The Artificial Stomach and Duodenum (ASD): A physiologically relevant *in vitro* dissolution tool

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Abstract

The artificial stomach and duodenum (ASD) is a physiologically relevant *in vitro* dissolution tool that simulates the pH, mixing conditions, fluid composition and fluid flow in the gastric and duodenum compartments (*1*). This tool is designed to generate gastric and duodenal concentration profiles to capture dissolution, precipitation and supersaturation phenomena under conditions that simulate the *in vivo* environment. Duodenal concentration profiles generated from ASD experiments have the potential to provide a rank order prediction of *in vivo* absorption of compounds as a function of formulation, gastric pH, or gastric emptying time. In this work, we report the IVIVC we established between ASD duodenal concentration profiles generated in our lab, and human *in vivo* duodenal concentration profiles that were obtained upon oral administration of solution doses of ketoconazole and dipyridamole to healthy volunteers (*2*). As an example that demonstrates the application of this tool, we also report on the rank order IVIVC we established between the plasma exposure of dipyridamole (*3*) and ASD duodenal concentration profiles we generated, under standard and elevated gastric pH conditions.

Introduction

The Artificial Stomach and Duodenum (ASD) is an *in vitro* system designed to study the dissolution behavior of compounds under conditions that simulate the dynamic fluid flow and pH conditions of the upper gastrointestinal tract as well as the potential for precipitation of a dissolved compound as it moves from the acidic pH of the stomach compartment to the near neutral pH of the duodenum. The system is based on the premise that by simulating physiological pH and mass transfer conditions, it is possible to compare the relative dissolution behavior and precipitation of different compounds, formulations, or salt forms. The ASD dissolution system described in this work is modeled after a system previously discussed by Carino (*1*) and simulates human physiology.

The goals of this work were two-fold. The first goal was to compare human *in vivo* duodenal concentration profiles (*2*) of two mono-basic compounds, ketoconazole and dipyridamole, with the duodenal concentration profiles generated by the ASD under similar conditions of dosing. This was done to assess whether the operating conditions of the system are appropriately desiged to mimic human *in vivo* dissolution.

The second goal was to demonstrate the ability of the system to predict the effect of gastric pH on the duodenal concentration profile of a basic compound. A previously reported study of the effect of gastric pH on the plasma exposure of dipyridamole in geriatric patients (*3*) was used for this comparison. The rank order of plasma exposure values of dipyridamole obtained *in vivo* under standard and elevated gastric pH conditions were compared with the rank order of the ASD duodenal concentration profiles.

Material and Methods

Materials

Dipyridamole, minimum 98% TLC, Sigma Brand, and L-Glutamic HCl, Ketoconazole, USP, and all other chemicals were purchased from Fisher Scientific.

ASD Operating Parameters

The ASD dissolution system (Fig. 1) consists of two compartments, representing the stomach and the duodenum. The default experimental parameters are set to simulate the fasted state human physiology and mimic the model described by Carino et al (*1*). Initially, the stomach compartment contains 50 mL

of gastric fluid containing HCl (10^{-2}N HCl or 10^{-5}N HCl depending on whether a gastric pH of 2 or 5.5 is being simulated) and 0.034M NaCl, to which 200mL of dosing fluid is added. The duodenum compartment contains 30 mL of fasted simulated intestinal fluid (ASD fasted SIF) formulated for the ASD. This fluid contains 5mM sodium taurocholate and 200mM potassium chloride in 50 mM pH6.5 phosphate buffer. During the experiment, fresh gastric fluid is pumped into the stomach at 2 mL/min. Fresh ASD fasted SIF is pumped into the duodenum at 2 mL/min. The temperature of all fluids in the system is maintained at 37°C. A computer controlled transfer pump empties the simulated stomach contents into the duodenum following first order kinetics, with a half life of 15 minutes. The volume of the duodenum is kept constant at 30mL using a vacuum line. The contents of both compartments are kept well mixed during the experiment using magnetic stirrers. *In situ* fiber optic probes and pH probes are placed in both compartments to collect real time UV absorbance during the experiment. It has been established using solutions of known concentration containing suspended particulate matter, that within reasonable limits, the presence of these particles does not affect the UV absorbance due to the dissolved compound. Prior to running the experiment, the probes are calibrated using a methanol solution of the compound at a known concentration. Alternatively, samples may be drawn manually from the duodenum compartment, filtered and analyzed by HPLC. The duration of a typical experiment is 150 minutes; however, some experiments for this research were shortened to 70 minutes.

