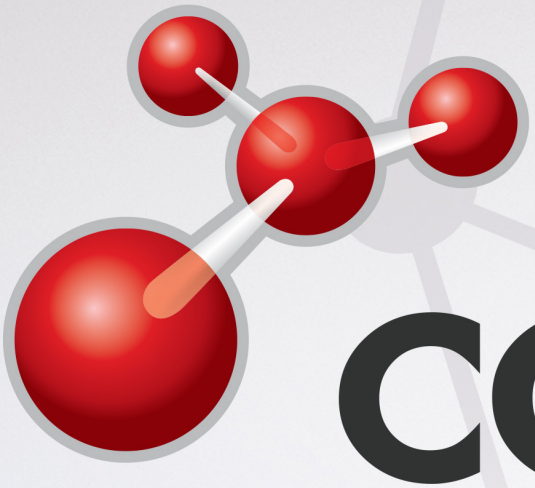


core Rx®

DEVELOPMENT AT OUR CORE

SCREENING PERMEABILITY AS A TOOL IN FORMULATION SELECTION

Janice Cacace, Ph.D.

Screening Permeability as a Tool in Formulation Selection

Using membrane flux systems and side by side diffusion cells, CoreRx was able to discern which factors were most important for bioavailability of a model Class IV compound. This enabled us to increase bioavailability of the compound by ~70%.

Introduction

The bioavailability of insoluble compounds remains one of the biggest challenges to drug delivery. While there are strategies that can be applied at lower dosage levels, it is particularly difficult to formulate for improved bioavailability at high dosage levels. Improving bioavailability can be crucial to creating a dosage form that is easy to use and meets requirements for patient compliance.

Bioavailability and its importance

The term **bioavailability** is used to describe the fraction of an administered dose of unchanged drug that reaches the systemic circulation. By definition, when a medication is administered intravenously its bioavailability is 100%. However, when a medication is administered via other routes (such as oral), its bioavailability decreases (due to incomplete absorption or first-pass metabolism). The measurement of the amount of the drug in the plasma at periodic time intervals indirectly indicates the rate and extent at which the active pharmaceutical ingredient is absorbed from the drug product and becomes available at the site of action.

Bioavailability can be affected by many things including:

1. Solubility
2. Permeability
3. Stability of the API in the GI tract
4. Membrane transport
5. First pass metabolism

Based on the FDA classification system the following classes of compounds can benefit from a solubility or permeability modifying formulation:

- Class II: low solubility and high permeability. The API's in this class generally have aqueous solubility <1 mg/mL and Log P between 1 and 3. These account for ~70% of API's being manufactured. Class II API's can be split into two subcategories:
    - IIa. solubility limited bioavailability. These are limited by dissolution rate rather than actual solubility
    - IIb. solubility limited absorbable dose. In this case, the full dose is not expected to dissolve before exiting the small intestine. Below the solubility limit, all the dose could dissolve but above the solubility limit, the fraction dissolved will diminish with increasing dose. Therefore, the fraction that has not dissolved, will not be absorbed. This can frequently be related to pH dependent solubility as well.

- Class III: high solubility and low permeability. The API's in this class generally have aqueous solubility >1 mg/mL and Log P <1. These are generally too hydrophilic to permeate lipid membranes. These account for ~5% of API's being manufactured.

- Class IV: low solubility and low permeability. The API's in this class generally have aqueous solubility <1 mg/mL and Log P >3. These API's are generally too hydrophobic to pass through lipid membranes, and present the most difficult challenge in terms of formulation. These account for ~20% of API's being manufactured.

Permeability Assessment
If you are experiencing low bioavailability, one of the first things to investigate is what's the rate limiting step? Is it solubility, dissolution rate, or diffusion?

One of our best tools at CoreRx for rapid screening of the factors affecting bioavailability is the membrane flux system. This coupled with a fiber optic UV monitoring system can be used to take readings directly from solubility samples, dissolution baths and both receiver and donor cells for permeability testing.

