# Innovative *in vitro* methodologies for establishing therapeutic equivalence

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**ABSTRACT**  *To improve the quality of pharmaceutical products in their markets, several Latin American countries have begun to require that new generic products demonstrate bioequivalence against innovator or reference products. However, given the number of products involved, it is not feasible to rely on clinical studies to comply with this requirement. Instead, it makes sense to adopt or develop strategies that are appropriate to the characteristics of the region.*

*To streamline drug development and accelerate patients' access to quality drug products, 15 years ago the United States Food and Drug Administration (FDA) decided to grant exemptions from clinical bioequivalence studies (i.e., biowaivers) for certain types of drug products based on the Biopharmaceutics Classification System (BCS). Biowaivers can significantly reduce development time and cost and can also prevent unnecessary human exposure to potentially dangerous drugs while providing a robust, consistent standard for therapeutic equivalence of generic drug products.*

*In addition, the limited success of translating* in vitro *dissolution data into* in vivo *performance can be enhanced using innovative tools such as the* in vitro *dissolution and absorption systems (IDAS). By integrating* in vitro *dissolution and permeability tests, these systems can provide useful insights for formulation development. A thorough assessment of the potential of* in vitro *techniques, along with formalization of their use through regulatory science initiatives when appropriate, may lead to cost-effective tools to help address some of the quality and regulatory challenges faced in the Latin American and Caribbean region.*

**Key words**  Biopharmaceutics; dissolution; permeability; absorption; therapeutic equivalency; quality control.

Cost control pressure, poor manufacturing practices, and inadequate regulation in the Latin American and Caribbean region contribute to the prevalence of substandard and counterfeit medications. Two examples of the potentially disastrous consequences of insufficient testing of pharmaceutical products were experienced in Panama when, in 2007, 365 deaths resulted from counterfeit glycerin (diethylene glycol) added to 260 000 bottles of cold medicine (1) and, in 2014, nine neonates died after receiving nutritional heparin containing benzyl alcohol (2). Attempts to control pharmaceutical product quality by analyzing one or more product lots during the registration process (e.g., every 5 to 7 years), with minimal or no additional evaluation of product lots produced in the intervening years, do little to mitigate the concern that the product lots actually ingested by patients can be different from those submitted for registration. On the other hand, the indiscriminate use of quality control tests not sensitive enough to monitor substandard or counterfeit medications can compound the problem because they may provide a false sense of security. To increase access to the therapeutic benefits of pharmaceutical products there is a pressing need to identify alternative tools and approaches that

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permit evaluation and monitoring of the bioequivalence of products in the market.

## Establishing Equivalence of Generic Drug Products

The United States Food and Drug Administration (FDA) and its counterparts in all highly regulated pharmaceutical markets demand that generic drug products meet the same standards of quality, efficacy, and safety as their reference listed drug (RLD) counterparts. Consistent with this premise, procedures have been established to demonstrate that generic products are therapeutically equivalent and interchangeable with RLDs. Therapeutic equivalence (TE) is generally achieved by assuring pharmaceutical equivalence (PE) and bioequivalence (BE) of the generic to the RLD products (3). Because implementation of BE requirements based on clinical studies would be prohibitively expensive, there is an incentive to develop or identify *in vitro* methods that can be used to evaluate and monitor the registration lots and also ensure the inter-lot consistency of a product.

## *In Vitro* Tools for Establishing Equivalence

In general, orally administered solid drug products disintegrate and dissolve in the stomach and are absorbed in the intestine. The solubility and permeability of the active ingredient and the drug product dissolution—the key determinants of intestinal absorption—are extremely difficult to determine *in vivo*. Therefore, the use of *in vitro* tools is necessary to study these characteristics.

Since drug product dissolution is a prerequisite for the process of absorption, dissolution tests are widely performed as an integral component of the battery of routine quality control tests for solid oral dosage forms. Thus, in principle, the establishment of *in vitro–in vivo* correlations (IVIVCs), whereby the dissolution rate may serve as a surrogate for systemic exposure (e.g., bioavailability, "area under the curve" (AUC), or $C_{max}$), is highly desirable because it could reduce the number of BE studies required for generic drug product development and registration (4). Although IVIVCs can be very advantageous to quality control

efforts, they are difficult to demonstrate, partly because *in vitro* dissolution tests do not mimic either the rate or mechanism(s) of drug release *in vivo*. The implementation of *in vitro* methodologies as alternatives to clinical BE studies has been facilitated by progressive regulatory guidelines such as the Biopharmaceutics Classification System (BCS) and could benefit from the inclusion of innovative technical approaches, such as the *in vitro* dissolution absorption systems (IDAS) discussed below.