Validation of Operating Conditions

Dosing solutions for the low dose of dipyridamole (30 mg) and ketoconazole (100 mg) were made by adding the compound to 50 mL 0.01N HCl/0.034M NaCl in water and stirring. Two hundred milliliters of deionized (DI) water was added after complete dissolution of the compound was observed. In this case, the 200 mL of DI water is considered the dosing fluid. Both low doses of dipyridamole and ketoconazole were solutions at the onset of the ASD experiment.

The high doses of dipyridamole (90 mg) and ketoconazole (300 mg) were made by adding the compound to 50 mL 0.01N HCl/0.034M NaCl in water and 200 mL of phosphate buffer 25 mM pH 2 followed by stirring until complete dissolution was observed. It was reported the high dose of the compounds were solubilized using HCl. We were not able to achieve complete dissolution using 0.01N HCl alone. For this reason the dosing fluid for the high dose is phosphate buffer 25 mM pH 2. Again, both high doses were solutions at the onset of the ASD experiment.

The ASD experiments were run for 70 minutes. Samples were removed from the duodenal compartment at approximately 3, 6, 9, 12, 15, 20, 30, 40, 50, 60, and 70 minutes, filtered, and submitted for HPLC analysis. The duration of the experiment and the sampling times were consistent with the *in vivo* experiment that was being used for the validation.

The solubility of dipyridamole and ketoconazole in fasted simulated intestinal fluid was also measured using 2.79 mg and 5.98 mg of the compound respectively, and 1 mL of the fasted simulated intestinal fluid in a screw capped vial. The contents of the vials were mixed by gentle stirring for approximately four hours on a stir plate. At the end of the mixing period, the undissolved solids were filtered from the solution by using a 0.45 μm centrifugation device and the dissolved concentration of compound in the solutions analyzed by HPLC.

Effect of Gastric pH on the Duodenal Concentration Profile

The work of Russell et al. (*3*) was used as reference *in vivo* data for this assessment. These authors showed that the bioavailability of dipyridamole is a function of gastric pH, and that elevated gastric pH results in a decreased bioavailability of this monobasic compound. Assuming that this difference in bioavailability is a function of the dissolution of the compound and the resulting absorption from the duodenum, ASD experiments were conducted using the same dose of dipyridamole (50 mg) as reported by these authors. Three experiments were run. The first and second were run at gastric pH of 2 and 5.5 respectively. pH 2 corresponds to normal gastric pH and pH 5.5 simulates the conditions of achlorhydria and famotidine treatment. The third experiment used the gastric fluid of pH 5.5, but with 1.36g of L-glutamic acid co-dosed to decrease the gastric pH as reported by Russell et al.

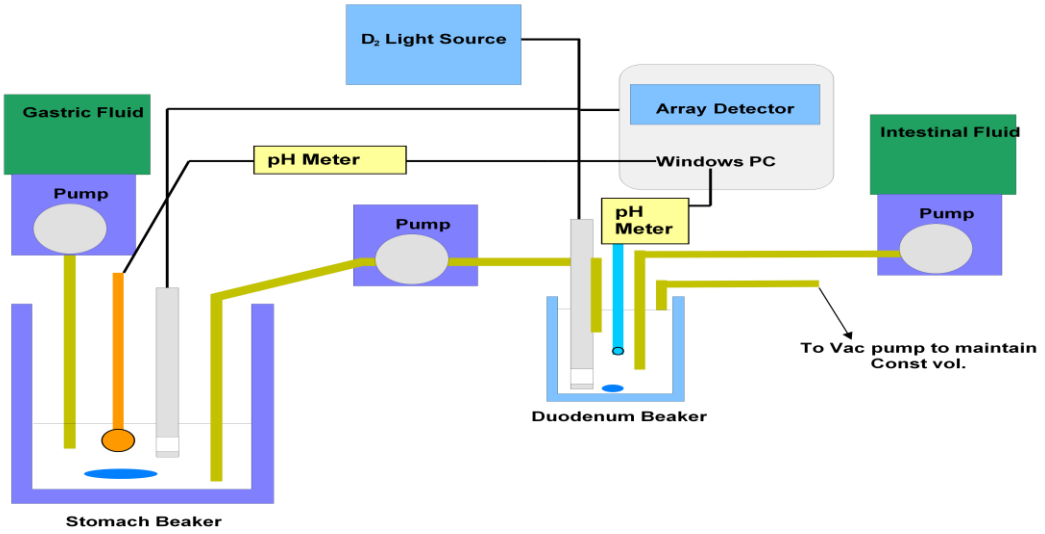

Figure 1: Schematic of ASD Set Up

Results and Discussion

Validation of Operating Conditions

The results of the ASD and in-vivo experiments showing comparative dissolution profiles of dipyridamole and ketoconazole in the duodenal compartment are summarized in Figures 2 and 3 respectively. The ASD profile peaks as the stomach compartment empties into the duodenum and then decays as the duodenum contents are emptied to maintain a constant volume of 30mL. The pH of the stomach contents at the onset of the experiments was between 2.4 and 3.0, similar to reported values.