With the fiber optic UV monitoring system, drug substance detection is performed in-situ with no need for sampling or secondary analysis. This provides several advantages over traditional HPLC analysis. These include:

  - Increased throughput
  - Elimination of costs associated with HPLC analysis for solvents, columns, etc.
  - Rapid prototype screening without HPLC methods in place
  - Decreased variance from sampling and secondary sample preparation
  - Elimination of need for media replacement
  - Run duration can be from 30 min to over 24 hours with no gaps in data collection
  - Sampling can be performed at any interval from every 5 seconds to several hours
  - Increased discriminatory capacity to detect changes in the drug product
  - Immediate turnaround of data with no requirement for post-run processing

The membrane flux system's small volume module allows *in situ* concentration monitoring, and at the same time evaluation of the absorption potential of a compound as you separate the receiver and donor chambers with a membrane.

The drug product is diluted in a "Donor" cell which is separated from a "Receiver" cell by a PVDF membrane impregnated with GIT-0 lipid solution (Pion Inc., Billerica, MA). The Cells are filled with 20 mL of a dissolution media; as the API dissolves in the Donor cell it diffuses across the membrane to the Receiver to simulate absorption in the intestine. The concentration in the Receiver cell is measured to determine the rate of diffusion in micrograms per minute per square centimeter (µg/min/cm2), also known as the Flux.

A typical flux system can be seen in Figure 1. These cells can be adapted to use a probe in both the receiver and donor cells that can take continuous readings for an extended period of time. Figure 2 is a modified version that enables the use of larger volumes of fluid in the receiver vessel. This arrangement is useful for highly insoluble molecules to create a flow through system, with a larger volume of receiver fluid to help promote diffusion and minimize saturation.

Figure 1: Side by side diffusion cells

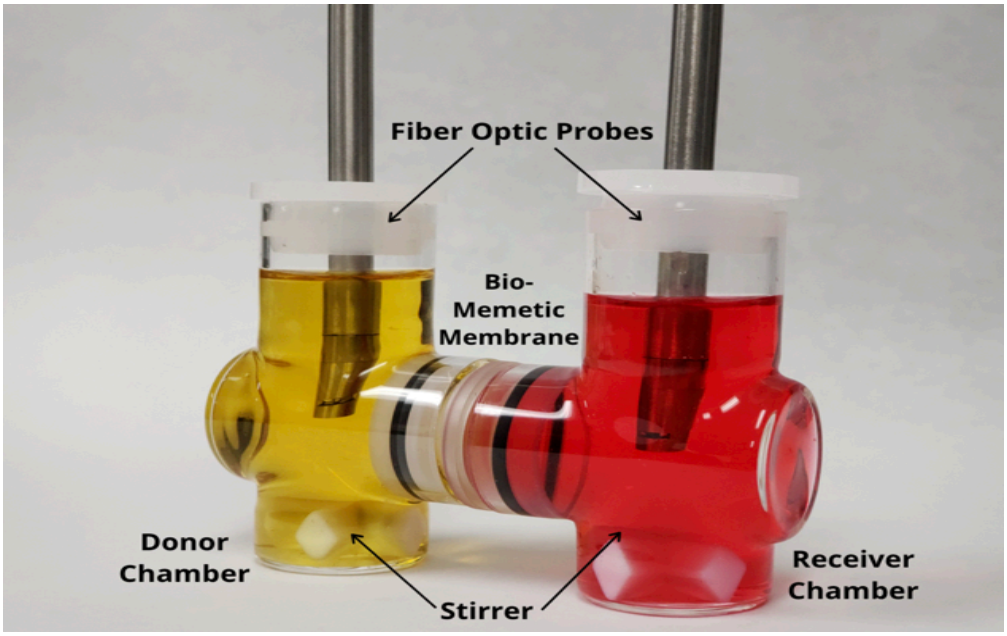
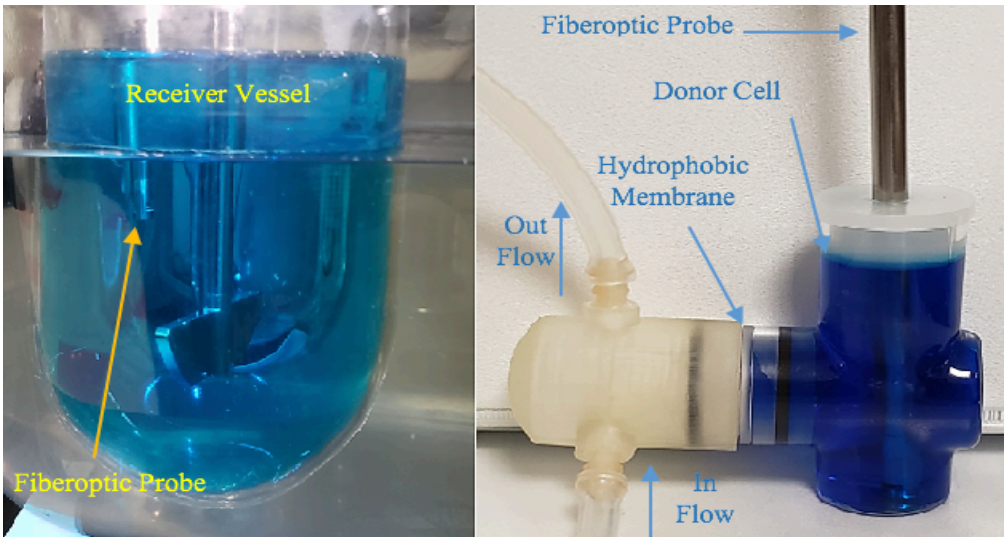


