IN VITRO RELEASE TEST (IVRT) FUNDAMENTALS: SCIENTIFIC AND PRACTICAL CONSIDERATIONS

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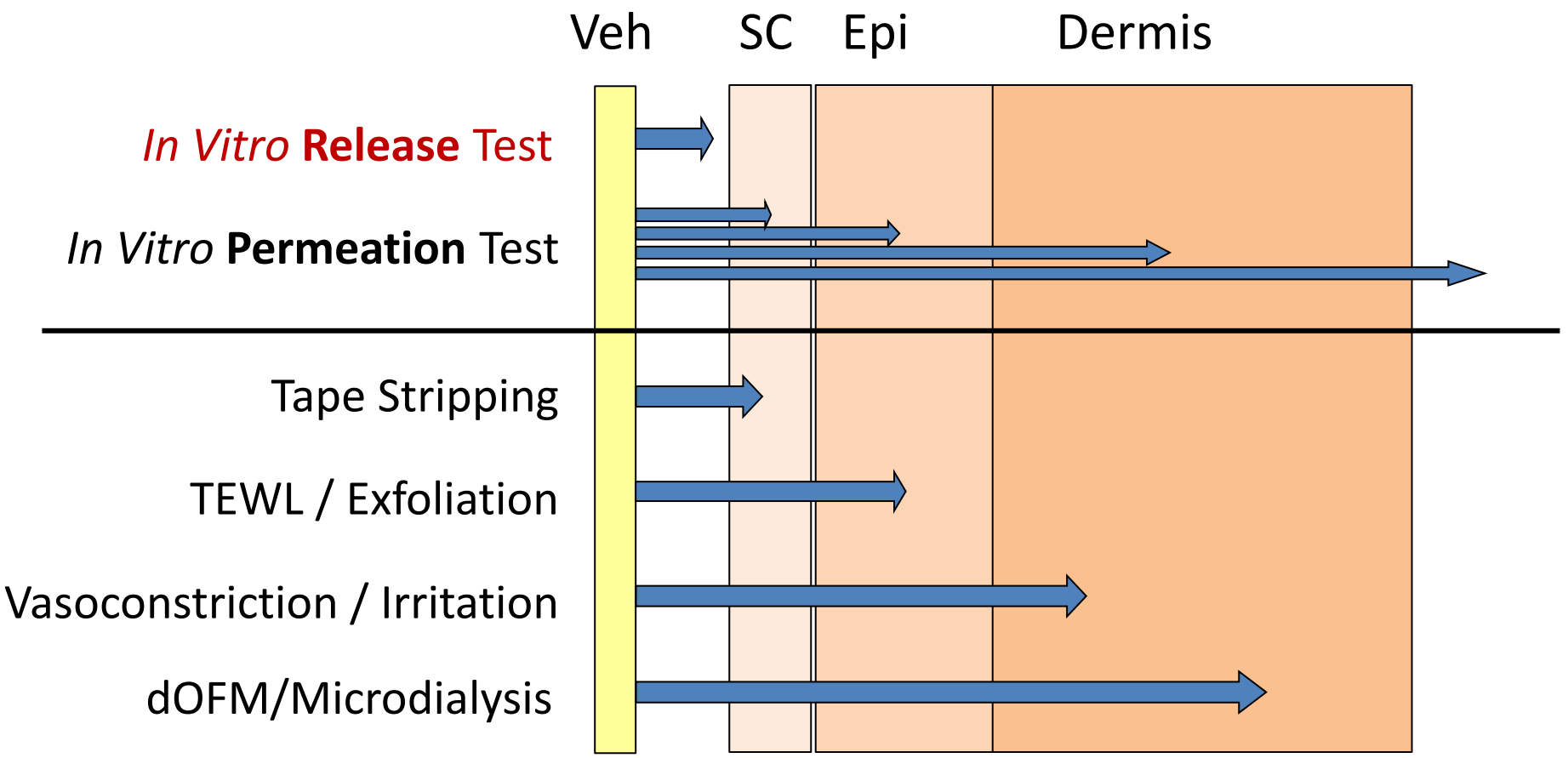
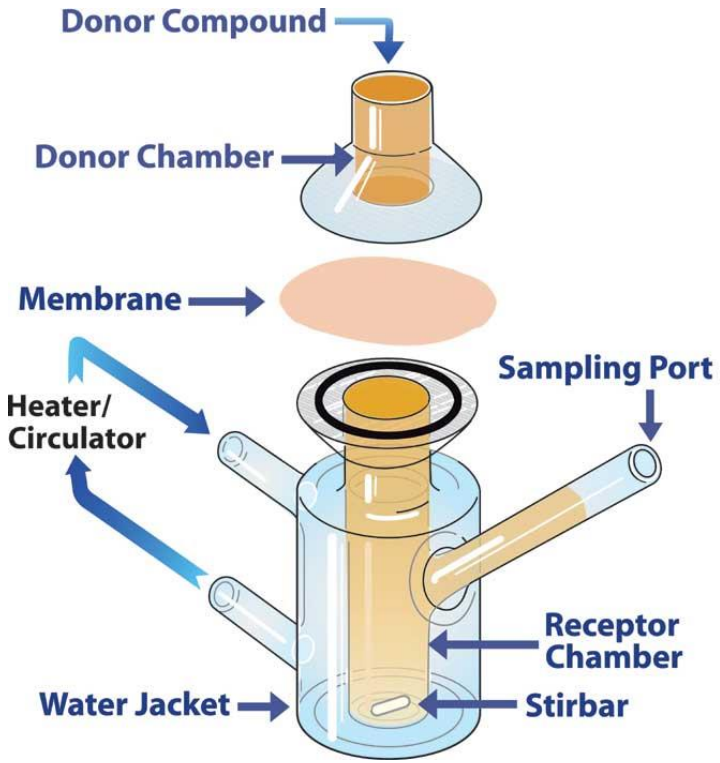
Topical Product Performance Tests



In Vitro Release Test (IVRT)



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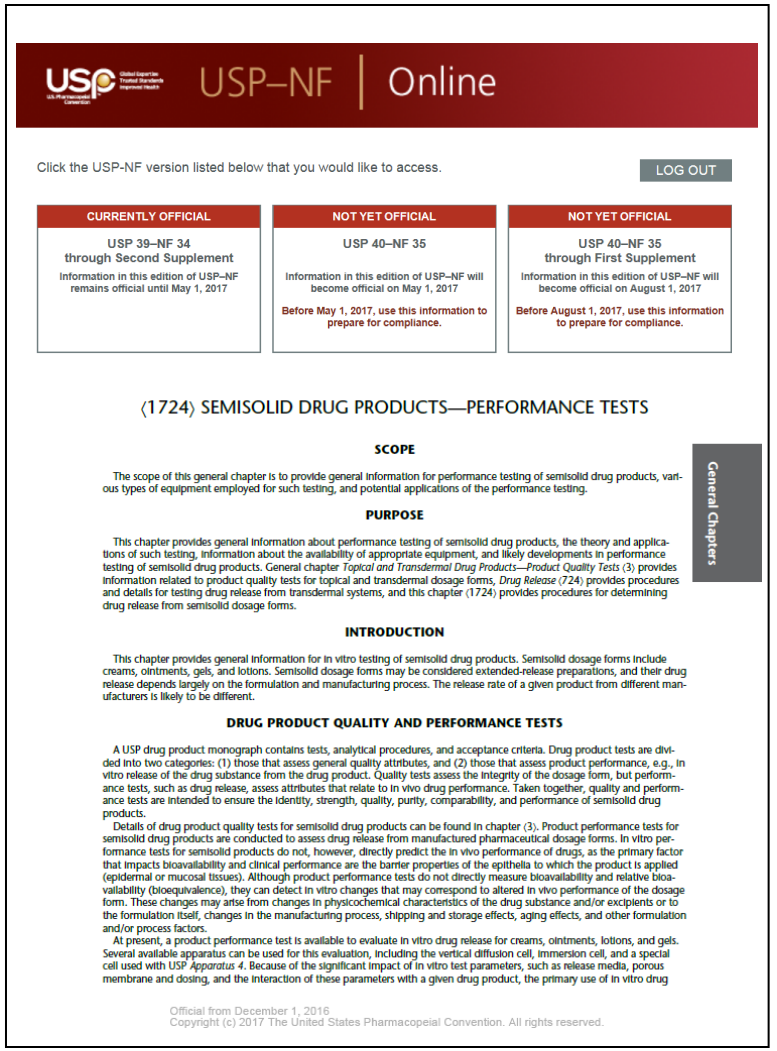

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⟨1724⟩ SEMISOLID DRUG PRODUCTS—PERFORMANCE TESTS

General Chapters

SCOPE

The scope of this general chapter is to provide general information for performance testing of semisolid drug products, various types of equipment employed for such testing, and potential applications of the performance testing.

PURPOSE

This chapter provides general information about performance testing of semisolid drug products, the theory and applications of such testing, information about the availability of appropriate equipment, and likely developments in performance testing of semisolid drug products. General chapter *Topical and Transdermal Drug Products—Product Quality Tests* ⟨3⟩ provides information related to product quality tests for topical and transdermal dosage forms, *Drug Release* ⟨724⟩ provides procedures and details for testing drug release from transdermal systems, and this chapter ⟨1724⟩ provides procedures for determining drug release from semisolid dosage forms.

INTRODUCTION

This chapter provides general information for in vitro testing of semisolid drug products. Semisolid dosage forms include creams, ointments, gels, and lotions. Semisolid dosage forms may be considered extended-release preparations, and their drug release depends largely on the formulation and manufacturing process. The release rate of a given product from different manufacturers is likely to be different.

DRUG PRODUCT QUALITY AND PERFORMANCE TESTS

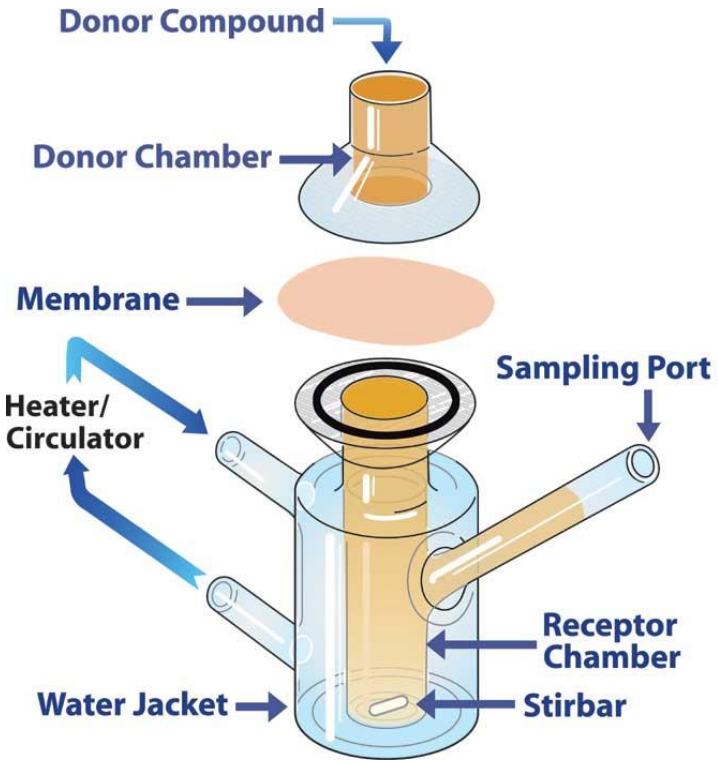
A USP drug product monograph contains tests, analytical procedures, and acceptance criteria. Drug product tests are divided into two categories: (1) those that assess general quality attributes, and (2) those that assess product performance, e.g., in vitro release of the drug substance from the drug product. Quality tests assess the integrity of the dosage form, but performance tests, such as drug release, assess attributes that relate to in vivo drug performance. Taken together, quality and performance tests are intended to ensure the identity, strength, quality, purity, comparability, and performance of semisolid drug products.

Details of drug product quality tests for semisolid drug products can be found in chapter ⟨3⟩. Product performance tests for semisolid drug products are conducted to assess drug release from manufactured pharmaceutical dosage forms. In vitro performance tests for semisolid products do not, however, directly predict the in vivo performance of drugs, as the primary factor that impacts bioavailability and clinical performance are the barrier properties of the epithelia to which the product is applied (epidermal or mucosal tissues). Although product performance tests do not directly measure bioavailability and relative bioavailability (bioequivalence), they can detect in vitro changes that may correspond to altered in vivo performance of the dosage form. These changes may arise from changes in physicochemical characteristics of the drug substance and/or excipients or to the formulation itself, changes in the manufacturing process, shipping and storage effects, aging effects, and other formulation and/or process factors.

At present, a product performance test is available to evaluate in vitro drug release for creams, ointments, lotions, and gels. Several available apparatus can be used for this evaluation, including the vertical diffusion cell, immersion cell, and a special cell used with USP *Apparatus 4*. Because of the significant impact of in vitro test parameters, such as release media, porous membrane and dosing, and the interaction of these parameters with a given drug product, the primary use of in vitro drug

Official from December 1, 2016

In Vitro Release Test (IVRT)



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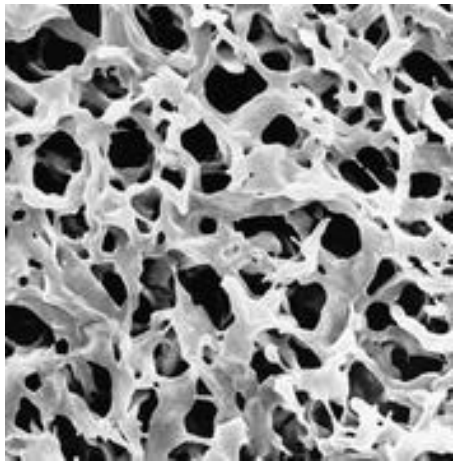


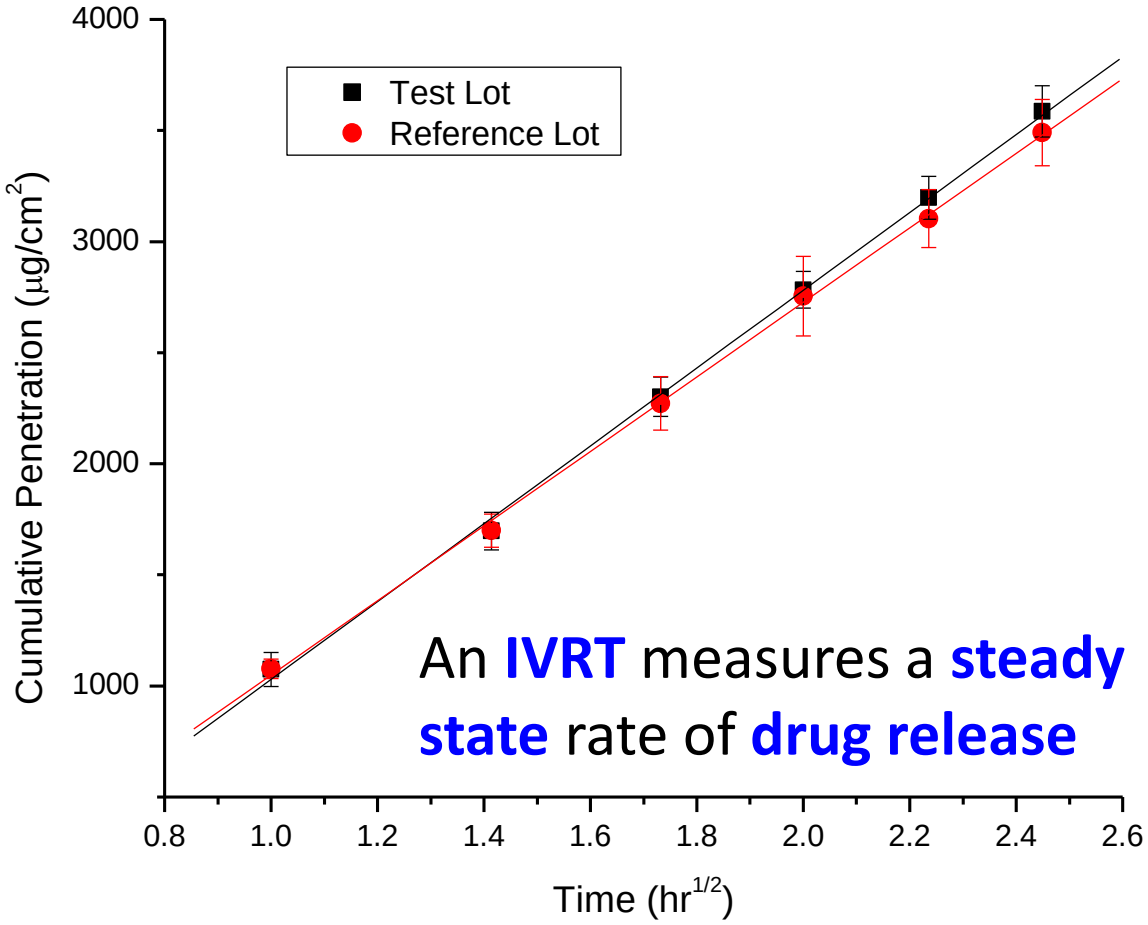
In Vitro Release Test (IVRT)

