulin release and consisted of multiple zero order and first order input processes, and a single first order output process. All CDs decreased the initial burst release of insulin by up to 30%

Recently, different matrices have been used in the development of extended-action insulin. Microparticle carriers and nanospheres made from synthetic biodegradable polymers such as poly(lactide-co-glycolide) (PLG), polylactide (PLA) and different polymers have been widely investigated for extended drug delivery measured the stability of insulin microencapsulated in the blended microparticles prepared from PEG and lactide polymers which were characterized by a uniform rate of protein release in vitro. Extensive degradation of the PLG/PEG microparticles occurred over 4 weeks, whereas the use of PLA/PEG blends resulted in stable microparticle morphology and much reduced fragmentation and aggregation of the associated insulin.

**CONCLUSION**

The success of commercial technologies and the emergence of new ones will be demonstrated only with time. The recent developments have been made to achieve them insulin liposomes were considered leading technology in this field. Since the discovery of insulin liposomes, the problems regarding the site specific targeting, drug entrapment, controlled release, storage stability and efficacy have been going to be solved. They have played a significant role in improving the drug delivery and dose regulation of very potent drugs. Simultaneously much advancement has been made to develop insulin liposomes to patient’s safety and compliance. It is expected that many conventional drugs will be benefited with their delivery in the liposomes form. The insulin in liposomal delivery system would be promisingly advantageous and hopes for further developments.

**REFERENCES**


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