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Brief/Technical Note

Development of a Continuous Dissolution/Absorption System—a Technical Note

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KEY WORDS: absorption; dissolution; permeability.

INTRODUCTION

It has been several decades since the compendial dissolution technique has been used to characterize the performance of oral drug products and has attempted to serve as a surrogate for *in vivo* bioequivalence testing (1). Provided a validated *in vitro–in vivo* correlation (IVIVC) is established, the demonstrative bioequivalence test based on clinical trials could be waived. This regulatory benefit increases the interest in linking dissolution results with pharmacokinetic performance. However, the outcomes based on previous research indicate that only a small portion of drugs (in Biopharmaceutics Classification System (BCS) class I, high solubility, high permeability) are successful in establishing IVIVCs and subsequently obtaining waives for bioequivalence tests (2).

The key parameters controlling oral drug absorption are the solubility/dissolution of the drug in the gastrointestinal (GI) track and the permeability of the drug through the GI membrane. The current compendial methods are simplified models to the human GI tract and are limited in their abilities to simulate the dynamic aspects of the *in vivo* processes that are associated with dosage form transit through a complex GI environment. Compartmental absorption and transit models have been developed for years (3,4). Some progress has been made to develop a dissolution apparatus based on multicompartmental models such as: (1) integrate dissolution testing with *in vitro* permeation process or (2) simulate drug dynamic transit in the GI tract. James Polli's group developed an integrated dissolution/Caco-2 system, which could be used to predict dissolution–absorption relationships, as well as the contributions of dissolution and intestinal permeation to overall drug absorption (2,5). The system has been applied to study piroxicam, metoprolol, and ranitidine formulations, and the results were in agreement with clinical studies. Some similar reports were also published recently by Makoto Kataoka's group on a novel dissolution/permeation (D/P) system (6–8). The D/P system included an apical side and a basal side with a Caco-2 monolayer in between. It was reported that drug dissolution and permeation could be simultaneously determined with this system. Another approach to assess bioavailability/bioequivalence *in vitro* is the TIM-1 system developed at TNO Nutrition and Food Research, Netherlands (9). This system is a multicompartmental, dynamic, computer-controlled system that closely simulates *in vivo* dynamic physiological processes in the human upper GI tract.

Those efforts make it possible to better simulate the GI tract and correlate *in vitro* drug performance to *in vivo* clinical trials. However, because of the complexity of these systems, some limitations such as result reproducibility and repeatability, cost of each test, ease of use of the method, and high throughput may prevent the wide use of these systems. A new dissolution/absorption system that takes the advantage of current progress and improves the reproducibility and repeatability of test results, at a low cost, would be of benefit.

This article is focused on the development of a new dissolution/absorption system. The system still depends on the idea of multicompartmental simulation and is able to link drug dissolution and absorption performance. On the other hand, the new system has some improvements: (1) it employs a dissolution section and an absorption section (two compartments) instead of using four compartments as in the TIM-1 system; (2) it applies an artificial membrane to link dissolution and absorption without using a Caco-2 monolayer; and (3) it accommodates current USP official dissolution apparatus easily into the system. Some preliminary tests with two drug products (Inderal propranolol capsule and Uricalm phenazopyridine tablet) have been done with this newly developed

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy.