- IVRT Key Features
  - The IVRT membrane is typically a synthetic filter membrane
  - The IVRT receptor solution/medium typically contains alcohol
  - The IVRT methodology can be arbitrary and product-specific
  - The dose is thick (pseudo-infinite) for steady-state kinetics
  - The dose is occluded to prevent drying/metamorphosis
  - The primary result is the steady-state release rate (slope)
  - Drug is released into the receptor solution in µg/mL range
  - The dosage form and the receptor solution are in contact
  - Validated IVRT methods can exhibit good reproducibility
  - There is no expectation of in vivo correlation or IVIVC
  - The release rate can be sensitive to changes in Q3 attributes

IVPT vs. IVRT

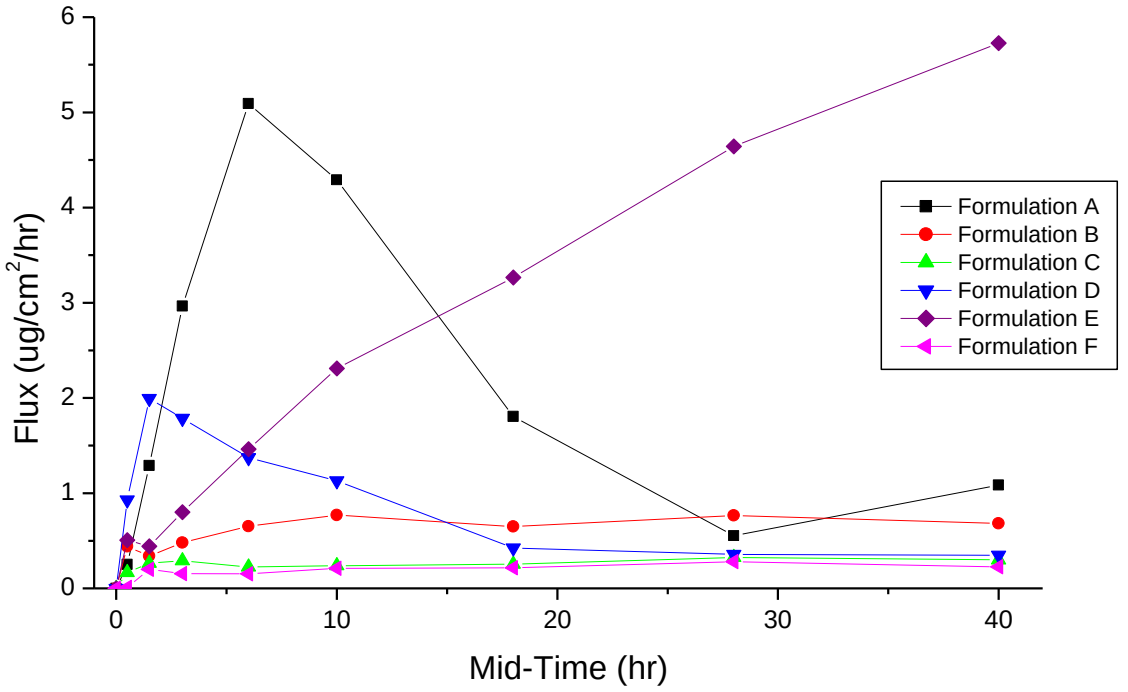
- IVPT (Permeation)
  - **Human Skin**
  - Unoccluded Dose
  - Finite Dose
  - Flux Profile (J_{max}, etc.)
  - Physiological Media
  - pg to ng Range
  - Product stays ‘dry’
  - *IVIV* Correlation
  - Donor Variability
- IVRT (Release)
  - **Synthetic Membrane**
  - Occluded Dose
  - Infinite Dose
  - Release Rate (slope)
  - Alcoholic Media
  - µg to mg Range
  - Product-Media Interface
  - Specific to the Formulation
  - Relative Consistency

IVRT vs. IVPT Data (example)



An **IVRT** measures a **steady state** rate of **drug release**

By comparison, an **IVPT** measures the **non-steady state** rate of **skin permeation**



USP General Chapter <1724> (IVRT)

- <1724> Semisolid Drug Products - Performance Tests
  - Describes appropriate diffusion cell apparatus models
  - Provides technical methodology for the use of each model
  - Provides recommendations and guidelines for the test
  - Became official as of August 1, 2013
  - Currently under revision

IVRT Method Development Parameters

- Determination of suitable method parameters for:
  - VDC Apparatus — Can often be standard
  - Dose Amount — Can often be standard
  - Stirring Rate — Can often be standard
  - Sampling Amount — Can often be standard
  - Sampling Schedule — Can often be standard (data dependent)
  - Receptor Solution — Method-specific and data dependent
  - Membrane — Method-specific and data dependent

- Determination of suitable IVRT performance for:
  - Linearity and Reproducibility of Release Rate
  - Sensitivity and Specificity of Release Rate
  - Receptor Solution Solubility for API(s)
  - Membrane inertness to binding of the API(s)

IVRT Method Development Considerations

- Theoretical Considerations: William Higuchi (1962)
  - William Higuchi (1962) confirmed the predictions of Takeru Higuchi's equations by fitting solutions with empirical data reported by Patel, Banker & DeKay (1961)
  - Further simplified the equations with square root approximations:

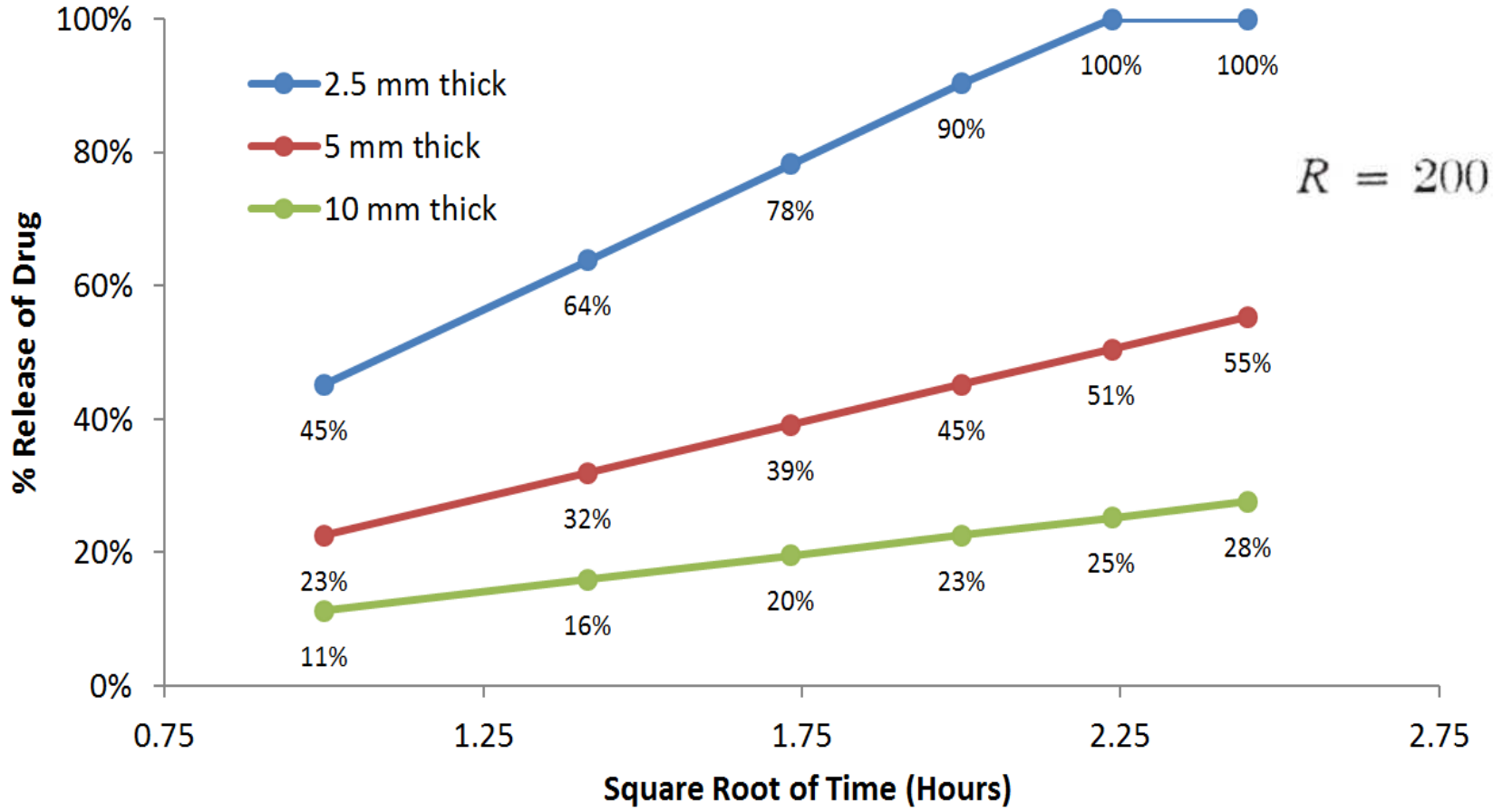
$$Q = 2\,C_0 \left(\frac{Dt}{\pi}\right)^{1/2} \qquad R = 200 \left(\frac{Dt}{\pi h^2}\right)^{1/2}$$

    - ***R*** = percent of drug released at time ***t*** per unit area of application with a thickness of ***h***
    - Assumes that ***R*** is less than ~ 30%

This does <u>not</u> mean that IVRTs with > 30% dose depletion are invalid.
An IVRT may be able to sustain steady state kinetics at > 30% dose depletion.

IVRT Dose Effect (Theoretical)

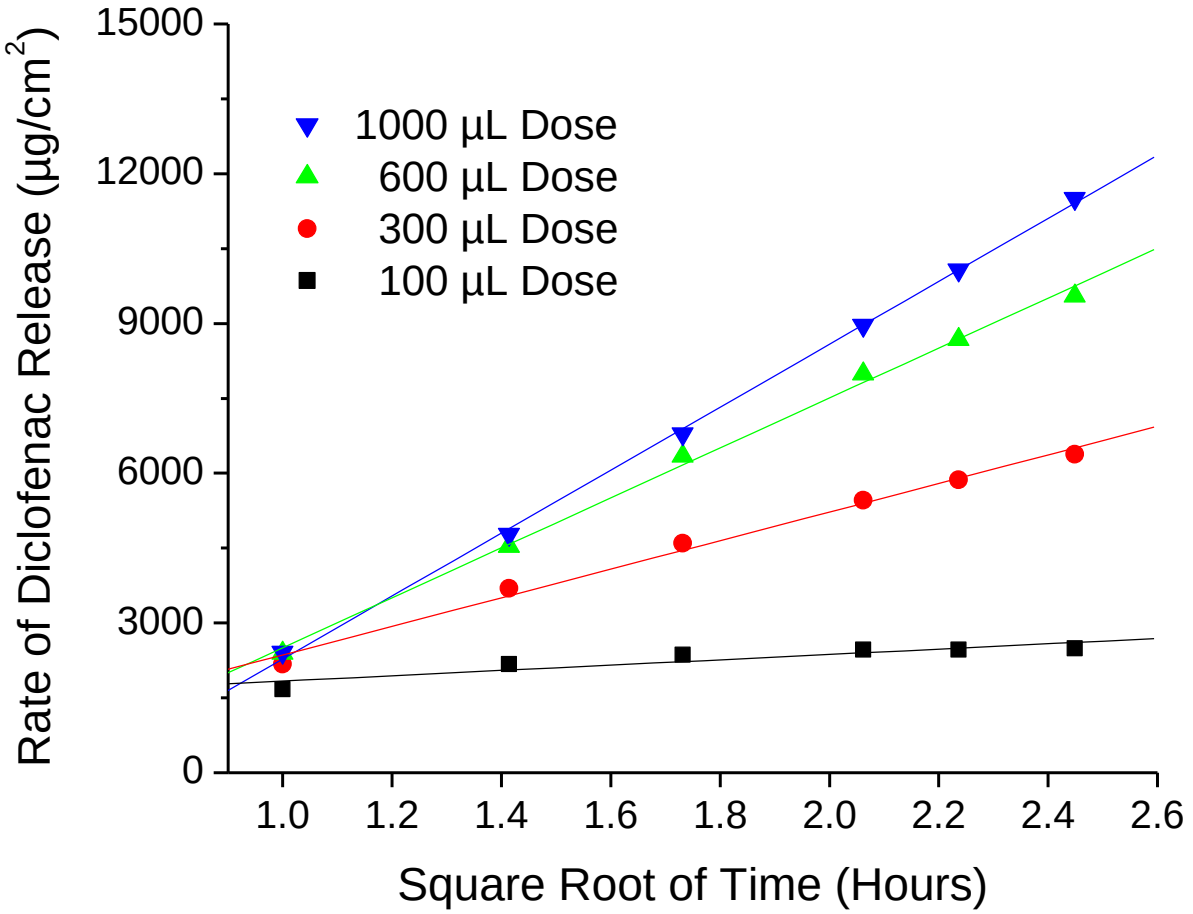
- William Higuchi's Prediction of Dose Effect
  - Assume ***D*** = 1 at ***h*** = 2.5 mm, 5.0 mm or 10.0 mm



$$R = 200\left(\frac{Dt}{\pi h^2}\right)^{1/2}$$

IVRT Dose Effect (Empirical)

- Diclofenac Sodium Gel, 3%



Diclofenac IVRT Characteristics

Dose ($\mu L/cm^2$)	Release Rate ($\mu g/cm^2/hr^{0.5}$)	Depletion of Dose (%)
100	536.3	82.9%
300	2862.9	70.9%
600	5002.1	53.1%
1000	6303.2	38.3%

Data from personal research performed in collaboration with Dr. Thomas Franz and Paul Lehman

