5. SUMMARY
The current research work was designed to rise above cited predicament. Microspheres based colon specific drug delivery systems were expected to effectively target bioactive compounds and increase residence time as well.

Microspheres are biologically inert, nonirritating, nonmutagenic, nonallergic, nontoxic polymeric drug delivery systems, which have exposed huge potential in the delivery of drugs at desired site. Microspheres can also be described as porous microspheres, which are fabricated by using cross-linked polymers (mainly substituted acrylates or styrene-divinyl benzene).

This study encompassed training and assessment of Microspheres based colon specific tablet formulations. Initially, Microspheres of Insulin was prepared by quasi-emulsion solvent diffusion method using Eudragit RL 100 and Eudragit L-100. The reason for preparing microsphere due to earlier reports, which suggested that drug carrier, systems less than 200 μm may efficiently be taken up by the macrophages present in colon tissue, thus exhibiting effective localized drug action at the desired site. A subsequent increase of residence time that may be postulated for Microspheres as compared to existing drug delivery systems may allow dose reduction and enhance therapeutic effect. Another reason for preparing Microspheres was their sponge like texture because of which they can easily be compressed to produce mechanically strong tablets.

Thereafter, the core tablets of Microspheres were prepared by direct compression method which were compression coated with pectin:HPMC mixture. The reason for selecting pectin was its selectively biodegradation in the colon by colonic flora. HPMC increased the mechanical strength of the tablet coat and helped in maintaining its integrity during its sojourn in the gastro-intestinal tract.

The drug Insulin selected for present study were identified using different methods reported in the literature viz. melting point determination, determination of absorption maxima (λ_max), loss on drying, and FTIR spectroscopy.

The thermogram of differential scanning colorimetry showed sharp endothermic peaks of Insulin at 174.23 °C, corresponding to the melting range of the drugs in crystalline form (Insulin- 172-174 °C). Absorption maxima (λ_max) of Insulin was noted to be at
wavelength 420 nm corresponding to the values reported in literature (Insulin - 420 nm). The loss on drying for Insulin was observed to be 2.516 % (limit NMT 10.0%).

FTIR spectra of the Insulin exhibited characteristic C-N, C-O, C-H, C=O (ester) stretching bands at 1134.07 cm$^{-1}$, 1193.85 cm$^{-1}$, 2929.67 cm$^{-1}$, 1718.45 cm$^{-1}$, respectively. FTIR spectra showed characteristic O-H, N-H, C=O (amide) stretching bands at 3326.98 cm$^{-1}$, 3413.77 cm$^{-1}$, 1654.81 cm$^{-1}$, respectively. Whereas, amide II band, C-N-H group and para-disubstituted aromatic rings were observed at 1560.30 cm$^{-1}$, 1259.43 cm$^{-1}$ and 837.05 cm$^{-1}$. The FTIR spectra drug confirmed their identity and purity.

Calibration curve of the drug was prepared in 0.1N HCl, phthalate buffer pH 4.5, phosphate buffer pH 6.8, phosphate buffer pH 7.4 and phosphate buffer pH 6.8 with pectinex ultra SPL. Calibration curve data of both the drugs were subjected to linear regression analysis. R-values were found to be 0.9995, 0.9997, 0.9998, 0.9998 & 0.9998 for Insulin in 0.1N HCl, phthalate buffer pH 4.5, phosphate buffer pH 6.8, phosphate buffer pH 7.4 and phosphate buffer pH 6.8 with pectinex ultra SPL indicating good linearity.

The solubility the drug was determined in different media. drug was found to be sparingly soluble in acidic medium and slightly soluble in basic medium. The solubility of Insulin in 0.1N HCl, phthalate buffer pH 4.5, phosphate buffer pH 6.8, phosphate buffer pH 7.4 was found to be 0.0163 gm/ml, 0.0083 gm/ml, 0.0069 gm/ml, and 0.0057 gm/ml, respectively.

