Selecting In Vitro Dissolution Methodologies For Amorphous Solid Dispersions

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Achieving good in vivo performance is a key attribute for ensuring safety and efficacy of oral solid dosage (OSD) forms intended for systemic delivery. Yet, new drug targets and mechanisms of action continue to drive drug candidates' physical properties toward poorly soluble biopharmaceutics classification system (BCS) II or IV designation.[1] Applying bioavailability enhancement techniques can help improve not only the water solubility and oral absorption of OSD drug products but also increase patient safety, efficacy, and compliance. With the availability of so many types of in vitro dissolution tests, though, how do you determine which one will be most effective in predicting in vivo performance of your BCS II or IV drug product?

WHAT FACTORS DETERMINE THE RIGHT METHODOLOGY FOR PREDICTING BIOPERFORMANCE?

Oral drug delivery is the most commonly used route of administration in the pharmaceutical industry. Oral drugs meant for systemic delivery need good oral bioavailability to ensure adequate drug concentrations at the site(s) of therapeutic action. Achieving good oral bioavailability requires dissolving in gastrointestinal fluids and permeating through the intestinal wall, which depend on a drug having adequate solubility and permeability. However, nearly 40% of currently marketed drugs and up to 80% of compounds currently under development are classified as having either low solubility/high permeability (BCS II) or low solubility/low permeability (BCS IV).[2,3]

Drugs with low solubility and/or permeability levels require formulation strategies that can overcome these challenges, in order to improve bioavailability. This will help bring new drugs to the market as well as improve existing medications by removing poor product characteristics, such as patient variability, as well as drug-drug and food-drug interactions. Methods to enhance bioavailability of a drug include improving its solubility and/or dissolution rate. There are several ways to do this, such as reducing particle size, developing a salt form of the drug, or creating different types of formulation, such as lipids or amorphous solid dispersions (ASDs).[4,5,6] A key advantage of the amorphous form of a drug is its higher solubility compared to the crystalline form.[7] However, the potential for precipitation to the more stable crystalline form is inherent for ASDs.[8] Biopredictive dissolution testing, which evaluates how the interplay between a drug formulation and GI fluid properties impacts bioperformance, can help assess bioperformance risk of drug product formulations, such as ASDs. It can be applied using a range of methods incorporating various apparatuses and test designs.

A desired goal of in vitro dissolution testing is to determine the rate of drug input into plasma over time, which is driven

by many factors, such as dissolution and precipitation in the stomach and/or the small intestine, gastric emptying and transit, and permeation into the intestinal membrane. Traditional in vitro dissolution methods for solid dosage forms common for quality control testing may not effectively predict oral bioperformance of drug products for which the rate of absorption is impacted by factors other than dissolution, such as products comprised of poorly soluble molecules.[9,10] For example, simple USP methods (Figure 1) typically use a single dissolution medium with non-physiological volumes and dose concentrations. They also do not take into account factors that can impact both dissolution rate and precipitation, such as GI transit or membrane permeation.

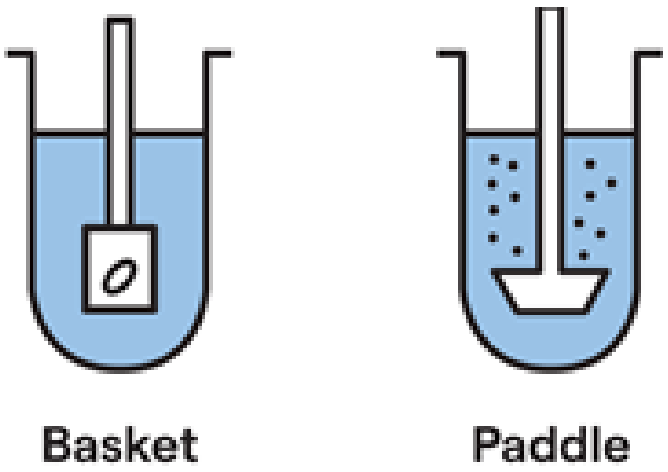

Fig 1: *Industry standard dissolution testing methodologies are USP Apparatus 1 (basket) and USP Apparatus 2 (paddle).*

