



Received on 17 March, 2013; received in revised form, 29 April, 2013; accepted, 21 June, 2013

DEVELOPMENT AND CHALLENGES FACING FOR INSULIN ORAL DELIVERY

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Keywords:

Oral insulin, Hypoglycemia, Diabetes, Permeability, Enzymatic degradation, Encapsulation efficiency, Bioavailability, Stability, Emulsion, Liposomes

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ABSTRACT: The Present study focuses on the challenges facing the development of oral insulin the fact we have no formulation in market, being an attractive and advantageous over the regular injections. Physiological and biological factors of the insulin has great influence for the development of an oral formulation with many difficulties regarding the stability over gastric pH and proteolytic enzymes, selection of the proper formulation and its components depends on these conditions. The development with insulin therapy are needed with better patient compliance, low toxicity, higher glycemic control, thereby Diabetes related complications occurrence can be prevented.

INTRODUCTION: In terms of efficacy, insulin in form of parental route is satisfactory, however it is associated with some severe adverse conditions like, peripheral hyperinsulinemia, smooth muscle cell proliferation, diabetic macro and micro angiopathy, lipotrophy or lipohyertrophy have become very common.

Insulin delivery by oral route is not effective due to its susceptibility to enzymatic degradation in GI & Permeability across intestinal epithelium is low ^{1, 2}. The proper combination of Multifunctional polymers will result a better biocompatible, biodegradable, hydrophilic and protective characteristic with better absorption orally in the GI ³.

Oral insulin reduces hyperinsulinemic effect, after intestinal absorption, channelled directly to liver following the route of physiological pancreatic insulin ⁴.

Normal therapy of insulin for T₂D patients does not receive it in a timely manner ⁵. In older patients it becomes difficult to administer by themselves. Various carriers for oral delivery includes Polymeric hydrogels ^{6, 7}, Microspheres, Nanoparticles ⁸, Microemulsions, Liposomes ⁹⁻¹², Emulsions, Insulin pellets.

The other novel approaches include Inhaled insulin-intrapulmonary, intranasal, Buccal tablets, sublingual, Transdermal patches etc. Long acting synthetic chemicals in case of these formulations are proved to cause breast cancer during recent studies ¹³. The use of enzymatic inhibitor to protect insulin has high incidence of systemic intoxications, disturbance in intake of nutritive proteins and pancreas malfunction ¹⁴.

QUICK RESPONSE CODE	DOI:
	10.13040/IJPSR.0975-8232.4(7).2576-93
	Article can be accessed online on: www.ijpsr.com

Absorption enhancers also show various safety problems at certain concentrations when used in the formulations intended to increase the absorption of insulin at intestinal mucosa followed by an oral delivery¹⁵.

Prolonged residence in the intestine by polymeric drug carrier provides higher concentration of the drug on the mucosa, with sustained release of drug¹⁶. Hydrogels with appropriate Molecular weight may overcome some of the problems with protein delivery.

The major factor pharmacologically effecting orally delivered insulin is noted to be 60% due to insulin degradation, 23% to premature insulin release and 17% to lack of mucoadhesion¹⁷. Most of the strategies in recent years focus on suppressing either of these barriers to intestinal absorption: epithelial cell layer, proteolytic enzymes and chemical environment¹⁸. The obstacle in long term therapy would be the aggregation of insulin molecules and form fibrils.

Oral insulin would lead to a significant improvement for diabetes treatment. Past few decades, many attempts were done to develop non-invasive method for delivery of insulin by oral route, the most convenient and desired.

Novel Delivery:

1. **Nanoparticles:** Protection to the sensitive drugs, controlled release, site specific drug delivery, minimizing the side effects by using little amounts of the drug makes it advantageous and attractive technique for oral insulin with increased bioavailability & increased stability in GI¹⁹. Peyer's patches having M-cells on their surface are useful for nanoparticles transport through GI tract^{20, 21}. A great disadvantage using this formulation process i.e. drugs are destroyed as they are susceptible to high shear stress²².

a. **Modified Inter-Ionic gelation process:** This process of nanoparticles preparation avoids organic solvents, surfactants & steric stabilizers²³. Polymethacrylic acid-chitosan an anionic-cationic polymer complex results a strong interpolymer complex, protecting insulin from acidic pH²⁴. Phosphate buffer pH 7.4 shows

good dispersion capacity for PMCP particles. Complexation of these nanoparticles with cyclodextrin (CD) enhances the stability of the insulin and improves the shelf life, without affecting the biological activity of native proteins^{25, 26}.

Fluorescence spectroscopy studies show the interaction between hydroxypropyl β cyclodextrin and insulin which confirms the complex formation. Diffusion filling method for loading of nanoparticles results in 80% encapsulation efficiency of insulin determined using Lowry's protein assay. *In-vitro* release profile showed better protection of insulin & pH dependent release kinetics due to ionic nature of materials used (Polymethacrylic acid).

Results from the research shows 20% release of loaded insulin at pH 1.2 at first 2 h, completed in 3h at neutral pH. Biological activity of insulin was retained by the formulation, confirmed using ELISA test. Adhesive nature of the particles determined using mucoadhesive studies on isolated rat intestine, revealed more than $84 \pm 3\%$ (n=3) of applied particles are retained in the intestine.

b. **Layer by Layer Adsorption technique:** An advantage using this technique, can be prepared at room temperature in aqueous solution using appropriate pH, having high drug loading capacity & high encapsulation efficiency²⁷. Coating to nanoparticles is done by opposite charged biodegradable polyelectrolytes, water soluble chitosan (WSC) as polycation & synthetic poly (α , β -L-Malic acid) (PMA) as polyanion. PMA & WSC being weak electrolytes, their charge density and conformation is highly pH dependent, beneficial for release of polypeptide²⁸⁻³¹.

Nanoaggregates of insulin, prepared with addition of NaCl to insulin solution. NaCl solution range between 0.6–0.8 M gives better nanoaggregates; lower temperature ($15 \pm 1^\circ\text{C}$) ensures protein activity in the prepared Nanoaggregates. Salting out at lower pH resulted in the insulin particles with less size (**Fig. 1**).

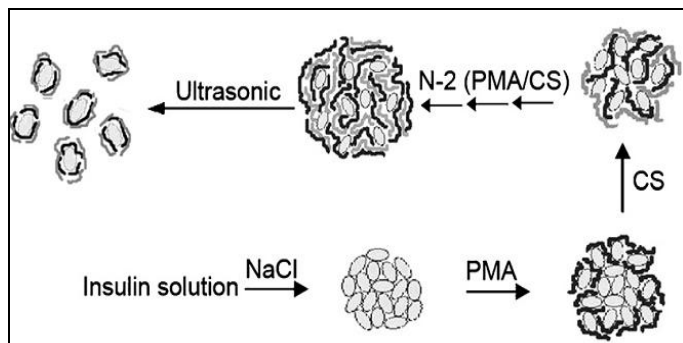


FIG. 1: SCHEMATIC REPRESENTATION OF LBL ADSORPTION OF NEGATIVELY (BLACK) AND POSITIVELY (GRAY) CHARGED POLYELECTROLYTES ON PROTEIN PARTICLES.

XRD spectra of nanoaggregates obtained using salting out method shows insulin as amorphous in nature. The pH & ionic strength of the polyelectrolyte solution is kept same as that of precipitating insulin aggregates (pH 1.1, 0.6 M NaCl) during LBL adsorption with polyelectrolyte to avoid insulin dissolution. During LBL adsorption the range of salt solution is kept 2.2–5.5, to bear enough charges on PMA, WSC, and Insulin particles. The adsorption of PMA & WSC onto the insulin particles requires 0.1 mM HCl containing 0.6 M NaCl or 3.0 M NaCl.

