TrabiORAL™ Final - Phase III

The platform developed by TrabiORAL™ has proven to be sound from various studies conducted so far on different molecules of varying sizes. Efficacy of the molecules such as Insulin (5.8 kD), Fusion protein (70 kD) and Humira (150 kD) was proven in all the studies but, one important drawback observed by Transgene team was that of TrabiORAL™ inability to demonstrate adequate bio-availability of those chosen molecules in the serum. Therefore, the scientific team at Transgene has started its efforts to address this issue surrounding the lack of adequate bio-availability.

TrabiORAL technology has been comprehensively modified to address the following issues:

1. Enzymatic barriers on the orally administered molecule from the time of its administration by mouth till its absorption from the GI tract.
2. Efficient protection of the chosen molecule from various enzymes at different sites of GI tract.
3. Timely and effective pH dependent release of above encapsulated moiety.
4. To overcome multilayered mucus across the GI tract.
5. Efficient and optimal binding of molecule conjugated to the ligand within TrabiORAL, the ligand present across the GI tract.
6. Efficient transportation of chosen molecule within the physiologically permissible time arising from the peristaltic movement.
7. Efficient transcytosis movement of the ligand bound molecules from the apical to baso-lateral layer of the intestinal epithelial cells.
8. Efficient transportation of the molecule across the baso-lateral layer of GI epithelium into vascular endothelium.
9. Protection of the molecule from the effects of hepatic enzymes as it passes from portal circulation into systemic circulation.
10. Finally, provide high bio-availability of the chosen molecule delivering it into serum without any modification or change to its structure.

TrabiORAL has been modified after extensive studies while retaining the basic architecture of ligand mediated transcytosis.

In our 'new' formulation:

1. We have added an additional Inner layer of encapsulation. Here the Ligand and the API are encapsulated **to form a predetermined particle size (second layer)**.

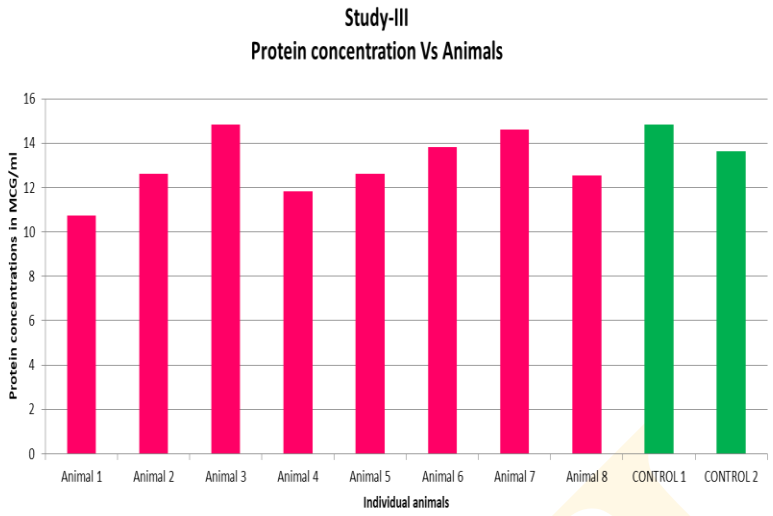
2. This **second particle is functionalized** to provide increased cell membrane permeability for facilitating the entry of the drug molecule / API into portal circulation.
3. The **Outer layer of encapsulation (first layer)**, similar to the encapsulation used in the earlier formulation, provides protection to withstand the harsh GI environment, and allows it to open up within an optimal pH range (6.2-7 pH) in the GI tract thereby allowing inner nanoparticle and free API to break free. It is to be noted that **this outer encapsulation has been enhanced and optimized for increased penetration of the tough mucus barrier**.
4. **Free API is to facilitate binding to its own receptor** on the brush border of the intestinal epithelium.
5. **Second encapsulation has been functionalized to enhance transcytosis across the baso-lateral layer of the GI epithelium into vascular endothelium.**
6. So, Ligand conjugation is carried out Twice in this new formulation; first with the API alone (prior to Inner encapsulation layer), and again on the surface of the inner encapsulation. This serves to bind the 'Inner Encapsulated particle' to the ligand receptors in the GI tract upon breaking up from the Outer encapsulation layer.

In summary, modification involved addition of functionalized double encapsulation for better presentation of the ligand A bound particles to its receptors on the brush border of intestinal epithelium. We have utilized two opposite mainstreams: muco-penetration and muco-adhesion in order to enhance the absorption efficiency.

Results of modified TrabiORAL™ - 2018-19

TrabiORAL has demonstrated in several successive studies, on normal healthy adult mice, a clear evidence of comparable levels of circulating (TT) protein within 30 mins of oral administration in serum samples (bio-availability) as compared those injected ones.