Newer in vitro dissolution methods, designed to capture some key rate-determining steps to absorption, are tests aimed at capturing more gastrointestinal variables, such as sequential exposure to different fluid pH and compositions.[10] However, while these tests can be more successful in predicting bioperformance, they can be more time-consuming and difficult to run. Selecting the simplest apparatus(es) that has the potential to be predictive of bioperformance requires an understanding of the factors that will affect your drug product performance in vivo. Likewise, the dissolution media you use during testing can be critical, as it can greatly impact performance. For example, it is important to select media tailored to the target population(s) of interest (i.e., fasted humans, fed humans, fasted dogs, etc.) and capture the range in GI fluid properties expected to affect dissolution for your particular drug product. A webinar highlighting a method for selecting the most relevant media based on these factors was recently presented by Lonza and can be found here.

At Lonza, we recommend the following steps for testing bioperformance using an in vitro dissolution test:

1. Predict in vivo problem statement and rate-determining steps to absorption.
2. Select in vitro dissolution apparatus.
3. Choose an in vitro dissolution media and test parameters.

Common in vivo problem statements for ASDs include precipitation/crystallization, slow dissolution rate, and solubility/permeability limited absorption. The following examples describe three scenarios for in vivo dissolution testing of these ASD problem statements.

PROBLEM STATEMENT 1: PRECIPITATION/CRYSTALLIZATION

Many ASD formulations are designed to supersaturate in GI fluids. While supersaturation can lead to improved absorption, it also increases the possibility of precipitation (i.e., conversion of the amorphous form to the more thermodynamically stable crystalline form).[12]

The gastric-to-intestinal transfer test is a practical test for assessing precipitation, particularly for ASDs that tend to precipitate in intestinal media after exposure to gastric media (Figure 2).[13] In this test, the dosage form is added to a simulated gastric medium, and drug concentration is monitored over a period of time, such as 30 min. Next, a

concentrated simulated intestinal buffer is added to the vessel to create a simulated intestinal medium and drug concentration is further monitored.[11] These tests can be conducted in USP 2 or Pion µDiss (Pion, Billerica, MA) vessels, allowing for multiple tests to be run simultaneously. In addition to media selection, key testing parameters include gastric and intestinal medium volumes and dose.

Depending on the drug dose, effective permeability, and fluid volumes, this test can represent a worst-case scenario for in vivo precipitation since fluid and solids remain in the vessel for the duration of the test. With their ease of use, high throughput, and likelihood to discriminate formulations in terms of in vivo precipitation (since they tend to represent a worst-case scenario), gastric-to-intestinal transfer tests can be useful for ranking formulations early in development.