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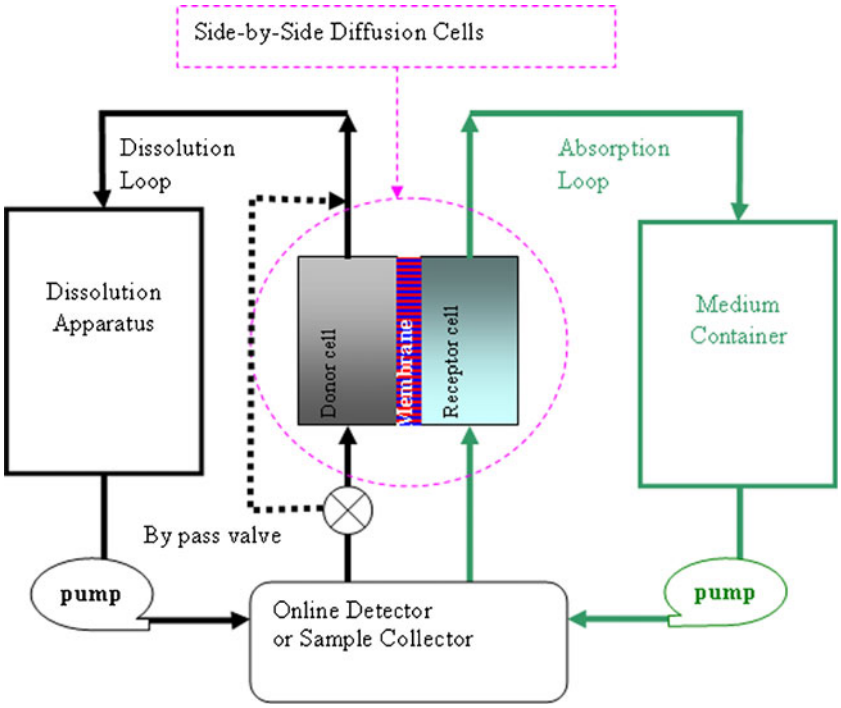

Fig. 1. Schematic illustration of the continuous dissolution/absorption system

system. The results indicate that this new system is flexible and easy to use, has low cost and maintenance, and may have potential to better correlate *in vitro* testing with *in vivo* clinical trials than current dissolution configurations.

EXPERIMENTAL

Development of Dissolution/Absorption System

The continuous dissolution/absorption system is illustrated in Fig. 1. The system consists of a dissolution apparatus, flow-through side-by-side diffusion cells, medium containers, online detector or sample collector, and other necessary accessories such as a pump and controls for temperature, pH of the dissolution medium, and medium agitation speed. The dissolution apparatus could be any apparatus commercially available on the market and uses either of seven USP official methods. In this study, the flow-through apparatus was used. The flow-through side-by-side diffusion cells contain a donor cell and a receptor cell (Spectrum Labs, P/N 132377). The donor cell and receptor cell, with a diameter of 38 mm and 10 mL of half cell total volume, are separated by a flat sheet of membrane. These two cells are clamped together. The cuprophan membrane was mounted between the donor and receptor cells in this study. The effective permeation surface area is 45.3 cm^2. The components are connected by the Teflon tubing with internal diameter of 1.5 mm to form the dissolution loop (D-loop) and the absorption loop (A-loop). During the test, drug products are dissolved in the dissolution apparatus following normal dissolution procedure. The dissolution medium is circulated in the D-loop from the dissolution apparatus through the online detector or sample collector,