IVRT Method Development Considerations

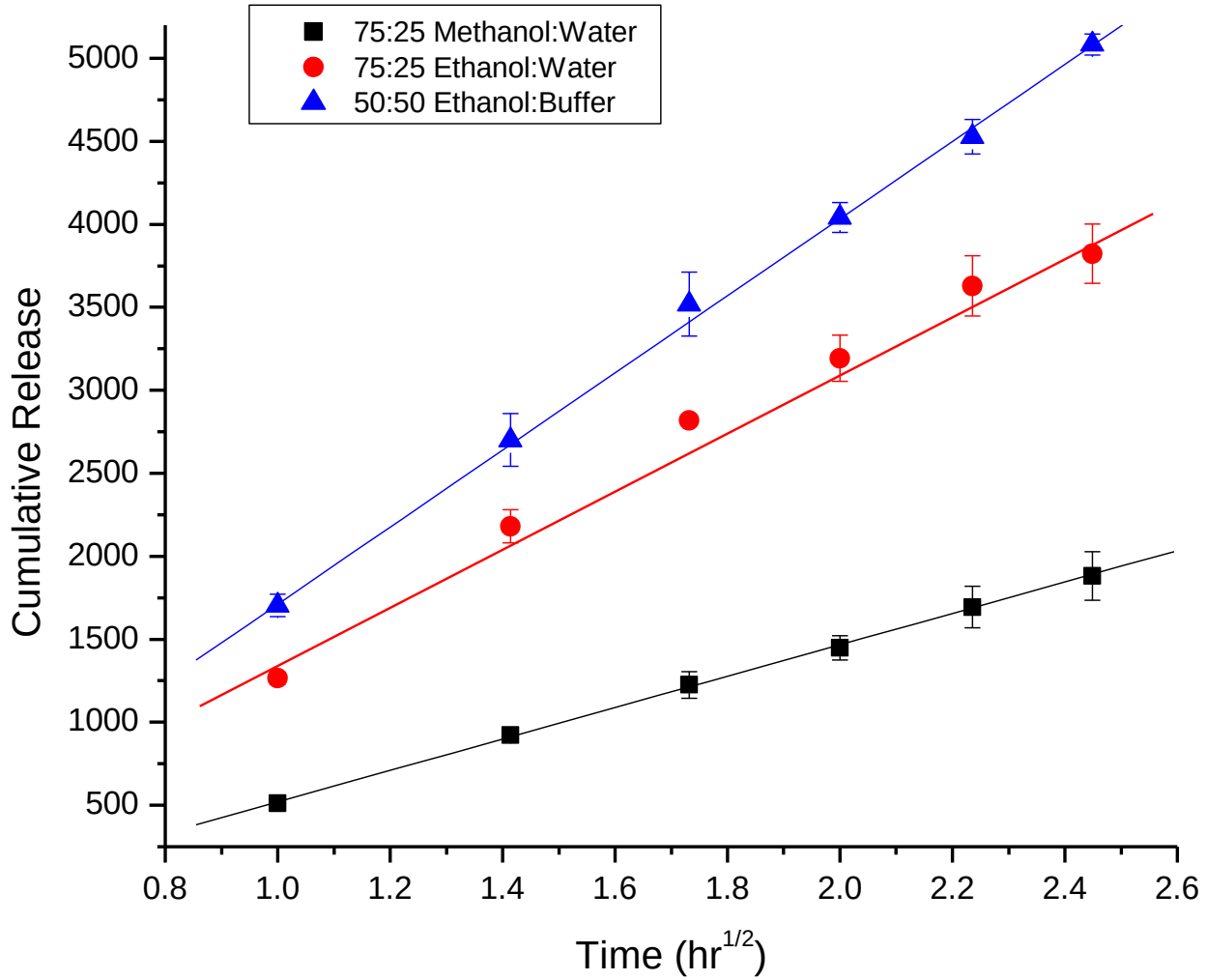
- Study Phase 1 – Receptor Solution Evaluation
  - Identify candidate solvents and solutions for the receptor solution
    - Organic/alcoholic solvents in which the API(s) has good solubility/stability
    - Focus initially on solvents that are miscible with aqueous solutions
    - Focus initially on solvents that are well suited to HPLC sample analysis
    - Acetonitrile, Ethanol, Methanol, Isopropanol, etc., are often suitable
    - API needs influence buffer salts and pH-control for the aqueous solution
    - Evaluate different ratios and species mixtures of the aqueous and organic
  - All other study parameters being kept consistent, evaluate a minimum of three different receptor solutions, each in triplicate diffusion cells.
  - Utilize a preliminarily developed HPLC analytical method for sample analysis, and begin to identify any issues (e.g., matrix effects) associated with a specific receptor solution matrix.
  - Evaluate solubility of the API(s) in the receptor solutions in triplicate.

IVRT Method Development Considerations

- Study Phase 1 – Receptor Solution Evaluation (continued)
  - Select appropriate receptor solution & test parameters based upon:
    - Top-end drop off (adjust sampling schedule as appropriate)
    - Bottom-end lag (adjust sampling schedule as appropriate)
    - Linearity of release (r^2)
    - Coefficient of Variation for linearity among replicate diffusion cells (%CV)
    - Magnitude of Release (Slope)
    - Coefficient of Variation for slope among replicate diffusion cells (%CV)
    - Identify a cutoff for acceptability of Slope %CV (e.g., < 10%)
    - Prefer higher ratios of excess solubility capacity in the receptor solution
    - Consider whether extent of API depletion impacts steady state kinetics
    - Consider matrix compatibility with the HPLC sample analysis method
  - Consider objective criteria to weight these different factors
  - Document the data evaluation and justify the final receptor selection

IVRT Method Development Considerations

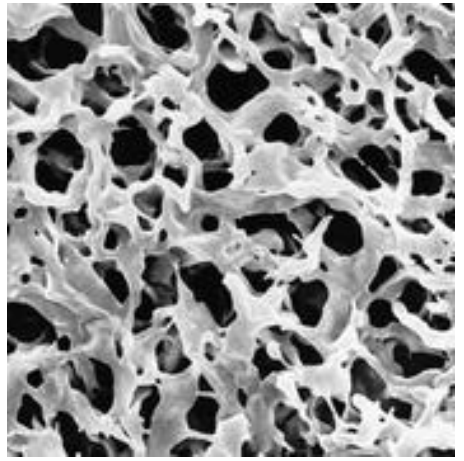
- Study Phase 1 – Receptor Solution Evaluation

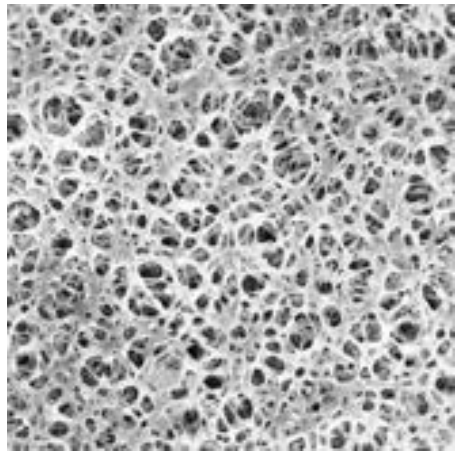


Data from personal research performed in collaboration with Dr. Thomas Franz and Paul Lehman

IVRT Method Development Considerations

- Study Phase 2 – Membrane Evaluation
  - Identify candidate membranes based upon the following
    - Pore size (~0.5µm ± 0.3µm is often suitable; e.g., 0.45µm)
    - Consider pore size relative to the viscosity of the formulation
    - Potential for receptor solution back-diffusion (and stirring rate)
    - Typical binding/inertness characteristics of the material
    - Hydrophobic vs. hydrophilic material of the membrane
    - Chemical compatibility of material with the receptor solution
    - Consistent commercial availability
  - Examples of suitable membranes include
    - Cellulose Acetate, Cellulose Nitrate, Mixed Cellulose Esters
    - Nylon aliphatic polyamides, Teflon polytetrafluoroethylene (PTFE)
    - Durapore polyvinylidenefluoride (PVDF), Versapor acrylic copolymer
    - Supor polyethersulfone, Tuffryn polysulfone



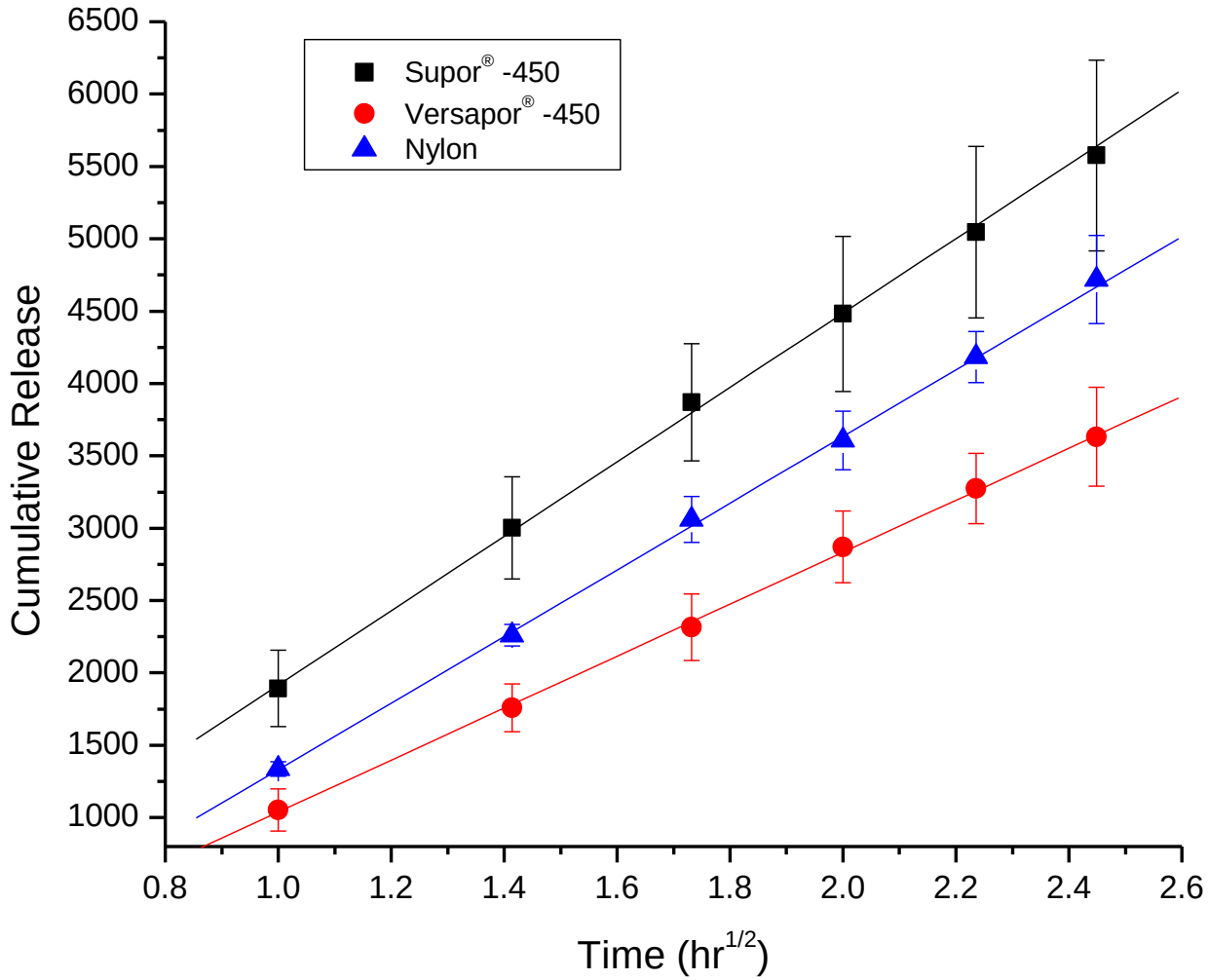


IVRT Method Development Considerations

- Study Phase 2 – Membrane Evaluation
  - All other study parameters being kept consistent, evaluate a minimum of three different membranes, each in triplicate diffusion cells.
  - Also, in triplicate, evaluate binding of the API(s) to each membrane while in the selected receptor solution for 6 hours at 32°C ± 1°C.
  - Select appropriate receptor solution and test parameters based upon:
    - Same IVRT performance criteria as for receptor solution evaluation
    - Identify a cutoff for acceptability of Inertness (e.g., 100% ± 5%)
    - Consider membrane compatibility with the HPLC sample analysis method
    - Prefer to avoid membranes where back-diffusion is apparent
  - Consider objective criteria to weight these different factors
  - Document the data evaluation and justify the final membrane selection

IVRT Method Development Considerations

- Study Phase 2 – Membrane Evaluation



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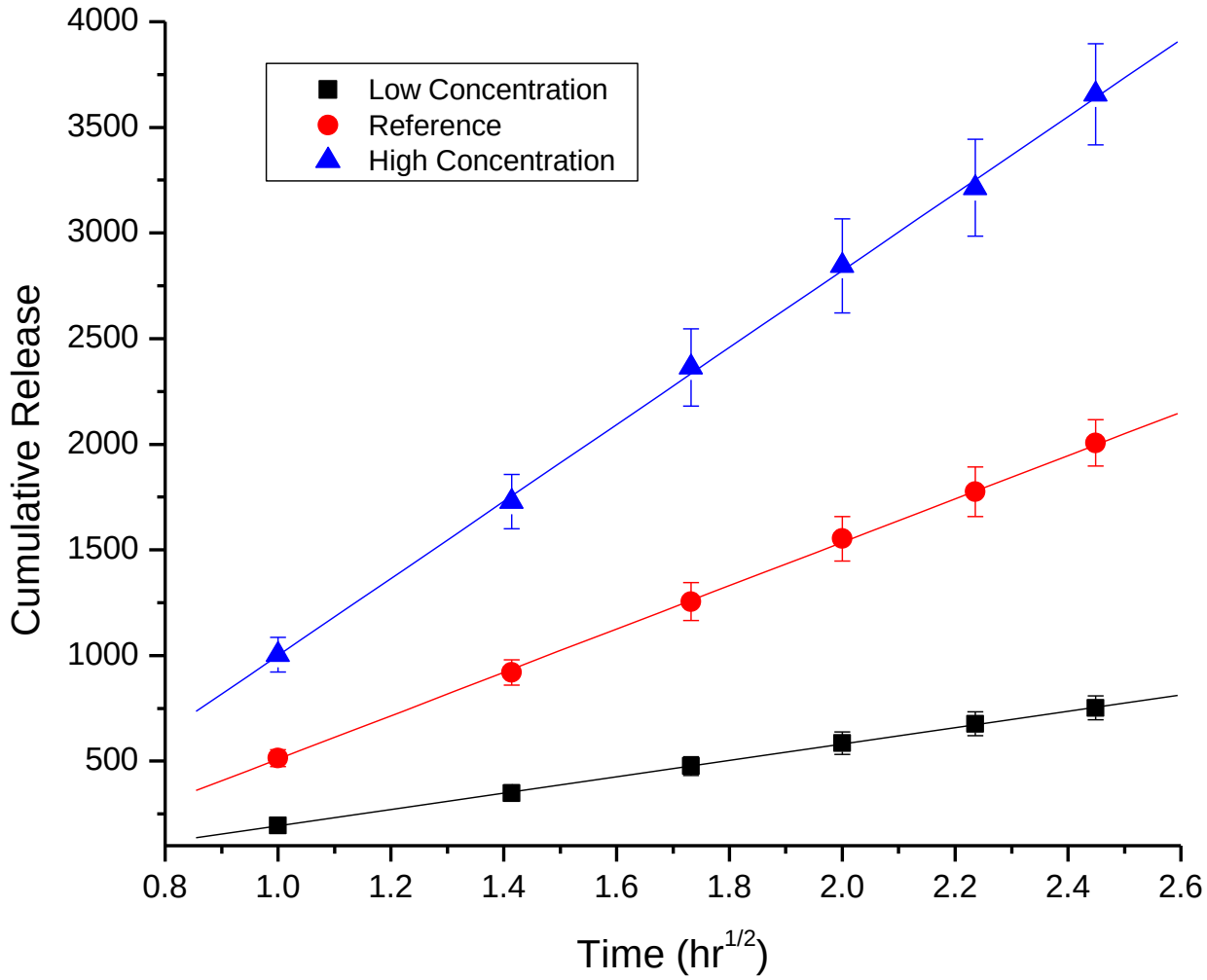
IVRT Method Development Considerations