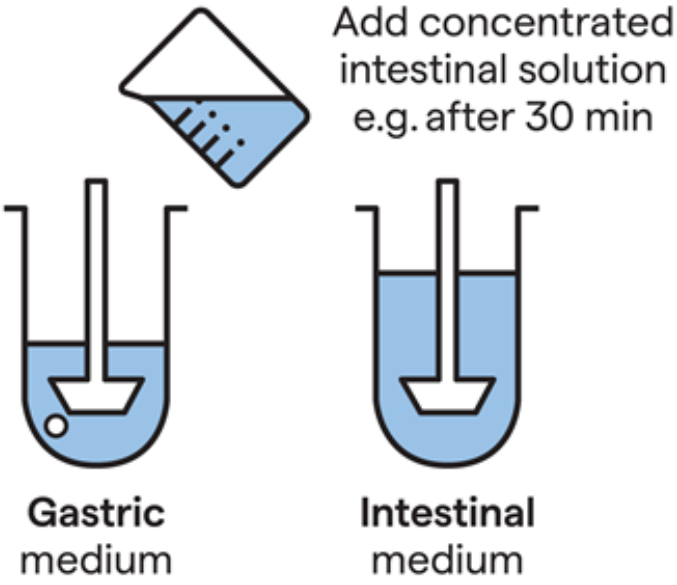

Fig 2: *Two-stage gastric-to-intestinal transfer test*

A more sophisticated option for assessing precipitation is a multicompartment/controlled transfer dissolution (CTD) test (Figure 3), which simulates a dynamic, physiologically relevant environment in terms of fluid volume, transit, and gastric and intestinal secretions.[14,15,16] In this test, the dosage form is placed in a compartment of simulated gastric medium and then fluid and dissolved solids are pumped into subsequent 'intestinal compartments' at a physiologically relevant gastric emptying rate (e.g., a 15-min emptying half time). At the same time, simulated gastric and intestinal secretion fluids are pumped into the relevant compartments as a function of time. Many multicompartment dissolution tests are developed by companies or universities in-house and can be labor- and time-intensive since typically only a single experiment can be conducted at a time. Key testing parameters for this type of test besides media selection include the volume of media being used and the transfer rates (i.e., pumping rates).

Compared to the static gastric-to-intestinal transfer test, multicompartment/CTD tests may be better suited for understanding a smaller number of formulations later in development due to their higher complexity and lower throughput, with a tendency toward better replicating the in vivo environment. A case study using the CTD apparatus demonstrated correct rank ordering of in vivo performance for an ASD tablet and crystalline drug in capsule dosed at low and high gastric pH in beagle dogs for a weak base drug with moderate crystallization propensity.[4]

A drawback for both types of tests is that they do not capture the in vivo absorption process, which can also drive down the supersaturation ratio and decrease the propensity for crystallization, particularly for high permeability (i.e., BCS II) drugs. Coupling these in vitro testing strategies with in silico modeling can be useful for capturing the sensitivity of formulations to absorption rate and other important physiological variables. Further, tests designed to capture the absorption process, such as membrane or biphasic tests can also be utilized.[17, 18, 19,20]

