Journal of Pharmaceutical Sciences 107 (2018) 453-465

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmacokinetics, Pharmacodynamics and Drug Transport and Metabolism

# *In Vitro–In Vivo* Correlations of Carbamazepine Nanodispersions for Application in Formulation Development

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ARTICLE INFO

*Article history:*
Received 22 March 2017
Revised 29 September 2017
Accepted 3 October 2017
Available online 16 October 2017

*Keywords:*
dissolution
absorption
*in vitro–in vivo* correlations (IVIVC)
*in vitro* models
nanoparticles
amorphous
oral absorption
bioavailability
solid dispersion

ABSTRACT

During formulation development, efficiently integrating *in vitro* dissolution testing can significantly improve one's ability to estimate *in vivo* performance and aide in the selection of premier drug candidates. The concept of *in vitro–in vivo* relationship/correlation has garnered significant attention from pharmaceutical scientists to predict expected bioavailability characteristics for drug substances and products. The present work illustrates a comparative evaluation of *in vitro* tests to access crystalline carbamazepine and various types of amorphous and crystalline dispersions of carbamazepine and Eudragit® L100 produced by spray drying, including a membrane-permeation dissolution methodology and nonsink dissolution. To establish the best model, parameters such as pH, membrane constitution, and dissolution media composition were investigated. The *in vitro* results were compared against *in vivo* mice pharmacokinetic studies and qualitatively, the membrane-permeation dissolution methodology correlated well with *in vivo*. Various correlations were performed in order to evaluate the optimal model for characterizing the relationship. Results exhibited a coefficient of determination ($R^2$) values of 0.90 and 1.00, depicting a linear relationship of the data in comparison. Therefore, for the current formulation system (drug/polymer/technique), membrane-permeation dissolution can guide formulation development and potentially reduce the number of animal and clinical pharmacokinetic studies required.


## Introduction

Oral drug bioavailability is a summation of processes comprised of the dissolution of a drug into gastrointestinal media, permeation or transport of the dissolved drug across the intestinal membrane, and metabolism of the drug in the gut wall or liver before it reaches the systemic circulation. The rate and extent to which a drug is absorbed into systemic circulation has a significant influence on the therapeutic efficacy and duration of action of a drug. The application of an *in vitro* test which can relate to the *in vivo* performance of a drug is an invaluable tool for drug development. *In vitro* dissolution data can be correlated to *in vivo* pharmacokinetic data, often denoted as an *in vitro–in vivo* correlation (IVIVC), and thereby provide the ability to predict the effect changes in a formulation will have on the absorption of the drug. Successful correlations can justify formulation changes without the need for additional *in vivo* testing. The concept of IVIVC has been well reported in the literature and implemented as a guidance document from the Food and Drug Administration (FDA) for use as a surrogate for human bioequivalence studies.[1-4] Commonly, IVIVC is performed by correlating *in vitro* dissolution data to *in vivo* absorption data obtained from a pharmacokinetic study. Classically, for acceptable IVIVC, the *in vitro* dissolution should behave similarly or be scalable to the percent of drug absorbed *in vivo*.[5] A majority of successful IVIVC examples for oral drug delivery are for modified-release dosage forms.[3] If the dosage form releases immediately or the drug has difficulty being absorbed, the rate-limiting step for the absorption of the drug is the absorption process, which can lower the correlation between *in vitro* dissolution and *in vivo* absorption.[6] Correlations may be influenced by the manner in which the *in vitro* tests are performed as well as the approaches taken to

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https://doi.org/10.1016/j.xphs.2017.10.014
0022-3549/

correlate with the *in vivo* results. Table 1 shows a comparison of the different dissolution tests available for designing an IVIVC method.[7-21]

Three correlation levels (A-C) are categorized in United States Pharmacopeia <1088>, with an additional level existing as a combination of levels (multiple C) defined in the FDA guidance.[1,2] The highest level of correlation is level A, which is established through a point-to-point comparison of *in vitro* dissolution data and *in vivo* input rates. If the correlation is linear, similarity can be ascertained by directly superimposing dissolution curves or by applying a correction/scaling factor to make the curves superimposable.[1] Preceding superimposing input curves, either deconvolution of *in vivo* data or convolution of *in vitro* data, is necessary. Deconvolution of plasma level data is executed with mathematical models that warrant comparison of the fraction of drug absorbed with fraction of drug dissolved.[22,23] Methods of deconvolution can be model dependent or independent. Traditionally, model-dependent methods such as Wagner-Nelson (single compartment) and Loo-Riegelman (2 compartment) are used to calculate the fraction of drug absorbed by taking into account metabolism and excretion.[24-26] Data-rich requirements of level A correlations encompass complete plasma levels and constitute it as the most useful and scientifically accepted category. In contrast, if the total dissolution data cannot be used to establish a correlation, single-point relationships can be instituted to develop level B correlations. Level B correlations use principles of statistical moment analysis to compare the *in vitro* mean dissolution time with either the *in vivo* mean residence time or mean dissolution time.[2] However, because level B correlations do not exclusively reflect *in vivo* plasma levels, their application is rarely exploited to prove bioequivalence of formulations. Analogous to level B correlations, level C correlations denote data reduction.[27] A level C correlation determines a single-point relationship between an *in vitro* dissolution parameter (i.e., percentage dissolved at a specific time point) and an *in vivo* pharmacokinetic parameter (i.e., area under the curve [AUC], maximum concentration [$C_{max}$], etc.).[1] Level C correlations are often applied during formulation development and quality control procedures.[28] The application of a single level C correlation has limited practicality in foreseeing the *in vivo* performance of a drug formulation; however, multiple level C correlations may be as suitable as a level A correlation.[1] A distribution of IVIVC proposals divulged multiple level C IVIVC accounted for 11% of the total regulatory submissions.[29]

Challenges associated with the development of a predictable IVIVC model are related to the *in vitro* dissolution methodology. Dissolution testing for quality control of drug product is often performed with an aqueous buffer solution including non-physiological surfactant concentrations to evaluate changes in dosage forms after manufacturing. While at times this is sufficient for achieving IVIVC, such testing solutions may not be well suited for IVIVC testing, and more complex media which simulate the physiological environment in the gastrointestinal tract are required to obtain *in vitro* dissolution which is representative of the process which takes place *in vivo*.[30] If the dissolution media significantly affect the relationship of dissolution *in vitro* to that of dissolution *in vivo*, then methods like biphasic dissolution systems may not be suitable, as inclusion of biorelevant surfactants will influence the miscibility between the organic and aqueous phases. However, despite challenges associated with biphasic dissolution systems, membrane-permeation dissolution devices analyze a dosage form's dissolution and permeability simultaneously, making it useful for predicting the *in vivo* absorption for drugs which have rate-limited absorption in the drug delivery system.[31]

In a recent study, Duarte et al.[32] reported a novel method for producing nano-solid dispersions using solvent-controlled precipitation and isolation by spray drying with a model drug, carbamazepine (CBZ). Using this process, Eudragit® L100 was selected as the polymer of choice due to its ability to stabilize the formation of the amorphous solid dispersion after solvent precipitation. Using this process, both an amorphous solid dispersion and a crystalline solid dispersion were developed by altering the drug-to-polymer ratio during production. Duarte et al.[32] tested the pharmacokinetics of nanoamorphous and nanocrystalline dispersions alongside a microamorphous solid dispersion produced by conventional spray drying and pure crystalline CBZ in mice. As seen in Figure 1, the orally administered mice pharmacokinetic study showed the nanocrystalline and nanoamorphous formulations had higher bioavailability than the microamorphous formulation which appeared to be greater than pure crystalline drug. The rank order of performance was established based on AUC values to be: pure CBZ < microamorphous < nanoamorphous < nanocrystalline (Table 2). Before pharmacokinetic studies, Duarte et al.[32] evaluated the performance of CBZ formulations with an *in vitro* microcentrifuge dissolution study. Results suggested the establishment of an IVIVC with the current microcentrifuge dissolution technique might be challenging.

In this study, it was hypothesized that a membrane-permeation dissolution/absorption method could accurately predict the pharmacokinetic performance of the CBZ formulations. To achieve an acceptable level IVIVC, parameters in the membrane-permeation dissolution/absorption method were optimized to yield the most accurate results. Results from the membrane-permeation dissolution/absorption method were compared to results acquired in microcentrifuge and ultrafiltration dissolution techniques as well as *in vivo* pharmacokinetic studies. In addition to qualitative analysis, IVIVC were made to quantitatively present the *in vitro* test with the highest correlation to the *in vivo* results.