The compatibility of drug with Eudragit RL100, Eudragit L100, and PVA was assessed by FTIR spectroscopy by keeping the samples at 40°C and 75% RH and at room temperature for 1 month. In FTIR spectra characteristic N-H stretching band at 3413.77 cm$^{-1}$, O-H stretching band at 3326.98 cm$^{-1}$, and carbonyl stretching band at 1654.81 cm$^{-1}$ were seen and in case of Insulin, characteristic C=O stretching band was observed at 1718.45 cm$^{-1}$, which are in agreement with the reported values. Eudragit RL 100 showed an ester C=O stretching peak around 1726.17 cm$^{-1}$ and Eudragit L 100 showed carbonyl stretching at 1718.46 cm$^{-1}$ & bond characteristic to carboxylic group in the range 2437-3473 cm$^{-1}$ as reported in the literature. The results showed that no chemical interaction or changes took place in the mixtures of the drug and
various excipients alone or in combination, as exhibited by the FTIR spectra, thus indicating compatibility of the drugs with all excipients.

Quasi-emulsion solvent diffusion method was used for preparation of Microspheres. The drug and polymer in the ratios 5:1, 4:1, 3.33:1, 2.86:1 were taken to prepare different microspheres formulations. In each formulation, the amounts of polymer (200 mg), dichloromethane (5ml), PVA (0.5% w/v) were kept constant. The microspheres formulations were prepared using mechanical stirrer (Remi RQ1217-D) at a stirring rate of 500 rpm for Eudragit RL-100 based Microspheres and 1000 rpm for Eudragit L-100 based Microspheres for 8 hours. The various microspheres formulations namely SP1, SP2, SP3, SP4 containing Drug:Eudragit RL-100 in the ratios 5:1, 4:1, 3.33:1, 2.86:1, respectively and PS1, PS2, PS3, PS4 containing drug : Eudragit L-100 in the ratios 3:1, 6:1, 9:1, 12:1, respectively were prepared.

The effect of various variables like drug to polymer ratio, stirring rate, volume of internal phase, amount of emulsifying agent on the nature of Microspheres was studied. The morphology of the Microspheres was studied by scanning electron microscopy (SEM). The Microspheres were observed to be spherical and uniform with no drug crystals on the surface. It was noted that drug-polymer ratio has considerable effect on the morphology and size of Microspheres. It was observed that as the ratio of drug to polymer was increased, the particle size decreased. The mean particle size of formulations SP1-SP2, PS1-PS4 in the ratios of 5:1, 3.33:1 were found to be between 60-44μm, 53-34 μm respectively. This could probably be due to the fact that in high drug to polymer ratios, the amount of polymer available per microspheres was comparatively less. Hence less polymer surrounded the drug resulting in smaller Microspheres.

The effect of stirring rate on the size of Microspheres was studied by photo microscope RXLr-3T (Radical, India). The formulation with the lower drug to polymer ratio (i.e., 5:1) was chosen to investigate the effect of stirring rate on the morphology of Microspheres. The stirring rate was varied in the range of 300 to 500 rpm for Eudragit RL-100 based formulations and 500 to 1000 for Eudragit L-100 based formulations. The dispersion of the drug and polymer into the aqueous phase and the formulation of microspheres were found to be dependent on the agitation speed. As the speed was...
increased, smaller spherical Microspheres with uniform size were formed. When the rate of stirring was increased 300 - 500 rpm Eudragit RL-100 based Microspheres, the spherical Microspheres were formed with mean particle size of 71µm - 60 µm for the formulation SP1. When the rate of stirring was increased 500 - 1000 rpm for Eudragit L-100 based Microspheres the spherical Microspheres were formed with mean particle size of 74 µm - 52 µm for formulation PS.

The result suggests that the amount of dichloromethane need to be controlled within an appropriate range to effect not only the formation of quasi-emulsion droplets at the initial stage but also the solidification of drug and polymer in the droplets. Microspheres were formed when 3 to 5 ml of dichloromethane was used.