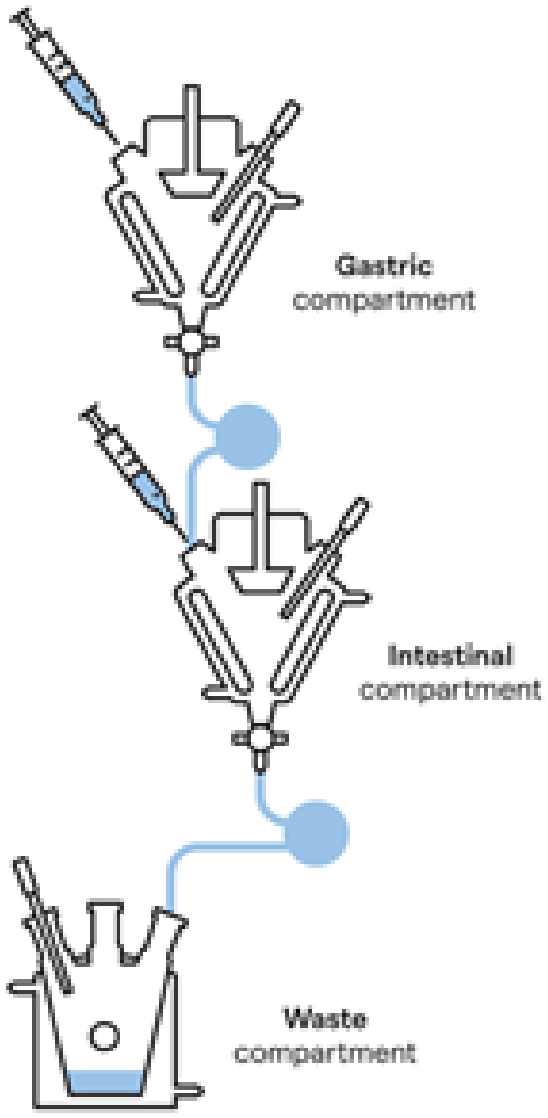

Fig 3: *Lonza multicompartment/controlled transfer dissolution (CTD) test*

PROBLEM STATEMENT 2: SLOW DISSOLUTION RATE

For some ASDs, dissolution can be a rate-determining step to absorption (i.e., when the dissolution rate is slow relative to the permeation rate and the dose is relatively low). A slow dissolution rate may be a concern for ASDs that dissolve via an erosion mechanism, whereby the drug and polymer erode from the particle surface, generating supersaturated drug concentrations. In this case, the dissolution rate is influenced not only by the formulation properties and drug loading, but also by the available particle surface area in contact with GI fluids, dictated by the ASD particle size distribution and any particle aggregation that may occur in GI fluids.[22,23]

A sink test is recommended for measuring differences in the dissolution rate between samples. A sink test represents conditions when dose/medium volume is low compared to the drug solubility, which would be the solubility of the amorphous form for an ASD. A sink test allows determination of dissolution rate differences between samples/formulations when dissolution rates are at their maximum values, and therefore contributions arising from factors such as differences in particle size distribution can be adequately assessed.

If the gastric dissolution rate is of interest, the sink test can be performed in the desired gastric medium. However, if the intestinal dissolution rate is of interest, an intestinal only test in a single intestinal medium can be completed or else a gastric-to-intestinal transfer test can be performed as described above. Gastric transfer is typically completed when prior exposure of the sample to gastric medium is expected to affect the dissolution rate of the sample in the intestinal medium. This can be driven by the drug properties (acid/base) and/or excipient properties (enteric or neutral polymer).

Although sink conditions may not be biorelevant for some, for example, high dose BCS II or IV compounds, sink tests can be highly discriminating for dissolution rate limited compounds and therefore can be useful for ranking formulations of these compounds to select those that might have the most robust in vivo performance. In addition, the sink dissolution data can be highly valuable for inputting into predictive modeling software that can assess sensitivity of the impact of the dissolution rate on absorption in conjunction with in

vivo processes, such as gastric emptying, intestinal transit, GI hydrodynamics, and absorption rate. A case study using a gastric-to-intestinal transfer test coupled with predictive modeling software was useful in elucidating the performance differences between three different ASD formulations administered to beagle dogs, supporting the hypothesis that one ASD formulation was limited by the dissolution rate.[24]

PROBLEM STATEMENT 3: SOLUBILITY/PERMEABILITY LIMITED ABSORPTION

Absorption of ASDs can be solubility/permeability limited when the dissolution rate is considerably faster than the permeation rate and the dose-to-solubility ratio is higher than the intestinal fluid volume.[21] When ASDs dissolve, different drug-containing species can form, such as freely dissolved drug (neutral and ionized forms); drug bound to bile-salt micelles and other lipidic structures; and in some cases, nano-sized species (i.e., "drug-polymer colloids") arising from amorphous-phase separation once the amorphous solubility of the drug has been reached in the GI fluid. Understanding the presence and abundance of each of these drug-containing species can be important, as they differ in diffusivity, release, and permeation behavior.

For some ASDs, transport across the unstirred water layer (UWL) in the small intestine is a rate-determining step to absorption. In these cases, micelle-bound drug and nano-sized drug species can boost dissolution rate and enhance transport across the UWL by acting as a drug "shuttle" to provide a reservoir at the epithelium surface, thereby improving the rate of drug absorption.

A good way to evaluate the impact of different drug-containing species on drug absorption is using a membrane flux test.[27,29,30] A membrane flux test is a dissolution/permeation test that can mimic the process of simultaneous drug dissolution and absorption in vivo. A membrane flux test can capture the contributions to flux from all drug-containing species and be used to make predictions about the rate-limiting step for absorption in vivo with respect to dissolution rate, UWL diffusion, and cell membrane permeation.