## Biopharmaceutics Classification System

The BCS provides a scientific framework for drug classification based on measurements of drug solubility and permeability, the primary determinants of drug absorption, and serves as an important tool for the application of *in vitro* measurements in support of BE requirements. The BCS is an excellent example of a successful regulatory initiative that has resulted in biowaivers, exemptions from clinical BE studies, for dozens of oral products. For over 15 years, the FDA permitted biowaivers for Class I drugs in rapidly dissolving immediate-release solid oral dosage forms (5), and in the recently updated BCS guidelines (6) it expanded biowaiver eligibility to Class III drugs. According to a recent survey, Classes I and III combined account for 63% of the top 200 selling drugs (7), suggesting that full implementation of the BCS could result in a major reduction in the number of clinical BE studies. In addition to reducing costs, *in vitro* studies prevent unnecessary exposure of healthy subjects to potentially adverse drug events and even deaths (7, 8) without compromising the high quality standards necessary to safeguard public safety.
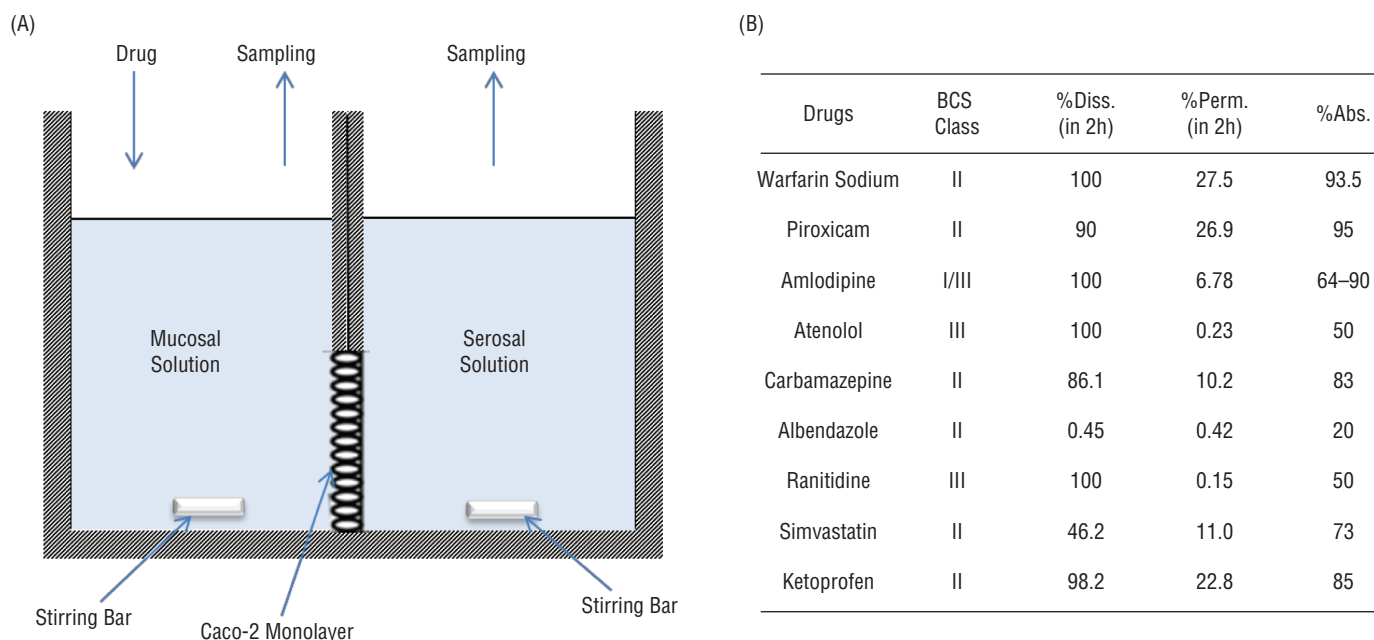
## *In Vitro* Dissolution and Absorption Systems