## Materials and Methods

### *Materials*

#### *Chemicals*

CBZ (5H-dibenzo(b,f)azepine-5-carboxamide, anhydrous form III, purity >97%) and Eudragit® L100 (methacrylic acid:methyl methacrylate copolymer [1:1]) were provided by Hovione FarmaCiencia SA (Loures, Portugal). Hovione purchased CBZ from TCI Company Limited (Tokyo, Japan) and Eudragit® L100 from Evonik Röhm GmbH (Darmstadt, Germany). Solvents and chemicals for *in vitro* dissolution testing and HPLC analysis were analytical or American Chemical Society grade. Analytical grade solvents and chemicals—acetonitrile, methanol, and sodium phosphate monobasic—were purchased from VWR (NJ). ACS grade or equivalent solvents and chemicals—sodium chloride (NaCl), 1-decanol, sodium hydroxide (NaOH), and hydrochloric acid—were purchased from VWR (NJ). Trifluoroacetic acid (liquid chromatography-mass spectrometry grade) was purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water (resistivity, 18 MΩ cm) was obtained from a Milli-Q® purification system (EMD Millipore, Burlington, MA). Fasted state simulated intestinal fluid (FaSSIF) and canine FaSSIF powder were purchased from Biorelevant.com (London, UK). Acceptor Sink Buffer (pION ASB-7.4, Double-Sink™ buffer, P/N 110139) was purchased from Fisher Scientific (Pittsburgh, PA). Porcine intestines were purchased from a local source. Polyethersulfone (PES; diameter, 25 mm; pore size, 0.03 μm; nominal thickness, 110 to 150 μm) polymer membrane filters were purchased from Sterlitech Corporation (Kent, WA).

*FaSSIF Preparation.* Two preparations of FaSSIF were used during dissolution testing of CBZ formulations. Traditional concentrated FaSSIF was prepared for dissolution experiments that did not

**Table 1**
SWOT Analysis of *In Vitro* Techniques for IVIVC of Oral Dosage Forms

| Technique | Strength | Weakness | Opportunity | Threat | References |
|---|---|---|---|---|---|
| USP Apparatus I | Compendial apparatus, simple, robust, pH adjustment capabilities | Requires large amount of sample, questionable hydrodynamics, basket screen obstruction by sample | Generates cumulative dissolution results, simultaneous disintegration and dissolution analysis, floating dosage form analysis | Nonphysiologically relevant mixing, reduced dissolution resulting from clogging mesh | USP[7] |
| USP Apparatus II | Compendial apparatus, well-controlled and well-defined mixing dynamics, robust, pH adjustment capabilities, standardized, simple operation | Requires large amount of sample, low-density solids may float and/or stick to the paddle, no information on drug absorption/permeation, nonuniform shear rates, fixed volume | Predicting *in vivo* hydrodynamics based on Reynolds number | Flow fluctuations significant enough to displace tablets, cone formation at base of vessel | USP[7] |
| USP Apparatus III | Compendial apparatus, allows easy transfer to different dissolution media, simulate pH change | Typically for nondisintegrating dosage forms, low-volume system | Fractionated dissolution results, run in different dissolution media | Disintegration of small particles, foaming, artificially high dissolution resulting from dissolution media evaporation during extended testing | USP[7] |
| USP Apparatus IV | Compendial apparatus, allows easy transfer to different dissolution media, pH adjustment capabilities | Pump precision, clogging of filters, large volumes may be required | Operate in open or closed system, may allow for physiologically relevant Reynolds number and hydrodynamics, Fractioned dissolution results, IVIVC | Impact of fluid sheer on dissolution | USP[7] |
| USP Apparatus II + biphasic dissolution | Evaluates drug absorption, aqueous layer does not saturate, dissolution-partition analysis | Dissolution medium limited to no surfactants, Emulsification of 2 phases | Complete dissolution | Artificial dissolution resulting from the lack of physical barrier between aqueous and organic phases | Gibaldi and Feldman[8] |
| USP Apparatus II/IV | Compendial apparatus, biphasic dissolution, pH adjustment capabilities, evaluated drug absorption and partitioning | Emulsification of 2 phases, surfactant limitations, no membrane in biphasic system | Evaluate drug absorption, improved hydrodynamics, IVIVC | Entrapment of air decreases sensitivity/performance of fiber-optic probes, complex optimization of procedure limits generalization of technique | Vangani et al.[9] |
| Microcentrifuge dissolution | Small quantity of sample used, relatively low cost | Limited relevance to biological system | High-throughput screening for early drug formulation selection | Artificially high dissolution, small-scale automated systems | Curatolo et al.[10] |
| Ultracentrifuge dissolution | Minimal sample requirements, drug/polymer species separation | Cost of equipment, challenging method development due to partitioning of species | Free/dissolved drug analysis, bile-micelle partitioning studies, drug and polymer colloid evaluation | High speed effect on drug release | Gao et al.[11] |
| Ultrafiltration dissolution | Small volume, limited sample requirements, separation of drug/polymer species, minimal equipment requirements | Membrane pretreatment required, membrane MWCO | Evaluate drug permeation, IVIVC, free/dissolved drug analysis | Drug binding to membrane resulting in low concentrations in filtrate | Zhang et al.[12] |
| Multicompartment dissolution apparatus/transfer model | Three compartments to mimic dissolution and absorption in gastrointestinal tract, pump-controlled transfer, volume control of each compartment, pH adjustment capabilities | Complex setup, pumps may be required, precipitation, stir rate optimization required, filter compatibility | Analysis of drug release from a floating system, IVIVC establishment, analysis of weakly basic drugs | Low drug absorption due to loss by precipitation in other compartments | Gu et al.[13] |
| 1X Bio dissolution | Discriminatory of *in vivo* performance for dissolution rate-limited absorption formulations | Requires accurate sampling of small aliquots for the dissolution at the solubility limit | Quantitative assessment of the predictability of the test to *in vivo* bioavailability | | Kuiper[14] |
| TNO gastrointestinal model (TIM-1) | Wide application range, physiologically relevant, individual analysis of each compartment | Extremely complex system, software requirements, throughput limitations | Model gastrointestinal physiology of animals, alternative to human/animal trials, accelerate product development, IVIVC | Computer-controlled, mechanical interaction | Minekus et al.[15] |
| FloVitro dissolution apparatus | Multiple chamber dissolution/absorption setup, real-time UV analysis | Hydrodynamic limitations, pump controlled, clogging | Developed to model deconvoluted *in vivo* data, biomodeling, simulated absorption phase, level A IVIVC | Limited by relevance of hydrodynamics, not a monographed dissolution method | Sirius Analytical/DOW Chemical Company[15] |
| Dialysis membrane dissolution | Controlled MWCO, ease of sampling, separation of sample from buffer | Limitations associated with drug diffusion rate in/out membrane, limited to single-phase system, nonautomated | Can be combined with compendial apparatus, versatile, can be used to study drug release from dosage form, real-time UV analysis | Reproducibility challenges due to variability in membrane, drug binding to membrane, drug release from bag is | Alonzo et al.[17] |

(*continued on next page*)

**Table 1** (*continued*)

| Technique | Strength | Weakness | Opportunity | Threat | References |
|---|---|---|---|---|---|
| | | | | strongly affected by stir rate and permeability, artificial release kinetics | |
| Biomimetic polymer membrane permeation dissolution | Evaluates dissolution and permeation process, flexibility in testing conditions, representative of human dissolution process, minimal sample requirements, pH adjustment capabilities, chemical/pH stability | Lacks transporter proteins, low cell volume | Research into membranes which best simulate human intestine, dissolution testing as an *in vivo* simulation, reduction in animal studies, mimic dissolution in stomach and intestine, IVIVC | Overestimation of absorption as technique lacks elimination function | Puppolo et al.[18] |
| Caco-2 cell membrane permeation dissolution | Indicator of intestinal permeability and absorption, incorporates tight junctions and uptake/efflux transporters, cell-based membrane | Dissolution time is limited <2 h, >20-d cell culture requirements, labor-intensive, high cost per assay, cell monolayer instability, specific media requirements due to membrane stability, interlaboratory variation, elevated transepithelial electrical resistance | *In vitro* dissolution screening tool during formulation development, IVIVC | Paracellular and active transport across Caco-2 cells is less efficient than *in vivo*, underestimation of certain drugs due to overexpression of Pgp efflux pump, unstirred aqueous layer depth dependent on stir rate | Yee[19] |
| MDCK cell membrane permeation dissolution | Reduced incubation time (<5 d) relative to Caco-2 cells, indicator of intestinal permeability and absorption, cell-based membrane | Labor-intensive, high cost per assay, low expression of efflux pumps | Acceptable to FDA for purpose of BCS classification | Insignificant correlation with *in vivo* results for drugs limited by permeability, not suitable for screening compounds with efflux challenges | Thiel-Demby et al.[20] |
| PAMPA membrane permeation dissolution | High-throughput, 96-well plate assay, transcellular transport, low cost | Lipophilic compound sensitivity, depth challenges associated with unstirred aqueous layer and agitation rate | Drug discovery, combination with cell-based approach to incorporate active and paracellular transport | Underestimation of permeability due to unstirred aqueous layer depth, drug permeability dependent on lipid composition | Kansy et al.[21] |

BCS, Biopharmaceutics Classification System; SWOT, Strength Weakness Opportunity Threat; USP, United States Pharmacopoeia; MWCO, molecular weight cutoff; MDCK, Madin-Darby canine kidney cells; PAMPA, parallel artificial membrane permeability assay.




