SUMMARY AND CONCLUSION

T1D is characterized by immune mediated pancreatic islet β-cell destruction. The process is insidious and may evolve over many years, with the overt expression of clinical symptoms becoming apparent only when most β-cells have been destroyed. Much investigation has been directed at interdicting the T1D disease process either by intervening during the stage of evolution of the disease to prevent it from becoming clinically manifest or by arresting the progression of the disease around the time of disease onset. Most studies have been conducted shortly after disease onset, with the aim of preserving residual β-cell function and thus facilitating glycemic management while also decreasing the likelihood of both hypoglycemia and long-term complications. A smaller number of studies have been conducted in individuals at high risk of T1D, with the goal of arresting immune destruction and thus preventing or delaying the clinical onset of T1D. Based on studies in animal models of T1D, particularly the NOD mouse and the Biobreeding rat, many strategies have been proposed to alter the course of T1D. Indeed, in the NOD mouse, there have been several hundred interventions that have altered the course of the disease.

A number of different immunological intervention strategies designed to preserve residual β-cell function have been studied in humans. Most studies have been conducted soon after the onset of T1D.

The concept beyond the application of combination of monoclonal antibodies and DPP-IV inhibitors is to restore the immune tolerance as well as residual β-cells mass in T1D individuals. Individually, both monoclonal antibodies and DPP-IV inhibitors are well documented in the prevention of T1D at preclinical and clinical level. The combination of monoclonal antibodies and DPP-IV inhibitors sounds good in T1D treatment, as the T1D is a state of failure of immune tolerance to self-antigens and loss of β-cells. Thus in the present work an attempt was made to develop anti-CD4 monoclonal antibody conjugated sitagliptin loaded nanoparticles. In this system simultaneous targeting of CD4⁺ T-cells that are involved in the T1D disease progression and increasing the endogenous GLP-1 half-life thereby initiating the restoration of β-Cell mass can presumed to be occur.
SUMMARY AND CONCLUSION

With the extensive literature survey the pre-formulation studies was carried out initially. As a part of development and manufacture of formulations, the analytical and bio-analytical method was developed using HPLC. Both the analytical and bio-analytical methods were suitable for estimation of drug without any interference of polymer or plasma components at wavelength of 267nm. The C$_{18}$ column was used as stationary phase with mixture of ACN and 0.5% TEA (pH 6.8) as mobile phase. The ratio of mobile phase ACN: 0.5% TEA was maintained at 50:50 and 23:77 for analytical and bio-analytical, respectively. The curve of linearity was developed for both analytical and bio-analytical method by plotting AUC versus concentration and response factor versus concentration, respectively.

The analytical method was developed for anti-CD4 mAbs using shimadzu LC20AD HPLC system and the LC solutions software. Princeton SPHERE C$_{18}$ column was used as stationary phase with 20mM KH$_2$PO$_4$ (pH-6.8) : ACN (50:50, v/v) as mobile phase at a flow rate of 1.0 ml/min. Plotting AUC versus concentration developed the curve of linearity.

To study the mode of compatibility between drug and polymer of choice, the FTIR and DSC were applied after storing the physical blend of drug and polymer at isothermal stress conditions. The study revealed the polymer was compatible with drug. With acceptable preformulation parameters the polymeric nanoparticulate system of SP was formulated by simple nanoprecipitation cum solvent evaporation technique. Nine batches from A1 to C3 were prepared to understand the process parameters. A1, A2 and A3 batches were formulated with 0.5% surfactant, B1, B2 and B3 batches were formulated with 1% surfactant and C1, C2 and C3 batches were formulated with 1.5% surfactant.

The so obtained formulations were subjected to in vitro characteristic studies like the process yield, particle size, zeta potential, DSC analysis, drug loading and entrapment efficacy, surface morphology, in vitro release pattern, and accelerated stability studies.
SUMMARY AND CONCLUSION

The process yield for all the batches was more than 70%. Particles size was determined for all the developed formulations. The formulated nine batches (A1 to C3) reported particle size less than 150 nm. Among all nine formulated batches minimum particle size (133.8 nm) and maximum percentage drug entrapment efficiency (76.79 ± 2.17) was achieved with the B3 batch. The zeta potential for all batches ranged from -17.4 to -23.7. DSC analysis revealed that the peak at 215.26°C exhibited by SP was not visible in the SP loaded NPs, indicating that SP was encapsulated by the polymer in the NPs. The entrapment efficiency for all batches ranged from 71.72 to 77.09%.