Figure 2: Modified Flux apparatus



Case study for evaluation of bioavailability and enhancement using a membrane flux system and side by side diffusion cells

Compound CRXA is a highly lipophilic molecule with low aqueous solubility (~2 x 10^{-4} mg/mL), and Log P >5. Dose was estimated to be 750 mg. Bioavailability of the native compound was low at ~20%. Due to the high dose, this was categorized as BCS Class IV. Given the compound's high dose and low solubility, the target dosage form was a suspension.

What is the drug's ability to permeate through a membrane?

The first step that CoreRx took was to evaluate passive diffusion across a membrane at different concentrations. If increasing API concentration caused an increase in receptor concentration, then it would indicate the diffusion was concentration rate limited.

If that was the case, we could screen for solubility modifiers. If any of these produced an increase in the diffusion, then the diffusion would be solubility limited. And either the type or level of modifier could be compared in vivo.

If these studies found that neither solubility nor diffusion was the rate-limiting steps, then the issue would likely be due to membrane transport or some other biologic barrier to diffusion. The test was conducted over 4 hours at 37° C and the slopes between 100 and 150 minutes were used to calculate the flux. Results are presented in Figures 3 and 4.

The data indicated a clear effect for the flux based on the dose concentration in the donor cell. Through this screening, we were able to verify that the permeability was a function of concentration dependent solubility. From this, we expected that particle size might also have some impact on the in vivo absorption.