**Figure 1.** Pharmacokinetic profiles in mice after oral administration of nanocrystalline (diamonds), nanoamorphous (squares), microamorphous (triangles), and pure crystalline CBZ (circles). The limit of quantification for the immunoassay is depicted by the dashed line. The broken dashed line corresponds to the maximum obtainable drug concentration if a 60% yield for the extraction process is assumed. The bars represent the standard deviation ($n = 3$). Points with no error bars present are from $n \leq 2$ animals. Reproduced from Duarte et al.[32] 2016 with permission from Elsevier.

incorporate a pH shift. Approximately 1 L of FaSSIF was prepared by dissolving 0.420 g of NaOH, 3.9540 g of sodium phosphate monobasic (monohydrate) ($NaH_2PO_4 \cdot H_2O$), and 6.186 g of NaCl in 0.9 L of purified water. Adjustment to pH 6.5 was made, as necessary, with 1 N NaOH or 1 N HCl and volume was increased to 1.0 L. Approximately 2.24 g of FaSSIF powder was added to 0.5 L of the buffer, stirred until complete dissolution occurred, and made up to 1.0 L with the remaining buffer solution. The resulting FaSSIF solution was allowed to equilibrate for at least 2 h before use. Ten times concentrated FaSSIF was prepared in the same manner as the traditional concentrated FaSSIF except with 10-fold higher amounts of the weighed additives for use in pH shift dissolution experiments. Once diluted during the pH shift experiments, the resulting concentrations of components are equivalent to traditional FaSSIF.

*Canine FaSSIF Preparation.* Approximately 1 L of canine FaSSIF was prepared by dissolving 8.70 g of NaOH, 39.50 g of $NaH_2PO_4 \cdot H_2O$, and 34.80 g of NaCl in 0.9 L of purified water. Adjustment to pH 5.2 was made, as necessary, with 1 N NaOH or 1 N HCl and volume was increased to 1.0 L. Approximately 87.0 g of canine FaSSIF powder was added to 0.5 L of the buffer, stirred until complete dissolution occurred, and made up to 1.0 L with the remaining buffer solution. The resulting FaSSIF solution was allowed to equilibrate for at least 2 h before use.[33]

## *Methods*

### *CBZ Solid Dispersion Preparation*

Three CBZ formulations—(1) nanoamorphous (20:80 CBZ:Eudragit® L100), (2) nanocrystalline (60:40 CBZ:Eudragit® L100), and (3) microamorphous (20:80 CBZ:Eudragit® L100)—were supplied by Hovione FarmaCiencia SA. Formulations were produced using solvent-controlled precipitation coupled with spray drying in a microfluidization process and provided by Duarte et al.[32] Varying the drug-to-polymer ratio during processing, Duarte et al. was able to produce amorphous and crystalline formulations.[32] Briefly, solvent-controlled precipitation studies were performed using PureNano™ Microfluidics Reaction Technology (MRT, CR5 Reactor model) by Hovione FarmaCiencia SA.[32] Following a single passage, suspensions were spray dried in a Mini Spray Drier B-290 (BÜCHI,

**Table 2**
Rank Order of Formulation Performance *In Vivo* and *In Vitro* Based on the $AUC_{0\text{-}120\ min}$

| Rank Order | *In Vivo* | *In Vitro* Technique | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Microcentrifuge | Ultrafiltration | FaSSIF pH 6.5/Decanol | FaSSIF pH 6.5/ASB | FaSSIF pH 5.5/Decanol | Canine FaSSIF pH 5.2/Decanol | Canine FaSSIF pH 5.2/ASB |
| 1 | Nanocrystalline | Nanoamorphous | Nanoamorphous | Nanocrystalline | Nanocrystalline | Nanocrystalline | Nanocrystalline | Nanoamorphous |
| 2 | Nanoamorphous | Microamorphous | Microamorphous | Nanoamorphous | Nanoamorphous | Pure CBZ | Nanoamorphous | Nanocrystalline |
| 3 | Microamorphous | Nanocrystalline | Nanocrystalline | Pure CBZ | Microamorphous | Nanoamorphous | Microamorphous | Microamorphous |
| 4 | Pure CBZ | Pure CBZ | Pure CBZ | Microamorphous | Pure CBZ | Microamorphous | Pure CBZ | Pure CBZ |

Flawil, Switzerland) equipped with a 2-fluid nozzle by Hovione FarmaCiencia SA.[32]

### In Vitro *Dissolution Testing*

*Microcentrifuge Dissolution.* Microcentrifuge dissolution testing was performed by Duarte et al.[32] to qualitatively rank the order of formulation performance. Duarte et al. used a dissolution methodology described by Friesen et al.[32,34] The procedure was performed by Duarte et al.[32] and can be seen in the following discussion. Nonsink dissolution experiments were executed at a CBZ concentration of approximately 425 μg/mL (saturation solubility, 268.72 μg/mL[35]) in 2-mL Eppendorf tubes in a 37°C water bath. pH was adjusted using 0.9 mL of intestinal fluid (FaSSIF, pH = 6.5) which was added to the simulated gastric fluid (0.01 N HCl, pH = 2) after 50 min of dissolution. Samples were acquired by centrifuging the dissolution solution at 13,000 rpm for 1 min, followed by a 15-fold dilution into methanol. The remaining solution was redistributed by vortexing and placed into the water bath until subsequent time points. The concentration of CBZ was determined by HPLC (discussed in section High-Pressure Liquid Chromatography). The area under the dissolution curve was established using the trapezoidal method.

*Ultrafiltration Dissolution.* A nonsink ultrafiltration dissolution method was used to investigate CBZ free drug concentration during testing of CBZ amorphous solid dispersions and crystalline CBZ. The ultrafiltration method employed Amicon® Ultra-0.5-mL centrifugal filters (EMD Millipore, Darmstadt, Germany) equipped with Ultracel® low binding regenerated cellulose membranes (EMD Millipore) with a 3-kDa cutoff. Filters were passivated overnight in a saturated CBZ solution. Appropriate amounts of CBZ formulations and crystalline CBZ were added to 6-dram glass vials to achieve a concentration of approximately 425 μg/mL such that nonsink conditions were fulfilled. pH was adjusted using 5 mL of simulated gastric (0.01 N HCl, pH = 2) and 5 mL intestinal fluid (FaSSIF, pH = 6.5) after 50 min of dissolution. Solutions were equilibrated at 37°C before addition. After addition of dissolution media, the vials were placed in an incubated shaker maintained at 37°C and operating at 250 rpm. At selected time points (i.e., 5, 10, 15, 30, 60, and 120 min), 0.5-mL aliquots were removed from the dissolution mixture, placed into an Amicon® Ultra-0.5-mL centrifugal filters, and centrifuged using an Eppendorf Microcentrifuge 5418 (Eppendorf, Hamburg, Germany) for approximately 5 min at 13,000 g. The supernatant was collected into an HPLC vial and the concentration of CBZ was analyzed using the HPLC method described in section High-Pressure Liquid Chromatography.