Coating with polyelectrolyte solution results insulin loss, that insulin molecules are not included in aggregates, due to reversibility a part of aggregates are dissolved can be another reason. SEM analysis of six layered insulin polyelectrolytes (PMA/WSC)³ shows the size range of 100–250 nm for the nanoparticles (**Fig. 2**). Higher to pH 5, release rate of insulin increases with increase in pH up to pH 8³².

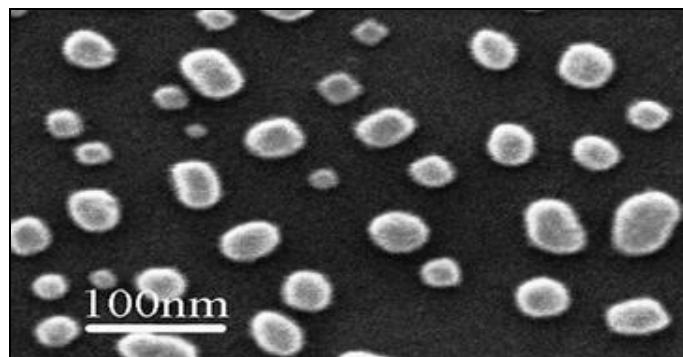


FIG 2: SEM PHOTOGRAPH OF INSULIN NANOPARTICLES WITH SIX POLYELECTROLYTE ADSORPTION CYCLES AFTER ULTRASONIC TREATING FOR 2 MIN AT 10°C.

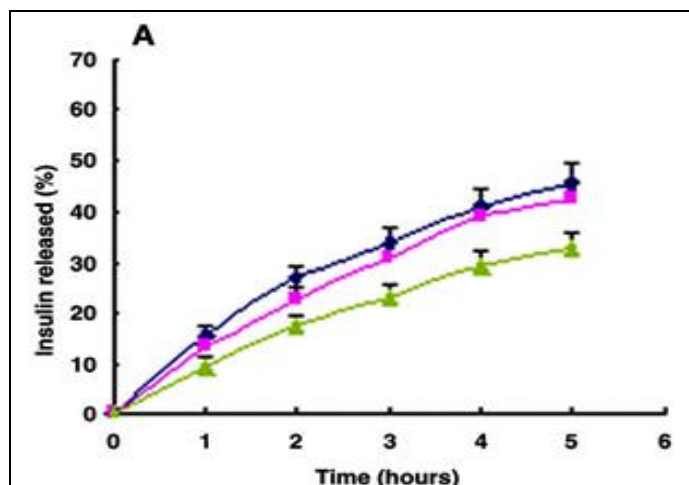
Kinetic studies results shows a rapid release of insulin followed by a slow decline at 8 h. Number of polyelectrolyte layer adsorbed onto the surface of

nanoparticles, influences release of insulin, multilayer impede swelling in the medium, resulting a slow release rate.

- c. **Poly Electrolyte Complexation method:** The method has an advantage of not necessitating organic solvents and sonication process thereby damage to protein and peptide is minimized during the nanoparticles preparation³³. Quaternized derivatives of chitosan (Triethylchitosan (TEC) & Dimethyl-ethylchitosan (DMEC)) used as polymeric coating material by PEC method for oral administration^{34,35}.

Principle involved in PEC method is to use electrostatic interactions between the positively charged polymer & negatively charged insulin (INS). Properties of PEC nanoparticles is influenced by the pH of the INS solution, desirable INS nanoparticles are derived at pH 8.0–8.5³³. pH of the polymer solution is expected to be 5.5 for better results.

Zeta potential of INS-CS, INS-TEC, INS-DMEC nanoparticles are positively charged with values of 17.6, 25.1 and 26.2 mv respectively, pDI of less than 0.3 shows homogenous size distribution, an index for stability by the particles³⁶. Association efficiency by the equation given by Fernandez-urrusuno³⁷ shows TEC & DMEC with higher association efficiency than CS nanoparticles, due to stronger positive charges by TEC & DMEC. *In vitro* release profile for nanoparticles in phosphate buffer saline, pH 7.4 is 32.7%, 42.5% & 45.7% for INS-CS, INS-TEC and INS-DMEC respectively (**Fig. 3**).



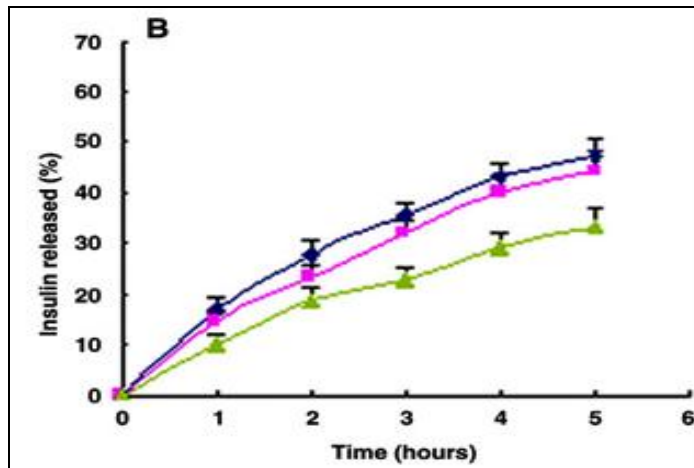


FIG. 3: INVITRO RELEASE PROFILES OF INS FROM NANOPARTICLES IN PHOSPHATE BUFFER SALINE pH 7.4 (A) AND pH 6.8 (B) —◆— DMEC-INS NANO —■— TEC-INS NANO —▲— CS-INS NANO

The results from the research prove the procedure to be suitable to encapsulate insulin, sensitive to stress factors³⁸.

d. Ionotropic Pregelation technique:

Nanoparticles developed by this technique are multilayer complexes, outermost coat consisting of a protease-protective protein layer²³. Nucleus of the particles is composed of alginate, dextran sulfate & insulin following Complexation with chitosan and poloxamer 188, coated with bovine serum albumin.

Alginate in the presence of multivalent cations forms stable & reversible hydrogel due to intra & inter molecular cross linking of polymer chains. Alginate hydrogels, stabilized by chitosan for formation of nanoparticles, reduces transepithelial electrical resistance, enhancing insulin absorption via paracellular pathway by opening tight junctions between epithelial cells^{39, 40}. Box- Behnken experimental design proves that nanoparticles mean diameter depends upon concentration of calcium chloride and chitosan, lowest concentration of chitosan (0.04%) & calcium chloride (0.20%), gives a particle with minimum size (394 nm). Increasing in the albumin concentration decreases particle size.

Larger structures are due to lateral association of a number of alginate chains, at higher concentrations of calcium⁴¹. Reduction in electrical repulsion related to albumin concentration has influence for the decrease in

particle size, depending on whether the electrical repulsion increases or decreases⁴², particles tend to swell or shrink. Calcium & Albumin concentrations influence entrapment efficiency of insulin, 90% at higher concentrations of calcium chloride.

Swelling of nanoparticles at higher concentrations of chitosan, break the ionic interaction between insulin & alginate/dextran nucleus affects insulin release⁴³. Weakening of the electrostatic interaction between insulin and alginate/dextran nucleus at high concentrations of albumin leads to higher insulin release⁴⁴. At low concentrations of chitosan and albumin in nanoparticles retained insulin completely.

Mucoadhesive properties of alginate and chitosan adheres particles to intestinal mucosa, releases insulin directly at site of absorption⁴⁵. Zeta potential varied in the range of -36.6 to -44.5 mv with a pDI of 0.20 to 0.24% with increase in calcium chloride concentration. Higher surface area per volume & higher mucoadhesive strength by the particles having small size is desirable to increase the contact with the intestinal mucosa⁴⁶.