An increase in amount of polyvinyl alcohol (emulsifying agent) from 0.5 % to 1.0 % w/v resulted in decreased production yield and increased mean particle size. The amount of emulsifying agent significantly effected the production yield and mean particle size. Due to non-ionic nature of the emulsifier some hydrophobic region might have formed which dissolved some of the drug and polymer resulting in lower production yield. An increased amount of emulsifying agent decreased the production yield from 79% to 61%, 73% to 65% for the formulations SP1, PS1 respectively. The increase in the amount of emulsifying agent resulted in larger Microspheres, probably due to increased viscosity, wherein larger emulsion droplets formed resulting in larger Microspheres. An increased amount of emulsifying agent increased the mean particle size from 60 µm to 71 µm, 53 µm to 64 µm for the formulations SP1, PS1, respectively.

The prepared microspheres formulations were characterized for angle of repose, Carr’s Index, Hausners ratio, production yield, actual drug content, encapsulation efficiency and mean particle size. The value of angle of repose, Carr’s Index and Hausners ratio was found to be between 14.14-25.050, 2.39-13.46 % and 1.02-1.14, respectively which showed excellent compressibility and good flowability.

The production yield was found to be between 72-76% for SP1-SP4, 70-79% for PS1-PS4. The actual drug content was found to be between 74-91% for SP1-SP4, 62-81% and 67-83% for PS1-PS4. The encapsulation efficiency ranged from 82-98%. The mean particle size was found to be between 60-44 µm for SP1-SP4, and 53-34 µm for PS1-PS4. The data obtained for various formulations in respect to production yield, actual
drug content, and encapsulation efficiency were subjected to t-test at 95% level of significance. No significant difference in relation to these parameters was observed amongst various formulations at p <0.05.

The different Microsphere formulations of Insulin was subjected to in-vitro release studies using USP XX1V dissolution assembly. It was observed that for each formulation the drug release decreased with increase in the amount of polymer. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases. The release showed a bi-phasic pattern with an initial burst effect. In the first hour drug release of different Microsphere formulations SP1- SP4, and PS1- PS4 was noted to be between 15-30% and 19- 29 %, respectively. This may be attributed to the drug present in the pores of the Microspheres. The overall cumulative percent release for different Microsphere formulations SP1- SP4, & PS1- PS4 at the end of 8 h was found to be between 61-94 %, & 56-86 %, respectively.

The correlation coefficient and release rate constant values for zero order, first order, Higuchi and Korsemeyer models were computed. The correlation coefficient values of different Microsphere formulations namely SP1- SP4, & PS1- PS4 were found to be between 0.9538-0.9733, & 0.9647-0.9720, respectively for zero order model; between 0.9748-0.9854, & 0.9832-0.9890, respectively for first order model; between 0.9783- 0.9884, and 0.9863-0.9921, respectively for Higuchi model. The R values were much closer to 1 for the Higuchi kinetics. From the correlation coefficient values it is concluded that the drug release from different Microsphere formulations follow Higuchi model. Higuchi model explained the matrix diffusion mechanism of drug release. The correlation coefficient values for Higuchi model confirmed that drug release followed matrix diffusion mechanism or Higuchi pattern release. The mechanism of drug release of the all Microsphere formulations was studied by fitting the release data to Korsemeyer equation. The n values for formulations SP1- SP4, and PS1- PS4 was found to be between 0.7476-0.6989, and 0.7231-0.6199, respectively. The n value for Korsemeyer-Peppas model was found to be between 0.5-1 indicative of non-fickian diffusion.
Chapter 5

The in-vitro dissolution data was subjected to statistical analysis using ANOVA. The p value was found to be 0.5930, and 0.5207 for SP1- SP4, and PS1- PS4, respectively. This indicated significant difference (p>0.05) amongst various formulation in relation to dissolution behaviour.

The core tablets consisting of Microspheres containing 40 mg Insulin Na-CMC and magnesium stearate were prepared by direct compression method. The core tablets were evaluated for various parameters like weight variation, thickness, hardness, friability, and drug content.