- Study Phase 3 (Optional) – Evaluation of Sensitivity and Specificity
  - Utilize the selected membrane and receptor solution, representing the developed IVRT method.
  - All other study parameters being kept consistent, evaluate three different strengths of the product, each in triplicate diffusion cells.
  - The reference product strength ± 50% is a suitable set
    - (e.g., nominal 2.5%, 5%, and 7.5% product strengths)
  - Utilize a preliminarily developed HPLC analytical method for sample analysis, and identify any issues (e.g., curve range, peak shape and injection volume, etc.) associated with a specific product strength.
  - Finalize the method and progress into IVRT Validation if the method appears to be:
    - Linear, Precise, and Reproducible
    - Sensitive (suitable differentiation in release rates between strengths)
    - Specific (e.g., $r^2 > 0.95$ for correlation of strength to release rate)

IVRT Method Development Considerations

- Study Phase 3 (Optional) – Evaluation of Sensitivity



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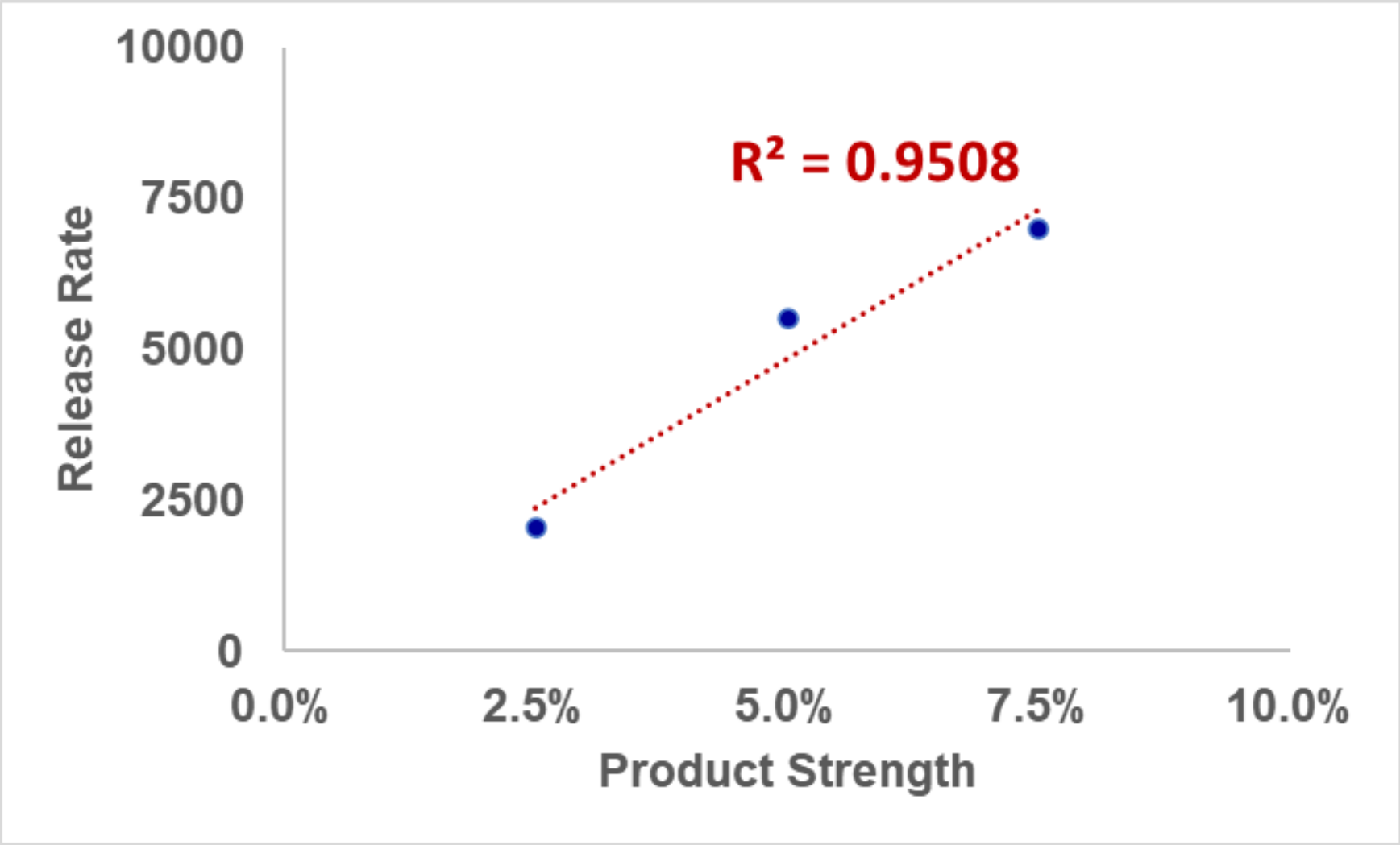
IVRT Method Development Considerations

- Study Phase 3 (Optional) – Evaluation of Specificity



IVRT Method Development Considerations

- A single lot of Reference (or Test) product is typically used throughout the Method Development study.
- IVRT performance criteria for selection of a receptor solution, membrane, or sampling schedule are typically objective and pre-defined.
- IVRT parameter selection criteria and relative weighting of factors are typically objective and pre-defined, with allowance for justifications based upon data.
- Objective analyses of results are documented to support the selection of a method parameter.
- Justifications are documented for the selection of receptor solutions and membranes for evaluation, as well as for the final developed method.
- The final IVRT method with all relevant parameters that define the developed method are finalized, to proceed into validation.
  - The final HPLC method is also finalized, to proceed into validation.

IVRT Method Development Approach (Example)

- Example Study Phase 0
  - HPLC analytical method development
- Example Study Phase 1
  - 1 run of 3 cells with the reference product and receptor solution 1
  - 1 run of 3 cells with the reference product and receptor solution 2
  - 1 run of 3 cells with the reference product and receptor solution 3
  - Receptor solution solubility characterization for each receptor solution
- Example Study Phase 2
  - 1 run of 3 cells with the reference product and membrane 1
  - 1 run of 3 cells with the reference product and membrane 2
  - 1 run of 3 cells with the reference product and membrane 3
  - Membrane binding/inertness characterization
- Example Study Phase 3 (Optional)
  - 1 run of 3 cells with the reference product — Preliminary Assessment of Reproducibility
  - 1 run of 3 cells with the 'low' test product — Preliminary Assessment of Sensitivity & Specificity
  - 1 run of 3 cells with the 'high' reference product — Preliminary Assessment of Sensitivity & Specificity

IVRT Method Validation Considerations

1. Apparatus Qualification

- Cell Capacity
- Cell Orifice Diameter
- Receptor Medium & Membrane Temp.
- Stirring Speed
- Dispensed Sampling Volume
- Environmental Conditions

2. Laboratory Qualification

- Inter-run Variability
- Intra-run Variability
- Product Sameness Test

3. HPLC Method Validation

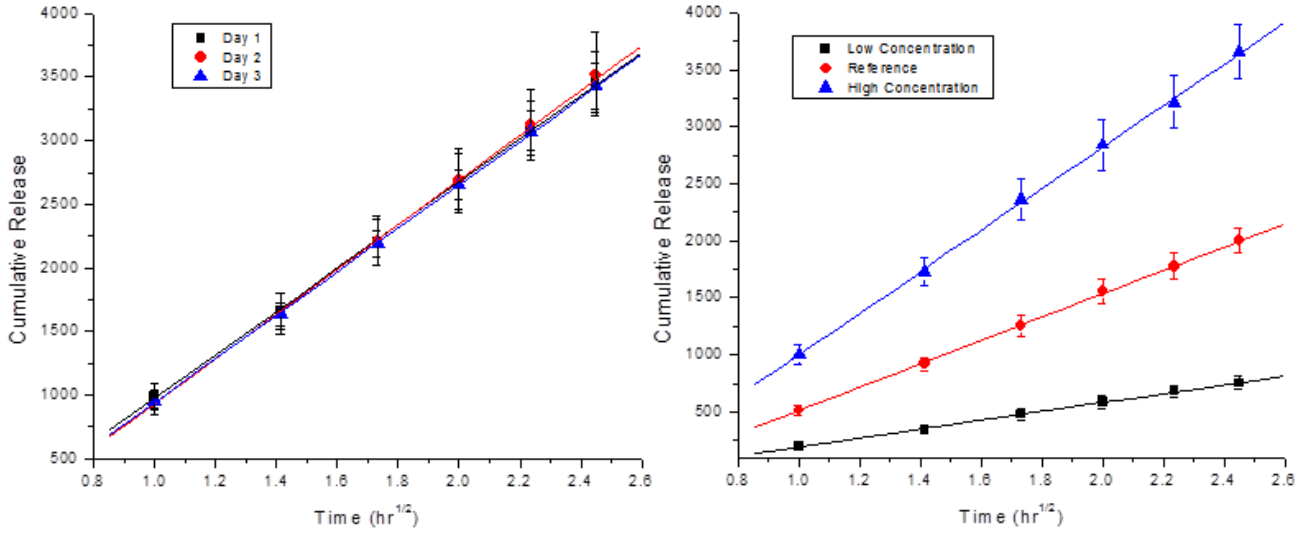
- Selectivity and Specificity
- Linearity
- Accuracy, Precision and Robustness
- Stability

4. IVRT Method Validation

- Linearity and Range
- Precision and Reproducibility
- Sensitivity
- Specificity
- Selectivity
- Robustness
- Etc.

NOTE:

These lists are *Not All Inclusive*



Data/figures from personal research collaborations with Drs. Isadore Kanfer, Frank Sinner, Thomas Franz and Paul Lehman

IVRT Apparatus Qualification

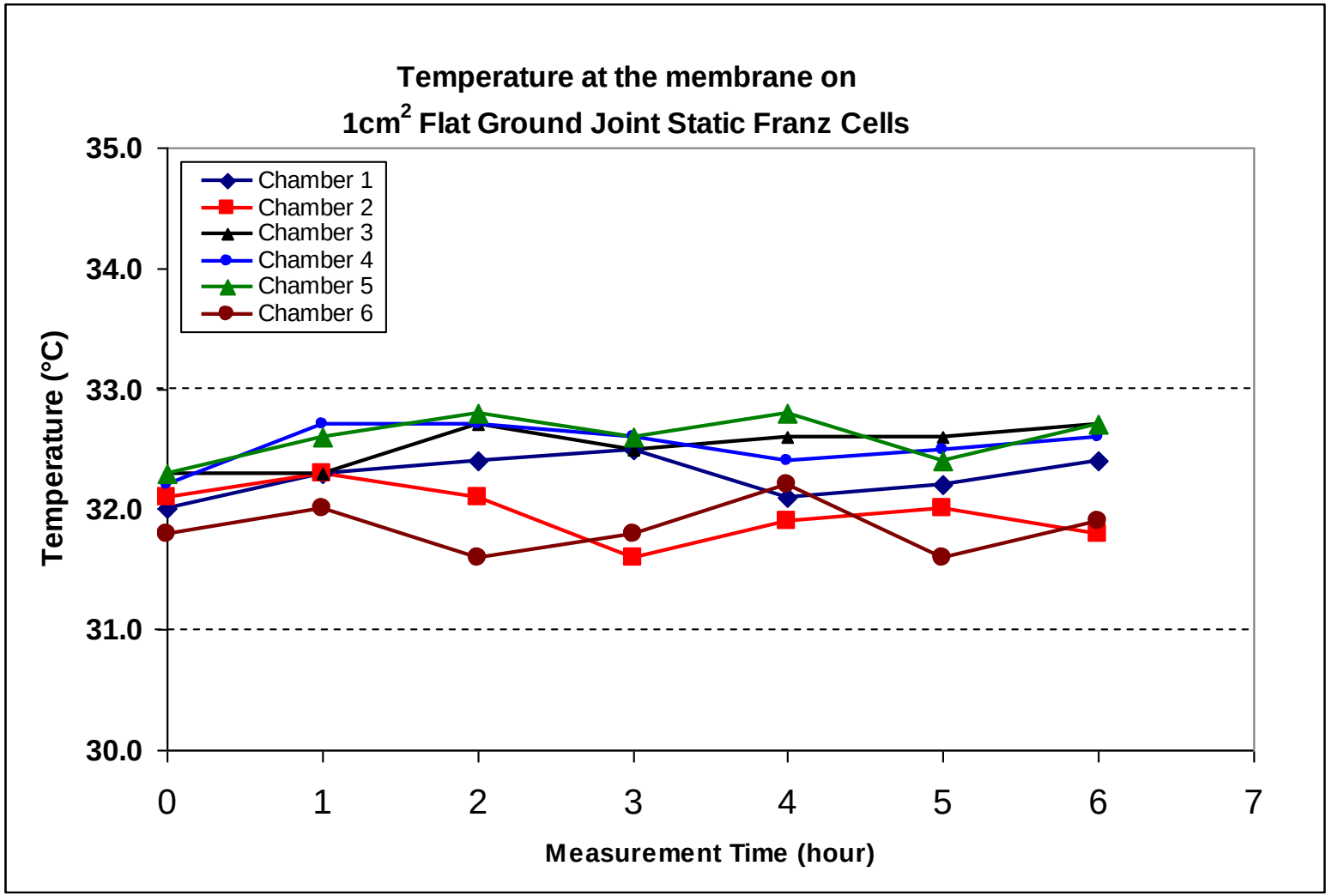
- USP <1724> specifies that
  - *The diameters of the orifices of the donor chamber and receptor chamber, which define the dosage delivery surface area for the test, should be sized within ±5% of the specified diameter.*
  - *The diameter of the donor and receptor chamber orifices may vary depending on the application.*
  - *The receptor chamber orifice should never be smaller than the orifice of the donor chamber but should be fabricated to the same size as the donor chamber orifice.*
  - *The design of the VDC should facilitate proper alignment of the donor chamber and the receptor orifice.*
  - *The receptor chamber should be manufactured consistently with uniform height and geometry.*
  - *All the cells should have the same nominal value, and the true volume should be measured for each individual cell.*

IVRT/IVPT Apparatus Qualification

- Qualification of the IVRT apparatus is described in USP <1724>
  - *Unless the method specifies otherwise, the qualification of the apparatus has been verified when*
    - *analysts determine that the **test temperature** and **stirring rate** are within their specified requirements and*
    - *a satisfactory performance verification test (i.e., drug release rate) results.*
- Equipment Manufacturers may provide
  - Supporting documentation (e.g., certificates of conformance)
  - Guidelines for IQ, OQ, and PQ of VDC apparatus and accessories
  - Recommended schedules for maintenance and re-qualification