Delivery of TT with size of 150 kDa is used since its success as a large size paves way for generation of oral delivery platform to be employed for many other proteins much smaller in size such as Insulin (6 kDa) HBs Ag (MW 19 kDa) hence, more predictable.



Study	Average protein concentration in Oral dosage (µg/ml)	Average protein concentration in SC (µg/ml)
STUDY-1	7.89	7.82
STUDY-2	7.85	7.78
STUDY-3	7.76	7.71

TrabiORAL – Oral vaccines (Conclusive proof of oral delivery) 2018-19

In the recent studies, Tetanus toxoid with MW of 150 kDa has been deliberately chosen for a study on its appearance in serum in view of its large size.

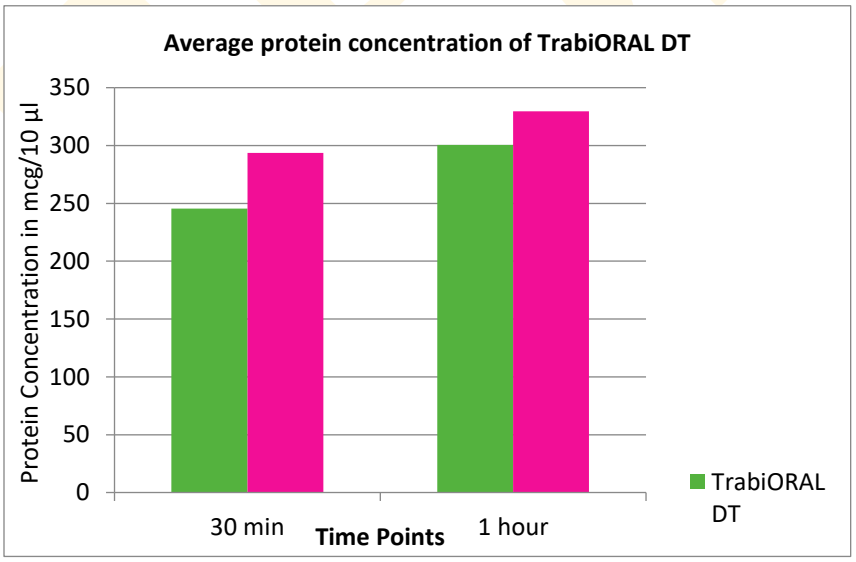
- TrabiORAL has demonstrated remarkable and comparable serum levels of orally administered TT (10x) to those of SC injected animals in three successive studies.
- Appearance of TT in serum samples following oral dosing has been detected at the end of 30 min reaching comparable levels to SC injected samples after 1 hr time point.
- We did not notice significant elevation of serum levels in our oral formulation with 15x as compared to 10x.

- Immuno-Protective antibody titres were detected comparable to SC injected controls at the end of 45 & 60 days following the administration of second dose on day 30.
- The data demonstrated consistent and reproducible results of circulating TT in serum samples at the end of 1 hr following oral dosage with comparable antibody titres following second dosage on Day 30 and the results are conclusive.

TrabiORAL™ - Diphtheria vaccine (70 kD) (10x)

Subsequent to the success with TT of 150 kD size, we wanted to demonstrate the TrabiORAL's ability to provide good serum levels (bio-availability) on smaller size DT (70 kD) molecule also.

	30 min	1 hour
TrabiORAL DT	245.51 µg/10 µl	300.37 µg/10 µl
Control	293.51 µg/10 µl	329.51 µg/10 µl



The results on two studies once again in healthy adult mice have confirmed the excellent bio-availability of Diphtheria toxoid too like in the case of much bigger molecule such as TT (150 kD).

TrabiORAL – Insulin (5.8 kD)

Following the successful demonstration of TrabiORAL's ability to deliver large molecule such as Tetanus toxoid (150kD) we moved on to demonstrate its versatility in the oral delivery of a much smaller molecule - Insulin (5.8 kD).

The studies were designed to demonstrate improved Bio-availability of orally administered Insulin with SC Insulin as a control in STZ induced diabetic SD rats. This is followed by OGTT study in normal adult SD rats.

Modified formulation (2019) Study No: TBL EX 21 PH – 003 A

Study A involved four groups of STZ induced SD rats with seven animals in each group.