The *in vitro* dissolution absorption system 1 (IDAS1) is the product of major modifications made to a device (the dissolution/permeation, or D/P, system) originally developed by Prof. Yamashita at Setsunan University in Japan (9). IDAS1 represents a physiologically relevant *in vitro* system for the evaluation of drug formulations. It comprises four components: dissolution-permeation chamber

(Figure 1A), magnetic stirring bar motor, motor controller, and temperature block. The dissolution-permeability chamber consists of two half-chambers separated by a cell membrane, typically polarized epithelial cells such as human intestinal cell Caco-2 cells or Madin-Darby canine kidney cells grown on a microporous membrane. While the internal design of the IDAS1 chamber is very similar to that of the D/P chamber, they differ in the chamber locking mechanism, external size and shape, type of motor (step versus brushless), ability of the controller to display the stirring speed (rpm), and the use of a heating block as opposed to an incubator in the D/P system, which avoids the need to open the incubator repeatedly for sample withdrawal.

The potential utility of IDAS1 has been shown in numerous studies performed with the precursor D/P chamber system (9–14). *In vivo*, drug product dissolution does not need to be completed before drug absorption can start; however, product dissolution and drug permeability are measured independently, largely under nonphysiological conditions. Thus, the strength of IDAS1 is its ability to allow the concomitant evaluation of dissolution and permeability. *In vitro* data obtained in a system that replicates the *in vivo* dissolution-permeability interplay should be more translatable into *in vivo* product performance. Whereas other *in vitro* systems for permeability measurement require administration of the drug as a solution or suspension, IDAS1 is unique in that the contents of a capsule or crushed tablet may be applied in solid form into the mucosal chamber.

To increase the physiological relevance of these experiments, biorelevant media are often used to mimic the gastrointestinal environment. The apical (mucosal) chamber buffer can contain bile acids and lecithin at concentrations to produce fasted (FaSSIF) or fed (FeSSIF) state simulated intestinal fluids, and the basolateral (serosal) chamber contains 4.5% albumin to mimic the blood plasma. Prior data with the D/P system (9) and our own data with IDAS1 not only provided evidence of the impact of dissolution rate on permeation but also found a good correlation between *in vitro* permeation and fraction absorbed in humans, suggesting that this system is useful for the study of oral absorption of drugs with poor aqueous solubility (9). Kataoka et al. reported that the albendazole

**FIGURE 1.** **(A) Schematic representation of the *in vitro* dissolution absorption system 1 (IDAS1); (B) correlation between IDAS1 dissolution and permeability values, depicted as percent per 2 hours, and *in vivo* human absorption**

(A)



(B)

| Drugs | BCS Class | %Diss. (in 2h) | %Perm. (in 2h) | %Abs. |
|---|---|---|---|---|
| Warfarin Sodium | II | 100 | 27.5 | 93.5 |
| Piroxicam | II | 90 | 26.9 | 95 |
| Amlodipine | I/III | 100 | 6.78 | 64–90 |
| Atenolol | III | 100 | 0.23 | 50 |
| Carbamazepine | II | 86.1 | 10.2 | 83 |
| Albendazole | II | 0.45 | 0.42 | 20 |
| Ranitidine | III | 100 | 0.15 | 50 |
| Simvastatin | II | 46.2 | 11.0 | 73 |
| Ketoprofen | II | 98.2 | 22.8 | 85 |

permeation (percent dose absorbed at 2 hours) *in vitro* under simulated fed and fasted conditions was in agreement with *in vivo* findings and concluded that the system can be used to study food's effect on drug absorption (10).