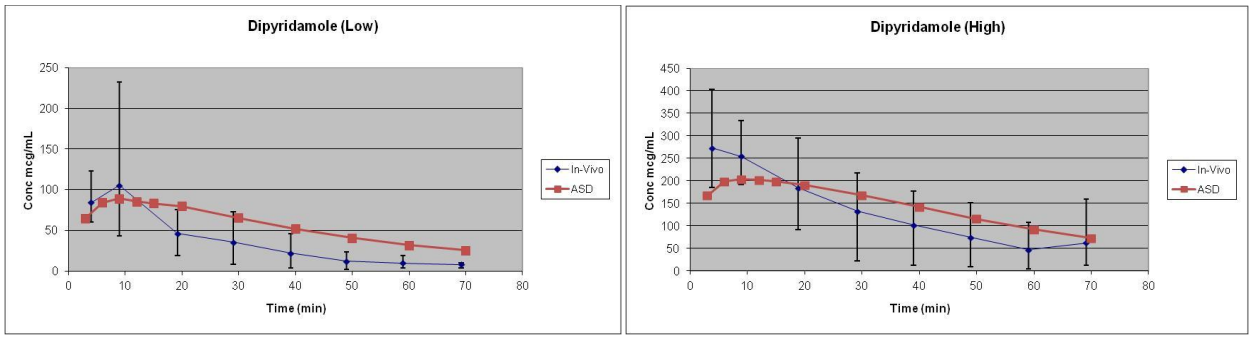

Figure 2: (Left) Shows low dose dipyridamole duodenal dissolution profile of the ASD compared with in-vivo results. (Right) The ASD and in-vivo duodenal dissolution profile comparison for the high dose of dipyridamole.

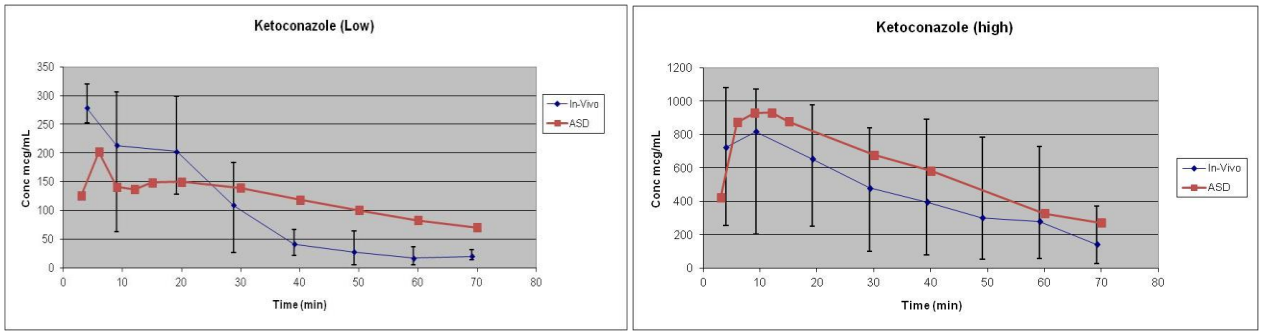

Figure 3: (Left) Shows low dose ketoconazole duodenal dissolution profile of the ASD compared with in-vivo results. (Right) The ASD and in-vivo duodenal dissolution profile comparison for the high dose of ketoconazole.

In general, for low and high doses of both compounds, the ASD data is in agreement with the in-vivo data.

The solubilities of dipyridamole and ketoconazole in fasted simulated intestinal fluid were 0.017 mg/mL and 0.007 mg/mL, respectively. In comparing the solubility data and the ASD data, it is clear that *in vivo* concentration data in the duodenum cannot be estimated from thermodynamic solubility values alone.

Effect of Gastric pH on the Duodenal Concentration Profile

The dipyridamole plasma exposures obtained by Russell et al (*3*) is shown in figure 4. As seen from the figure, all subjects with elevated stomach pH had lower plasma concentrations of dipyridamole when compared to those who had normal stomach pH.