A membrane flux test developed at Lonza (Figure 4) consists of a donor compartment and receiver compartment separated by a membrane.[27] The receiver solution acts as a sink for the drug, allowing for flux measurements based on formulation performance in the donor compartment. Important testing parameters to consider include the dose concentration, surface-area-to-volume ratio, and dissolution media.[27] Key advantages of Lonza's membrane flux test include a high membrane surface area to donor volume (SA/V) similar to that of the small intestine (when approximated as a smooth tube), high throughput, and low material requirements.[27]

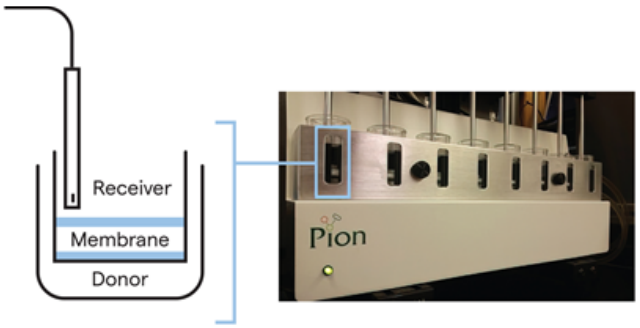

Fig 4: *Membrane flux test developed internally at Lonza[27] (surface area = SA, donor volume = V)*

A case study using the membrane flux test showed correct ranking of in vivo performance of three different ASD formulations that were solubility-permeability limited in rats.[25] In this case, only one dissolution media provided the correct ranking, further emphasizing the importance of dissolution media selection.

CONCLUSION

Advances in biological science and technology are leading to the discovery of novel drug candidates aimed at addressing unmet medical needs across the global market. Many of these products are OSDs classified as poorly soluble, calling on the need for formulation strategies that can increase bioavailability.

In vitro dissolution testing can help assess bioperformance, but only if the most appropriate testing method and solution for your problem statement and rate-determining steps are selected. Doing so not only enables rapid development of robust oral dosage forms, such as ASDs, but also aids formulation scientists in selecting formulations that will perform well in vivo without the need for multiple clinical studies. This lessens the number of formulation iterations, which can help reduce the cost and time of development.

Lonza's in-house experts have a wide range of experience in designing and implementing various testing methods that can accurately identify and help a drug's performance in the human body, ultimately setting a path toward clinical and commercial success for our clients.

REFERENCES

1. **Amidon, G.L., Lennernäs, H., Shah, V.P. et al. A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability. Pharm Res 12 (1995), 413–420.**
2. **Moreton, Chris, Ph.D. (February 18, 2021). Poor Solubility – Where Do We Stand 25 Years After The 'Rule Of Five'? American Pharmaceutical Review. https://www.americanpharmaceuticalreview.com/Featured-Articles/573402-Poor-Solubility-Where-Do-We-Stand-25-Years-after-the-Rule-of-Five/**
3. **Kalepu, S., & Nekkanti, V. Insoluble drug delivery strategies: review of recent advances and business prospects. Acta pharmaceutica Sinica. B, 5, 5 (1995), 442–453.**
4. **Mudie, D.M.; Stewart, A.M.; Rosales, J.A.; Biswas, N.; Adam, M.S.; Smith, A.; Craig, C.D.; Morgen, M.M.; Vodak, D.T. Amorphous Solid Dispersion Tablets Overcome Acalabrutinib pH Effect in Dogs. Pharmaceutics, 13 (2021), 557.**
5. **Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today. 12, 23-24 (Dec. 2007):1068-75.**
6. **Fasinu P, Pillay V, Ndesendo VM, du Toit LC, Choonara YE. Diverse approaches for the enhancement of oral drug bioavailability. Biopharm Drug Dispos. 32, 4 (May 2011):185-209.**
7. **Almeida e Sousa, L.; Reutzel-Edens, S.M.; Stephenson, G.A.; Taylor, L.S. Assessment of the amorphous "solubility" of a group of diverse drugs using new experimental and theoretical approaches. Mol. Pharm. 12 (2015), 484–495.**
8. **David E. Alonzo, Yi Gao, Deliang Zhou, Huaping Mo, Geoff G.Z. Zhang, Lynne S. Taylor, Dissolution and Precipitation Behavior of Amorphous Solid Dispersions, Journal of Pharmaceutical Sciences, 100, 8 (2011), Pages 3316-3331, ISSN 0022-3549.**
9. **DM Mudie, GL Amidon, GE Amidon, "Physiological Parameters for Oral Delivery and In Vitro Testing", Molecular Pharmaceutics, 7, 5 (2010):1388-1405**
10. **James Butler, Bart Hens, Maria Vertzoni, Joachim Brouwers, Philippe Berben, Jennifer Dressman, Cord J. Andreas, Kerstin Julia Schaefer, James Mann, Mark McAllister, Masoud Jamei, Edmund Kostewicz, Filippos Kesisoglou, Peter Langguth, Mans Minekus, Anette Müllertz, Ronald Schilderink, Mirko Koziolek, Philipp Jedamzik, Werner Weitschies, Christos Reppas, Patrick Augustijns, In vitro models for the prediction**