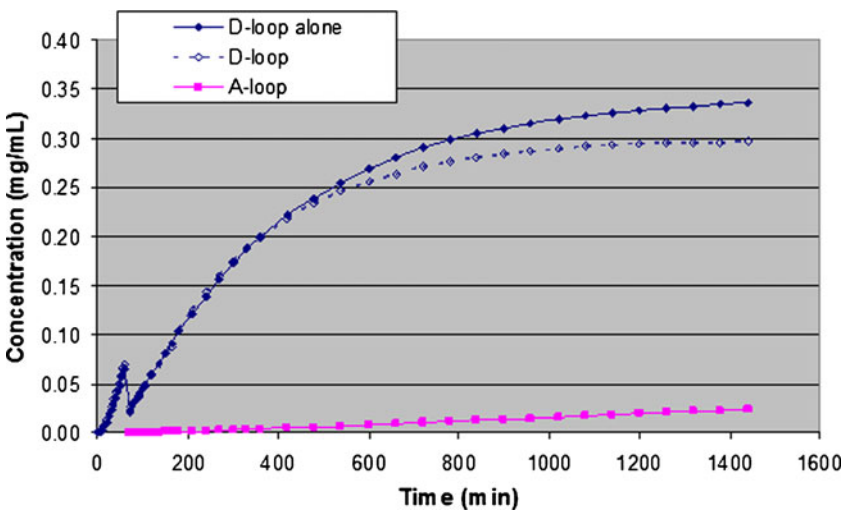

Fig. 2. Propranolol HCl dissolution/absorption profiles

Table I. Summary of the Drug Information and Test Results

		Propranolol HCl	Phenazopyridine HCl
BCS Class		I	II
Solubility (mg/mL)	pH1.2 (SGF)	NA	0.619±0.007
	pH6.8 (SIF)	NA	0.077±0.003
% Dissolved (D-loop control)	1 h	6.6±1.2 (6.1)	35.9±10.7 (37.8)
	12 h	84.5±3.8 (90.6)	34.7±6.2 (43.8)
	24 h	94.2±3.9 (105.0)	30.8±3.4 (41.3)
$P_{app}\times10^{-6}$ (cm/s)		12.2±0.9	26.6±0.8

BCS Biopharmaceutics Classification System, *SGF* simulated gastric fluid, *SIF* simulated intestinal fluid, *D-loop* dissolution loop, P_{app} permeability coefficient, *NA* not applicable

the donor cell of the flow-through side-by-side diffusion cell, and then back to the dissolution apparatus (vessel or medium reservoir). There is a valve in the D-loop to direct the dissolution medium to flow through the donor cell or to bypass it depending on the experimental design. In the A-loop, the medium is also pumped through the online detector or sample collector and receptor cell of the flow-through side-by-side diffusion cell and then flows back to the medium container. The dissolved drug in the donor cell is available to permeate into the receptor cell through the membrane. The drug substance in both the D-loop and A-loop could be monitored as a function of time either online or offline simultaneously.

Artificial Membrane Permeability

The apparent permeability coefficient, P_{app}, can be calculated based on Fick's law under the assumption that transport equilibrium is not attained (*i.e.*, $C_D > C_A$) (10).

$$P_{app} = \frac{dM/dt}{A \times (C_D - C_A)} \quad (1)$$

where dM is the drug accumulation in the A-loop during time interval dt, A is the effective surface area of the membrane, and C_D and C_A are drug concentrations in the D-loop and A-loop.

Materials

- Inderal LA propranolol hydrochloride capsule, 160 mg, Wyeth Pharmaceuticals.
- Uricalm maximum strength phenazopyridine hydrochloride tablet, 99.5 mg, Alva-Amco Pharmacal Cos. Inc.
- Simulated gastric fluid (SGF): 36 mg NaCl and 126 mL HCl in 18 l deionized (DI) water.
- Simulated intestinal fluid (SIF): 129.2 g monobasic potassium phosphate in 19 l DI water, adjust pH to 6.8 using 50% NaOH solution.
- Cuprophan membrane, Code# 32-4160-7, Agilent Technologies Inc. The membrane was soaked in SIF for more than an hour before use.

Instrument and Method

A USP apparatus 4 (CE 7 Smart with CP7 piston pump, Sotax AG, Switzerland) with 22.6 mm (Sotax part #8820) flow cells was used during the study. Each cell was prepared by placing a 5-mm ruby bead in the apex of the cone to protect the inlet tube, and 8.0 g of 1-mm glass beads was added to the cone area to form a glass bead bed. The tablet or capsule