*Membrane-Permeation Dissolution.* A nonsink membrane-permeation dissolution method was used to emulate the *in vivo* performance of CBZ amorphous solid dispersions and crystalline CBZ. A 5-mL Side-By-Side Cell (PermeGear Inc., Hellertown, PA) was outfitted with either PES membranes or porcine intestines to determine the free drug content and predict the *in vivo* absorption of CBZ. The dissolution apparatus is comprised of 2 cell compartments, a donor, and an acceptor compartment. Simulated gastric and intestinal fluids were employed in the donor cell compartment. In pH shift experiments, 4.5 mL solution of 0.01 N HCl, pH = 2, was used to replicate gastric conditions. After 30 min, the donor compartment media was shifted to either human and canine FaSSIF solutions at pH = 6.5 or 5.2, with the addition of 0.5 mL of 10 times concentrated FaSSIF or canine FaSSIF solution (Biorelevant, London, UK), to simulate gastrointestinal conditions. See above sections FaSSIF Preparation and Canine FaSSIF Preparation for preparation of FaSSIF solutions. The acceptor cell compartment was composed of 5 mL of either 1-decanol or an ASB (Pion Acceptor Sink Buffer; Fisher Scientific). 1-Decanol was selected for the acceptor cell solution because it is immiscible with the aqueous dissolution media and acts a sink for the CBZ. The temperature of the donor and acceptor cell compartments was maintained at 37°C and the cells were agitated at a rate of 300 rpm using a 60-position stir plate (Variomag-USA, Port Orange, FL). Membranes were inserted between the donor and acceptor cell. Appropriate amounts of CBZ formulations and crystalline CBZ were added to the donor cell to achieve a concentration of approximately 425 μg/mL and ensure nonsink conditions were fulfilled. Dissolution testing was performed over a time period of 210 min and 100-μL samples were obtained at intervals such as 0, 5, 15, 30, 35, 40, 45, 60, 90, 120, 150, and 210 min. Samples acquired from the acceptor cell were analyzed directly by HPLC to establish the concentration of CBZ (discussed in section High-Pressure Liquid Chromatography). The area under the dissolution curve was established using the trapezoidal method.

### *High-Pressure Liquid Chromatography*

The following discussion describes the HPLC procedure used to determine CBZ concentration during *in vitro* membrane-permeation dissolution testing. The concentration of CBZ was measured using an Alliance 2695 HPLC system (Waters Corporation, Milford, MA) equipped with a 2996 photodiode array detector (Waters Corporation). Analysis was performed using a Waters XBridge C18 column (4.6 × 50 mm, 3.5 μm) (Waters Corporation). CBZ was analyzed using a gradient elution, a flow rate of 1.0 mL/min, column temperature of 25°C, a 10-μL injection, and detection wavelength of 285 nm. The method employed a gradient elution of 0.1% trifluoroacetic acid in water (mobile phase A) and 0.1% trifluoroacetic acid in acetonitrile (mobile phase B) and a gradient as follows: t = 0 min: 60% B, t = 0 min → t = 3 min: 60% B → 95% B, t = 3.01 min: 60% B, and t = 5 min: 60% B. Chromatograms were collected and analyzed with Empower version 2.0 software (Waters Corporation). The concentration of CBZ from *in vitro* membrane-permeation dissolution samples was quantified based upon a single point.

## Results and Discussion

Prior *in vitro* dissolution studies by Duarte et al.[32] inaccurately predicted the rank order performance of formulated CBZ

formulations when results were compared to pharmacokinetic studies. To develop a method which could overcome the limitations of the previously performed *in vitro* dissolution, several methods and techniques were implemented and evaluated. The following results and discussion present the techniques applied to evaluate CBZ formulation performance including *in vitro* dissolution studies and membrane-permeation studies. Membrane-permeation studies were found to more closely reflect the *in vivo* performance of CBZ formulations, and therefore that technique was further studied with various conditions to develop a method that accurately reflected the pharmacokinetic data in the following section. The final section discusses the metrics evaluated to establish a strong correlation between the developed *in vitro* test and observed *in vivo* results.

### *Evaluation of* In Vitro *Dissolution Techniques*

#### *Microcentrifuge Dissolution*

Previously, Duarte et al.[32] reported an *in vitro* microcentrifuge dissolution study on manufactured formulations performed in centrifuge tubes and employing a pH shift with gastric and FaSSIF dissolution media. As can be seen in Figure 2, the rank order of the formulations differs from that established *in vivo*. According to *in vitro* microcentrifuge dissolution data, the predicted performance of *in vivo* results would be pure CBZ < nanocrystalline < microamorphous < nanoamorphous (Table 2). Quantitatively it is clear this does not reflect the *in vivo* absorption observed in mice (Fig. 1); however, at the time this was the only dissolution technique available for evaluation. Nevertheless, Figure 2 depicts the processed drug formulations exhibited several fold higher dissolution rates relative to pure CBZ crystalline powder.

The effectiveness of a microcentrifuge dissolution filtration method depends strongly on the drug/polymer system, the centrifuge speed, and the type/pore size of membrane filters.[36] Microcentrifuge dissolution results of ketoconazole-povidone and ketoconazole-hydroxypropylmethylcellulose acetate succinate (HPMCAS) spray-dried dispersions indicated a heterogeneous supernatant was present following centrifugation, which inferred the presence of precipitated species.[36] During the dissolution of amorphous solid dispersions, nanoprecipitates can form. Isolation of the free drug from the nanoprecipitates can be used to provide results which reflect the *in vivo* absorption of the dosage form.[37-39] The data suggest that to isolate free/dissolved drug from nanoprecipitates and aggregate species in a microcentrifuge dissolution method, a secondary filtration step may be required to better represent *in vivo* performance. Having said that, microcentrifuge dissolution is not an ideal technique to evaluate the described CBZ formulations.

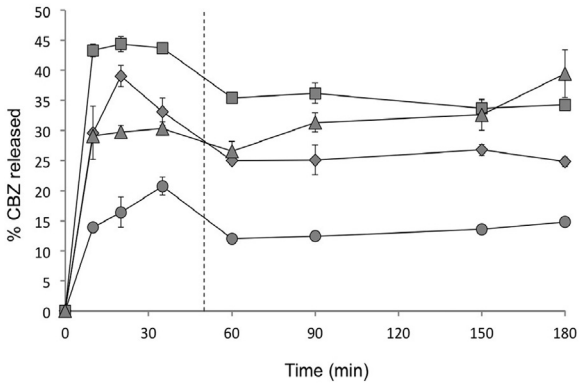


**Figure 2.** Average percentage of CBZ released during microcentrifuge dissolution of nanocrystalline (diamonds), nanoamorphous (squares), and microamorphous (triangles) CBZ formulations and crystalline CBZ (circles). The dashed line corresponds with the pH transition. Error bars represent the standard deviation ($n = 3$). Reproduced from Duarte et al.[32] 2016 with permission from Elsevier.

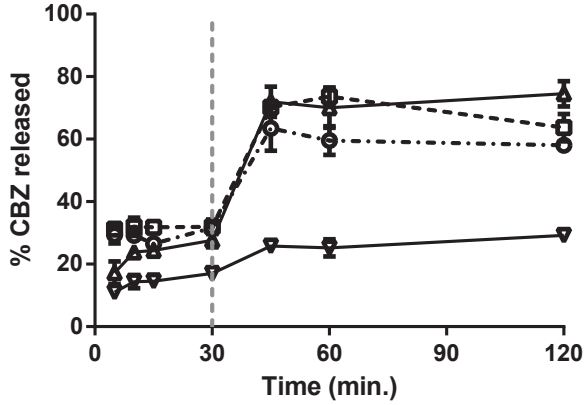


**Figure 3.** Ultrafiltration dissolution profiles for nanocrystalline (open circle, dashed line), nanoamorphous (open square, dashed line), and microamorphous (open upright triangle) CBZ formulations and crystalline CBZ (open inverted triangle). Dashed line represents the pH transition that took place at 30 min. Error bars represent the standard deviation ($n = 3$).

#### *Ultrafiltration Dissolution*

Ultrafiltration is a technique which can be used to filter small dissolved molecules from larger molecules like proteins and other polymers. Analogous to microcentrifuge dissolution, the process of ultrafiltration dissolution functions to separate small particulates from dissolved matter primarily based on size. However, ultrafiltration dissolution is capable of removing species or complexes >3 nm allowing analysis of a solution closely representative of free/dissolved drug.[40,41] Thus, ultrafiltration dissolution studies were performed in an equivalent manner as microcentrifuge dissolution studies. Membranes were passivated before analysis to prevent adsorption of CBZ. Analysis of the dissolution profiles in Figure 3 appear to align with those observed using microcentrifuge dissolution. Although the values after the pH shift were found to be high when performing the ultrafiltration dissolution test, the same rank order of formulation performance was obtained (Table 2).