The results shows, 0.20% calcium chloride, 0.04% chitosan & 0.46% bovine serum albumin produces nanoparticles with optimum size of 402 nm, pDI 0.91% & zeta potential of -39.8 mv having an entrapment efficiency of 96% with complete retention of insulin at gastric conditions.

e. Reverse Micelle – Solvent evaporation method:

This technique for the preparation of nanoparticles uses organic solvents, dichloromethane, ethylacetate and dehydrated alcohol⁴⁷. Soybean phosphatidylcholine (SPC) used to complex with insulin using anhydrous co-solvent lyophilization process improves the solubility of insulin. Micellar solution is obtained by adding SPC-INS complex to dichloromethane or ethylacetate containing polymer *Poly(lactic-co-glycolic acid)* (PLGA), pouring of this solution to aqueous solution of 2% poly vinyl alcohol forms a stable o/w emulsion, evaporation of the organic solvent precipitates polymer & complex in the emulsion droplets resulting nanoparticles (Fig. 4).

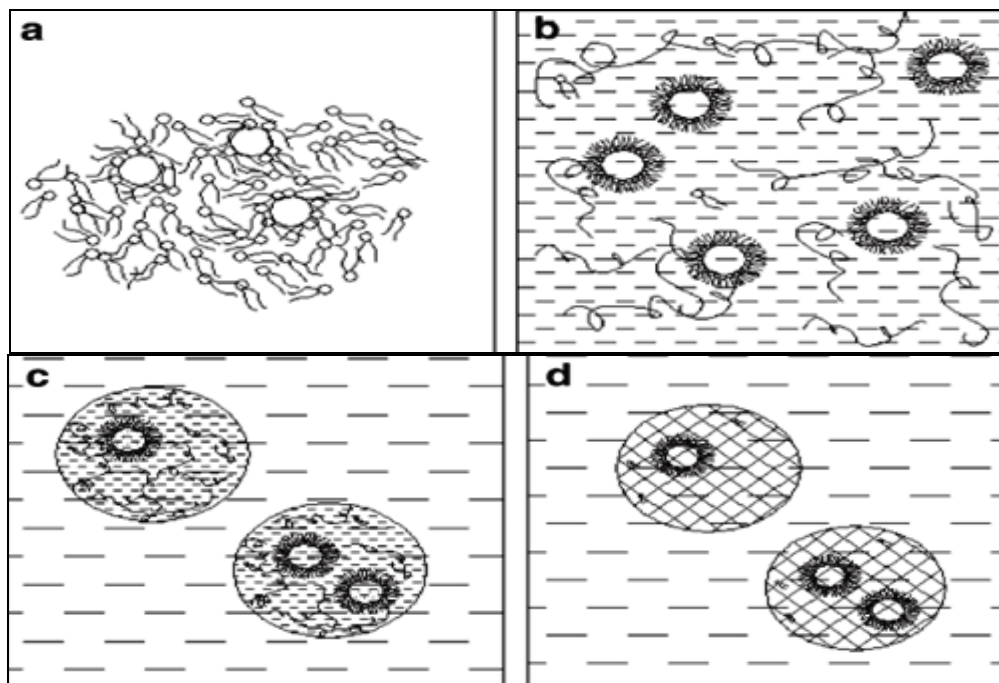


FIG 4: DIAGRAMMATIC REPRESENTATION OF THE FORMATION OF NANOPARTICLES LOADED WITH INS-SPC COMPLEX. (A) INS-SPC COMPLEX. (B) REVERSE MICELLAR SOLUBILIZATION OF INSULIN WITHIN ORGANIC SOLVENT CONTAINING POLYMERS. (C) EMULSION DROPLET CONTAINING MICELLES AND POLYMERS (O/W). (D) SOLIDIFIED NANOPARTICLES.

XRD pattern of SPC-INS complex shows insulin to be either molecularly dispersed or amorphous in nature. Infra-red spectroscopy results show weak interactions between insulin and SPC. Higher viscosity of emulsion droplets with increase in polymer concentration in organic phase, results less partition of drug into aqueous phase, but 2.5:1 ratio of PLGA/SPC resulted particle having ideal properties. The entrapment efficiency of 90% is seen with PLGA/ SPC ratio 5:1. Polymer precipitation is seen with organic phase having low boiling point and limited water solubility due to higher evaporation rates, addition of acetone in proper concentration without decreasing the interfacial tension promoted drug entrapment.

Entrapment efficiency is not affected with the polymer composition & molecular weight but increase in the particle size is seen with increase in molecular weight⁴⁸⁻⁵⁰. Weak affinity between SPC-INS complex and the polymer control the drug release, unavoidably results in burst release. Reduction with initial & final release over 24 h is seen with increase in the molecular weight of PLGA 50/50^{51, 52}. Decrease in the fasting plasma glucose level to 57.4% within the first 8 h is seen which continued to be for 12 h after administration of 20 IU/Kg SPC-INS nanoparticles in diabetic rats with a relative bioavailability of 7.7% (Fig. 5).

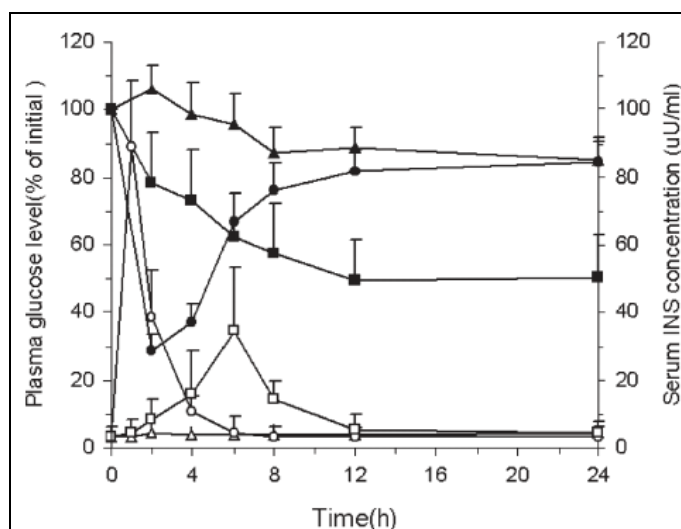


FIG. 5: GLUCOSE LEVELS IN PLASMA AFTER ORAL ADMINISTRATION OF INS-SPC NANOPARTICLES IN DIABETIC RATS: NPs 20 IU/Kg (■), Ins solution control 20 IU/Kg (▲), Ins solution 1 IU/Kg s.c. (●). The insulin levels in serum after oral administration Ins-SPC nanoparticles in diabetic rats: NPs 20 IU/Kg (□), Ins solution control 20 IU/Kg (Δ), Ins solution 1 IU/Kg s.c. (○). Data represents the mean ± S.D., n=6 per group. Statistically significant difference from control (* $p < 0.05$, ** $p < 0.001$).

The results from the study reveals the specific drug targeting characteristics of the nanoparticles prepared using this technique with favorable absorption at posterior segment of intestine, where abundant peyer's patches and less proteolytic enzymes exists^{53, 54}.

Microcapsules: Microcapsules give an opportunity for the drug to be released in a controlled fashion for longer periods. The non-toxic nature, good bioavailability and compatibility & approval by food & drug administration, PLA & PLGA are used extensively for microcapsule preparation⁵⁵. Double emulsion, organic phase separation, super critical fluid & spray drying are the various techniques used to prepare microcapsules^{56,57}.

When double emulsions are usually prepared by Mechanical stirring, Homogenization or Ultra sonication method, disadvantages like difficulty to control size distribution of microcapsules & lower bioavailability⁵⁸, difficult to control drug release behavior, lower drug encapsulation efficiency, poor reproducibility is seen. Shiroso porous glass (SPG) technique is promising one overcoming all the disadvantages of double emulsion with additional advantages i.e. with help of membrane of various

pore sizes, diameter of double emulsion & microcapsules can be controlled, shear sensitive protein & peptide can be encapsulated as emulsification is carried out with low shear.