IVRT/IVPT Apparatus Qualification

- Qualification of Temperature Control for VDC Models B & C



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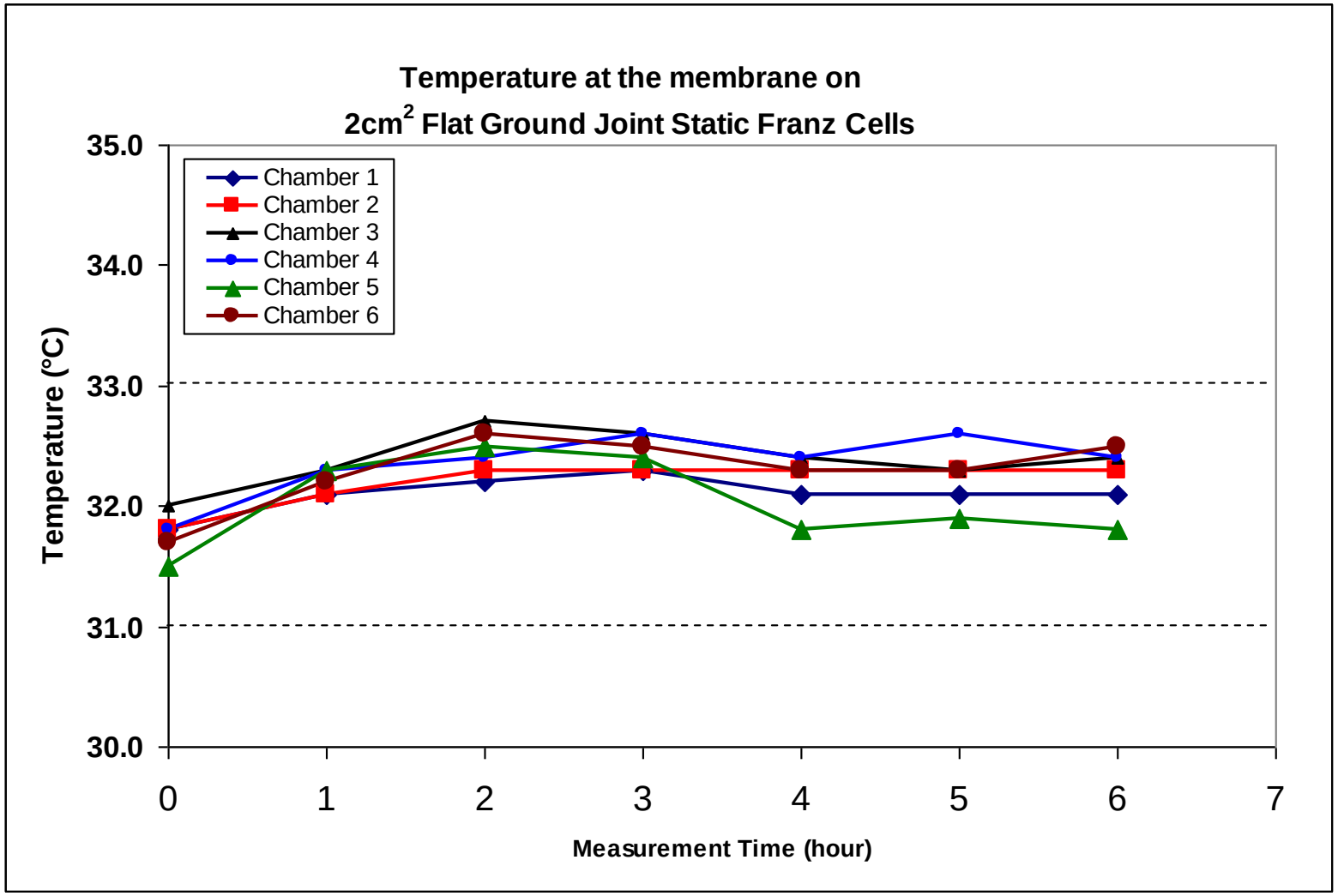
IVRT/IVPT Apparatus Qualification

- Qualification of Temperature Control for VDC Models B & C



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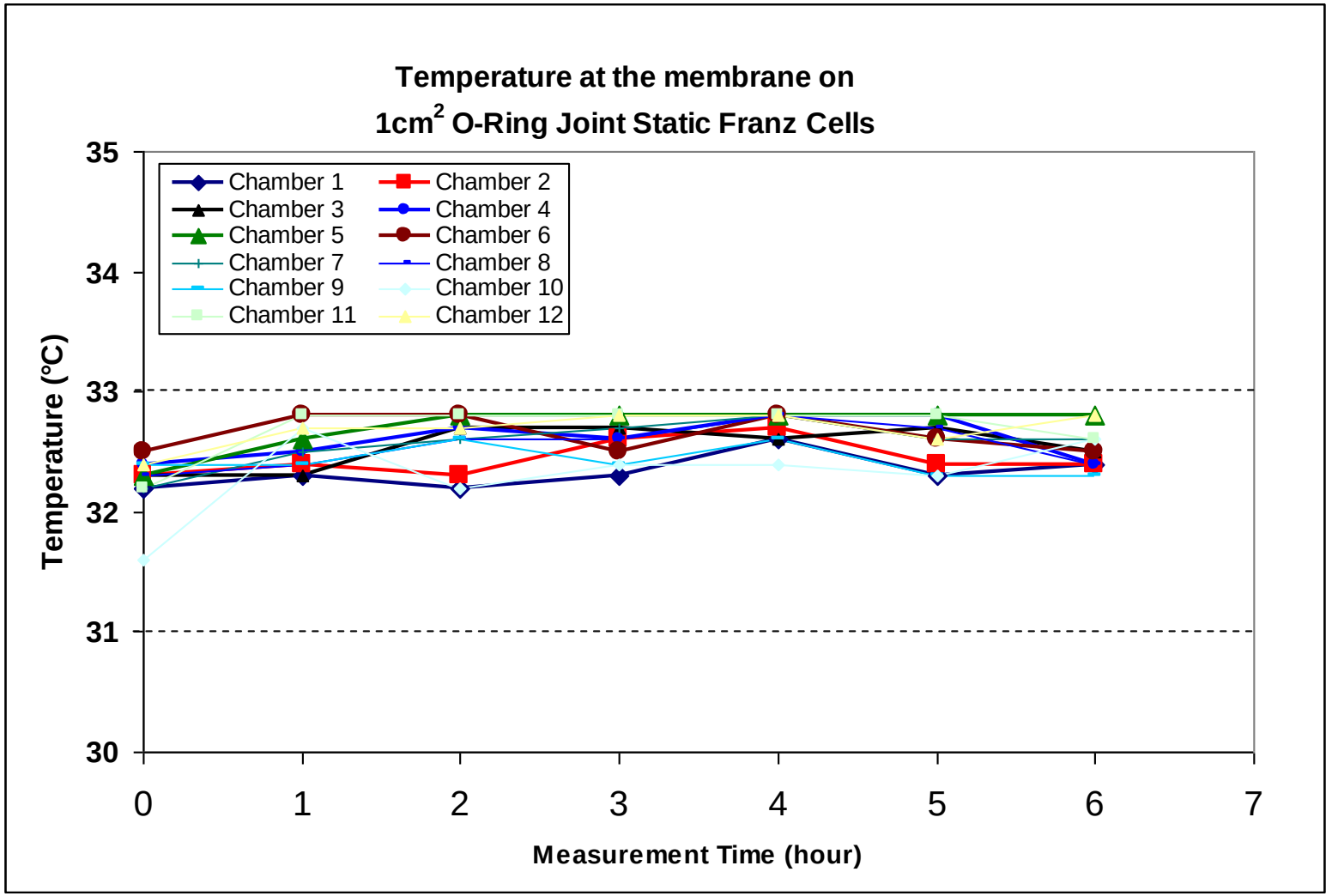

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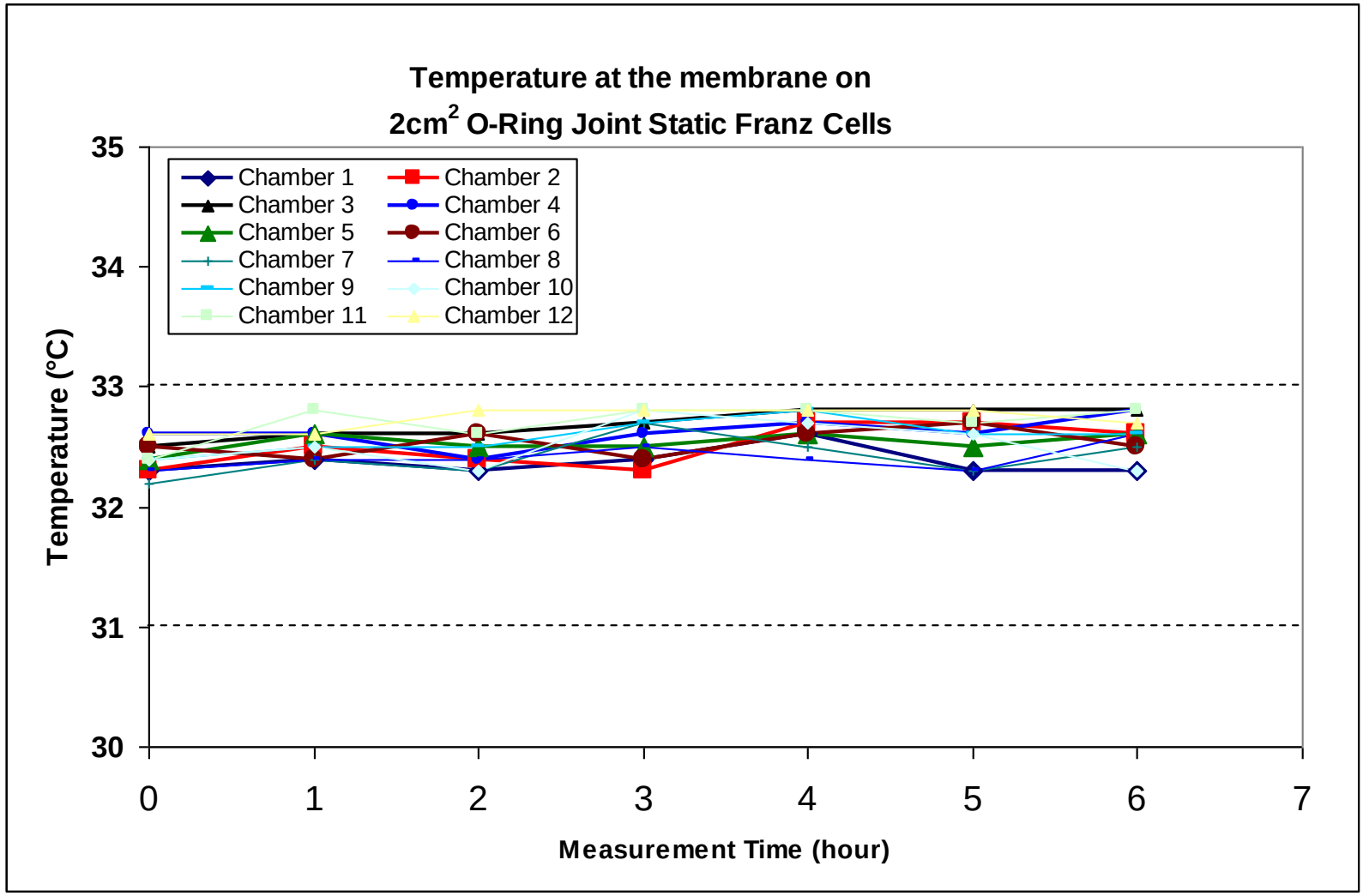

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IVRT/IVPT Apparatus Qualification

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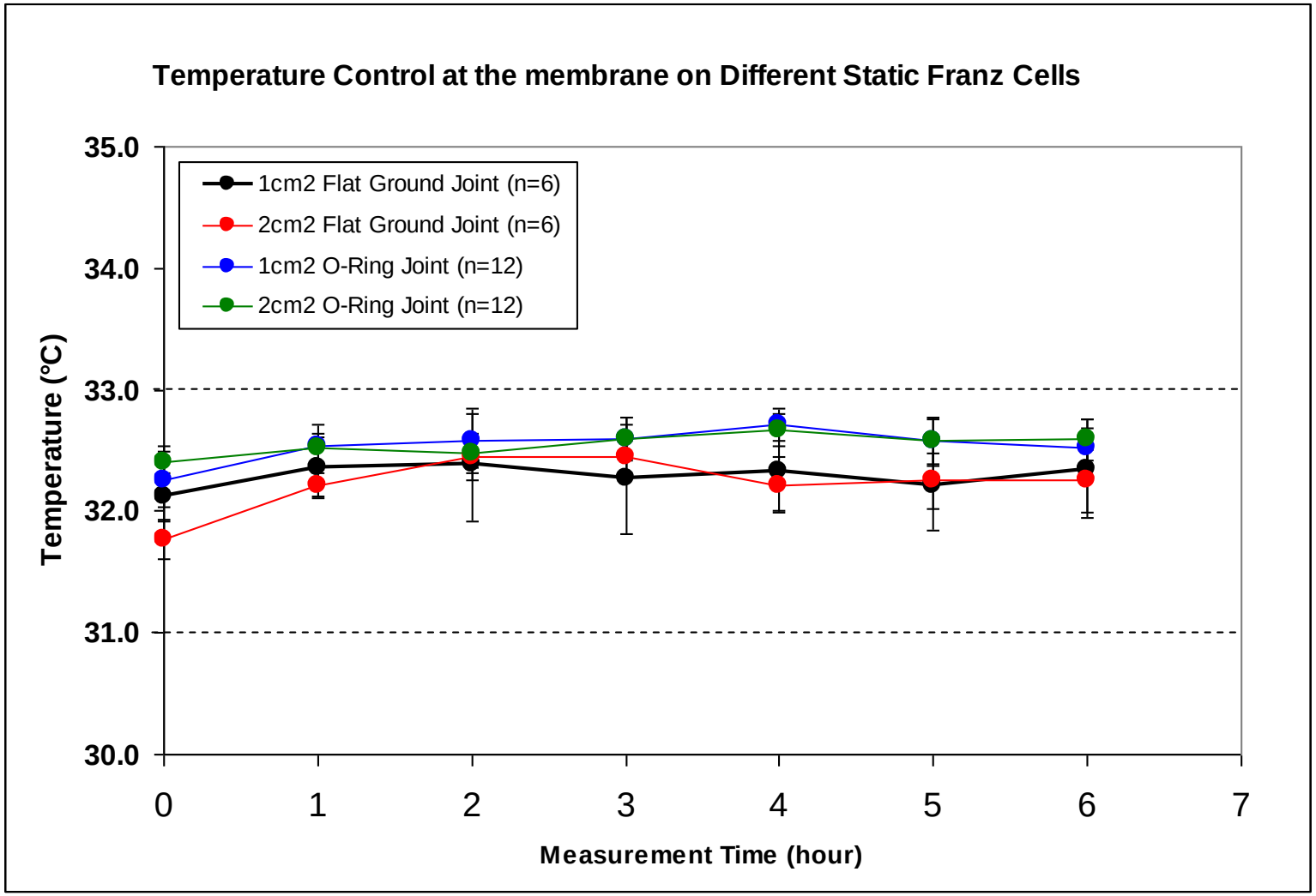

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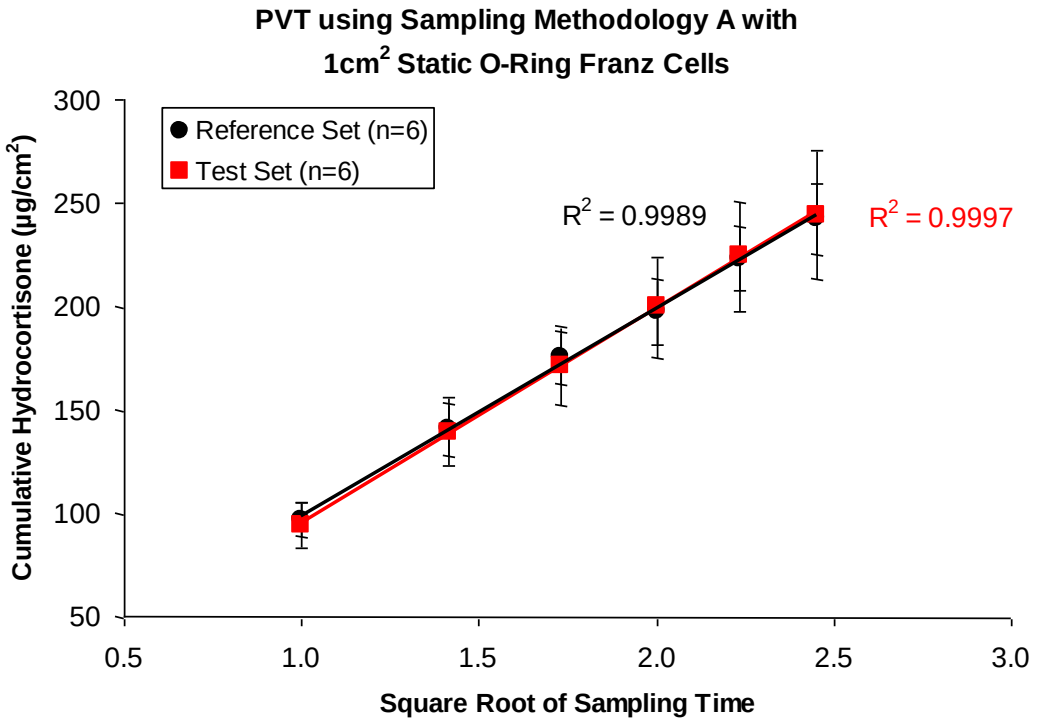
IVRT/IVPT Apparatus Qualification