Figure 3: Flux of API concentration versus time

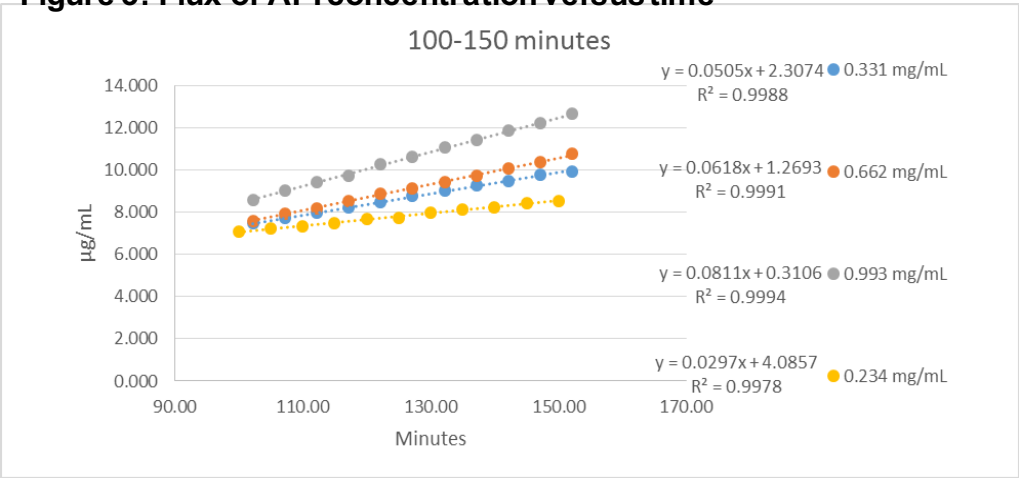
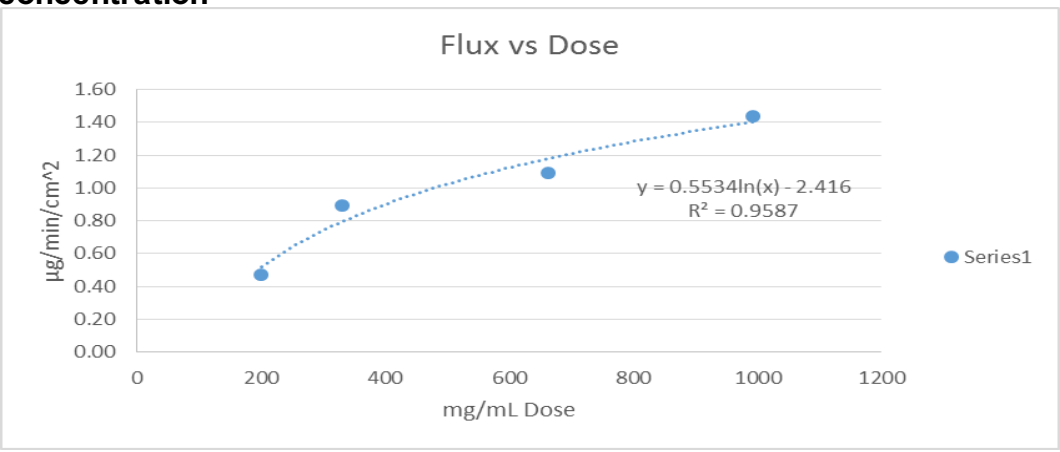


Figure 4: Flux of API versus concentration



Is the diffusion rate limited due to particle size?

Assuming the API exhibited passive diffusion, the diffusion should show a difference in rate if the experiment was repeated with API of different particle size.

Particle size reduction does not change the API solubility. It changes (increases) surface area, which greatly affects the dissolution rate, and in most cases, the bioavailability.
In addition, there are processes like microfluidization wherein the API particle size can be reduced, particle morphology can be changed, and surface modification can occur by the use of surfactants or permeability enhancers.

During microfluidization (see Figure 5), the particle size can be tunable by adjusting process pressure and number of passes. As presented in Figure 6, there was a rapid decrease in the particle size of CRXA, which decreased from 38 µm, to <5 µm in ~7.5 minutes.

The flux data for these two different sized particles of CRXA is presented in Figure 7. This clearly shows that the decrease in particle size was accompanied by an increase in membrane permeability.