A good correlation between *in vitro* permeation and systemic absorption (AUC and $C_{max}$) in the rat was observed for fenofibrate (11, 13). The study compared six fenofibrate tablet formulations. The *in vitro* data showed a good correlation with $C_{max}$ but not AUC in humans, and the most likely explanation for the apparent lack of correlation for AUC is micellar entrapment of the drug. This conclusion is supported by a recent study that compared two capsule formulations containing nanosized and micronized fenofibrate formulations (15). Using IDAS1, Class II drugs that were completely dissolved within 2 hours reached the highest experimental percent permeation values, ranging between ~23 and ~28 (Figure 1B). This is consistent with *in vivo* data since the absorption in humans of these compounds was at least 85% (16), the threshold defined by both the FDA and the European Medicines Agency for complete absorption (5, 17). For compounds with substantial but incomplete dissolution (e.g., simvastatin and carbamazepine) experimental percent permeation at 2 hours

was ~10, and their absorption in humans is slightly lower (73% and 83%, respectively) (16, 18–19). Albendazole, known to have a low (20%) absorption in humans (16), had very low dissolution and a low percent permeation at 2 hours (0.42) in IDAS1. Since the main absorption barrier for Class III drugs is the cell membrane, values for percent permeation at 2 hours were low for atenolol and ranitidine (0.23 and 0.15, respectively) despite complete dissolution, consistent with a 50% oral absorption in humans for both drugs (16). These data illustrate the potential versatility of this type of system, which, as detailed above, has been successfully applied to the evaluation of several aspects of formulation development and/or characterization.

The IDAS1 device also makes it possible to obtain dissolution/absorption profiles for candidate formulations. Dissolution can be affected by excipients, crystal polymorphism, pH, and buffer composition, whereas absorption can be affected by formulation excipients and buffer composition. These variables can also affect the solubility of the active ingredient(s). For example, the active ingredient may have limited solubility in biological buffers, but the solubility limit may never be reached if the absorption rate equals or exceeds the rate

of dissolution. This situation can be expected with some BCS Class I and II drugs, but it is very unlikely for Class III and IV drugs, which have poor absorption. Data from IDAS1 will allow scientists to assess the effect of excipients on dissolution and permeability and to evaluate multiple iterations of formulation modifications at a higher throughput.

The *in vitro* dissolution absorption system 2 (IDAS2) is a more recent innovation (20). It consists of a novel, specially designed dissolution-vessel lid with an attached permeability chamber that is compatible with commercially available dissolution apparatuses (Figure 2A). Experiments with IDAS2 are performed in two consecutive phases. In Phase 1, a tablet or capsule is added to the dissolution vessel that contains a biorelevant medium; the contents are stirred at a predetermined speed (e.g., 25 to 50 rpm) and allowed to undergo dissolution for 15 to 30 minutes. If the dissolution medium is acidic (e.g., fasted-state simulated gastric fluid, FaSSGF), it must be modified after Phase 1 to mimic the intestinal content. To this end, in a single step a concentrated buffer is added to increase the pH to 6.5 and introduce bile acids and lecithin at appropriate concentrations to mimic the intestinal contents under fed or fasted conditions. Immediately after this

transfer, the IDAS2 chamber containing the cell monolayer is introduced into the dissolution medium, and the dissolution-permeation phase (Phase 2) is initiated. Donor samples are withdrawn from the bulk fluid in the dissolution vessel at various time points during both phases to ascertain dissolution rate and donor concentration. Multiple samples from the receiver compartment are taken from the IDAS2 chamber (basolateral, serosal) during Phase 2 of the assay. A unique feature of IDAS2 is that it permits the assessment of intact, clinical-size dosage forms. In addition, the use of 250 mL dissolution media replicates the dose/volume ratio expected in the gastrointestinal tract after the administration of a tablet (or capsule) with a glass of water.

Recent experiments with propranolol and warfarin tablets in IDAS2 highlight the potential utility of the system. The initial dissolution rate of propranolol was slightly higher than that of warfarin, but both products were completely dissolved in 30 minutes (Figure 2B). Despite having a higher intrinsic permeability due to its initially slower dissolution, the percent permeation of warfarin was almost the same

as that of propranolol. However, when the dissolution of warfarin reaches a solubilized amount comparable to that of propranolol (i.e., after 30 minutes), as expected warfarin had a much faster rate of permeation than propranolol (Figure 2C). This example demonstrates the ability of IDAS2 to reveal the dissolution-permeability interplay.

