**P-043** **Design improvements to *in vitro* gastrointestinal models to evaluate effectiveness of insulin encapsulation in nanoparticles**

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## INTRODUCTION AND OBJECTIVES

Encapsulation within nanoparticles has shown promise for overcoming the problems associated with oral insulin delivery. These nanoparticles act as a barrier for the insulin to protect the drug from proteolytic degradation and enhance its absorption across the intestinal epithelium. Their effectiveness is often evaluated based on *in vitro* and *in vivo* release studies; however, problems have occured where *in vitro* results differ greatly from *in vivo* results. For example, in one study, pharmacological availability of insulin in the orally-delivered nanoparticles was as high as 42% of free insulin administered by subcutaneous injection, while an *in vitro* simulation of the GIT suggested that nearly all insulin should have been released from the particles shortly after they entered the small intestine and thus should have been unavailable pharmacologically (Reis 2008). These conflicting results demonstrate a problem with the *in vitro* GIT models as they do not accurately mimic *in vivo* conditions. It would be worthwhile to optimize the *in vitro* models as they are an inexpensive, simple, and ethical means of evaluating the efficacy of a potential oral insulin delivery system.

Existing GIT models vary greatly in complexity, ranging from simple batch vessels to continuous-flow multi-compartmental. One of the most notable examples of a continuous-flow multi-compartmental system is the TIM-1 and TIM-2 developed by the TNO Nutrition and Food Research Institute in the Netherlands (Minekus 1995; Minekus 1999). This is an extremely elaborate and complex system; however, there are times when a simpler approach is applied. In general, the nanoparticles are first placed in a small flask containing a buffer at pH 1-2 to simulate the conditions of the stomach. After a specified amount of time, the nanoparticles are then transferred to a second flask buffered to an approximation of the pH in the small intestine (usually pH 6-7). The overall purpose of this study was to begin the process of improving these models in terms of the accuracy in which they capture *in vivo* conditions by looking at both model design and choice of simulated intestinal fluid, which will ultimately enhance the reliability of the information they provide.

## MATERIALS AND METHODS

### *Materials*

Fully sealed 500 mL Tedlar® (polyvinyl fluoride) gas sampling bags featuring a syringe port with a Teflon®-lined septum were purchased from Concept Controls Inc. (Canada). A brass bulkhead union with a 16 mm outer diameter was purchased from Swagelok Co. (Canada). Low viscosity (~250 cP at 25 °C for 2% solution) sodium alginate, extracted from brown algae, was from Sigma-Aldrich Co. (USA). Novolin ge Toronto human biosynthetic insulin (100 IU/mL) was purchased from a local pharmacy. A Micro BCA Protein Assay Kit was purchased from Thermo Scientific Inc. (USA). All other chemicals used were of reagent grade.

### *Methods*

To allow for filling and emptying of the initially airtight Tedlar® plastic bag, a hole was cut in the centre of the top face of the bag and a brass bulkhead union with a 16 mm OD was installed. The bag contents were agitated via a 10 cycle per minute horizontal rocking motion provided by a Roto-Shake Genie (Scientific Industries Inc., USA).

The mixing rate of the gastrointestinal simulator was compared to that of a 100 rpm magnetically-stirred flask through a simple tracer experiment. Each vessel was loaded with 200 mL of distilled water to which 0.5 mL of 1.0 M hydrochloric acid. The pH of the water was recorded every five seconds until it reached a steady value.

Insulin-loaded alginate microparticles were prepared based on drop-wise addition of an alginate-insulin solution into a solution of a divalent cation (calcium), inducing rapid gelation (Kierstan et al. 1977). To begin, a 2% (w/v) sodium alginate solution containing 2 mL of insulin solution was prepared. The pH of the mixture was adjusted to approximately 4.5-4.9. After holding in a vacuum chamber to remove entrained air bubbles, the microparticles were formed by drop-wise addition of the alginate-insulin solution through an 18-gauge needle at a speed of 0.5 mL/min into a 100 mM solution of calcium chloride pump with an air-jet impinging on the needle.

Insulin release profiles from alginate microparticles in simulated digestive fluids resulting from agitation with the gastrointestinal simulator and the magnetically stirred flask setup were compared. In both cases, particles (17 g) were first placed in simulated gastric medium (200 mL) for 60 minutes and subsequently transferred to simulated intestinal medium (200 mL) for 100 minutes. Insulin concentration was quantified via a Micro BCA Assay.