The combination of both the techniques i.e. double emulsion & SPG is used to obtain PLA/PLGA microcapsules where the primary emulsion is permeated through uniform pores of SPG membranes (pore size 2.8 μm) under pressure of nitrogen gas into outer aqueous phase to form double emulsion with uniform droplet size followed by evaporation of dichloromethane & toluene to form microcapsules⁵⁹. SEM analysis shows the microcapsules to be spherical in shape with a smooth surface & size distribution to be narrow. The result showed that microcapsule with PLA/PLGA ratio to be 1:1 has high encapsulation efficiency, PLGA being the inner layer & PLA as outer layer (Fig. 6).

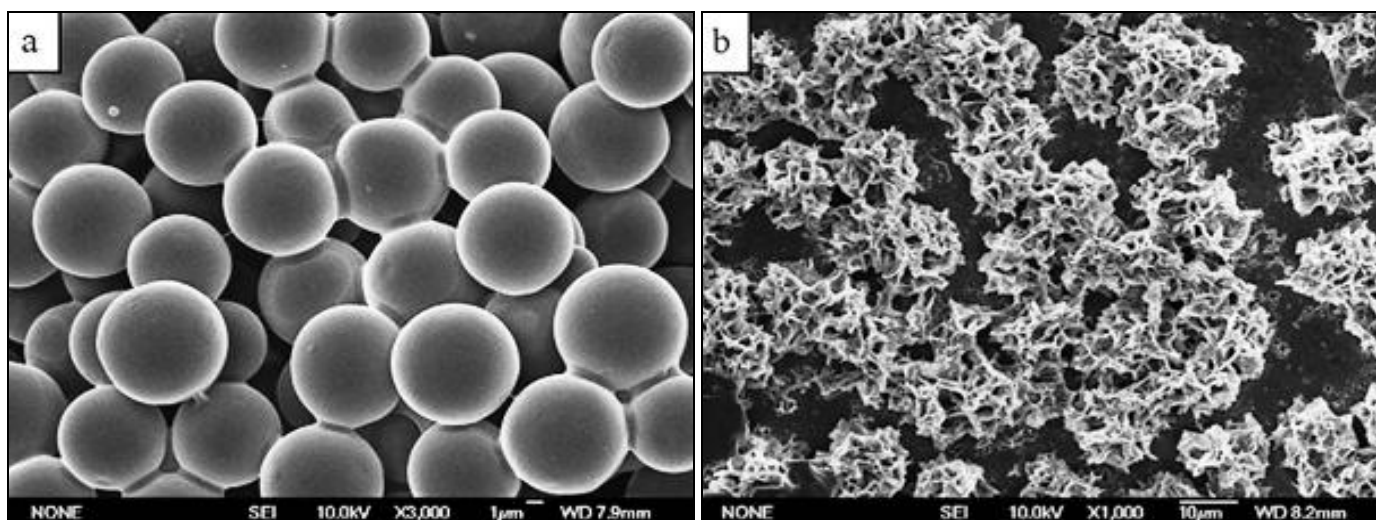


FIG. 6: SEM PHOTOGRAPH OF THE ORIGINAL PLA/PLGA MICROCAPSULES (A) AND THE DEGRADED PLA/PLGA MICROCAPSULES (B) PREPARED USING SPG MEMBRANE EMULSIFICATION.

The release profile by *Invitro* studies shows that the microcapsules has initial burst followed by control release⁶⁰. Increase in encapsulation from 65.61% to 92.21% is achieved with NaCl concentration in outer phase increased from 0 to 5.0 wt%, as formation of microcapsules & encapsulation efficiency is affected

by osmotic pressure & pH values. Decrease in encapsulation of drug from 65.61% to 14.19% is seen with increase in inner water phase volume from 250 to 625 μl , this is mainly due to coalescence between inner & outer water phase (Table 1).

TABLE 1: EFFECT OF MICROCAPSULES SIZE ON THE DRUG ENCAPSULATION EFFICIENCY AND INITIAL RELEASE

Emulsification technique	Mean size (μm)	Encapsulation efficiency (%)	Initial rhI burst ^a (%)
Membrane	3.2	48.96	46.94
	5.8	65.61	34.43
	9.0	81.28	14.50
Stirring	15.9	32.85	-

^a rhI released during the first 24 h

An initial little by little increase in release with time, followed by small intestine burst is seen with low loading amount of recombinant human insulin, the pores and interconnecting channels increased with initial release of recombinant human insulin, these channels help in release of left over insulin from microcapsules. Highest drug encapsulation efficiency of 91.82% is obtained with optimized preparation process discussed.

The results show that pore size of membrane affects the size of microcapsules as there exist a linear relationship⁶⁰.

Emulsions: Protection of proteins and peptides by proteolytic enzymes offers an advantage of emulsions oral delivery of insulin^{61, 62}. Small amounts of protease inhibitor & absorption promoter in multiple emulsions do not affect the normal digestive process, easy to drink and handle due to its low viscosity⁶³. Emulsion with fine particles effect bioavailability⁶⁴, can be absorbed for ileum and colon regions of the intestine. Emulsions incorporating free fatty acids, high concentration in omega-3 fatty acids, oleic acid (OA) and decosahexanoic acid, shows enhanced insulin action⁶⁵, reduction of inflammatory phenomena⁶⁶, high pharmacological availability of insulin following colonic and rectal administration⁶⁷, incidence of cardio vascular diseases is reduced⁶⁸ and with the use of cyclic monoterpenes drug absorption promoting action is expected.

Transmucosal bioavailability of insulin can be improved by:

- i. Minimizing aggregates formation by using specific insulin species.
- ii. Inhibiting enzymatic degradation
- iii. Increasing membrane permeability by oleic acid & certain fatty acids⁶⁹⁻⁷⁴.

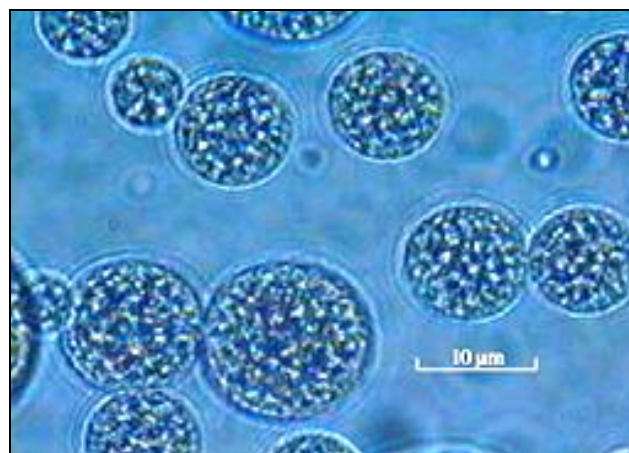
a. Emulsion by Two step Homogenization:

Insulin is kept in the inner aqueous phase for improving the protection against proteolytic enzymes which is made up of 0.1 M Hcl & phosphate buffers saline pH 7.4, with gelatin (5%) as stabilizing agent in most of the formulations involved in various researches. Oily phase of emulsion varied with different formulations, egg yolk polysaccharide & span 80 with varied concentrations 0.13%, 5% and

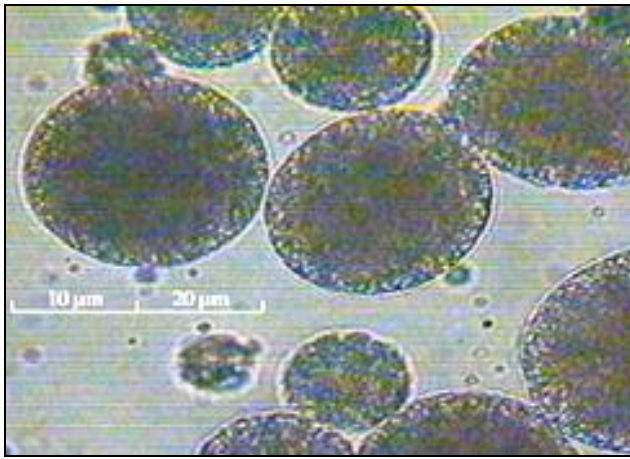
20% and 60-75% triglycerides, in some cases with triglycerides being replaced by oleic acid from 0%-5% in range, fish oils and Medium chain triglycerides (MCT) being used in other formulations. Absorption enhancer is kept in oily phase in all the formulations. Purified water with a hydrophilic surfactant, 3%-4% tween 80 used as outer aqueous phase.