Evolving experience with IDAS1 and IDAS2 suggests that they have the potential to become important tools in formulation development and regulatory oversight of drug product quality. Their roles are naturally complementary; IDAS1 is primarily useful in excipient selection and formulation development/optimization, whereas IDAS2 is mainly deployed in the comparison of multiple intact products (tablets or capsules). Even when clinical BE studies are necessary, the information derived from IDAS1 and IDAS2 could be used to inform the experimental design of the studies.

## Regulatory Science Initiatives for Enhanced Quality

Sensitive and reproducible *in vitro* methodologies such as dissolution

testing, along with the BCS classification, have proved successful in numerous jurisdictions in helping to assure equivalence, facilitate product development, and reduce regulatory burden. Given the pervasiveness of the problem, we believe that two approaches could be undertaken immediately to help mitigate the consequences of poor quality and counterfeit pharmaceutical drug products. First, tools should be put in place that are capable of detecting biopharmaceutically relevant differences between different products of the same drug, or large inter-lot variations in the same product, in order to permit both regulatory bodies and manufacturers to detect and correct important deviations in product or lot characteristics that may result in differences in therapeutic performance. Second, more effective programs should be designed and implemented to monitor the similarity between registration lots and commercial lots of pharmaceutical products in order to assure continuity of product therapeutic performance. Since the success of this strategy depends on the availability of tools and mechanisms capable of detecting therapeutically meaningful differences in a time frame compatible with

**Figure 2.** **(A) Schematic representation of the *in vitro* dissolution absorption system 2 (IDAS2); (B) dissolution profiles of propranolol and warfarin tablets, as percent of dose over time; (C) permeation profiles of propranolol and warfarin associated with the dissolution of their respective tablets**
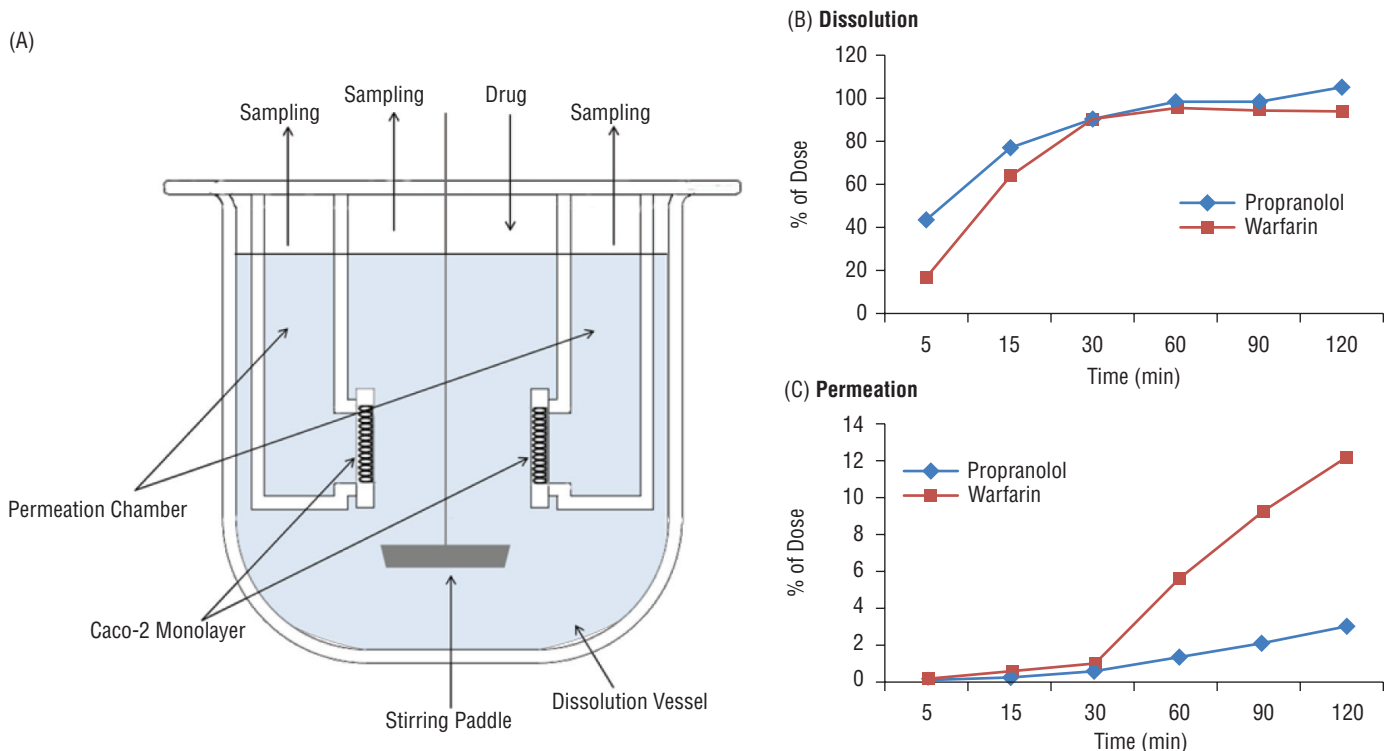
decision-making timelines, it is essential to achieve a reasonable balance among tool sensitivity, biopharmaceutical relevance of the test (e.g., simulation of *in vivo* performance), and speed (e.g., days as opposed to weeks).