In a second release experiment, various intestinal buffers were compared. Two buffer formulations were based on a phosphate buffer (PB-A, PB-B) and three formulations

were based on a biocarbonate buffer (KBB-A, KBB-B, KBB-C). PB-A is a simple phosphate buffer and PB-B is the simple phosphate buffer combined with the electrolytes of the simple bicarbonate buffer. The only difference in the bicarbonate buffers is the ratio of sodium chloride to calcium chloride. The ratios are as follows: KBB-A 47:1; KBB-B 10:1; KBB-C 1:1. In all cases, a 150 minute release was conducted in a 100 rpm magnetically-stirred Erlenmeyer flask containing 30 mL of release medium and 3.2 g of particles. Insulin concentration was quantified via a Micro BCA Assay.

## RESULTS AND DISCUSSION

The mixing rates for each agitation system were evaluated based on pH tracking. For the simulator, roughly 120-140 seconds elapsed before the pH reached a steady-state at 2.8, compared to 20-25 seconds for the flask. As mixing in the GIT is known to be poor, the mixing provided by the simulator was considerably more representative of *in vivo* conditions than was the mixing provided by the 100 rpm magnetically-stirred flask.

Insulin release profiles from alginate microparticles in simulated digestive fluids resulting from the two different vessel and agitation configurations are compared in Figure 2. The profiles reveal that there was not a significant difference between the two setups under gastric conditions. Regardless of mixing style, protons were able to infiltrate the gel matrix and stabilize it, preventing insulin release after the first 5-10 minutes. Once the particles were transferred to intestinal media, the profiles diverged markedly. The flask approaches full release at 120 minutes, while release in the simulator does not begin to plateau until the end of the 160-minute sampling period.

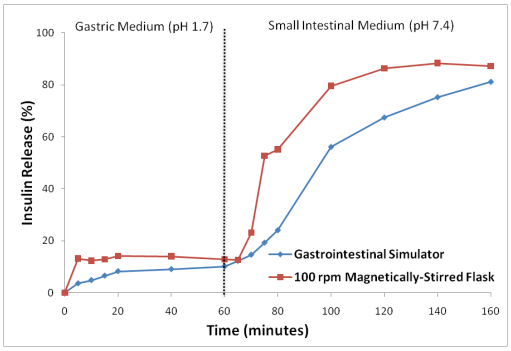


**Figure 1: Comparison of the insulin release of gastrointestinal simulator with 100 rpm magnetically-stirred flask**

These observations indicate that the profile of insulin release from alginate microparticles is strongly dependent upon the physical conditions of the vessel used to perform the experiment. The more stagnant conditions provided by the gastrointestinal simulator resulted in a slower breakdown of particle structure (and thus insulin release) than did the well-mixed environment provided by the rigid flask with magnetic stirring at 100 rpm.

Figure 2 displays the results obtained for the second release study involving varying buffer composition.

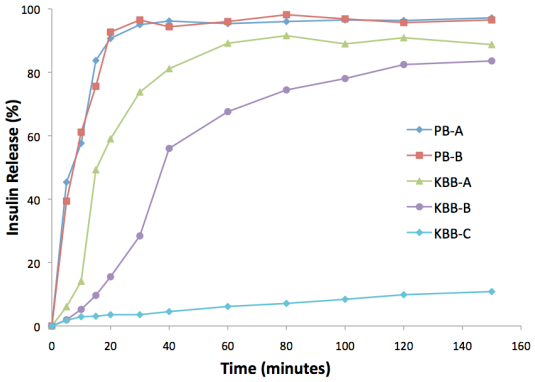


**Figure 2: Comparison of release profiles resulting from the use of various intestinal simulation media**

With respect to buffer type, the phosphate buffers promoted a faster rate of release than the bicarbonate buffers. In regards to electrolyte composition, release was slowed in the bicarbonate buffers as the sodium to calcium ratio decreased. The presence of physiological electrolytes had no clear effect on the release behaviour in the phosphate buffers.

## CONCLUSIONS

In this study, it has been shown that the design of a simulated GIT model is crucial in obtaining results from *in vitro* drug release studies. *In vitro* models used in previous studies of insulin-loaded nanoparticles have yielded results that were in disagreement with *in vivo* observations. This discrepancy is likely due to over-mixing of the model system and choice of intestinal medium. From these results, it can be concluded that the simulator is a better representation of *in vivo* conditions than is the typical 100 rpm magnetically-stirred flask.

## REFERENCES


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