of in vivo performance of oral dosage forms: Recent progress from partnership through the IMI OrBiTo collaboration, European Journal of Pharmaceutics and Biopharmaceutics, 136 (2019): 70-83, ISSN 0939-6411,

11. Mudie DM, Samiei N, Marshall DJ, Amidon GE, Bergström CAS. Selection of In Vivo Predictive Dissolution Media Using Drug Substance and Physiological Properties. AAPS J. 22, 2 (January 2020):34. doi: 10.1208/s12248-020-0417-8.

12. David E. Alonzo, Yi Gao, Deliang Zhou, Huaping Mo, Geoff G.Z. Zhang, Lynne S. Taylor, Dissolution and Precipitation Behavior of Amorphous Solid Dispersions, Journal of Pharmaceutical Sciences, 100, 8 (2011): 3316-3331, ISSN 0022-3549.

13. Patrick J O'Dwyer, Chara Litou, Karl J Box, Jennifer B Dressman, Edmund S Kostewicz, Martin Kuentz, Christos Reppas, In vitro methods to assess drug precipitation in the fasted small intestine – a PEARRL review, Journal of Pharmacy and Pharmacology, 71, 4, (April 2019): 536–556

14. Deanna M. Mudie, Stephanie Buchanan, Aaron M. Stewart, Adam Smith, Kimberly B. Shepard, Nishant Biswas, Derrick Marshall, Alyssa Ekdahl, Amanda Pluntze, Christopher D. Craig, Michael M. Morgen, John M. Baumann, David T. Vodak, A novel architecture for achieving high drug loading in amorphous spray dried dispersion tablets, International Journal of Pharmaceutics: X, 2 (2020): 100042, ISSN 2590-1567,

15. Stephen R. Carino, David C. Sperry, Michael Hawley, Relative bioavailability estimation of carbamazepine crystal forms using an artificial stomach-duodenum model, Journal of Pharmaceutical Sciences, 95, 1 (2006): 116-125, ISSN 0022-3549,

16. Tsume Y, Patel S, Wang M, Hermans A, Kesisoglou F. The Introduction of a New Flexible In Vivo Predictive Dissolution Apparatus, GIS-Alpha (GIS-α), to Study Dissolution Profiles of BCS Class IIb Drugs, Dipyridamole and Ketoconazole. J Pharm Sci. 109, 11 (November 2020): 3471-3479.

17. Pathak, S. M.; Schaefer, K. J.; Jamei, M.; Turner, D. B. Biopharmaceutic IVIVE-Mechanistic Modeling of Single- and Two-Phase In Vitro Experiments to Obtain Drug-Specific Parameters for Incorporation Into PBPK Models. J. Pharm. Sci. 108, 4 (2019): 1604–1618.