The average weight of the core tablet formulations CPDRS1-CPDRS4 and CPDS1-CPDS4 was found to be between 438 - 470 and 442 - 461 mg, respectively. The variation in weight was within the range of ±5% complying the pharmacopoeial specifications. The hardness was found to between 4.1 kg/cm2 - 4.8 kg/cm2 indicating satisfactory mechanical strength. The friability of the core tablet formulations were found to be between 0.23 % - 0.79 %. The friability was below 1% which indicated good mechanical resistance. The thickness was found to between 2.76-3.01 mm.

The coated tablets were prepared by compression coating with Pectin:HPMC (80:20) mixture as outer shell. The coated tablet formulations were evaluated for various parameters like weight variation, thickness, hardness, and friability.

The average weights of the coated tablet formulations CPDRS1-CPDRS4 and CPDS1-CPDS4 were found to be between 637 - 670 and 648 - 663 mg, respectively. The variation in weight was within the range of ±5% complying the pharmacopoeial specifications. The hardness was found to between 5.8 kg/cm2 - 6.8 kg/cm2 indicating satisfactory mechanical strength. The friability of the core tablet formulations were found to be between 0.41 % - 0.79 %. The friability was below 1% which indicated good mechanical resistance. The thickness was found to between 3.40 - 3.67 mm.

The developed formulations (CPDRS1-CPDRS4 and CPDS1-CPDS4) were subjected to in-vitro drug release studies using USP XXIV dissolution assembly at the stirring rate at 50 rpm and temperature at 37±0.5 oC. The dissolution studies were carried out at first hour in 0.1N HCl, second and third hour in phthalate buffer pH 4.5, fourth and fifth hour in phosphate buffer pH 6.8, sixth hour in phosphate buffer pH 7.4 and after 6th hour in mixture of phosphate buffer pH 6.8 and pectinex Ultra-SPL (1% v/v) in order to
simulate the enzymatic action of the colonic bacteria were used.
It was observed that no drug was released in the first six hours. After the lag time of 6 hours, the drug started releasing at 7th hour due to the presence of the pectinex Ultra-SPL. Formulation CPDRS1, CPDRS2, CPDRS3, CPDRS4 released 76%, 99%, 98%, 99% of Insulin, respectively at the end of 12-14 h. Formulation CPDS1, CPDS2, CPDS3, CPDS4 released 72%, 99%, 97%, 93% of Insulin, respectively at the end of 12-14 h.

The results of in-vitro drug release studies showed that pectin: HPMC (80:20) coat could protect the core for 6 hours which correspond to the time to reach the colon and then under the influence of the enzyme, the system started delivering the drug to the proximal colon, main site for bacterial carbohydrate metabolism.

Stability study was carried out at 40 0C ± 2 0C and 75 % ± 5% RH for 3 months. The changes in drug content of different formulations were noted. The obtained data was subjected to t-test at 95% level of significance. No significant difference in relation to drug content was observed amongst various formulation at p <0.05.

The results of the in-vitro drug release and stability studies indicated that the formulations CPDRS1 could be the potential formulation for targeting the Insulin to the colon.

This study presents a new approach for the preparation of modified Microspheres as well as a new delivery system with a great potential for colonic drug delivery. The unique compressibility of Microspheres offers a new alternative for producing mechanically strong tablets. The colon specific tablet formulations were prepared following two approaches. Both the approaches, using the triggering mechanism of micro-flora activation, represented interesting forms for delivery if the drugs to the proximal part of the colon, avoiding release in the small intestine. The particulate form (Microspheres) has been used to provide more uniform distribution of the drug in the colon and help the drug to spread on the colon surface in an appropriate way.

The obtained Microspheres exhibited spherical shape, good flow ability and excellent compressibility properties. The compression coated formulations prepared using pectin and HPMC in the ratio of 80:20 protected the drug from being released in the stomach and small intestine under in-vitro conditions mimicking mouth-to-colon transit.
This study presents a novel colon specific drug delivery system containing Insulin Microspheres. It is concluded that the Microspheres prepared by quasi-emulsion solvent diffusion method can be used successfully for colon specific drug delivery.