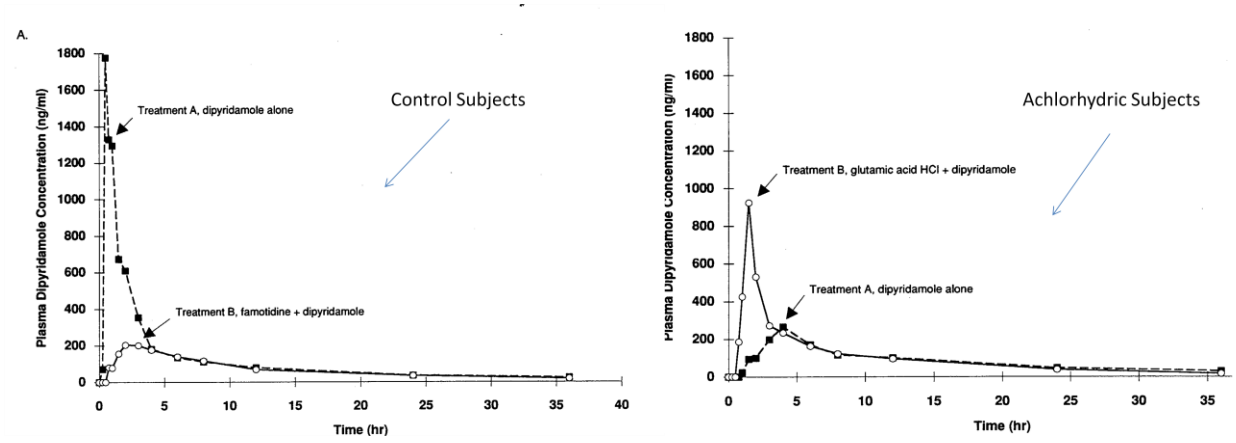

Figure 4: Dipyridamole plasma exposures from reference (*3*). Left: Control subjects with normal gastric pH (Treatment A) and with famotidine treatment to simulate achlorhydria (Treatment B). Right: achlorhydric subjects without L-Glutamic acid HCl (Treatment A) and with L- glutamic acid HCl treatment to decrease gastric pH (Treatment B).