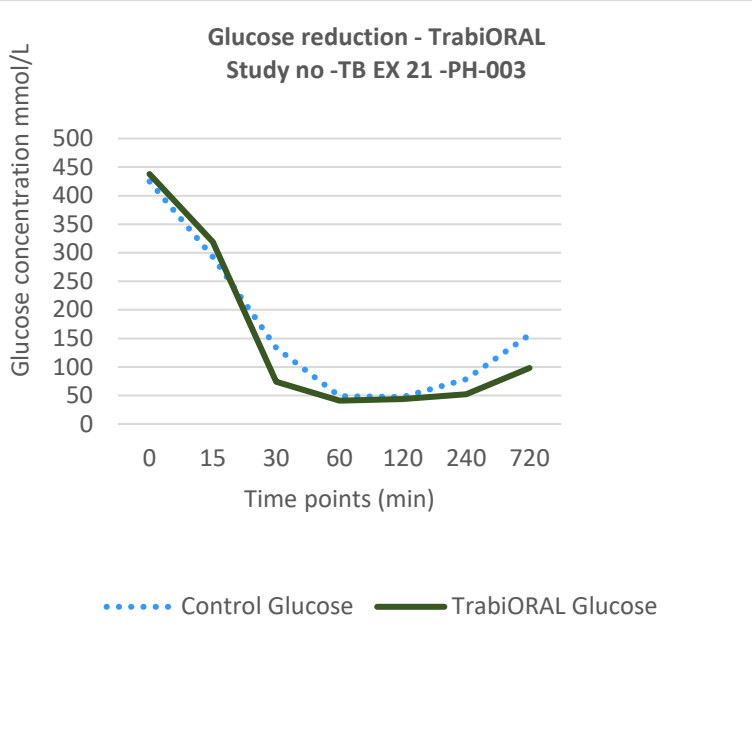
This study A involved measuring serum glucose levels at various time points (0, 15, 30 mts, 1 hr, 2 hrs, 4 hrs, 12 hrs and 24 hrs) in STZ induced SD rats.

In this study A, it involved not only measuring serum Insulin concentrations (bio-availability) at different time points coinciding with those of Glucose estimations but also using SC administered Insulin as control in another group of animals.

Study B involved three groups of normal adult rats for OGTT assay with administration of oral Glucose after determining the Tmax from Study A.

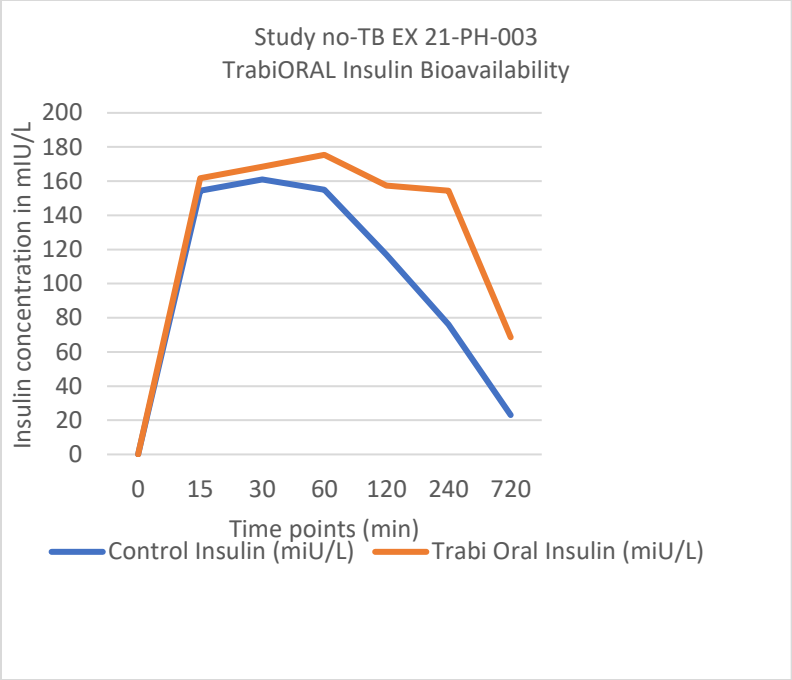
Study B measured serum Glucose levels after dosing TrabiORAL and a buffer with oral glucose lavage as per the achieved t-max in study A.

These two sets of studies were conducted involving a total of 46 animals for comparative evaluation of serum glucose and Insulin levels in Study A followed by oral OGTT assay in Study B.



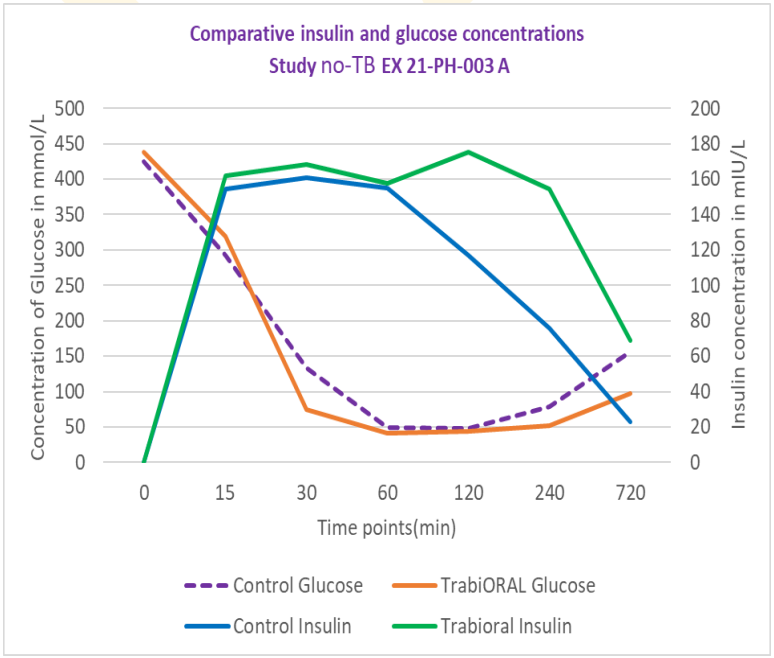
Conclusion:

- Plasma Glucose reduction in TrabiORAL administered animals at the end of 30 mts is comparable to that of SC injected Control group.
- The decrease of blood glucose levels was in consonance with the serum Insulin levels.



Conclusion:

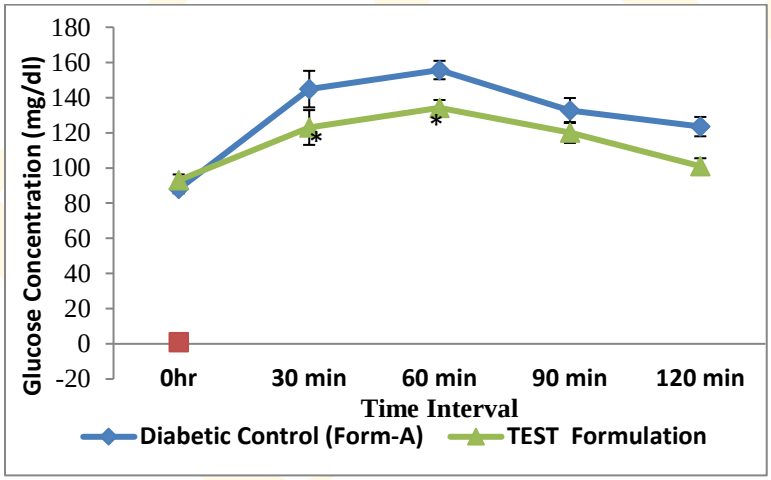
- Bio-availability of TrabiORAL Insulin is comparable with that of the control (SC) Insulin.
- Serum Insulin concentration in the TrabiORAL Insulin administered animals was significantly prolonged upto 4 hrs as compared to 1 hr in control group (SC).
- Even at the end of 12 hrs of TrabiORAL administration, serum Insulin levels remain significantly elevated than control group.



Conclusion:

- TrabiORAL insulin has demonstrated significant reduction of blood glucose levels within the first 15 min with a further fall at the end of 30 mts with lowered serum glucose levels observed at the end of 12 hours also unlike SC injected control group with serum glucose levels raising significantly by the end of two hours itself.
- The blood glucose reduction at the end of 30 mts of oral administration is comparable to the control (SC) insulin.
- Comparable bioavailability of TrabiORAL insulin with that of Control group (SC) is also observed in the serum at the end of 15 mts.
- Sustained presence of TrabiORAL Insulin in serum samples is detected from 15 mts after oral administration till the end of 4 hrs unlike injected Insulin falling at the end of 1 hr itself.

Modified formulation (2019) Study No: TBL EX 21 PH – 003 B – OGTT study



Conclusion on OGTT study

- Oral Glucose Tolerance Test was performed on normal healthy adult rats.
- Blood samples were analyzed for serum glucose levels at 0, 30, 60, 90 and 120 minutes.
- "From the results it is observed that, (TrabiORAL) test formulation by per oral route has shown significant reduction in glucose levels up to one hour (30 and 60 min) compared to respective diabetic control group of rats. Among the six rats taken for the study one rat showed deviation at 30 min time interval in diabetic control and test formulation."

SUMMARY – TrabiORAL™

1. TrabiORAL™ has succeeded in meeting its primary objective of achieving excellent bio-availability.
2. Concentration of the molecule in the formulation by TrabiORAL technology varies as per the size of the molecule. In other words, formulation strategy varies with the size of the molecule, available end terminal sequences, solubility etc.
3. Also, final formulation of TrabiORAL™ is to be modified as per its end clinical target and preferred route of entry i.e., portal circulation or lymphatic; systemic delivery or local delivery (partially) (IBDs) etc.
4. TrabiORAL™ has unequivocally demonstrated its versatility in the oral delivery of molecules of different sizes ranging between 5.8 kD to 150 kD.
5. TrabiORAL™ is protected by a series of patents covering countries such as USA, France, Germany, Britain, Italy, Australia, Japan etc.