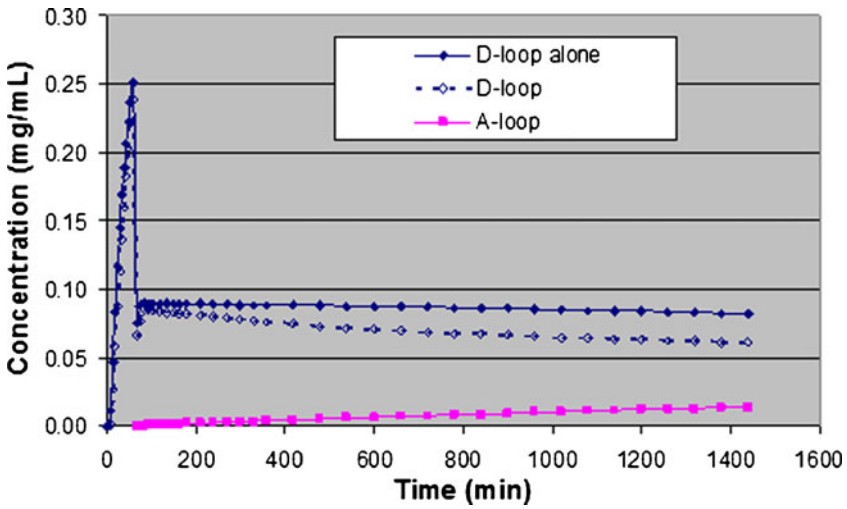

Fig. 3. Phenazopyridine HCl dissolution/absorption profiles with one tablet in each flow-through cell

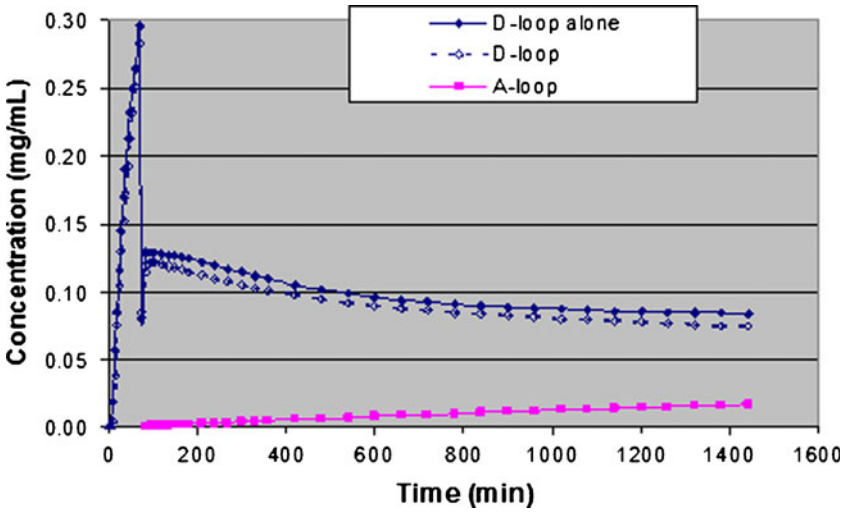

Fig. 4. Phenazopyridine HCl dissolution/absorption profiles with two tablets in each flow-through cell

samples were positioned on the glass bead bed. Mechanical calibration consisted of measuring the flow rate through each cell.

The dissolution tests were conducted in a closed loop at a flow rate of 4 mL/min at 37°C. The absorption tests were run simultaneously with dissolution testing using the SIF medium circulating in the loop at 4 mL/min and room temperature.

Following the BCS guidance (11), the solubility of phenazopyridine HCl was determined with the shaking speed set at 100 using an Orbital Shaker (Thermo Forma 420, Waltham, Massachusetts 02454, USA). The solution saturated with phenazopyridine HCl was shaken at 37°C for at least 24 h before being tested by UV–vis (Agilent 8453, Santa Clara, California 95051, USA) at 422 nm.

Propranolol hydrochloride is a water soluble drug. Based on Sigma product information, its solubility in water is 10 mg/mL (12).