Control Parameter	Description	Target Specification	Average Value	Precision	Result
Manufacturing Specification - Flat Ground Joint	11.28mm Diameter Donor Orifice	11.28mm ± 5%	11.37mm	0.09% CV	PASS
Manufacturing Specification - Flat Ground Joint	11.28mm Diameter Receptor Orifice	11.28mm ± 5%	11.38mm	0.20% CV	PASS
Manufacturing Specification - O-Ring Joint	11.28mm Diameter Donor Orifice	11.28mm ± 5%	11.28mm	0.49% CV	PASS
Manufacturing Specification - O-Ring Joint	11.28mm Diameter Receptor Orifice	11.28mm ± 5%	11.42mm	0.59%CV	PASS
Manufacturing Specification - Flat Ground Joint	15.00mm Diameter Donor Orifice	15.00mm ± 5%	15.04mm	0.36% CV	PASS
Manufacturing Specification - Flat Ground Joint	15.00mm Diameter Receptor Orifice	15.00mm ± 5%	14.94mm	0.43% CV	PASS
Manufacturing Specification - O-Ring Joint	15.00mm Diameter Donor Orifice	15.00mm ± 5%	15.01mm	0.38% CV	PASS
Manufacturing Specification - O-Ring Joint	15.00mm Diameter Receptor Orifice	15.00mm ± 5%	14.99mm	0.24% CV	PASS
Dose Area - 1cm^2 O-Ring Joint	Actual Area of 1% HC Cream dose	Dose Area Precision of ± 5%	1.34 cm^2 Dose Area	2.48% CV	PASS
Dose Area - 1cm^2 Flat Ground Joint	Actual Area of 1% HC Cream dose	Dose Area Precision of ± 5%	1.08 cm^2 Dose Area	2.22% CV	PASS
Manufacturing Specification - Magnetic Impeller	Stirring at 600 rpm	600 rpm ± 10%	601.5 rpm	0.54% CV	PASS
Manufacturing Specification - Stir Bar (1cm^2)	Stirring at 600 rpm	600 rpm ± 10%	598.5 rpm	0.29% CV	PASS
Manufacturing Specification - Stir Bar (2cm^2)	Stirring at 600 rpm	600 rpm ± 10%	599.6 rpm	0.25% CV	PASS
Manufacturing Specification - 1 cm^2 Flat Ground Joint	Receptor Volume Control	5.5mL ± 5%	5.5mL	1.69% CV	PASS
Manufacturing Specification - 1 cm^2 O-Ring Joint	Receptor Volume Control	6.0mL ± 5%	5.9mL	3.59% CV	PASS
Manufacturing Specification - 2 cm^2 O-Ring Joint	Receptor Volume Control	7.0mL ± 5%	6.9mL	2.50% CV	PASS
Manufacturing Specification - 2 cm^2 Flat Ground Joint	Receptor Volume Control	7.0mL ± 5%	7.0mL	0.58% CV	PASS
Membrane Temperature - 1 cm^2 Flat Ground Joint	Temperature Control over 6 hr duration	32°C ± 1°C	32.3°C	± 0.32°C	PASS
Membrane Temperature - 1 cm^2 O-Ring Joint	Temperature Control over 6 hr duration	32°C ± 1°C	32.5°C	± 0.14°C	PASS
Membrane Temperature - 2 cm^2 O-Ring Joint	Temperature Control over 6 hr duration	32°C ± 1°C	32.5°C	± 0.12°C	PASS
Membrane Temperature - 2 cm^2 Flat Ground Joint	Temperature Control over 6 hr duration	32°C ± 1°C	32.2°C	± 0.14°C	PASS
Membrane Temperature - 1 cm^2 Flat Ground Joint	Linear drug release over 6 hr duration	Mean $r^2 > 0.90$	$r^2 > 0.99$	All Cells	PASS
Membrane Temperature - 1 cm^2 O-Ring Joint	Linear drug release over 6 hr duration	Mean $r^2 > 0.90$	$r^2 > 0.99$	All Cells	PASS
Membrane Temperature - 2 cm^2 O-Ring Joint	Linear drug release over 6 hr duration	Mean $r^2 > 0.90$	$r^2 > 0.99$	All Cells	PASS
Membrane Temperature - 2 cm^2 Flat Ground Joint	Linear drug release over 6 hr duration	Mean $r^2 > 0.90$	$r^2 > 0.99$	All Cells	PASS

Data from personal research performed in collaboration with Dr. Thomas Franz and Paul Lehman

IVRT Laboratory Qualification

- IVRT Performance Verification Test (PVT)
  - Can be based upon the recommended approach described in USP Pharmacopeal Forum Vol. 35(3) [May-June 2009] Draft General Chapter 725, comparing two sets of six VDC dosed with Hydrocortisone cream, 1% evaluated using the statistical approach described in USP <1724>.



Data from personal research performed in collaboration with Dr. Thomas Franz and Paul Lehman

IVRT Method Validation Considerations

- The HPLC method used for IVRT validation is validated in a manner compatible with FDA or ICH guidelines
- IVRT validation characterizes critical method parameters
  - Apparatus Qualification
  - Membrane Qualification
  - Receptor Solution Qualification
  - Receptor Solution Sampling Qualification
  - Environmental Control
  - Linearity and Range
  - Precision and Reproducibility
  - Dose Depletion
  - Discrimination Sensitivity, Specificity, and Selectivity
  - Robustness

IVRT Method Validation Considerations

- Membrane Qualification
  - Membrane inertness is evaluated for drug binding in the receptor solution at a concentration relevant to the average terminal drug release concentration.
  - Determinations are typically based upon three replicate membrane incubations for the IVRT duration (e.g., 6 hours) at 32°C ± 1°C.
  - Three replicate control incubations are performed in parallel, without membranes, to monitor for drug loss that is not associated with membrane binding.
  - Aliquots of these solutions are collected before and after the duration of incubation, to assess any decrease in drug in solution.
  - The recovery of drug in solution with an inert membrane is ideally 100% ± 5% at 6 hours relative to controls.

IVRT Method Validation Considerations

- Receptor Solution Qualification
  - The solubility of drug in the IVRT receptor solution is evaluated in triplicate by attempting to dissolve the drug in the receptor solution at ~ 1mg/mL (or as appropriate for the IVRT) and assaying for soluble concentrations of drug.
  - The solubility should, at minimum, be in excess of the terminal (e.g., 6 hr) IVRT receptor solution sample concentration for the 150% strength product.
  - The solubility should ideally be 10-fold greater than the terminal (e.g., 6 hr) IVRT receptor solution sample concentration for the reference product.

IVRT Method Validation Considerations

- Receptor Solution Sampling Qualification
  - The accuracy and precision of receptor solution sample collection at each time point is appropriately qualified.
  - Evidence to qualify a sampling procedure typically illustrates that:
    - the sampling technique can reliably collect a consistent volume of the sample from the well-mixed volume of the receptor compartment at each sampling event.
    - no artifacts are likely to be created by the sampling technique (e.g., due to carry-over between samples in automated sampling systems or due to sampling from an un-mixed volume in the sampling arm of a vertical diffusion cell).
  - Information should be included describing the apparatus manufacturer's specification for the accuracy and precision of receptor solution sampling, when available.

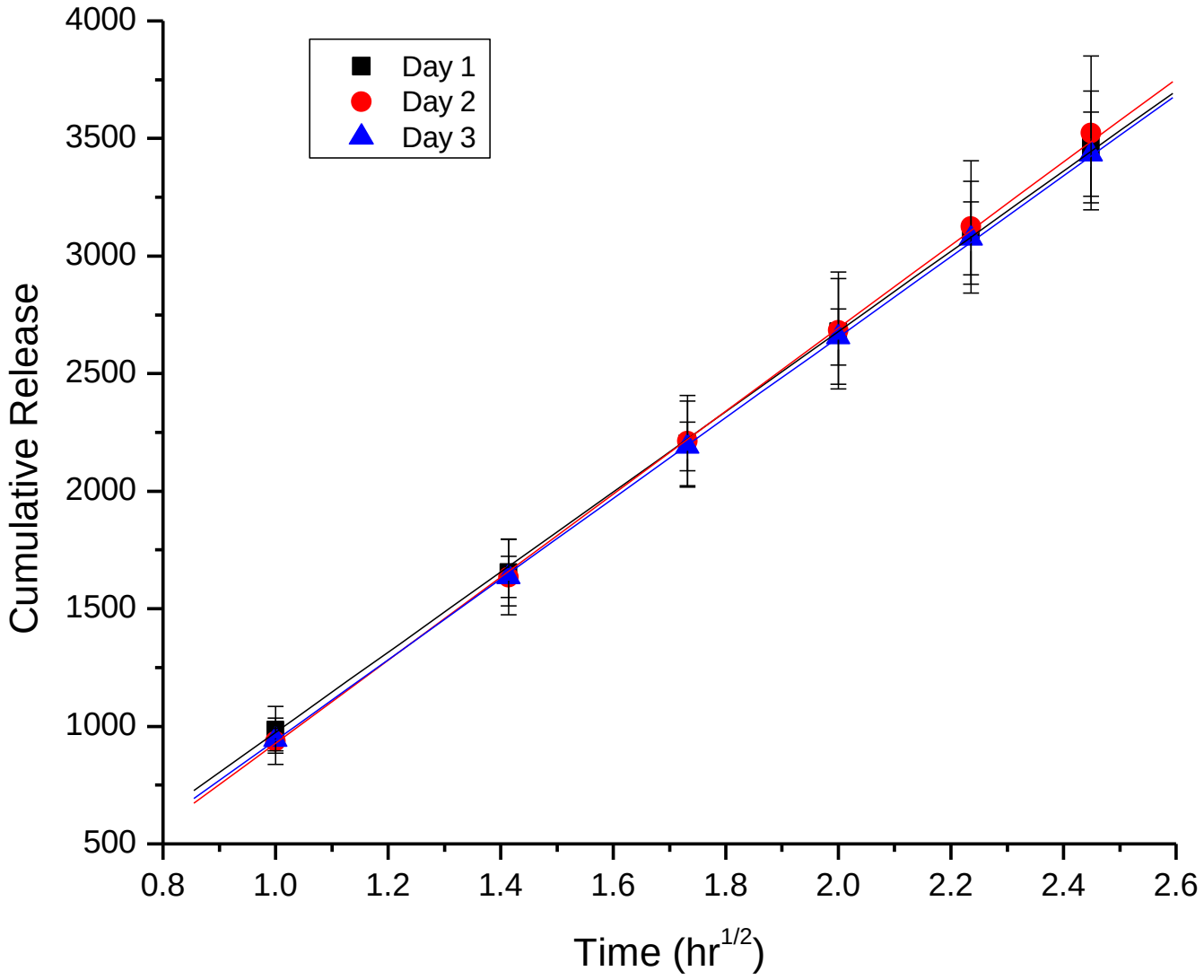
IVRT Method Validation Considerations

- Environmental Control
  - Ambient laboratory temperature and humidity during the study is monitored and reported.
  - A controlled environmental temperature is typically feasible (e.g., in the range of 21°C ± 2°C).
  - A humidity range of 50% ± 20% relative humidity is ideal, if feasible.

IVRT Method Validation Considerations

- Linearity and Range
- Precision and Reproducibility
- Dose Depletion
  - Determinations are typically based upon a minimum of 3 runs of 6 diffusion cells each, all dosed with the same lot of reference product.
    - 1 run of reference product on **day 1**
    - 1 run of reference product on **day 2**
    - 1 run of reference product on **day 3**
  - Different operators/analysts for the IVRT test can be utilized across different days

IVRT Method Validation Considerations



Data from personal research performed in collaboration with Dr. Thomas Franz and Paul Lehman

IVRT Method Validation Considerations

- Linearity and Range
  - The linearity (r^2 value) of the release rate (slope) is calculated across the range of the sampling times, which corresponds to the IVRT study duration
  - Linearity is compared within and across all runs.
  - The r^2 value is ideally ≥ 0.97 across the IVRT study duration (range).

IVRT Method Validation Considerations

- Precision and Reproducibility
  - The Precision and Reproducibility of the release rate (slope) is calculated for each diffusion cell.
  - The mean, standard deviation, and percent coefficient of variation (%CV) among slopes are calculated within and across all runs.
  - The intra- and inter-run CV is ideally ≤ 15%.

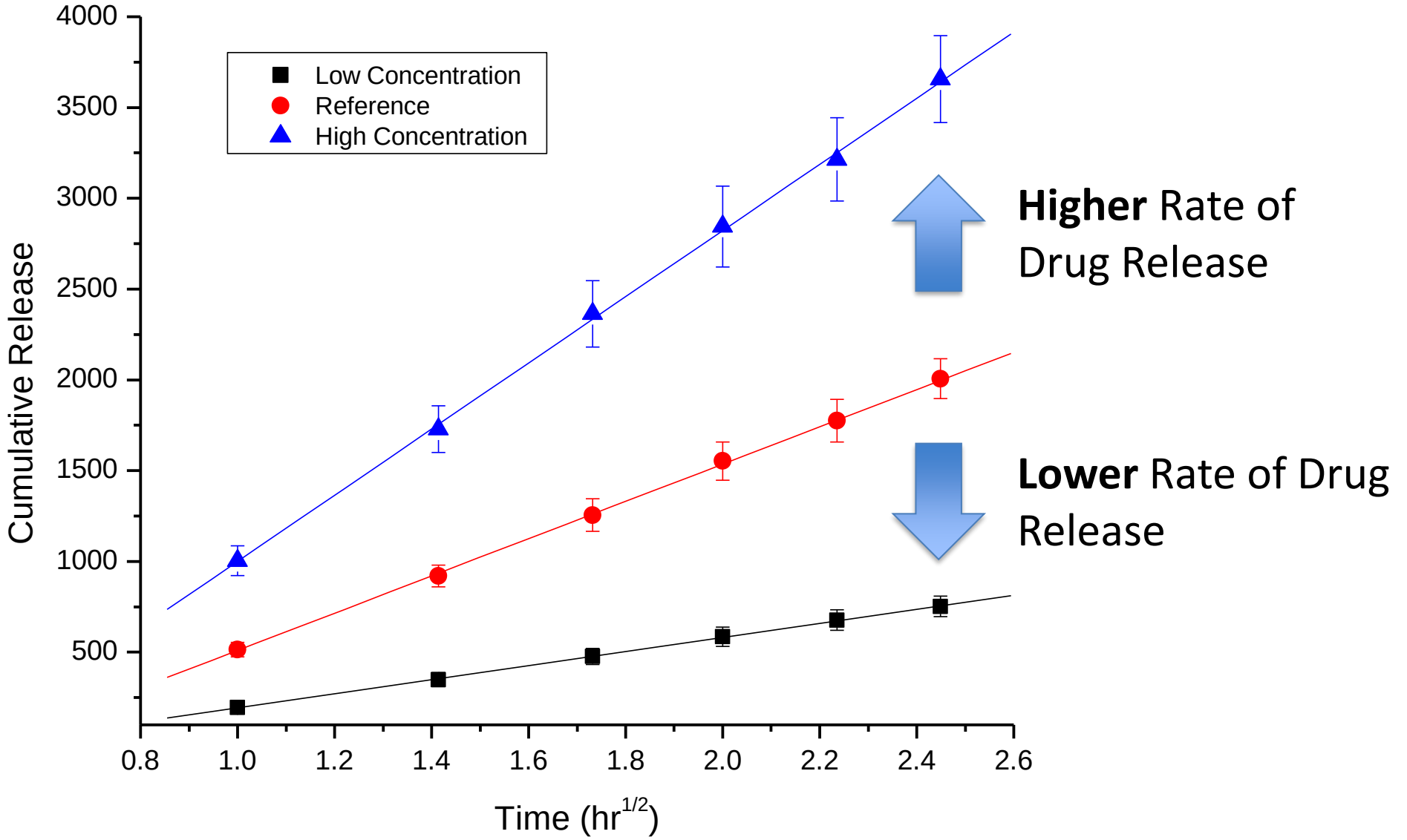
IVRT Method Validation Considerations

- Dose Depletion
  - The average recovery of released drug in the receptor solution is characterized in each diffusion cell as the accumulated amount of drug in the receptor solution over the IVRT duration.
  - This is expressed as a percentage of the amount of drug in the applied dose.
  - The mass balance distribution of drug between the product dose and the receptor solution, and associated recovery (dose depletion) are calculated and reported.