The ratio of the phases varied from 1:4:5 (v/v/v), 0.9:2.1:3.0 (v/v/v). Spray dried w/o/w emulsion is prepared using spray dryer with 3000 RPM as the speed of atomize⁷⁵. Emulsion prepared at $\pm 15^{\circ}\text{C}$ gives larger globules ($28 \pm 11\mu\text{m}$ in diameter) but at 25°C gives globules with small size ($9 \pm 6\mu\text{m}$ in diameter)^{76,77}, influence on the rate of hydrolysis is also seen with the temperature changes⁷⁵. Agitation time varied with the RPM used i.e. 30 min with 3000 RPM & 5 min with 18,000 RPM for the first, 20 min with 900 RPM & 4 min with 12,000 RPM for the second step. Encapsulation efficiency & biological efficiency of the insulin as well as globule size of the emulsion depends on the nature of the lipid that constitutes the oily phase^{78,79}.

Results of oral administration are analyzed using one-way analysis of variance (ANOVA) test for multiple comparisons in every research conducted. Use of fish oils produces a thick primary emulsion which does not allow the formation of multiple emulsions, due to higher interfacial tension by longer hydrocarbon chain length [80]. Viscosity of emulsion & diameter of inner droplet of emulsion is influenced by the volume ratio of outer aqueous phase⁸¹ (**Fig. 7**).



A



B

FIG 7: MICROSCOPIC PICTURE OF INSULIN MCT-ME (A) INSULIN FO-ME (B) AFTER PREPARATION

Decrease in the volume ratio of outer aqueous phase, the oleic acid amount & agitation time, increase in the gelatin concentration in the inner aqueous phase influenced the viscosity of emulsion i.e. increased. Enhancement in stability of multiple emulsion is seen with formation of insulin-low HLB complex⁷⁶ and increase in gelatin concentration & decrease in insulin and the oleic acid amount, shortening of agitation time and volume ratio of aqueous phase⁸² and composition of the oil phase⁷⁹.

Protection of insulin by enzymatic proteolysis can be increased with increase in viscosity surrounding insulin by gelatin in inner aqueous phase and also by increase in the resident time of inner droplets at the mucosal membrane. Transport enhancement effect was seen with soybean oil as the oily phase and the effect increased with hydrolysis time, linoleic acid & its acylglycerols present in soybean oil; act as representatives of the hydrolysates. Monolinoleoylglycerol acts as main transport enhancer, obtained by the hydrolysis of trilinoleoylglycerol⁸³. The permeability coefficient in case of rehydrated emulsion in the presence of Fructooligosaccharide (FO) decreased, but with linoleic acid it is not affected.

Increase in permeability can also be due to lipase generated fatty acids or monoglycerides⁸⁴. Administration of emulsion with aqueous phase diluted under hypo-osmotic conditions seems to decrease glycemia than that diluted at iso-osmotic conditions & is more pronounced with emulsions containing sodium taurocholate (TC) as emulsifier.

Cyclic monoterpenes like menthol and limonene promotes the hypoglycemic effect of insulin at ileum region but not at colon^{85 - 87}, from colon region insulin can be absorbed only with the use of absorption enhancers.

Hypoglycemic effect increased with increase in oleic acid concentration form 0% to 2% in emulsion with no membrane damage. Rehydrated w/o/w emulsion filtered with 0.8 μm shows less effect, might be due to the presence of Fructooligosaccharide. Emulsions containing 2% fatty acids shows stronger hypoglycemic effects, due to disruption in the configuration of lipid region in the intestinal brush border membrane⁷³. Emulsion with zinc insulin showed no reduction of glycemia when compared with that containing sodium insulin after 120 min of glucose administration⁸⁸. Apical microvillus membrane in the small intestine provides major physical barrier to drug absorption

Bioavailability of insulin can be enhanced by inhibition of insulin degradation, forming of reverse micelles by interaction of bile salts with cell membrane as channel to increase membrane permeation & dissociation of molecular aggregates through micellar solubilization^{71, 89}. Multiple emulsion containing sodium taurocholate slightly increased the release of encapsulated insulin. Higher concentration of Aprotinin & sodium taurocholate is necessary to increase intestinal absorption of multiple emulsion⁸⁸. Unsaturated fatty acid is useful carrier for enhancing insulin absorption via intestinal tract. The amount of oleic acid in the emulsion is an influencing factor for the hypoglycemic effect⁸¹.

Emulsion containing soybean oil phase with a mixture of Fructooligosaccharide and Triacylglycerol, suppress transport of insulin. The lowered levels of blood sugar depend on the size of oil droplets. A stable unloaded Fructooligosaccharide emulsion with similar characteristics to that of Medium chain triglycerides can be obtained with low oil content after the viscosity of primary emulsion is reduced⁷⁹.

b. Emulsion by Membrane Emulsification:

Coating of insulin with surfactant, converts into a lipophilic complex, can be dispersed in the oil phase of oil-in-water (o/w) emulsions. Enhancements in the stability & control with the particle size can be expected with membrane

emulsification^{60, 90}. Stable emulsion is prepared using a homogenizer at 26,000 RPM for 1 min. followed by rapid freezing in liquid nitrogen a lyophilization for 24 h. soybean oil is added to surfactant-coated insulin, and dispersed thoroughly by ultra-sonication. The obtained s/o/w emulsion is adjusted to a constant particle size through a SPG membrane.

The activity of the insulin is retained when solubilized in s/o/w emulsion^{91, 92} with no coalescence or break down of the droplets during storage. Sharp particle size (1.1 μm) obtained by SPG membrane showed high stability by depressing the Ostwald ripening effect. The measurement for leakage of insulin from emulsion with the help of FTIC-labeled insulin (FTIC-INS), surfactant coated FTIC-INS did not leak from the emulsion as being a lipophilic complex.

The s/o/w emulsion on oral administration, readily soluble in micelles i.e. along with micelles from mucous membrane the coated insulin is slowly absorbed with hypoglycemic effects for long time (Fig 8).

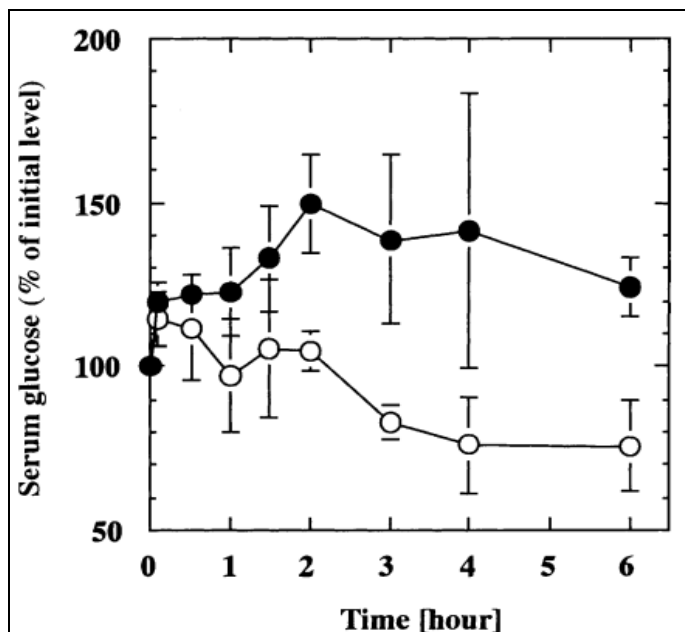


FIG 8: CHANGES IN SERUM GLUCOSE LEVELS AFTER ORAL ADMINISTRATION OF INSULIN EMULSION OR SOLUTION: S/O/W EMULSION (\circ), INSULIN SOLUTION (\bullet). Each value represents mean \pm S.D. (n=4)

The emulsion by s/o/w showed enhanced absorption of insulin followed by oral administration⁹³.