RESULTS AND DISCUSSIONS

Operation of Dissolution/Absorption System

An artificial membrane was used in this study, since a previous publication has reported that permeability measurement with an artificial membrane is a potentially high-throughput and low-cost alternative for *in vitro* assessments of drug absorption (10). Cuprophan membrane is a regenerated cellulose-based membrane with the thickness of 10 μm, and the diffusive permeability is 0.042 cm/min for NaCl and 0.005 cm/min for vitamin B_{12}. Propranolol HCl and phenazopyridine HCl were used as target drug products as examples of BCS I and II drugs (13). The permeability of these two drugs determined by filter-immobilized artificial membranes was reported as 14×10^{-6} and 30×10^{-6}cm/s for propranolol and phenazopyridine, respectively (14,15). The propranolol

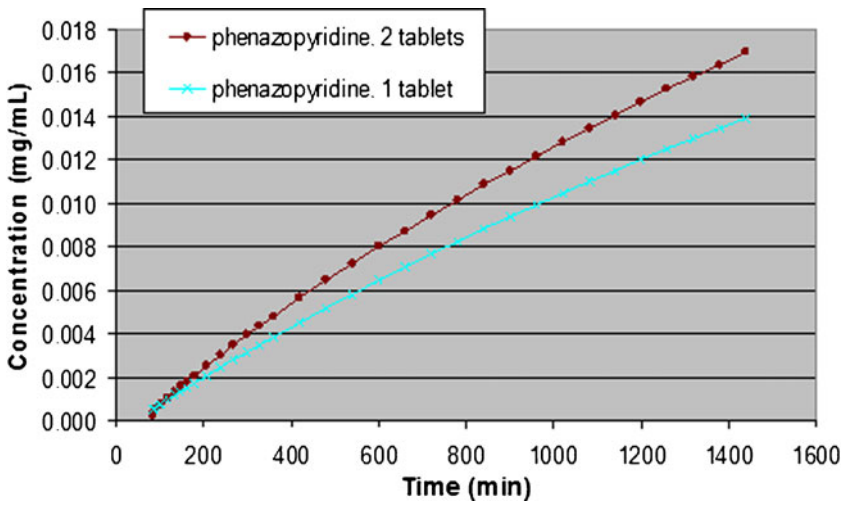

Fig. 5. Comparison of phenazopyridine HCl absorption profiles with one and two tablets in each flow-through cell

permeability using Caco-2 monolayer is reported as 25×10^{-6} and 42×10^{-6} cm/s in different publications (12,16).

To best simulate physiological parameters in a human GI tract, the test conditions including volume and composition of fluid in each compartment and test time for different stages were chosen based on Mudie *et al.* (17). The dissolution medium (in the D-loop) was 150 mL SGF flowing through the sample and bypassing the diffusion cell in the first hour. Then, 350 mL SIF was added, the pH was adjusted to 6.8, and the test was continued for an additional 23 h. This process mimics the drug transit time from stomach to small intestine. The medium in the A-loop was 450 mL SIF. Since the medium in the D-loop bypassed the diffusion cell in the first hour; there was no absorption during the first hour of the test. The experiment was conducted with a paired D-loop and A-loop connected by a side-by-side diffusive cell. Samples in both loops were analyzed simultaneously with online UV–vis (Agilent 8453 spectrophotometer, Santa Clara, California 95051, USA) at 290 nm for propranolol and 422 nm for phenazopyridine with a 1-mm UV flow cell in the D-loop and 10 mm UV flow cell in the A-loop. To compare the dissolution profile with traditional dissolution testing, samples were also tested with only the D-loop.

In Vitro Test with the Dissolution/Absorption System

Figure 2 shows the dissolution and absorption profiles of propranolol HCl. For better comparison, the concentration data are used. The percentage of drug dissolution at various time points in the D-loop is summarized in Table I. The product label claims that propranolol HCl is a highly soluble drug, and Inderal LA product is formulated as a long-acting capsule. The dissolution profile confirms this drug design and shows the drug-extended release in 24 h. The dissolution profile (dashed line with open diamonds) shows a difference from results without an added A-loop (solid line with solid diamonds) because of drug diffusion to the A-loop. The small spike at 1 h in the D-loop is due to the medium change from 150 mL SGF to 500 mL SIF. The absorption profile starts at 1 h after the dissolution test with a linear increase (R^2=0.996). The apparent permeability coefficient, P_{app}, can be calculated based on Eq. 1, and the result is listed in Table I.