Predicting the biological performance of solubility-enabling formulations can be improved with the use of biorelevant *in vitro* testing. In order to improve the accuracy of physiologically based pharmacokinetic modeling, Gao et al.[11] applied an ultracentrifuge technique with biorelevant media to characterize the drug release from an amorphous dispersion. Ultracentrifugation of dissolution samples can resolve species that are not dissolved drug, which improves the relationship between dissolved and absorbed samples. Ultrafiltration of dissolution samples separates free drug from other drug species much like ultracentrifugation without the need for an ultracentrifuge with the capability of producing such high forces. The expectation is that ultrafiltration- and ultracentrifugation-based dissolution techniques would provide comparable results.

### In Vitro *Membrane-Permeation Dissolution*

Depending on the physicochemical properties of a drug and its dosage form, the absorption of a drug may be limited by the dissolution or absorption rate of the drug. To that extent, whether a drug is dissolution or absorption rate limited can significantly influence which *in vitro* tests will best reflect the *in vivo* behavior.[42] Because dissolution/permeation devices more closely mimic the absorption system *in vivo*, they have utility for assessing the

performance of a large array of compounds from different Biopharmaceutics Classification System classifications.[43] Figure 4 depicts a schematic illustration of an example membrane-permeation dissolution device. These systems allow adjustment of the dissolution media, the membrane for permeation, the agitation during the dissolution process as well as the acceptor sink solution, making it a versatile system for relating to *in vivo* absorption of many different drugs and dosage forms.[44] The usefulness of membrane-permeation dissolution systems has been demonstrated by establishing IVIVC in multiple studies.[31,45,46] Microcentrifuge and ultrafiltration dissolution techniques depicted the disconnect between the *in vitro* tests and *in vivo* results was due to more than the measurement of the free drug concentration alone, thereby suggesting the need for a system which would not only assess free drug concentrations but also consider the diffusion of a drug across a membrane. To study whether the membrane-permeation dissolution method could provide results that better reflected *in vivo* performance, a study was completed in 0.1 N HCl and FaSSIF pH 6.5 dissolution media. An acceptor solution of 1-decanol was used in this experiment to provide a sink for CBZ. A PES membrane was positioned between the donor and acceptor compartments. Qualitative analysis of the percentage of CBZ permeated depicted in Figure 5a demonstrates the nanocrystalline formulation outperformed the other 3 tested formulations. These results more closely agreed with the results found *in vivo*; however, the permeation of nanoamorphous, microamorphous, and pure crystalline CBZ all behaved similarly *in vitro*. *In vivo* results showed the nanocrystalline formulation having the highest bioavailability of all formulations but similar performance to that of the nanoamorphous formulation. Both microamorphous and pure crystalline preparations had substantially lower bioavailability.

### *Developing a Predictive Membrane-Permeation Dissolution Method*

In an effort to improve the relationship between *in vitro* and *in vivo* results, the *in vitro* method was adjusted to better reflect the biological conditions of the *in vivo* model, the mouse. Conditions that were altered to study the IVIVC included the dissolution media, the acceptor sink solution, and the membrane in which permeation took place. Figure 5 depicts the results obtained from the membrane-permeation dissolution method with each adjustment performed.

To further optimize the membrane-permeation dissolution method to resemble the physiological conditions of a mouse, the dissolution media after the pH shift was adjusted. The pH of the mouse intestinal tract is reported to be around pH 5.2, which could have significant implications for the release of the drug from the dosage form.[47] Additionally, mice, rats and dogs have total bile salt concentrations much greater than those of humans, which can significantly affect the absorption of drugs *in vivo*.[48,49] Although there are currently no literature reports suggesting a correlation between mice and canine intestinal fluid composition, the components of canine FaSSIF may relate closer to that of a mouse than human FaSSIF. The mechanisms of supersaturation and solubility of compounds can be significantly affected by the pH of the dissolution media.[50,51] Therefore, mimicking gastrointestinal pH and pH transformations has noteworthy implications in dissolution testing. The CBZ dispersions are formulated with Eudragit L100, a known enteric polymer whose dissolution occurs at approximately pH 6.0. While the pH of a mouse gastrointestinal tract may never reach this level, it is still likely to have a noteworthy effect on the release of drug from the dosage form. Figure 5c depicts the results from changing the pH of the dissolution to pH 5.5 while maintaining the same FaSSIF composition, PES membrane, and acceptor sink conditions. Figure 5d presents the results obtained by altering the dissolution media to pH 5.2, altering the FaSSIF composition to canine FaSSIF solution, changing the membrane to porcine intestine, and maintaining the same acceptor sink conditions. The percentage of CBZ that permeated in dosage forms containing Eudragit L100 is reduced while that of the pure crystalline CBZ is maintained around the same with the decrease in pH in the dissolution media. Comparing Figures 5c and 5d to the *in vivo* data, it is clear that only changing the pH of the dissolution media does not fully resolve the mismatch between the *in vitro* and *in vivo* data. Figure 5d exhibits a decreased percentage of permeated CBZ from all formulations containing Eudragit® L100 compared to the other tests at higher pH. Analogous to the *in vivo* data, nanocrystalline and nanoamorphous formulations behaved similarly, with the nanocrystalline material having the highest percentage of CBZ permeated. Additionally, the microamorphous and pure crystalline drug behaved comparably with the microamorphous preparation having higher permeability than the pure crystalline drug, but both permeating less than the nanosized dispersions. The overall rank-order from this *in vitro* test was pure CBZ < microamorphous < nanoamorphous < nanocrystalline. These

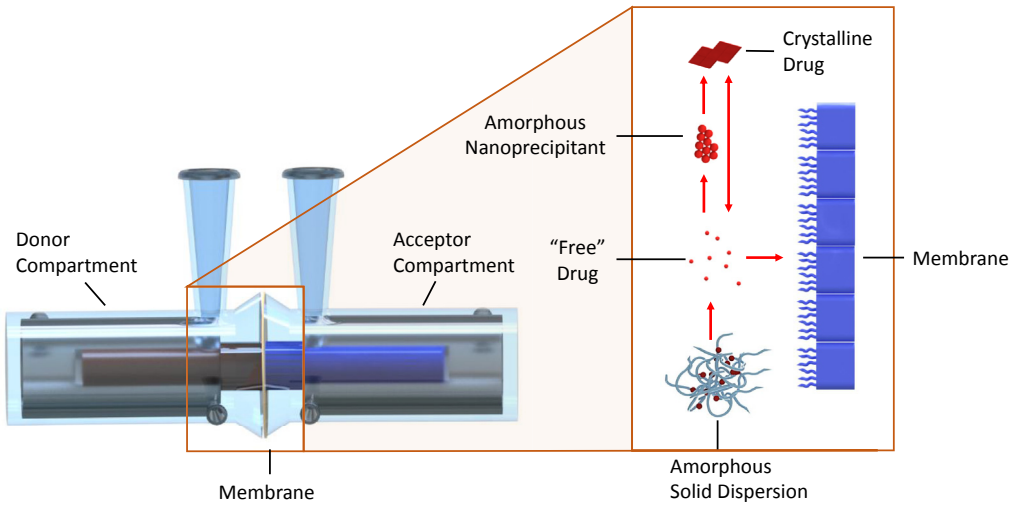


**Figure 4.** Schematic of a side-by-side diffusion cell for membrane-permeation dissolution experiments. Magnified section depicts possible drug-containing species that exist in the dissolution media.