Enteric coated Pellets: Suitability of the pellets to incorporate additional substances to enhance oral bioavailability gives advantage as a dosage form. By film coating of pellets, it is possible to control the release of active ingredients^{94, 95}. Twin screw powder feeder fitted with specially manufactured stainless steel die plate with cylindrical holes of 1 mm diameter is used for extrusion of insulin pellets⁹⁶. Spheronization is done using a device with a 320 mm rotating disc.

During granule preparation before extrusion the powder blend is added with Aprotinin as protease inhibitor and sodium cholate as intestinal absorption promoter. Mannitol is used as non-sugar soluble filler, water pellets are coated with shellac, with air temperature of 40°C, product temperature 35-38°C, spray pressure 0.7 bar, spraying rate 5-8 g/min to obtain a film covering 7mg/cm² to protect over gastric juices. Higher humidities during Spheronization cause 'snow balling' (Table 2). Insulin in the pellets is stable for 4 months, stored at 4-8°C, the formulations coated with Aprotinin⁹⁷.

Table 2: Insulin pellet composition, formulation with sodium cholate and Aprotinin (ICAP)

Compound	Content/100 g
D (-) Mannitol	61.80
Microcrystalline cellulose	26.49
Sodium cholate	10.00
h-insulin	1.06
Aprotinin	0.36
Phosphate buffer salts	0.29

Addition of protease to the dissolution medium of pellets at pH 7.5 showed a considerable degradation of insulin. The results shows insoluble protection covering against gastric juice is obtained by shellac coating at lower temperatures.

Inhibition of enzymatic degradation of the drug is ensured by fast dissolution of Aprotinin. 80% of the dosage form reached distal parts of the small intestine within 6 h. enhancement of absorption through rectal mucosa is seen with sodium cholate with a concentration more than 0.2% (w/v)⁹⁸. After oral administration, it is proved to reduce plasma glucose by 40% (Fig. 9).

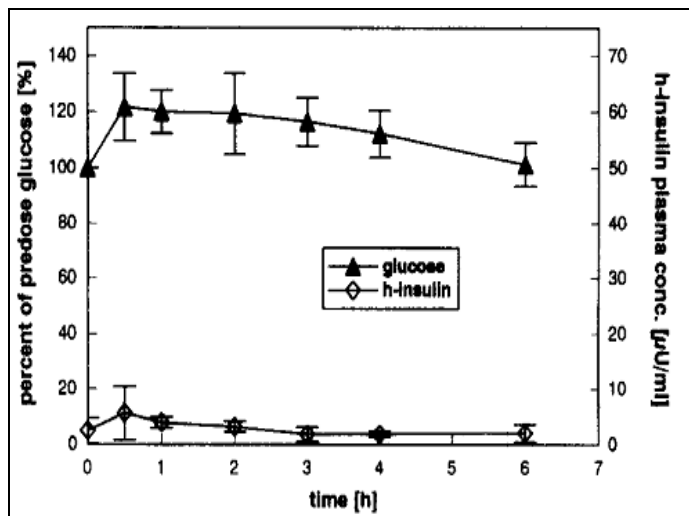


FIG 9: PLASMA LEVELS OF DIABETIC RATS AFTER ORAL ADMINISTRATION ICAP-INSULIN PELLETS (250 IU H-INSULIN AND 22000 KIU APROTININ/KG BODY WEIGHT) (n=4, mean±SD)

The main factor for deficient in insulin absorption is found to be the pancreatic luminal proteolysis in the duodenum by *invivoluminal* experiments, can be avoided by alterations in coating procedure of the pellets with thicker shellac films or with other polymer combinations⁹⁷.

Liposomes: The potential usefulness of Liposomes for encapsulating hydrophilic & hydrophobic drugs, biodegradable and are nontoxic in *in-vivo* has attracted them as drug carriers in past two decades⁹⁹⁻¹⁰². Various attempts are reported to apply liposomes for preparation of oral insulin¹⁰³⁻¹⁰⁸. A special method is required to develop liposomes, stable in GI tract by preventing the degradation by bile salts

TABLE 3: COMPOSITION OF VARIOUS LIPOSOMES (VALUE BEING EXPRESSED AS MOLAR RATIO)

	DPPC	Chol	SA	Cetyl-mucin (mg/ml)	DSPE-PEG
(+)- Lip	10	10	1	-	-
(N)-Lip	10	10	-	-	-
Mucin-Lip	10	10	1	1	-
PEG-Lip	10	10	-	-	1

Entrapment of insulin in liposomes is due to non-polar forces. Method proposed by Lowry¹¹⁵ is used to determine the concentration of insulin in liposomes. Size range of liposomes varied from 50-250 nm when prepared using DPPC/ DPPE / PSS (or) with PC / PI, but the liposomes prepared by Bangham method have a size range of 300-1 µm in all the cases. Entrapment of insulin onto liposomes increased with a rise of proportion of negatively charged Phosphatidyl ethanol also prevents the immune response to insulin.

which act as surfactants¹⁶. Lipid composition, surface charge and physical state are related to the hypoglycemic effect of liposomal insulin^{104-106, 109} and bioavailability is influenced by location of drug in liposomes¹¹⁰.

Various studies on the liposomes uses different composition, in a study, liposomes are made by mixing:

- Dipalmitoyl phosphatidyl choline (DPPC)/ Dipalmitoyl phosphatidylethanol (DPPE)/ Palmitoyl stearyl sucrose (PSS) in the ratio of 1:1:0.2 w/w/w, solid dissolving in chloroform.
- Phosphatidylcholine (PC) / Phosphatidyl-inositol (PI) in the ratio of 1:1 w/w (as fluid).

Insulin dissolved in 10 mM Tris-HCl buffer (pH 8.0) containing Ethylene diamine tetra acetic acid is added to dried lipid obtained after solvent evaporation under a stream of argon, to yield multilamellar liposomes.

In another study liposomes are prepared according to Bangham method with slight modifications¹¹¹ with various compositions as listed in **table 3** to form Neutral [(N)-Lip], positively charged [(+)-Lip], Mucin-Lip, PEG- Lip, PEG & Mucin is used for surface coating of materials (Table 3). Number of investigators demonstrated that diabetic animals can be administered with charged liposomes containing insulin in it¹¹²⁻¹¹⁴.

Increase in salt concentration results decrease in the entrapment efficiency, suggests that electrostatic mechanism is involved in binding of insulin to liposomes. Entrapment efficiency is seen to be 20 - 40 % for liposomes prepared using Bangham method.

On adsorption of insulin onto liposomes ζ- potential shifts to higher values¹¹⁰. Hydrolysis of liposomes is affected with the composition¹¹⁶.

Irregularities in the packing of “solid” substrate can be a reason for higher hydrolysis of DPPC in the liposomes¹¹⁷, as these solid liposomes are preferable substrate for pancreatic phospholipase A₂.

The degradation of insulin is completely suppressed by PEG-Lip in the intestinal fluid. 35 and 20% of insulin is degraded in 10 min for (+)-Lip and (N)-Lip, respectively there after no degradation is seen.

The *in-vivo* results showed hypoglycemic effects by liposomes composed of DPPC / DPPE / PSS within 3 h after administration, but no fall in plasma glucose is detected by Liposomes composed of PI / PC, acceleration of tryptic digestion of insulin by phosphatidyl inositol is a reason¹¹⁸.