The surface morphological studies of SP-NPs showed that the particles were almost spherical and were non-aggregated. The release pattern of drug from formulations was carried by dialysis bag method at 37±2°C using 20 ml of phosphate buffer pH 7.4 as the media for SP formulations. At the end of 96th h the release of drug from A1, A2, A3, B1, B2, B3, C1, C2 and C3 batches was 88.25, 85.18, 82.11, 87.29, 82.76, 78.24, 86.33, 79.41 and 72.49 % respectively on an average of three observations. To understand the release behavior, mathematical models like Zero order, First order, Higuchi, Peppas and Hixson-crowell study designs were applied. The release of drug from NPs was diffusion controlled as indicated by the higher r² values in the higuchi model. Since, the n values obtained from the Korsmeyer-Peppas model were less than 0.45, the mechanism of drug release from the NPs was Fickian.

As the formulations are recommended to refrigeration during storage the accelerated stability studies were carried at 25±2°C at 60±5% RH. The entrapment efficacy, average particle size, cumulative % release were determined as function of analysis to study the stability of formulations. There was no significant change in results of above parameters even at the end of 6 months on comparison to their day zero values. Thus formulations are termed to be stable.

Anti-CD4 mAbs were successfully coupled to batch B3 SP-NPs in two steps: introduction of –SH groups on the surface of NPs and covalent coupling of anti-CD4 mAbs to NPs–SH via the bifunctional crosslinker, sulfo-MBS. The amount of anti-CD4 mAb conjugated to NP was determined indirectly by measuring uncoupled mAb in the
SUMMARY AND CONCLUSION

supernatant by a HPLC method. The amount of anti-CD4 mAb conjugated to NPs-SH was found to be 359.41 μg/ml for selected 1 h time of incubation.

The so obtained anti-CD4 mAb conjugated SP-NPs formulation was subjected to in vitro characteristic studies like FTIR analysis, particle size, zeta potential, surface morphology, Hemolysis study, and in vitro cytotoxicity study.

The preliminary qualitative analysis for the coupling of anti-CD4 mAbs to NPs by FTIR analysis reveals that mAbs successfully coupled to NPs. It was observed that increase in particle size and decrease in zeta potential followed by anti-CD4 mAb conjugation to increase in particle size. Surface morphology studies by TEM reveals that mABs successfully coupled to NPs.

Hemolysis studies resulted that neither SP-NPs nor anti-CD4 mAb-SP-NPs induced agglutination of erythrocytes. In vitro cytotoxicity studies suggested that both SP-NPs and anti-CD4 mAb-SP-NPs were non-toxic to pancreatic cell lines. These results suggested that the developed NPs formulations safe for delivery.

BALB/c mice weighing 20-30g were divided into three groups each carrying six animals for investigation of pharmacokinetic profile of formulations on single i.p. infusion of SP solution, SP-NPs (B3 batch) and anti-CD4 mAb-SP-NPs. The pharmacokinetics of SP solution, SP-NPs and anti-CD4 SP-NPs differed significantly (P < 0.0001). The bioavailability of SP was significantly enhanced by SP-NPs (1.9 fold) and anti-CD4 mAb-SP-NPs (3 fold) (P < 0.0001) when compared with SP solution.

Similar grouping was made for tissue distribution studies in T1D induced BALB/c mice with each group carrying 12 animals. SP meteorically taken up into the pancreas and bring off a maximal concentration at 3 h for SP solution and 4 h for SP-NPs and anti-CD4 mAb-SP-NPs. The SP delivered through SP-NPs and anti-CD4-SP-NPs experienced enhanced distribution to the pancreas.
SUMMARY AND CONCLUSION

Adoptive transfer of diabetogenic splenocytes successfully induced autoimmune diabetes in BALB/c mice recipients. Followed by the four weeks of treatment after onset of diabetes, the response to treatment was monitored by BGLs, OGTT, insulin and active GLP-1 content during OGTT and cytokine analysis. Anti-CD4 mAb-SP-NPs significantly lowered the BGLs (P < 0.0001), significantly increased oral glucose tolerance, insulin and active GLP-1 levels during OGTT (P < 0.0001). The cytokine analysis reveals that the anti-CD4 mAb-SP-NPs treatment leads to the reduction of the cytokine proliferation in diabetes induced BALB/c mice.

Immunohistochemical analysis demonstrated that negative control mice had > 80% reduction of total β-cell mass relative to normal control mice. Treatment with anti-CD4 mAb-SP-NPs restored β-cell mass significantly (P < 0.001) but did not expand it beyond that of normal mice.

In conclusion overall, the anti-CD4 mAb conjugated sitagliptin loaded nanoparticles gets targeted to the pancreatic site with prolonged half-life. The dual action i.e. blockage of CD4+ T cells autoimmune response and increased GLP-1 half-life might have influenced the significant change in their β-cell dynamics thereby reversal of autoimmune diabetes. Thus the anti-CD4 mAb conjugated sitagliptin loaded nanoparticles are expected to have better therapeutic efficacy in treatment of T1D. However, further research at the pre-clinical or clinical level with the large group of subjects is in needed to understand the status of any dual action involved in the abrogation of T1D.