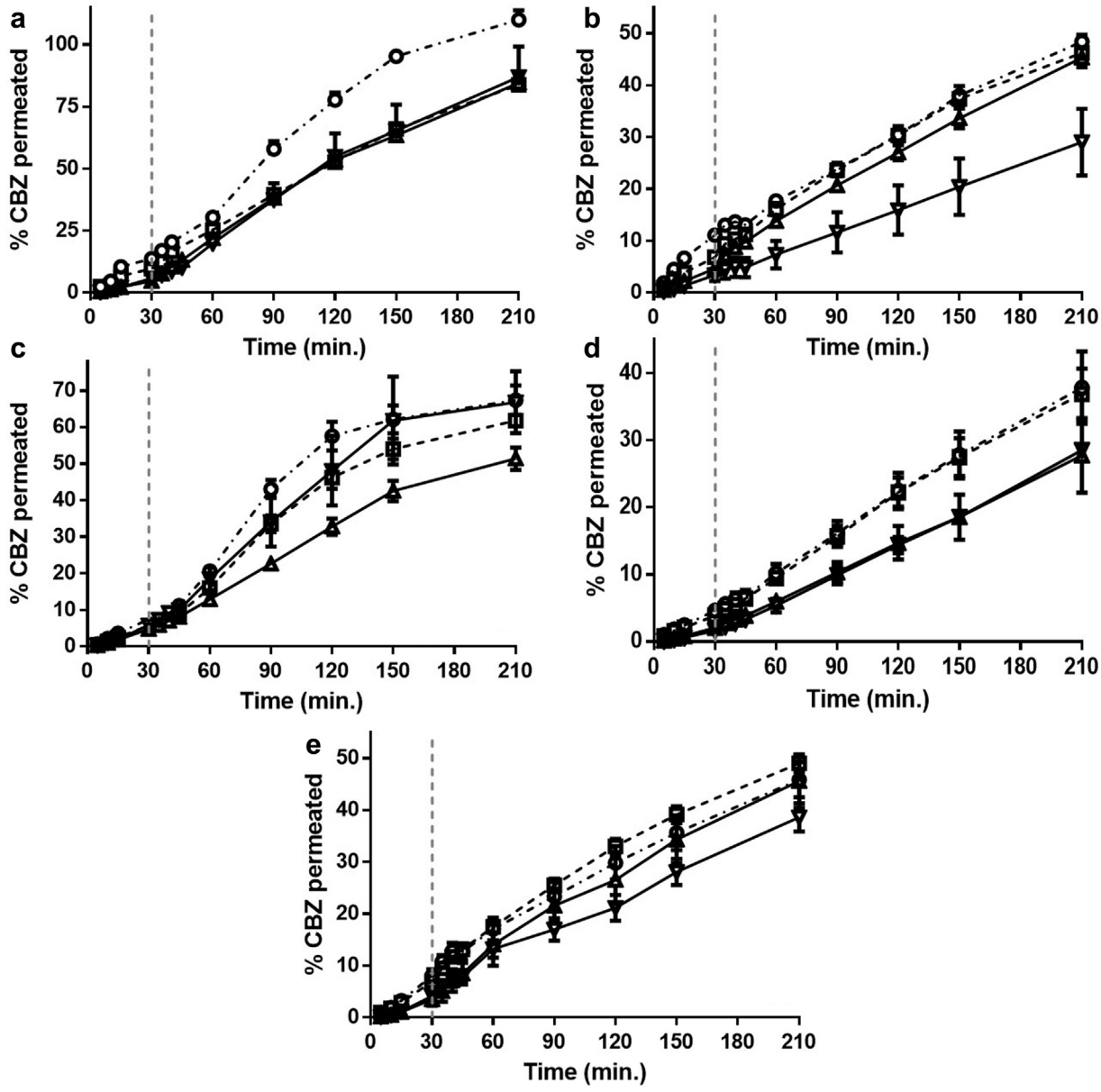


**Figure 5.** The percentage of CBZ permeated in a side-by-side diffusion cell apparatus with the following formulations: nanocrystalline (circles, dashed line), nanoamorphous (square, dashed line), microamorphous (upright triangle), pure crystalline CBZ (inverted triangle). The vertical dashed line represents the time of the pH transition. The following specific conditions were tested in each experiment: transition to FaSSIF pH 6.5 with 1-decanol used as acceptor solution, separated by a PES polymer membrane (a); FaSSIF pH 6.5 with ASB used as acceptor solution, separated by a PES membrane (b); FaSSIF pH 5.5 with 1-decanol used as acceptor solution, separated by a PES membrane (c); canine FaSSIF pH 5.2 with 1-decanol used as acceptor solution, separated by porcine intestine (d); canine FaSSIF pH 5.2 with ASB used as acceptor solution, separated by porcine intestine (e). Error bars represent the standard deviation ($n = 3$).

results align exactly with *in vivo* pharmacokinetic studies, thereby providing a method that qualitatively matches *in vivo* results (Table 2).

Adjustments to the acceptor sink solution were also made to evaluate the influence the acceptor solution has on the permeation of the CBZ formulations. The acceptor solution was exchanged for a marketed ASB (pION ASB-7.4, Double-Sink™ buffer, P/N 110139), which is maintained at a physiological pH and contains chemical scavengers intended to simulate serum proteins.[52,53] The dissolution media used was pH 6.5 FaSSIF and the membrane used was PES. The resulting percentage of CBZ permeated was much lower relative to previous studies enlisting 1-decanol as the acceptor sink solution. At the 60-min time point, each formulation exhibited nearly half the permeated amount compared to previous experiments (Fig. 5b). Additionally, the performance of the nanoamorphous, nanocrystalline, and microamorphous was all comparable while the pure crystalline was much lower. Although the separation of crystalline material from CBZ formulations closely resembled *in vivo* results, the performance of the processed formulations was difficult to distinguish. Previous experiments showed changes in the dissolution media and membrane type improved the correlation between the *in vitro* and *in vivo* results. Therefore, experiments were performed with the pH 5.2 canine FaSSIF and pig intestine membrane with the use of the ASB as the acceptor solution. The percentage of CBZ permeated over time is exhibited in Figure 5e. Overall, the percentage of CBZ permeated after the pH shift was greater for all formulations compared to the previously mentioned experiment using 1-decanol as the acceptor

sink solution. The rank order of the formulation performance was also altered, the nanocrystalline and nanoamorphous formulations behaved similarly to one another, with nearly overlapping permeation profiles. However, the nanoamorphous formulation consistently had slightly higher values, indicating it did not align with *in vivo* results (Table 2).

### In Vitro–In Vivo *Correlation*

While the membrane-permeation dissolution apparatus was able to resolve the rank order of the formulations *in vitro* as they appeared *in vivo*, conclusions as to the optimal conditions which best predict the *in vivo* response could not be made based on qualitative observations alone. In order to build a better understanding of which permeation and dissolution conditions provided an *in vitro* test which most closely reflected the *in vivo* performance of the formulations, development of an IVIVC was performed to facilitate quantitative evaluation. During formulation development, if an *in vitro* test correlates to the *in vivo* performance for an array of different formulations, the number of experiments which have to be performed in animal models can be reduced. Furthermore, a challenge often faced in early drug and formulation development is that large quantities of material are not available. Therefore, limited material amounts and cost drive interest in analytical characterization to small-scale techniques. In development work, encountered limitations can be overcome with small-scale systems by reducing the amount of material needed and increasing the sensitivity of analysis. A small-scale membrane-permeation dissolution apparatus that provides data reflective of *in vivo* drug performance can help overcome several challenges associated with formulation development. Understanding *in vivo* drug performance during developmental stages provides invaluable information to researchers and formulation scientists. To achieve a level A IVIVC, typically a deconvolution of the *in vivo* data is performed in order to obtain the *in vivo* input which can then be compared to the *in vitro* amount dissolved or permeated. One of the objectives of the present study was to produce a method which could be used by other researchers in a straightforward manner, as such we limited our correlation models to those which could be performed using Microsoft® Excel. Gómez and Valencia[54] developed correlations between the cumulative AUC *in vivo* and that of drug permeated during *in vitro* studies with several antiretroviral drugs. The correlation between different factors such as *in vivo* input and *in vivo* cumulative AUC was tested in order to develop a model which best reflected acceptable results.

The deconvolution of the *in vivo* data was performed by employing the Wagner-Nelson method to obtain the fraction absorbed over time for each formulation, depicted in Equation 1.

$$\%\ \text{Absorbed} = \frac{C(t) + k_e\, x\, AUC_{0-t}}{k_e x\, AUC_{0-\infty}} \times 100 \qquad (1)$$

where C (t) is the observed plasma concentrations and $k_e$ is the elimination rate constant. As the *in vivo* study did not have an intravenous solution in the study, an elimination rate constant of 0.02 $min^{-1}$ was used in the calculation based on values reported by Vuckovic et al.[55] Alterations in the particle size and crystal morphology of CBZ formulations clearly impacted the overall bioavailability of studied formulations. Due to limitations such as the lack of intravenous data and the inability to obtain an absolute bioavailability from *in vivo* data, the relative bioavailability of each formulation was compared to the nanocrystalline formulations and then used in Wagner-Nelson calculations by using the $AUC_{0-\infty}$ found for the nanocrystalline formulation in the calculation from Equation 1. The *in vitro* test that employed canine FaSSIF at a pH of 5.2 as the dissolution media, 1-decanol as the acceptor solution, and porcine intestines as the permeation barrier reflected the *in vivo* performance the best at a qualitative level. As such, this *in vitro* experiment was used to compare correlations found from different methods before comparing how well each *in vitro* test correlated to the *in vivo* results. Table 3 summarizes the relationships found for each correlation method tested for the same *in vitro* test, including that found for the Wagner-Nelson method.