18. Mudie, D.M.; Stewart, A.M.; Rosales, J.A.; Adam, M.S.; Morgen, M.M.; Vodak, D.T. In Vitro-In Silico Tools for Streamlined Development of Acalabrutinib Amorphous Solid Dispersion Tablets. Pharmaceutics 13 (2021): 1257. https://doi.org/10.3390/pharmaceutics13081257

19. Frank, K. J., Locher, K., Zecevic, D. E., Fleth, J. & Wagner, K. G. In vivo predictive mini-scale dissolution for weak bases: Advantages of pH-shift in combination with an absorptive compartment. Eur. J. Pharm. Sci. 61, 32–39 (2014).

20. Xu, H., Vela, S., Shi, Y., Marroum, P. & Gao, P. In Vitro Characterization of Ritonavir Drug Products and Correlation to Human in Vivo Performance. Mol. Pharm. 14, (2017): 3801–3814

21. Sugano, K. & Terada, K. Rate- and Extent-Limiting Factors of Oral Drug Absorption: Theory and Applications. J. Pharm. Sci. 104 (2015): 2777–2788.

22. Saboo S, Mugheirbi NA, Zemlyanov DY, Kestur US, Taylor LS. Congruent release of drug and polymer: A "sweet spot" in the dissolution of amorphous solid dispersions. J Control Release. 28;298 (2019 Mar):68-82.

23. Zheng K, Lin Z, Capece M, Kunnath K, Chen L, Davé RN. Effect of Particle Size and Polymer Loading on Dissolution Behavior of Amorphous Griseofulvin Powder. J Pharm Sci.108,1(2019 Jan):234-242.

24. Stewart A, Yates I, Mudie D, Pivette P, Goodwin A, Sarmiento A, Winter M, Morgen M, Vodak D. Mechanistic Study of Belinostat Oral Absorption From Spray-Dried Dispersions. J Pharm Sci. 108, 1 (January 2019):326-336.

25. **Stewart, A. M. et al. Impact of Drug-rich Colloids of Itraconazole and HPMCAS on Membrane Flux In Vitro and Oral Bioavailability in Rats. Mol. Pharm. (2017).**

26. **Ruochen Yang, Amanda K. P. Mann, Tu Van Duong, James D. Ormes, Grace A. Okoh, Andre Hermans, Lynne S. Taylor. Drug Release and Nanodroplet Formation from Amorphous Solid Dispersions: Insight into the Roles of Drug Physicochemical Properties and Polymer Selection. Molecular Pharmaceutics, 18, 5 (2021), 2066-2081.**

27. **Stewart AM, Grass ME, Mudie DM, Morgen MM, Friesen DT, Vodak DT. Development of a Biorelevant, Material-Sparing Membrane Flux Test for Rapid Screening of Bioavailability-Enhancing Drug Product Formulations. Mol Pharm. 5;14(June 2017): 2032-2046.**

28. **Aaron M. Stewart, Michael E. Grass. Practical Approach to Modeling the Impact of Amorphous Drug Nanoparticles on the Oral Absorption of Poorly Soluble Drugs. Molecular Pharmaceutics, 17, 1(2020), 180-189.**

29. **O'Dwyer, P. J., Imanidis, G., Box, K. J., & Reppas, C. On the Usefulness of Two Small-Scale In Vitro Setups in the Evaluation of Luminal Precipitation of Lipophilic Weak Bases in Early Formulation Development. Pharmaceutics, 12, 3 (2020), 272.**

30. **Kataoka M, Fukahori M, Ikemura A, Kubota A, Higashino H, Sakuma S, Yamashita S. Effects of gastric pH on oral drug absorption: In vitro assessment using a dissolution/permeation system reflecting the gastric dissolution process. Eur J Pharm Biopharm.101 (2016 Apr):103-11**