Figure 5: Microfluidizer Schematic

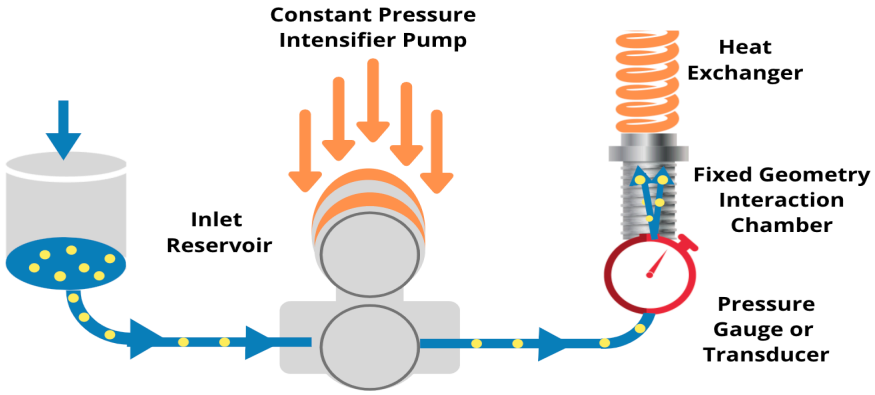


Figure 6: Particle size reduction versus time using the microfluidizer

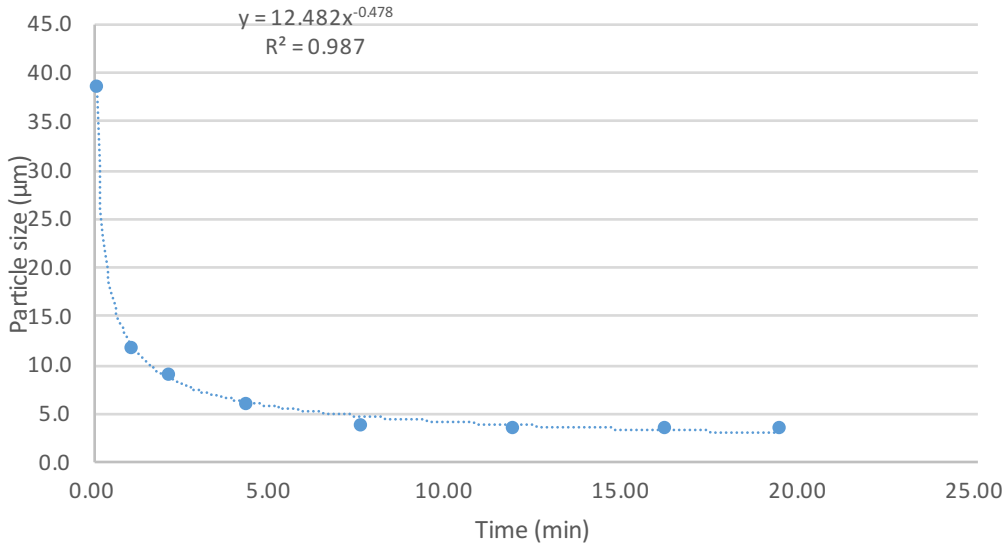
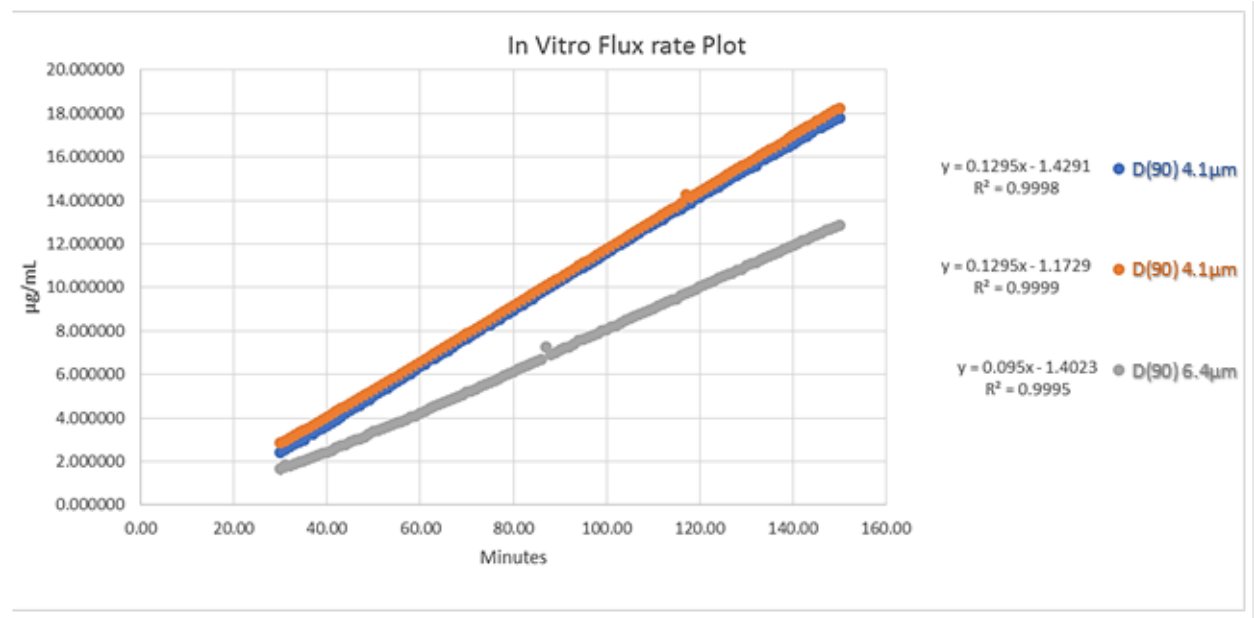