5.1 **Outcome of the research:**

Thus, it can be concluded that CPDRS1 formulation is shows better results and hence, Insulin as anti Diabetis can be successfully delivered as microspheres.

Effect of drug-polymer ratio on the size of Microspheres and Effect of amount of emulsifying agent on the production yield and size of Microspheres Optimization of Insulin loaded Eudrajit L microspheres Eudrajit RL and conclude that proper concentration of polymer and emulsification agents give us better formulation and production yield.

Thus, major Advantages of the oral insulin system include: Ease of preparation, Good and easily available of material, High encapsulation efficiency, Sustained drug release over several hours. Improved half-life, lower immunogenicity as compared to insulin, in the digestive tract and improved absorption, lower mitogenicity as compared to insulin, retains a similar pharmacological activity as insulin, and conserves safety profile and good clearance profile as compared to insulin.

5.2 **Limitations of work done:**

The question is then how the insulin molecules are actually transferred across this wall into the bloodstream; there is no insulin specific transfer mechanism. Potentially different routes can account for this transfer. The basic idea of the majority of the oral insulin development approaches presented in the following sections is to use one or more of these transfer mechanisms for other substances to bring insulin along with them into the Bloodstream. The approaches presented in the following sections differ significantly in this respect; this appears to have a profound impact on the rapidity of absorption/onset of metabolic effect and the amount of insulin that is intact absorbed into the bloodstream.

It appears as if, with most developments, the high barriers for oral insulin are associated with a considerable loss of insulin; only a relatively small number of insulin molecules make it successfully into the portal blood. Insulin measurements of insulin levels in the peripheral blood after administration of an oral insulin formulation do not reflect the
pharmacokinetic (PK) properties in the same manner that we are used to with SC insulin administration. This had to be taken into account when PK summary measures were discussed with oral insulin, at least when it came to parameters describing maximal concentration levels. This fact also had to be kept in mind when numbers about the relative bioavailability of an oral insulin formulation (comparison of serum insulin levels achieved after SC injection of a given dose of a prandial insulin formulation versus oral administration of a given dose of an oral insulin formulation) were provided; instead the relative biopotency should be reported. This requires performance of adequately designed glucose clamp studies. Unfortunately, most companies do not employ this standard approach to evaluate the pharmacodynamic (PD) properties and thereby the biopotency of their novel oral insulin formulations. In view of its mechanism of action, measurements of the suppression of hepatic glucose production by means of stable tracers would also be of high relevance. However, this was not employed in any of the studies reported thus far (only in a study performed), most probably due to cost reasons. The PD properties of oral insulin can also be assessed by meal challenge studies, i.e., measurement of the differences in postprandial glycemic excursions with standardized meals and different insulin administrations.

In view of the attractiveness of this route of insulin administration and all the failures in the past, it is obvious that the barriers built up by Mother Nature for oral insulin must be extremely high. The peptide insulin must survive transit through the gastrointestinal tract (GI) in order to allow absorption into the bloodstream. It is the job of the gut to destroy proteins into amino acids that are absorbed via the epithelium in the GI but to avoid uptake of potentially dangerous proteins. The low pH in the stomach and the activities of peptidases in the GI usually degrade/destroy all peptides.

If insulin molecules make it intact into the gut (experimentally, a solution containing insulin can be applied by means of a catheter directly into the gut), they have to pass the wall of the jejunum, which is a single thick mucus layer with a tight barrier of epithelial cells. Mucus is secreted by the underlying mucosal goblet cells and is continuously excreted at the apical side of enterocyte. It is a dynamic structure exhibiting a viscosity gradient such that the viscosity of mucus increases from the bottom (as a liquid to be excreted) to the top (as a gel to act as a permeability barrier). From a chemical point of
view, diffusion of insulin into the mucus lining the gut should be rather easy since both are of hydrophilic nature. However, intermolecular interactions between the functional groups of both insulin and mucins (such as COOH, OH, and NH2) cannot be underestimated, especially through hydrogen bonds between both proteins. From a physical point of view, diffusion of a protein such as insulin (which has a molecular weight of approximately 6000 Da) in the mucus layer is hampered because of the high viscosity of the latter. Therefore, the diffusion coefficient of native insulin in the intestinal mucus is most probably quite low.