IVRT Method Validation Considerations

- Discrimination Sensitivity, Specificity, and Selectivity
  - Determinations are based upon a minimum of 1 run of 6 diffusion cells for each of three strengths of product – the reference and two altered strengths.
    - 1 run of ‘low’ test product at -50% strength
    - 1 run of reference product at nominal strength
    - 1 run of ‘high’ test product at +50% strength
  - Allowance may be made if the high test product is not feasible to formulate without substantial reformulation.

IVRT Method: Sensitivity



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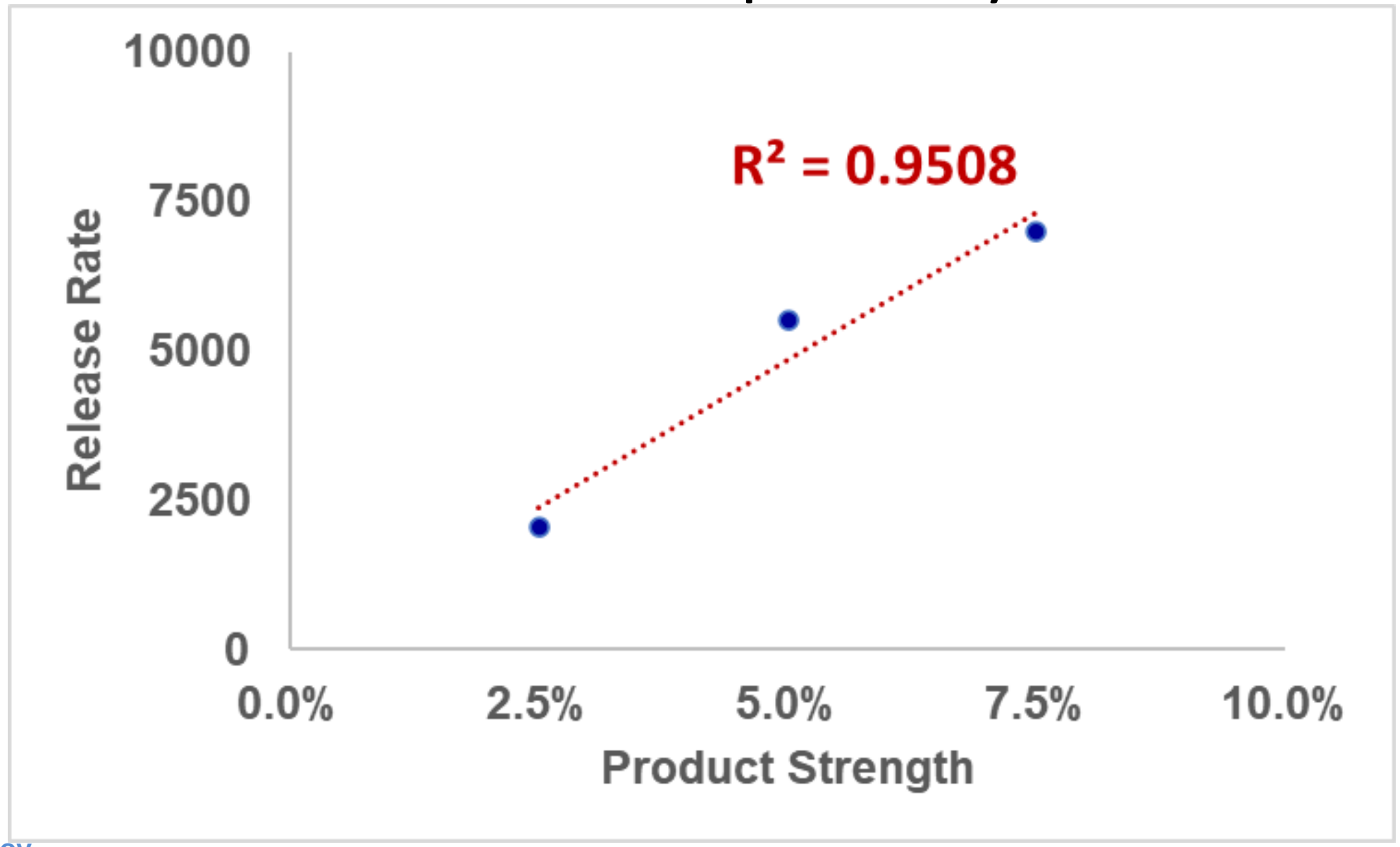
IVRT Method: Specificity



IVRT Method Validation Considerations

- Sensitivity and Specificity

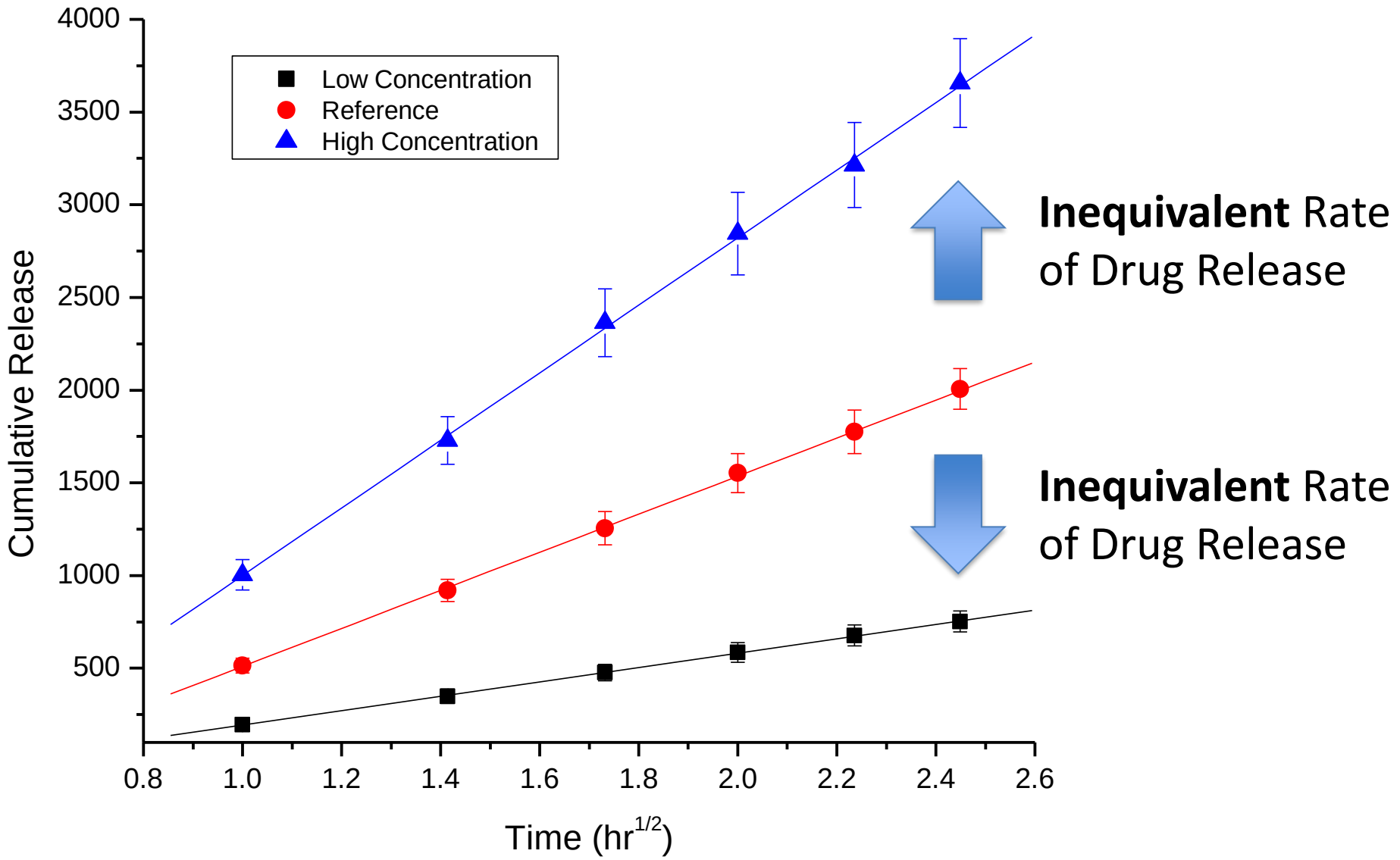
  Sensitivity is the ability to detect changes in the release rate as a function of drug concentration in the formulation.

  – If the IVRT method consistently identifies higher or lower rates of release for test products with increased or decreased drug concentrations, respectively, relative to the reference product run in parallel on the same day, the IVRT method will be determined to be sensitive.

  Specificity is the ability to monitor proportional changes in the release rate as a function of drug concentration in the formulation.

  – Specificity is reported as the linearity (ideally an r^2 value of ≥ 0.95) of the correlation of product concentration to average IVRT release rate (slope).

IVRT Method: Selectivity



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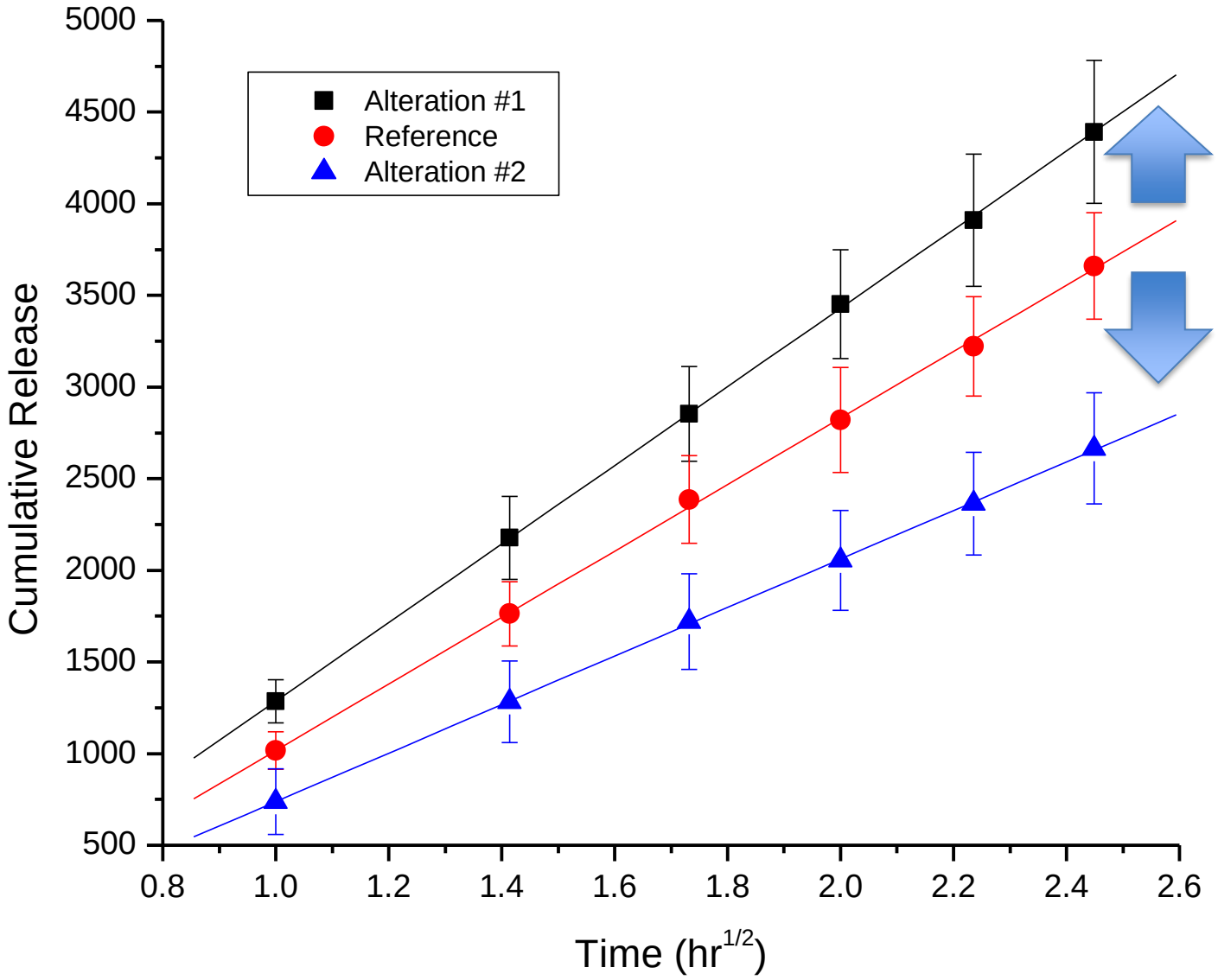
IVRT Method: Supplemental Selectivity