**Table 3**
Correlations Calculated for Different Dissolution Methodologies Between *In Vitro* Test With Canine FaSSIF pH 5.2 Dissolution Media, 1-Decanol Acceptor Solution, and a Porcine Intestine as the Permeation Barrier With *In Vivo* Results Reported by Duarte et al.[32]

| *In Vitro–In Vivo* Correlation Methodology | Correlation ($R^2$) |
|---|---|
| %Permeated to %dose absorbed (Wagner-Nelson) | 0.85 |
| %Permeated $AUC_{0-t}$ to *in vivo* $AUC_{0-t}$ | 0.70 |
| %Permeated to *in vivo* $AUC_{0-t}$ | 0.90 |
| %Permeated at 60 min to *in vivo* $AUC_{0-inf}$ | 1.00 |

A comparison between the percentage of CBZ permeated in the *in vitro* test (employing canine FaSSIF at a pH of 5.2 as the dissolution media, 1-decanol as the acceptor solution, and porcine intestines as the permeation barrier) and the percentage of CBZ absorbed *in vivo* determined by Wagner-Nelson provided evidence for a strong linear correlation ($R^2 = 0.85$); however, other methodologies were continued to be explored. Similar to Gómez and Valencia,[54] the cumulative AUC from the *in vitro* results were related to those from the *in vivo* results. Unlike the antiretroviral drug studied by Gómez and Valencia, the correlation between the *in vitro* and *in vivo* results was less effective using this method ($R^2 = 0.70$). Comparing the percentage of CBZ permeated *in vitro* and the cumulative AUC from the *in vivo* results resulted in the highest correlation calculated on a point-to-point basis between the 2 groups ($R^2 = 0.90$). This method of correlating *in vitro*-to-*in vivo* results was applied to each of the various *in vitro* experiments to quantitatively discriminate between each test.

It was not feasible to establish a relationship between the *in vitro* microcentrifuge dissolution results published by Duarte et al.[32] and the *in vivo* animal studies using the percent permeated to cumulative AUC correlation methodology due to differences in the sampling time points between the *in vivo* and *in vitro* studies. The correlation between the ultrafiltration dissolution method and *in vivo* animal studies is depicted in Figure 6. It is evident from the Figure 6 that a strong linear relationship between the *in vitro* and *in vivo* data is lacking. The coefficient of determination ($R^2 = 0.52$)

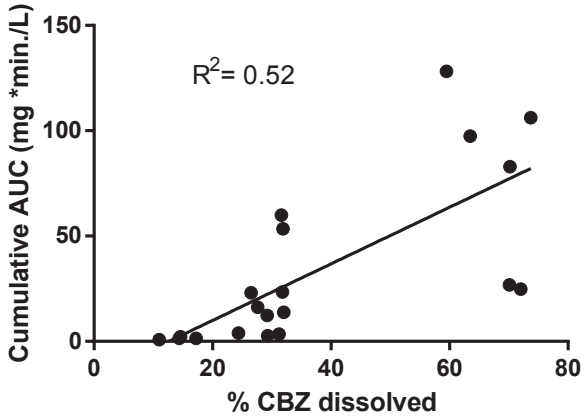


**Figure 6.** Correlation between *in vitro* ultrafiltration dissolution data and the cumulative $AUC_{0\text{-}60\ min}$ from *in vivo* pharmacokinetic data.

agrees with the qualitative observations that the *in vitro* test fails to adequately reflect the *in vivo* scenario.

Using the membrane-permeation dissolution system, the relationship between the percentage of CBZ permeated *in vitro* and the cumulative AUC *in vivo* is depicted in Figure 7 for each experimental condition. Each of the experiments with the membrane-permeation dissolution system resulted in a strong correlation with *in vivo* results. In agreement with qualitative observations, the permeation experiment performed with canine FaSSIF in combination with the porcine intestine and 1-decanol acceptor solution resulted in the highest correlation between the *in vitro* and *in vivo* results.

Although a point-to-point comparison between *in vitro* and *in vivo* is preferred, level C correlations consisting of a single point correlation are useful in displaying how well the *in vitro* test reflects the *in vivo* performance. The percentage of CBZ permeated in the membrane-permeation dissolution system at time 60 min was compared to the $AUC_{0\text{-inf}}$ between the various *in vitro* test

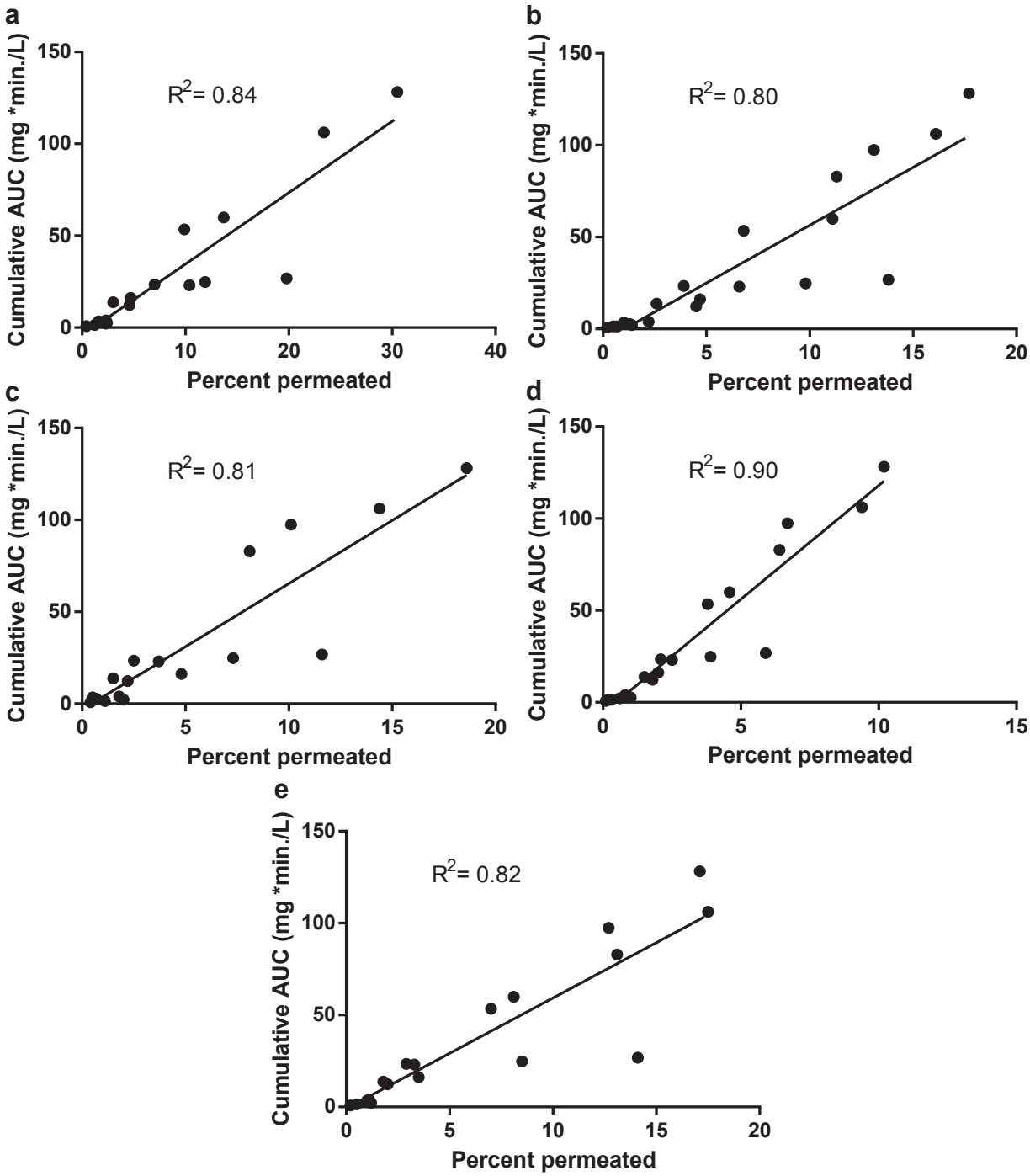


**Figure 7.** Correlation between *in vitro* dissolution-permeation data and the cumulative $AUC_{0\text{-}60\ min}$ from *in vivo* pharmacokinetic data. The specific conditions for each *in vitro* experiment were as follows: FaSSIF pH 6.5 with 1-decanol used as acceptor solution, separated by a PES polymer membrane (a); FaSSIF pH 6.5 with ASB used as acceptor solution, separated by a PES membrane (b); FaSSIF pH 5.5 with 1-decanol used as acceptor solution, separated by a PES membrane (c); canine FaSSIF pH 5.2 with 1-decanol used as acceptor solution, separated by porcine intestine (d); canine FaSSIF pH 5.2 with ASB used as acceptor solution, separated by porcine intestine (e).

conditions ($R^2 = 1.00$). Figure 8 depicts the results from the level C correlations, further confirming a strong correlation between the *in vitro* test employing canine FaSSIF at pH 5.2 and a porcine membrane with the results found *in vivo*.