Phenazopyridine HCl is a BCS class II drug; its solubility in SGF and SIF was determined and shown in Table I. The results indicate that phenazopyridine dissolved more in SGF and was very poorly soluble in SIF. Figure 3 shows the dissolution and absorption profiles of phenazopyridine HCl. The dissolution profile shows the drug dissolution is affected by drug solubility. About 36% of the drug is dissolved in the SGF medium, and the concentration reaches 0.25 mg/mL at the end of 1 h. After changing the medium to SIF, no more drug was released, and the drug dissolution which was limited by the low solubility in SIF was at a constant 0.08 mg/mL. The absorption profile is shown in Fig. 2 and has a linear increase (R^2=0.995). The P_{app} was calculated based on Eq. 1 and is listed in Table I. The permeability results are in agreement with reported values and also show the same permeability ranking order of propranolol HCl and phenazopridine HCl (14).

For the Uricalm drug product, the label requires to take two tablets to get the maximum strength, so the capability of this system to compare changes of drug bioavailability and absorption when using different drug dose amount was also investigated. Kataoka *et al*. reported that if a supersaturated drug solution exists in the intestinal fluid for a sufficient length of time, it may result in an enhanced flux across the intestinal wall and thus improve the absorption (8). Two phenazopyridine tablets were used in the D-loop, and the results are shown in Fig. 4. More phenazopyridine is released in the SGF in the first hour with a concentration of 0.30 mg/mL. After changing the medium to SIF, the phenazopyridine becomes supersaturated with the concentration higher than the solubility. The supersaturated solution lasted for about 10 h, then phenazopyridine was gradually precipitated, and the concentration was reduced to its solubility concentration (0.08 mg/mL). The absorption profile increased linearly (R^2= 0.993) with a calculated P_{app} of $25.7\pm1.4\times10^{-6}$ cm/s, the same as that when one tablet was used in the D-loop. Figure 5 shows the comparison of the absorption profiles with one and two tablets. The concentration is higher in the A-loop when a higher amount dose is used. The concentration in the A-loop shows a 30% increase when using two tablets instead of one tablet in the D-loop. This was also a simple direct support *in vitro* that drug absorption could be increased when drug bioavailability was higher.

CONCLUSIONS

A new dissolution/absorption system was developed based on the knowledge of multicompartmental models and linking current USP official dissolution technology with side-by-side diffusive cell to enable the prediction of dissolution and permeation contributions to drug absorption performance. BCS class I drug propranolol HCl and BCS class II drug phenazopyridine HCl were used to demonstrate the advantage of this new system. The results indicated that the dissolution/absorption system was able to link dissolution performance and drug absorption similar to other systems, but could be conducted more reliably and easily, at low cost, and potentially with high throughput. The comparison of different drug amounts showed the great potential of better surrogate for *in vivo* bioequivalence test. The dissolution/absorption system may provide a way to integrate the nonclinical and clinical fields to help not only on drug discovery for drug safety and efficacy but also on drug formulation to ensure the quality of the drug product.

This new dissolution/absorption system could provide an opportunity for potential further research including different membranes to simulate various absorption circumstances. The formulation effects (excipient, dosage form, claimed value, *etc.*) on drug absorption profiles could be tested. Other dissolution conditions such as medium composition, drug transit time (dissolution stage), agitation speed/flow rate, and apparatuses could be explored. The medium in the A-loop could be adjusted to mimic plasma composition. More BCS class I and II drug products, as well as drugs in BCS class III and IV, could also be studied.

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