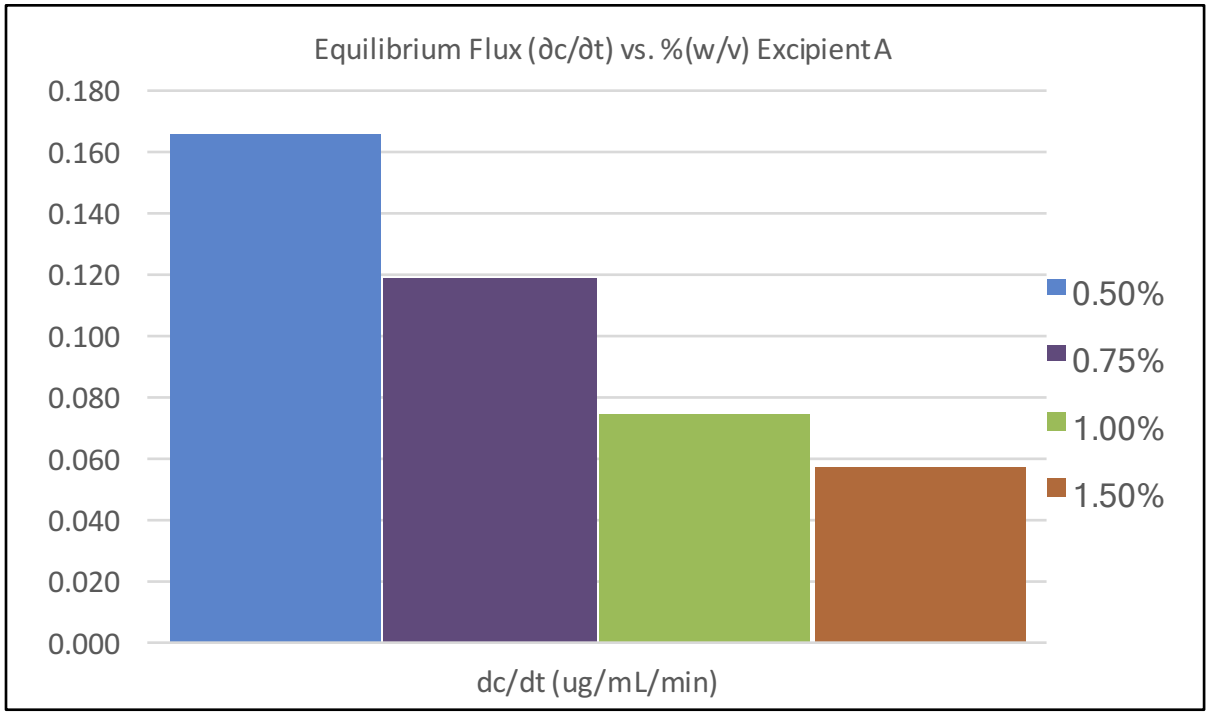
Figure 7



Do solubilizers affect the permeability?

Using the reduced particle size for CRXA, the flux was examined for the influence of surface active agents. As seen in Figure 8, it was found that one agent, in particular, showed a distinct relationship to the flux.