5.3 Industrial application and future done:

Various types of insulin are available in the market, including newer analogs (lispro, aspart, glargine). Although insulin analogs seem to be more physiological, controlled studies suggested either similar efficacy to regular insulin or only a minor benefit in favor of insulin analogs. Noninvasive insulin deliveries are now in development. In the future, patients with type 1 diabetes will receive insulin in optimal quantities at optimal times by way of optimal routes into the body because of needle-free routes of administration in order to achieve optimal blood glucose control. These new technologies will facilitate proper treatment of type 1 diabetes and improve the lives of affected patients. Among the noninvasive ways for controlling diabetes in the future, the Microspheres systems seem to be promising potential drug carriers for oral insulin delivery. The ultimate goal for the treatment of diabetes remains the development of a fully automated glucose-controlled by tablet formulation in this study.

The current research work was designed to rise above cited predicament. Microspheres based colon specific drug delivery systems were expected to effectively target bioactive compounds and increase residence time as well. The current formulation for oral insulin is a second generation tablet, which is declared to be simple to manufacture, uses readily available excipients, and has an attractive stability profile at ambient conditions. It appears as if We are intensively working on this development.

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The obtained Microspheres exhibited spherical shape, good flow ability and excellent compressibility properties. The compression coated formulations prepared using pectin and HPMC in the ratio of 80:20 protected the drug from being released in the stomach and small intestine under in-vitro conditions mimicking mouth-to-colon transit. This study presents a novel colon specific drug delivery system containing Insulin Microspheres. It is concluded that the Microspheres prepared by quasi-emulsion solvent diffusion method can be used successfully for colon specific drug delivery.
5.4 How the developed formulation overcome the potential side effect of insulin by enhance the therapeutic effectiveness.

A large number of drug delivery systems are presently available to treat diabetes. However, therapeutic efficacy of the drug is frequently effective by parental use that is not suitable for daily injection due to painful. The present research work was aimed to overcome this problem. Microsphere based colon specific drug delivery system(s) was expected to effectively target bioactive compounds and increase residence time as well.

Insulin is degraded very quickly by the stomach's acidic environment and proteolytic enzymes. The dream of an "Insulin tablet" has also not become a reality, the main problem being digestion and a lack of a specific peptide carrier system in the gut.

Researchers are currently examining whether insulin absorbed into a microsphere can bypass these enzymes and pass through the wall of the intestine. But this research is still in its early phases. The polymer in acid collapses into a tight ball that traps the insulin. In about 30 minutes, the tablet reaches the non-acidic intestine, where the polymer expands to release the insulin. so far it has been hard to predict how much insulin will be absorbed and how fast. Also, at least 85 percent gets wasted.

This study encompasses preparation and evaluation of microspheres based colon specific tablet formulations. Initially, microspheres of Insulin was prepared by quasi-emulsion solvent diffusion method using Eudragit RL 100 and Eudragit L-100. The reason for preparing Microsphere due to the fact that, drug carrier systems less than 200 μm may efficiently be taken up by the macrophages present in colon tissue, thus exhibit effective localized drug action at the desired site.

5.5 How the develop formulation is superior than as compared to conventional delivery systems.

Insulin conventionally deliver by the parenteral route which are obviously painful for the daily dose of the drug by injection, so for overcome of that convention potential situation we are highly recommend for these innovative work for brightness of the pharmacy field in the global.
A subsequent increase of residence time that may be postulated for microspheres as compared to existing drug delivery systems may allow dose reduction and enhance therapeutic effect. Another reason for preparing microspheres was their matrix like texture that can easily be compressed by direct compression for producing mechanically strong tablets.

The obtained Microspheres exhibited spherical shape, good flow ability and excellent compressibility properties. The compression coated formulations prepared using pectin and HPMC in the ratio of 80:20 protected the drug from being released in the stomach and small intestine under in-vitro conditions mimicking mouth-to-colon transit.

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