Realistic approaches to guard product quality must rely on an important *in vitro* component, but commonly used techniques (single point dissolution, drug content, etc.) lack sensitivity and/or relevance and/or speed to make them sufficiently useful for this purpose. Therefore, the development or incorporation of novel, physiologically relevant systems

like IDAS1 and IDAS2 would appear to be useful in any strategy that seeks to have a meaningful and long-term impact on the quality of pharmaceutical products in these markets. Following a more robust evaluation of the potential of IDAS1 and IDAS2, their utility, if warranted, could be formalized through regional regulatory science initiatives. Since these tools appear to be technologically and financially accessible, they could aid Latin American and Caribbean regulatory authorities' efforts to safeguard public health through improving both the quality of products in

the market and the speed of regulatory approval. Proper implementation of these *in vitro* tools would present additional cost-effective means to assist in formulation development and optimization, which could also help bolster the regional pharmaceutical industry.

**Conflicts of interest.** None declared.

**Disclaimer.** The authors hold sole responsibility for the views expressed, which may not necessarily reflect the opinion or policy of the RPSP/PAJPH or PAHO.

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**RESUMEN**

**Métodos *in vitro* innovadores para determinar la equivalencia terapéutica**

Para mejorar la calidad de los productos farmacéuticos comercializados en su mercado, varios países latinoamericanos han empezado a exigir que se demuestre la bioequivalencia de los nuevos medicamentos genéricos frente a los medicamentos innovadores o de referencia. Sin embargo, dado el gran número de medicamentos, resulta, poco factible realizar estudios clínicos para cumplir con este requisito pero tiene sentido incorporar o elaborar estrategias que sean acordes a las características de la región.

Para simplificar el desarrollo de fármacos y optimizar el acceso de los pacientes a medicamentos de buena calidad, hace 15 años la Administración de Alimentos y Medicamentos de los Estados Unidos de América (FDA) decidió conceder exenciones a la realización de estudios clínicos de bioequivalencia (es decir, bioexenciones) a algunos tipos de medicamentos conforme al Sistema de Clasificación Biofarmacéutica. Las bioexenciones reducen significativamente el tiempo y el costo de desarrollo, y también evitan la exposición innecesaria de seres humanos a medicamentos que podrían ser nocivos, a la vez que constituyen una norma robusta y uniforme que garantiza la equivalencia terapéutica de los medicamentos genéricos.

Por otra parte, los métodos innovadores, como los sistemas de disolución y absorción *in vitro*, permiten ampliar los resultados limitados obtenidos al aplicar los datos de disolución *in vitro* para simular los efectos *in vivo*. Dado que combinan las pruebas de disolución *in vitro* con las de permeabilidad, estos sistemas brindan conocimientos útiles para el desarrollo galénico. Es probable que la evaluación meticulosa del potencial de las técnicas *in vitro*, junto con su formalización mediante iniciativas de normalización científica cuando corresponda, permita concebir métodos eficaces en función de los costos que ayuden a encarar algunos de los retos relativos a la calidad y la regulación de los medicamentos que enfrentan América Latina y el Caribe.

**Palabras clave**    Biofarmacéutica; disolución; permeabilidad; absorción; equivalencia terapéutica; control de calidad.