Figure 8



Overall effect on bioavailability

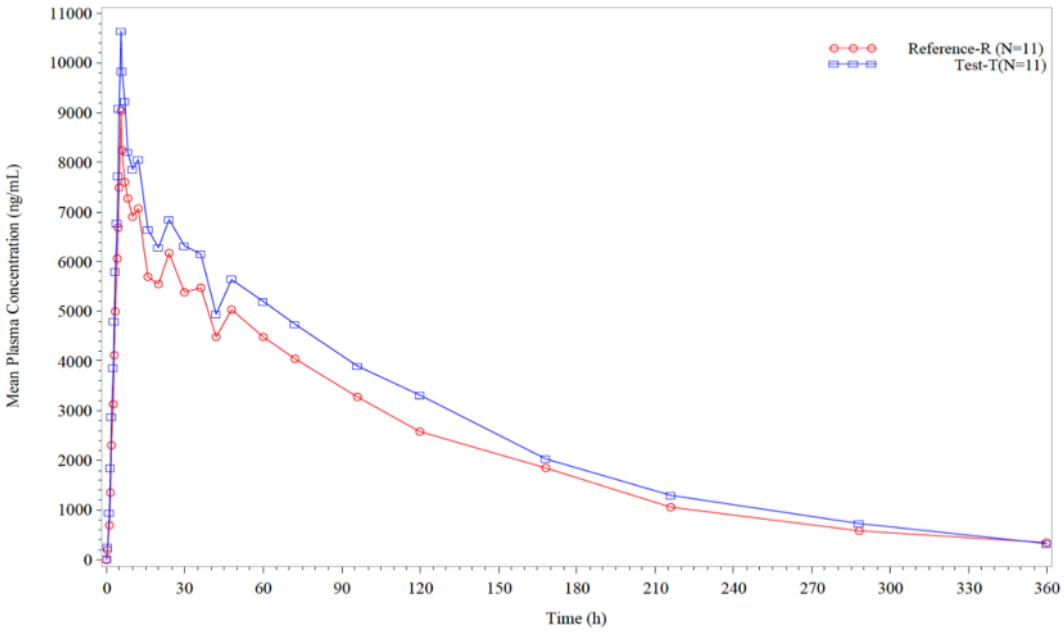
Using these results, our client took the high and low flux CRXA products into the clinic. It should be noted that modification of the excipient's concentration did not lead to a different dissolution profile.

Single dose pilot bioequivalence (BE) studies were conducted on healthy adult subjects to compare two formulations with different levels of surfactant.
Bioavailability was ~50% with the formulated microsuspension. However, as presented in Table 1 and Figure 9, this was increased to ~80% with the change in surfactant concentration in the fasted state.

Table 1: Results for the Bioequivalence study

	Fasting	
Parameter	Cmax	AUC0-Inf
	ng/mL	ng.h/mL
%Test/Reference	187.4	168.0

Figure 9



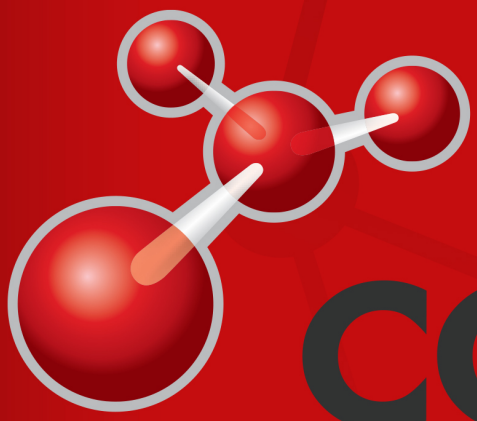
Conclusion

Based on the effects of dose and solubilizing agent evaluations, it appears that CRXA displays a combination of dissolution and membrane limited flux. In this case, the particle size and surface modifier had a large impact on bioavailability by affecting both solubility and permeability. By making this as a suspension dosage form and with the increase in bioavailability, it provided for improved delivery of a high dose product.

In Memory of Konstantin Tsinman 1968 -2020

Special thanks to Travis Webb and Brent Hilker, CoreRx.

Dr. Janice Cacace has a BS in Pharmacy from Purdue University and a Ph.D. in Pharmaceutics from the University of Florida. Her career has spanned over 30 years in the pharmaceutical industry in the areas of formulation, product development, consulting, and academia. 20 of these have been with consulting and contract development organizations. Over this time, she has gained a unique blend of new drug and generic drug development experience. She is currently Sr. Director of Development at CoreRx where she oversees product development and preformulation activities. This includes oversight of a diverse group of formulators with expertise in virtually every type of dosage form including solid and liquid oral, ophthalmic, parenteral, and semisolid oral and topical. She is a co-inventor of 8 drug delivery patents.



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