Liposomes surface coated by PEG showed longer duration, (+)-Lip have effect for 1 h and (N)-Lip showed no effect when administered orally because the adhesive ability of them was low towards negatively charged bio-membrane than that of (+)-Lip.

As liposomes in blood circulation undergo a series of transformation a part of hormone is inactivated (Fig. 10 & 11).

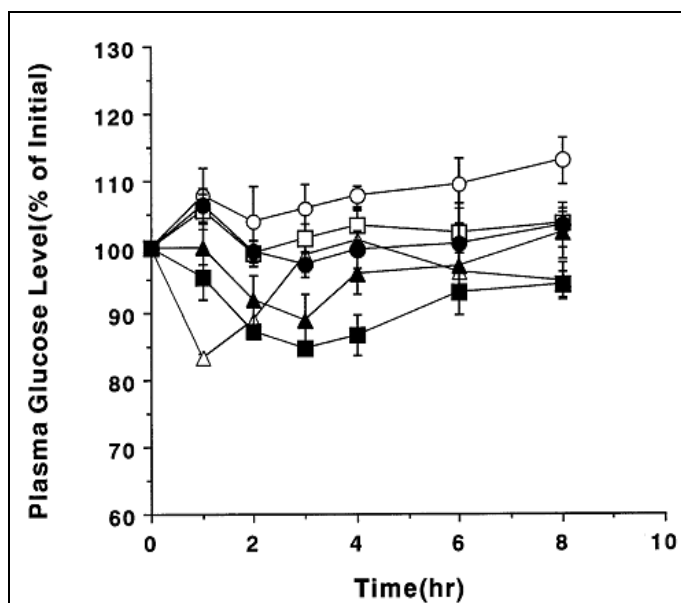


FIG. 10: DEGRADATION OF INSULIN IN THE INTESTINAL FLUID FROM RATS. ○, solution; △, (+)-Lip; □, (N)-Lip; ▲, Mucin-Lip; ■, PEG-Lip. Results are expressed as the mean 9 S.E.Lip; □, (n= 3-4). ■, PEG-Lip; ●, EMP-Lip.

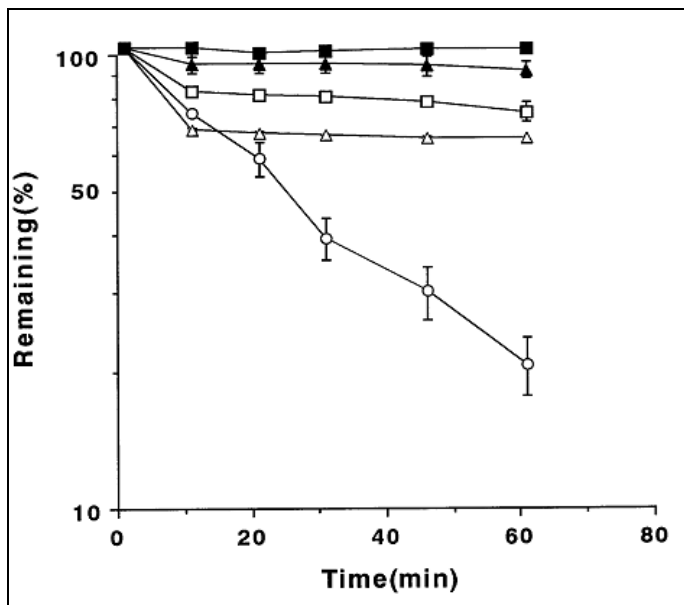


FIG. 11: PLASMA GLUCOSE LEVELS AFTER ORAL ADMINISTRATION OF INSULIN IN VARIOUS LIPOSOMES IN RATS. ○, solution; △, (+)-Lip; ▲, Mucin-Lip; Results are expressed as the mean 9 S.E. (n=4)

Release of insulin in acidic solutions by the action of bile salts on the liposomes is reduced by surface coating with PEG or Mucin, due to strong resistivity. Results show that ionic interaction is involved in binding of insulin to phospholipids rather than hydrophobic association¹¹⁹. Accumulation of liposomes on the brush border membrane, enables absorption of insulin & the uptake of liposomes itself by peyer's patches present in intestine also contribute to insulin absorption¹²⁰. Surface properties of liposome entrapped insulin plays a vital role in the therapeutic effects, which is mostly given by phosphatidyl ethanol.

Vesicles: Presence of hydrophilic core & hydrophobic bilayers, vesicles can deliver not only hydrophobic but also hydrophilic carriers, making them promising drug delivery carriers with enhanced permeability & retention properties^{121, 122}. An advantage of these vesicles, with change in molecular weight & block composition of the polymer, varied size and thickness of bilayers can be obtained.

The amphilic properties of pluronic block copolymers show strong affinity towards intestine & high permeation towards cell membrane^{8, 123}. In aqueous solutions PLA-F127-PLA block copolymers, found to be vesicular nanoparticles, potential for oral delivery of insulin of radii 56 nm.

Pluronic F 127 is attached on both ends with PLA to obtain an amphiphilic PLA-F127-PLA block copolymer, synthesized by ring opening polymerization of the L-Lactide monomer using stannous octate [sn (oct)₂] as catalyst & Pluronic F-127 as initiator¹²⁴. PLAF 127-29 block copolymer, 29 indicate the molecular weight of polymer (**Fig 12**), dissolved in THF and added to insulin drop to drop with gentle stirring.

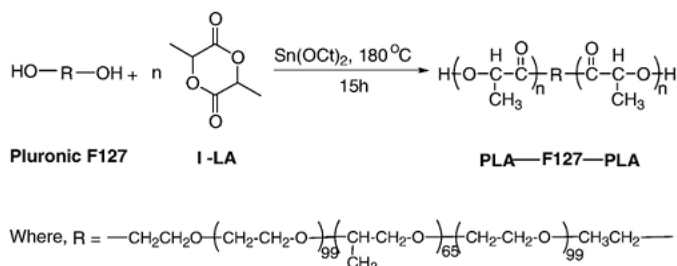


FIG. 12: SCHEMATIC REPRESENTATION OF SYNTHESIS OF PLA-F127-PLA BLOCKS COPOLYMER.

THF is removed by dialysis from the insulin-loaded polymer aggregates, lyophilization for 2 days results dried vesicles. The presence of appropriate PLA blocks (57.2 % in weight) in block copolymer PLAF 127-29 (Mn, 29 KD_a) is suitable to load insulin¹²⁴.

As the nanoparticles within the size range ~ 10–100 nm have higher circulation time & better bioavailability, PLAF 127-29 is desirable. PLAF 127-29 (0.08 wt%) has a calculated loading capacity of 0.3 g/g polymer. *In-vitro* release behavior is found to be biphasic with initial burst (50%) in first 30 min & a slow release in later stages for almost 2 h, however 82% of free insulin is released only 30 min later, 60% of insulin is released only 2 h later from PLAF 127-29 vesicles which is affected by factors like particle size & morphology, block composition, Mol. wt, degradation rate etc. Oral administration of PLAF 127-29 vesicles showed hypoglycemic effect with minimum glucose level, 25% of initial glucose level after 5 h & maintained this level for 18.5 h (**Fig. 13**).

The results show that PLAF 127-29 vesicles initially protected insulin from enzymatic degradation in GI tract & hypoglycemic effect for longer period is due to delayed GI transit, due to the small size of vesicles along with strong interaction of PE block with the intestinal wall. Vesicles are found to be promising carriers for oral insulin delivery with longer hypoglycemic effects¹²⁵.