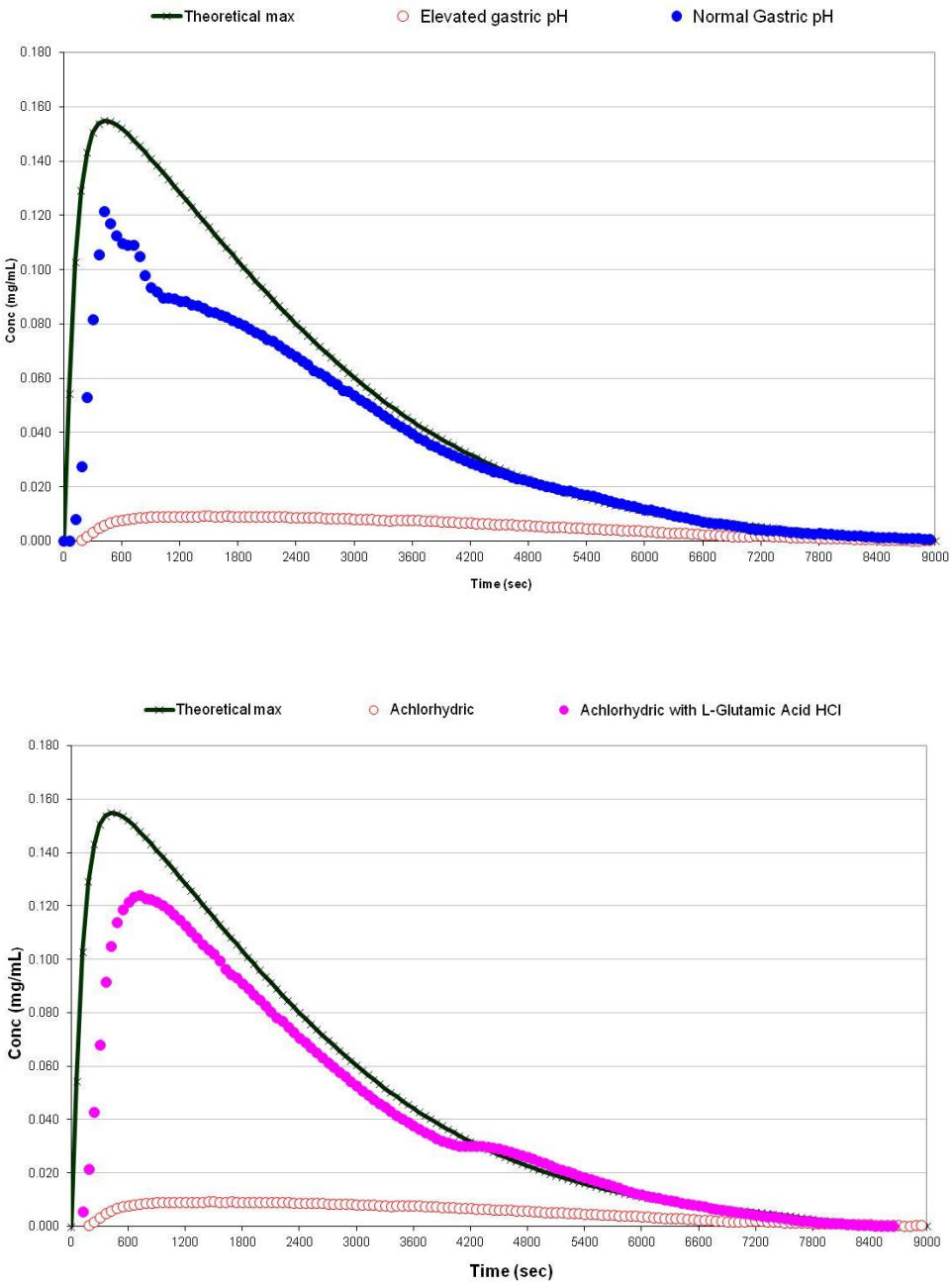

Figure 5: ASD duodenal concentration profiles for dipyridamole. Above: Simulating control subjects with normal gastric pH, and after famotidine pretreatment to simulate elevated gastric pH or achlorhydria. Below: Simulating achlorhydric subjects with elevated gastric pH and with L- glutamic acid HCl pre-treatment to decrease gastric pH.

Corresponding ASD duodenal concentration profiles are shown in Figure 5. As seen from the figure, the dipyridamole concentration profile in the duodenum is greater with normal gastric pH, than with elevated or achlorhydric gastric pH. It can also be seen from the figure that when the gastric contents are acidified with glutamic acid HCl pre-administred before dipyridamole dosing under elevated gastric pH conditions,

the duodenal concentration profile is similar to that with a normal gastric pH. These results are in rank order agreement with the results reported by Russell, et al (*3*).

Conclusions

The extent of dissolution of compounds in the duodenum compartment of the gastrointestinal tract is a very important parameter for absorption of orally administered compounds. An *in vitro* system that simulates the pH, fluid composition, mixing and emptying kinetics of the *in vivo* environment is essential for understanding dissolution under physiologically relevant conditions. From a comparison of the ASD and *in vivo* duodenal concentration profiles of two monobasic compounds dipyridamole and ketoconazole, this work demonstrates that the operating parameters of the ASD adequately simulate *in vivo* dissolution in these compartments.

One of the many important applications of the ASD is its ability to rank order the *in vivo* duodenal dissolution of compounds or formulations as a function of gastric pH. This would be very important for controlled release formulations that use enteric polymers and also for compounds that have pH-dependent solubility and dissolution.

Russell et al., (*3*) clearly demonstrated that the absorption and the resulting plasma exposure of dipyridamole was significantly compromised by elevated gastric pH arising from achlorhydria or co-administration of certain medications. The ASD data we that report in in the second part of our work demonstrates a rank order correlation with the *in vivo* plasma concentration data reported by these authors.

In conclusion, the ASD is a simple, tool that simulates the *in vivo* stomach and duodenum compartments. As illustrated through the dipyridamole example, it can be used to undertand the impact of gastric pH on the *in vivo* dissolution (and therefore absorption) of compounds. Although only one example of its application is discussed in this report, it can also be used to understand the effect of formulations, gastric emptying times and other parameters on *in vivo* dissolution.

Acknowledgements

Karl Kovach for analytical support and valuable input.

References

1. Carino, S. R., Sperry, D. C., and Hawley, M. (2006) Relative bioavailability estimation of carbamazepine crystal forms using an artificial stomach-duodenum model, *J Pharm Sci 95*, 116-125.
2. Psachoulias, D., Vertzoni, M., Goumas, K., Kalioras, V., Beato, S., Butler, J., and Reppas, C. (2011) Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults, *Pharm Res 28*, 3145-3158.
3. Russell, T. L., Berardi, R. R., Barnett, J. L., O'Sullivan, T. L., Wagner, J. G., and Dressman, J. B. (1994) pH-related changes in the absorption of dipyridamole in the elderly, *Pharm Res 11*, 136-143.