During early phase drug/formulation development, the application of *in vitro* dissolution testing that is capable of predicting *in vivo* bioavailability and dissolution performance can drive research in more efficient and effective directions. The primary object of the work performed by Duarte et al.[32] was to present a novel particle engineering technique for the production of nanosolid dispersions. As such, the focus of the work described by Duarte et al.[32] was to select the formulation that yielded the highest stability, dissolution, and bioavailability. Eudragit® L100 was selected as the preferred excipient for CBZ nanosolid dispersions because it permitted the highest drug loading and exhibited the best stability. Following production of formulations in an experimental design space study for solvent-controlled precipitation, selection of optimal candidates was based upon the drug's solid state and molecular arrangement within coprecipitated particles. Before animal studies, CBZ:Eudragit® L100 systems were

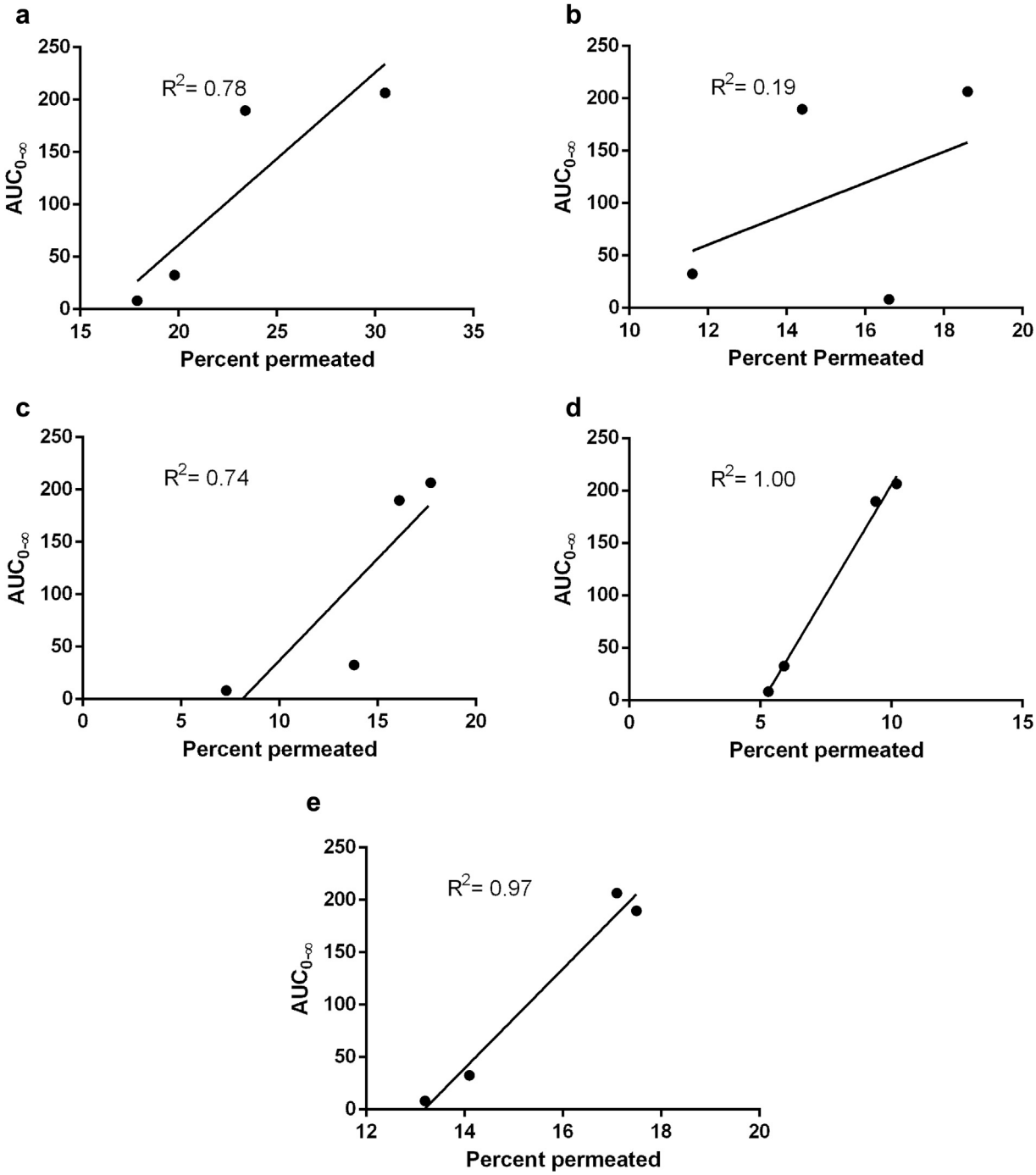


**Figure 8.** Level C correlation between the *in vitro* percentage of CBZ permeated at 60 min and the *in vivo* $AUC_{0\text{-inf}}$. The specific conditions for each *in vitro* experiment were as follows: FaSSIF pH 6.5 with 1-decanol used as acceptor solution, separated by a PES polymer membrane (a); FaSSIF pH 6.5 with ASB used as acceptor solution, separated by a PES membrane (b); FaSSIF pH 5.5 with 1-decanol used as acceptor solution, separated by a PES membrane (c); canine FaSSIF pH 5.2 with 1-decanol used as acceptor solution, separated by porcine intestine (d); canine FaSSIF pH 5.2 with ASB used as acceptor solution, separated by porcine intestine (e).

evaluated for dissolution performance using a microcentrifuge dissolution method. Although the microcentrifuge dissolution method was sufficient to meet the objectives of the study, the formulation development process may have been different with the application of the current membrane-permeation dissolution system. Microcentrifuge dissolution results described by Duarte et al.[32] depicted the amorphous formulations outperforming the nanocrystalline formulation as well as crystalline CBZ, which later disagreed with *in vivo* results. It can be suggested that if the membrane-permeation dissolution method was available, dissolution results would have been better aligned with *in vivo* studies and offered a better tool to guide process optimization. Ultimately due to desirable solid-state properties, Eudragit® L100 formulations were preferred to HPMCAS-MG formulations. However, at this point a decision to maintain HPMCAS-MG as a formulation candidate may have existed if it yielded formulations with equal or greater bioavailability compared to the Eudragit L100. Hence, the application of the described membrane-permeation dissolution system could have been exploited as a tool to understand formulation performance, select the polymer system, and optimize process parameters.

## Conclusion

The results of the present work establish a correlation between an *in vitro* membrane-permeation dissolution test and an *in vivo* mice pharmacokinetic study. The *in vitro* membrane-permeation dissolution technique resembles the dissolution and absorption processes that occur in the gastrointestinal tract following oral administration of a drug formulation. Results acquired from this apparatus and optimization of the methodology mimicked the *in vivo* performance in mice pharmacokinetic studies performed by Duarte et al.,[32] an improvement upon the original rank order for formulations obtained with the original microcentrifuge *in vitro* technique. Qualitative and quantitative analysis of *in vitro* permeation profiles with the membrane-permeation dissolution system demonstrated the application of canine FaSSIF at pH 5.2 with porcine intestine established the most successful IVIVC. It should be noted that throughout the development of this technique, a significant dependence on pH was observed. Initial experiments involved the application of FaSSIF at a pH relevant to human intestinal fluid. Experiments at the pH revealed a poor correlation to mice PK data for the discussed drug and polymer system. To establish IVIVR/IVIVC with the described membrane-permeation technique, the pH should be adjusted to correspond to the subject of *in vivo* studies. Application of the membrane-permeation dissolution system during drug and formulation development stages may permit a better prediction of the *in vivo* response, thereby guiding the development process in a cost- and time-efficient manner. Future testing to compare the *in vitro* performance of other drugs and formulation types using the membrane-permeation dissolution system with *in vivo* human pharmacokinetic data will help define the robustness of the membrane-permeation dissolution technique and further solidify the applicability of this technique in drug/formulation development and routine testing. Going forward the membrane-permeation dissolution system can provide scientifically sound data that can be used to guide decisions in formulation development.

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