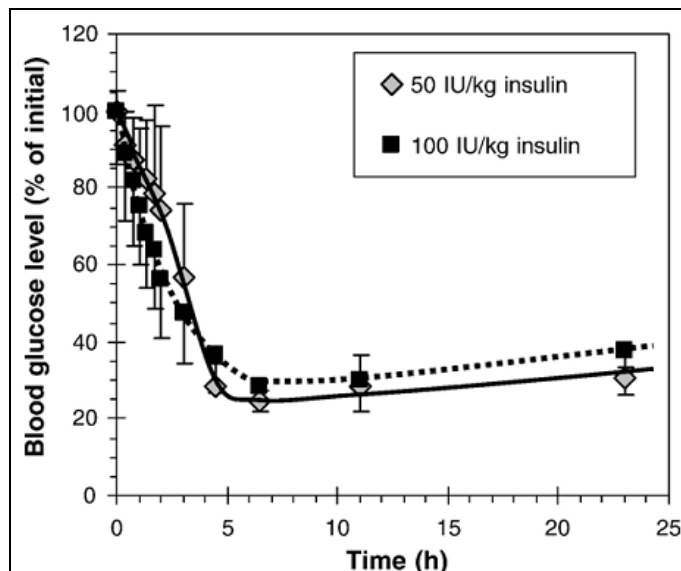


FIG 13: CHANGES IN BLOOD GLUCOSE LEVEL FOLLOWING ORAL ADMINISTRATION OF INSULIN-LOADED PLAF 127-29 VESICLES AT THE DOSES OF 50 IU/kg AND 100 IU/Kg. Results are mean ±S.D. (n=5)

Microspheres: Simplicity of preparation and the convenience of individualized dosage adjustments are the advantages of using microspheres. Reported in previous research, protection against enzymatic degradation can be attained by using protease inhibitors with insulin in microspheres¹²⁶. Microsphere are prepared by the methods previously discussed^{127, 128} using Eudragit S100 (s), L and LS in 1:1 ratio for the specific delivery of insulin at mid & lower intestinal regions and with or without Aprotinin. ANOVA method is used for statistical analysis of data followed by a student's unpaired t-test.

Incorporation efficiency for the microspheres is found to be more for L-IMS (80.2 % ± 8.4) with the smaller particle size compared to that of S-IMS (65.8% ± 5.4) & LS-IMS (78.1% ± 7.0). The lower insulin incorporation by S-IMS may be that during coating of microspheres, some amount of the polymer alone solidified. Release rate for *in vivo* studies show that at pH below 7.0, release is slow for S-IMS, less than 30% & for L-IMS it is about 70%. More than 90% of release is seen at pH 7.5 with all IMS.

The release of insulin by all three of the IMS is supposed to be at different sites through the small intestine. More than 90% of IMS is found at small intestine, 6 h after administration, shows rapid gastric emptying with similar profile for all three preparations.

Release by L-IMS is found to be at upper parts of the small intestine, where as that of S-IMS is at lower parts, LS-IMS also appeared in lower parts of intestine, until 1 h post administration although the ratio (%) is less than S-IMS. Hypoglycemic effects by L-IMS are found to be greater than LS-IMS

during initial 6 h of administration, S-IMS showed only after 2 h of administration. The effects by L-IMS appeared from lower intestinal absorption, as the release of insulin from L-IMS flowed to lower jejunum and ileum, having greater permeability¹²⁶ (Table 4).

TABLE 4: THE RATIO OF THE RESIDUAL IMS IN THE SMALL INTESTINE AGAINST THE IMS EMPTIED FROM THE STOMACH

Time (h)	L-IMS (%)			LS-IMS (%)			S-IMS (%)		
	Total	Upper	Lower intestine	Total	Upper	Lower intestine	Total	Upper	Lower intestine
1	8.2	8.2	0	19.0	6.1	12.9	20.7	3.0	17.7
2	7.3	6.1	1.2	4.2	1.8	2.4	20.2	5.8	14.4
4	2.3	1.2	1.1	12.9	4.2	8.7	18.7	3.1	15.6
6	2.5	0.4	2.1	5.8	1.5	4.3	4.0	0.3	3.8
8	0.3	0	0.3	1.1	0	1.1	2.8	0	2.8
10	0	0	0	1.6	0	1.6	4.1	0	4.1

LS-IMS & S-IMS release is appeared to be in more distal areas, absorption being extremely limited. Presence of Aprotinin enhanced biological effects in all three kinds by inhibiting trypsin & α -chymotrypsin, promoting insulin absorption in upper jejunum¹²⁹ & in ileum^{130, 131}.

The results state that L-IMS showed greater hypoglycemic effect with higher relative efficiency of 2.8 when compared to 2.3 & 1.5 by LS & S-IMS with Aprotinin¹³².

Intestinal patches: Patches with a proper polymeric drug carrier have the advantage of enzyme inhibitor and efficient paracellular permeation enhancement¹³³ with low doses of insulin to induce hypoglycemic effect¹³⁴. Fabricated patches of carbapol/pectin/SCMC with insulin concentration of 0.25-2.50 U/mg with radii of 1-4 mm is obtained and coated with ethylcellulose with a thickness of 50 μ m.

In another study, patches with 4% PCP-Cysteine, 26% insulin, 5% glutathione and 20% mannitol is found to obtain a patch with 2.5 mm diameter & 0.5-0.8 mm thickness with ethylcellulose coating on one side & Eudragit L 100-55 on the other side of the patch. The adhesion of these patches on the mucosa of the intestine depends on the amount of fluid content in the intestine, patch characteristics & amount of time spent in the intestine which is measured using method described by Bernkop-Schnurch¹³⁵. Unidirectional diffusion of insulin is obtained by the coating towards the mucosa, minimized the loss of insulin into the intestinal lumen with minimized enzyme penetration.

Adhesion force of patch decreased with increase in intestinal fluid volume, with a significant adhesion force of 1.0–2.7 N/cm² over a period of 4 h¹³⁶. The patches with Eudragit coating shows sustained release at intestinal pH conditions, 74.6 \pm 4.8 % release over 3 h with a zero order kinetic¹³⁷. The release from the patches with carbapol is over a period of ~ 4 h. Hypoglycemic effect is found to be dose dependent with high insulin uptake from patches the patches administered orally than that administered via jejunum^{138, 139}. Patches fully dissolved after 1–2 h that did not adhere to the mucosa.

Insulin levels dropped to 31.6 % of initial value after 6-8 h of administration & maintained for several hours by patches coated with Eudragit, site specific delivery into upper intestine (Fig. 14). Thiol groups in the patches attain improved mucoadhesion¹⁴⁰.

Water insoluble ethylcellulose layer provides the protection against enzymatic hydrolysis¹⁴¹ with a sustained release over a period of 6 h. The hypoglycemic effects by patches with carbapol was > 50% with doses in range of 5–10 U/Kg. Results state that Insulin absorption kinetics from patches depends on adhesion time and patch dissolution time, which indeed depends on the composition of the patch.





FIG. 14: IMAGES OF RAT MID-JEJUNUM SHOWING THIOMER PATCHES 6H AFTER ORAL ADMINISTRATION

DISCUSSION: From the literature survey, it is to be seen that wide range of research work has been carried out to achieve oral insulin delivery using different techniques. It is also proved that oral insulin drug delivery shows very poor absorption, less bioavailability, low stability and higher inter-patient variability. There are challenges that researchers need to overcome before going with newer delivery systems, also have to focus on the wide range of chemicals to be used in the formulation without altering the normal physiology of the GI tract, which can produce dose-dependent and reproducible effects in addition to increased bioavailability with fewer complications in the patients suffering with severe diabetes.

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How to cite this article:

Senthil C, Saikishan I, Jawahar N, Riswan B and Nagasamy V: Development and challenges facing for Insulin Oral Delivery. *Int J Pharm Sci Res* 2013; 4(7); 2576-2593. doi: 10.13040/IJPSR. 0975-8232.4(7).2576-93