Inequivalent Rate of Drug Release

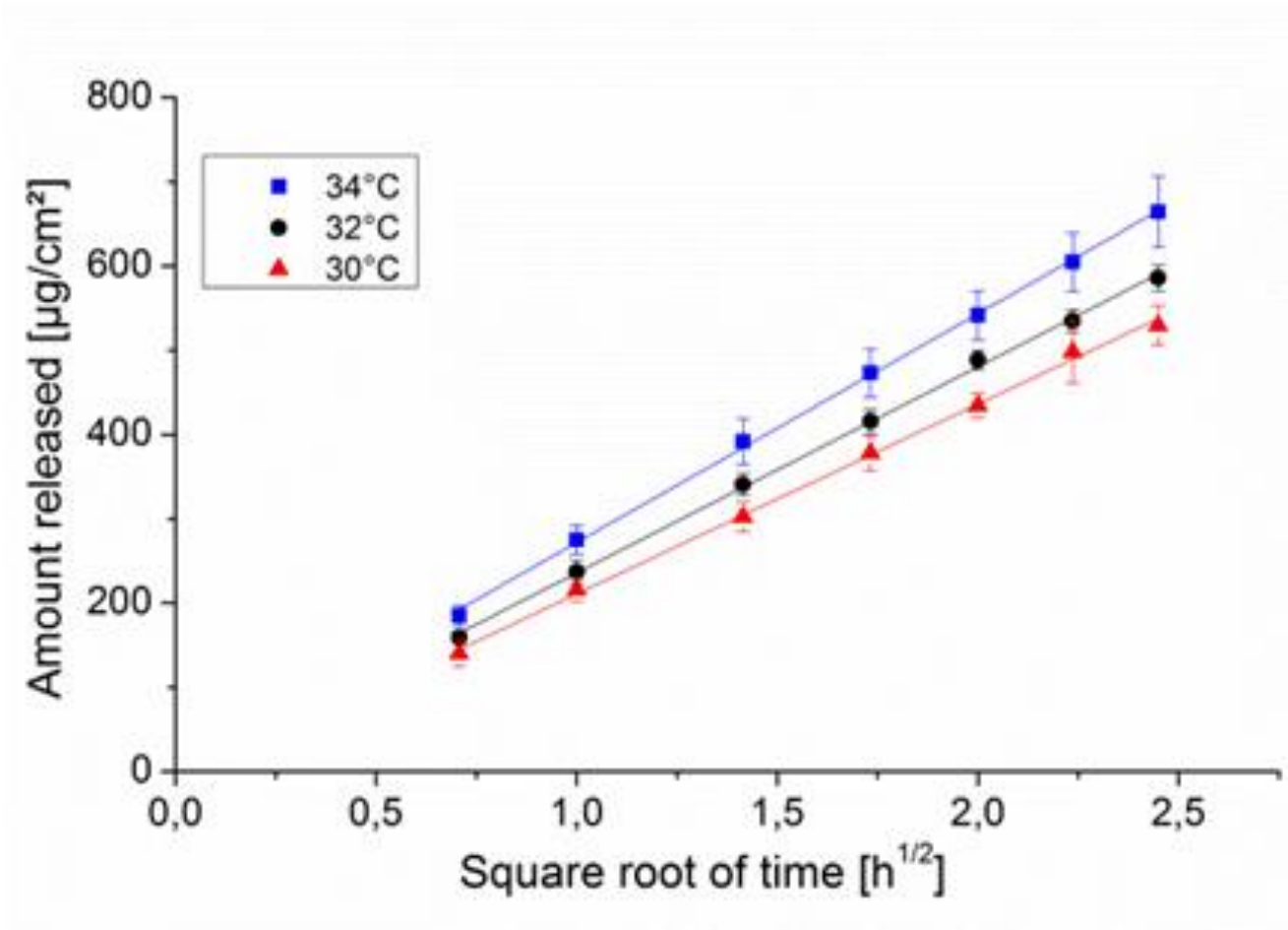
Inequivalent Rate of Drug Release

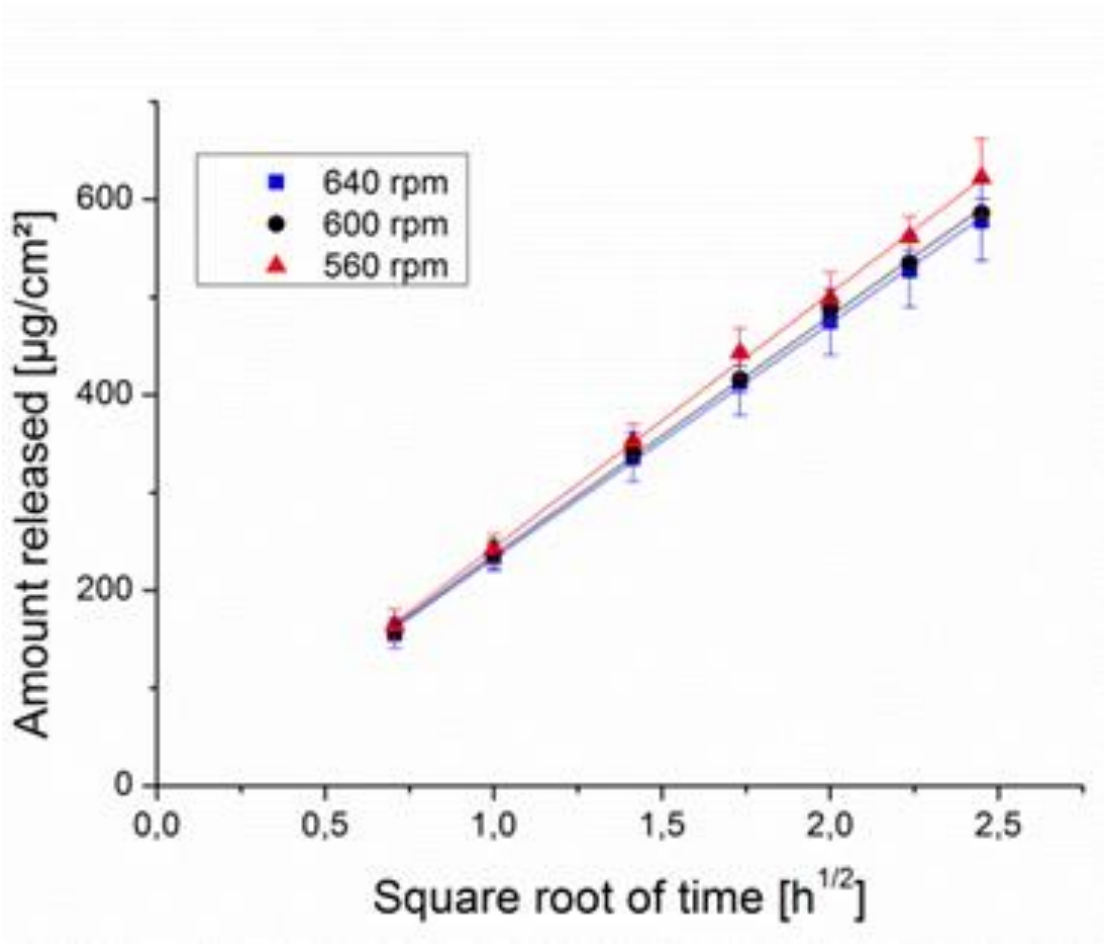
Data from personal research performed in collaboration with Dr. Thomas Franz and Paul Lehman

IVRT Method Validation Considerations

- Selectivity
  - Selectivity is the ability of the IVRT method to accurately identify **in-equivalent** product performance between the reference product compared with test products that have been intentionally altered.
  - Alterations include different drug concentration, excipients, excipient concentration, or manufacturing processes.
  - Determination of (in)equivalence is by the USP <1724> statistical approach.
  - The ability of the IVRT method to accurately identify **equivalent** product performance for the reference product to itself across different days/runs supports both, the reproducibility and selectivity of the IVRT method.
  - **Supplemental selectivity** is performed similarly, with test formulations at the same strength as the reference product, which have been altered in composition or manufacturing process, as relevant.

IVRT Method: Robustness





Data from personal research performed in collaboration with Dr. Thomas Franz and Paul Lehman

IVRT Method Validation Considerations

- Robustness
  - Determinations are based upon the release rate (average slope) of a minimum of 1 run of 6 diffusion cells for that variation, all using the same lot of reference product.
  - The IVRT method may be considered robust to a variation If the average slope of that robustness run is within ± 15% of the average slope of the Accuracy/Precision and Reproducibility runs.
  - If not, that parameter may be identified as a critical parameter of the IVRT method.
  - Robustness testing encompasses
    - Temperature variations (i.e., - 1°C and +1°C relative to 32°C ± 1°C)
    - Dose volume variations (e.g., +10% and -10% in the dose volume)
    - Receptor solution variations (e.g., change in composition and/or pH)
    - Mixing rate variation (i.e., differences in stirring speed, or without stirring).

IVRT Method Validation Approach (Example)

- Study Phase 1
  - HPLC analytical method validation
- Study Phase 2
  - Membrane binding/inertness characterization
  - Receptor solution solubility characterization
- Study Phase 3

– DAY 1:	1 run of 6 cells with reference product	Linearity, P&A, Reproducibility
– DAY 1:	1 run of 6 cells with 'low' test product	Sensitivity, Specificity, Selectivity
– DAY 1:	1 run of 6 cells with 'high' reference product	Sensitivity, Specificity, Selectivity
– DAY 2:	1 run of 6 cells with reference product	Linearity, P&A, Reproducibility
– DAY 2:	1 run of 6 cells with 'altered 1' test product	Supplemental Selectivity
– DAY 2:	1 run of 6 cells with 'altered 2' reference product	Supplemental Selectivity
– DAY 3:	1 run of 6 cells with reference product @ 32°C	Linearity, P&A, Reproducibility
– DAY 3:	1 run of 6 cells with reference product @ 31°C	Robustness
– DAY 3:	1 run of 6 cells with reference product @ 33°C	Robustness

- Study Phase 4

– 1 run each with reference product at +10% and -10% in the dose volume	Robustness
– 1 run each with reference product at +10% and -10% in the receptor composition	Robustness
– 1 run each with reference product at +10% and -10% in the receptor stirring (or none)	Robustness

IVRT Pivotal Study Considerations

- IVRT pivotal studies are routinely performed in a manner compatible with USP <1724>
- A single lot each of a designated Reference and Test product are evaluated and compared
- The objective is to determine whether the Test product is equivalent to the Reference product in performance
- The rate of release of the API measured in vitro serves as a surrogate for product performance
- It is understood that in vitro release as measured by this method does not directly correlate with clinical bioavailability or bioequivalence

IVRT Pivotal Study Considerations

- General Study Design and Procedures
  - One Test and one Reference product lot are each prepared for dosing to six replicate VDC utilizing a validated IVRT methodology
  - The diffusion cell membrane equilibrated to 32°C ± 1°C prior to dosing, confirmed, and maintained at this temperature throughout the test duration
  - Dosing is performed in a pattern alternating between Test and Reference
  - Following dose application, receptor solutions are collected at timed intervals over a defined period (e.g., 6 hours) as specified in the IVRT method
  - Receptor solution samples are stored according to a validated method and then analyzed for drug concentration by a validated HPLC sample analysis procedure
  - The rate of release of the API(s) (slope) is calculated for each VDC

IVRT Pivotal Study Considerations

- General Study Design and Procedures (Continued)
  - The in vitro release rates for the Test and Reference products are compared utilizing a Wilcoxon Rank Sum/Mann-Whitney rank test
  - A 90% confidence interval for the ratio of the median in vitro release rate for the test set over the reference set is computed, expressed in percentage terms
  - If this 90% confidence interval falls within the limits of 75% to 133.33%, the release rates of the test and reference sets are considered equivalent
  - Otherwise, the test and reference sets are not considered to have demonstrated equivalence at the first stage of testing
  - A second stage may be performed with an additional 12 replicate VDC dosed with each, Test and Reference product lots, and the statistics for equivalence are recalculated (18x18)
  - The results of the second stage of testing are not likely to change the determination of equivalence based upon the first stage, unless the 90% confidence interval from the first stage overlapped just slightly outside the 75% - 133.33% range by a few percentage points

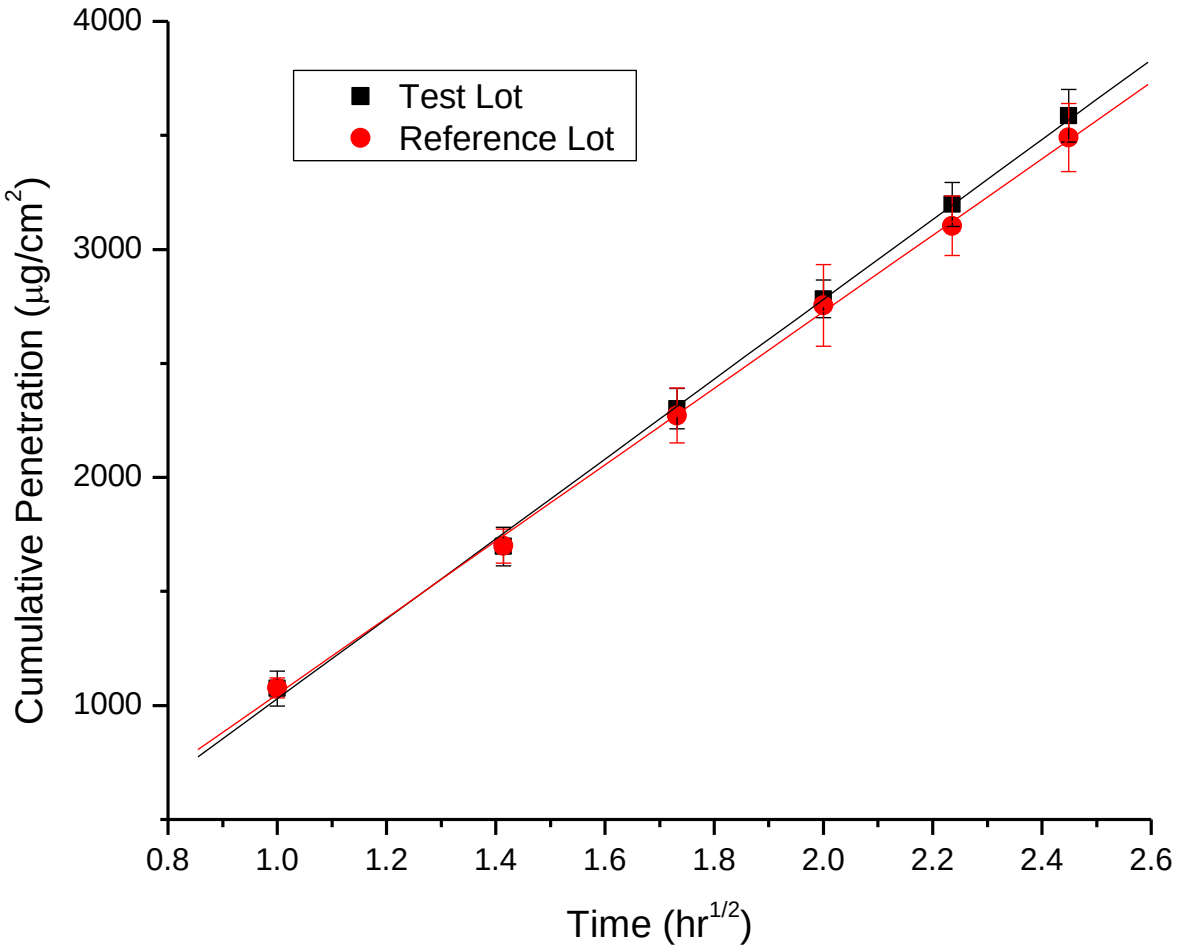
IVRT BE Study Considerations

- IVRT Pivotal Studies for the purpose of supporting bioequivalence may include:
  - Controlled Procedures for Blinding of Test and Reference Products
  - Controlled Procedures for Un-Blinding of Test and Reference Products
  - Statistical Randomization for Dosing Scheme
  - Statistical Randomization of Retention Product Tube selection
  - Controlled Room Archival Procedures for Retention Product

IVRT BE Study Considerations

- Handling and Retention Product Samples
  - Refer to 21 CFR 320.38, 320.63 and the following Guidances for Industry, as applicable, regarding considerations for retention of study drug samples:
    - Handling and Retention of BA and BE Testing Samples (May 2004)
    - Guidances for Industry: Compliance Policy for the Quantity of Bioavailability and Bioequivalence Samples Retained Under 21 CFR 320.38(c) (August 2020)
  - Refer to 21 CFR 320.36 for information on requirements for maintenance of records of BE testing.
  - Retention samples should be randomly selected from the drug supplies received prior to dispensing during the IVRT study in which the test and reference topical products are compared.
  - Experimental observations that may have the potential to influence the interpretation of the study results, as well as any protocol deviations, should be reported.

IVRT Pivotal Study (Example)



Reference Product	Test Product	Lower Limit	Upper Limit	Pass/Fail
(Details Redacted)	(Details Redacted)	100.881 %	109.068 %	Pass

IVRT Quality Management System (QMS)

- IVRT validation and pivotal studies are conducted within a QMS that includes documented procedures for
  - Study personnel identification, training, qualification, and responsibilities
  - Study management and study management personnel responsibilities
  - Quality control (QC) and QC personnel responsibilities
  - Quality assurance (QA) and QA personnel responsibilities
  - Utilization of SOPs, study protocols, and study reports
  - Maintenance and control of the study facility environment and systems
  - Qualification and calibration of instruments and computerized systems
  - Good documentation practices
  - Maintaining suitable records that facilitate the reconstruction of study events
  - Archival of study records

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