



Biopharmaceutical Relevance of In Vitro Release and Skin Permeation Studies in Topical Product Development

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Human Skin: an effective barrier



«Brick and mortar» model of the *stratum corneum* (horny layer) according to **Elias** (1981), to understand barrier function:

Topical Skin Delivery

Key: to deliver effective amount of drug into the right target site of the skin

Topical dosage forms are design to deliver **therapeutically effective amount of drug** into the targeted skin layers for the treatment of skin diseases.



Skin Delivery: a sophisticated phenomenon!



The Efficacy of Topical Semisolid Dosage Forms

Keywords: Diffusion and Partitioning

Topical dermatological products are mostly semisolid formulations including ointments, creams, lotions and gels.

The pharmacological action for a topical semisolid dermatological formulation depends on the efficacy of the **consecutive three main steps**:

Release and Diffusion

- the release of the drug molecule from the vehicle or delivery system (diffusion and release)

Diffusion

- the transport of the drug molecule through the stratum corneum (**diffusion**)

Partitioning

the uptake of drug in viable epidermis and dermis (partitioning)



Topical semisolid dosage formscomplex formulations!

- However, semisolid dosage forms are complex formulations.
 - They differ in excipient composition or dosage form (gel versus cream, for instance), amongst which the partitioning and diffusion of the drug substance into and across the skin may be altered.
 - The rate of release of drug molecule from a semisolid topical formulation could be affected by its physical and structural properties.

Topical semisolid dosage formscomplex formulations!

The physical and structural properties of a topical semisolid dosage form depend upon various factors:

- Size and shape of dispersed particles
- Droplet size of the internal phase
- Physical state of drug substance (polymorphism)
- Interfacial tension between the phases
- Partition coefficient of the drug substance between the phases
- Rheology of the product
- Manufacturing process.

Measurement of drug release

The first step: drug release from the semisolid topical products

□ The drug substance **must be released**, before it can diffuse into and become bioavailable in the skin.

- □ The release of a drug substance from the dosage from is crucial for efficacy of the topical dermatological semisolid products.
- □ The measurement of drug release from the formulation is important to topical drug product development for ensuring its quality.

In vitro Release Test (IVRT)

- □ In vitro release testing (IVRT) is used to monitor the release and diffusion of drug substances from semisolid dosage forms.
 - The principle is to determine the diffusion of the drug substance from a semisolid matrix across a membrane into appropriate receptor media.
- Although the release rate measured by IVRT may not reflect the in vivo fate of drug delivery, IVRT can determine
 - differences between release rates which may be occur due to formulation changes and
 - differences in various physicochemical properties of the drug product.

In vitro Permeation Test (IVPT)

- IVPT are also widely used for the development of topical dermatological products.
- The flux measurement following IVPT provide useful in vitro data during the development of a formulation for the selection of the best formulation.
- IVPT provide a more significant insight on the products in vivo performance.

IVRT & IVPT: Apparatus



USP <1724> Semisolid Drug Products-Performance Tests 2013

Apparatus: Franz Diffusion Cells

- Diffusion cells, such as Vertical Franz Diffusion Cells model is regarded as the most valid *in vitro* model for evaluating *in vitro* drug release and skin permeation from topical preparations.
- Experimental set-up
 - Receptor media selection: the solubility of drug substance in the receptor phase must be sufficient to maintain sink conditions.
 - Membrane selection: synthetic membranes act as inert support rather than a barrier (IVRT), human, porcine skin or artificial membranes resembling human skin, full thickness or dermatomed skin samples (IVPT)
 - Donor: dose application (finite/infinite vb)
 - Sampling periods and volume
- Validation method: mechanical calibration and performance verification testing
- Data analysis: Higuchi kinetic model , steady-state flux measurement

IVRT & IVPT: Experimental Setup

The most discriminant test conditions are recommended in a IVRT for semisolid dosage forms.



IVRT: Validation

□ Recently, an integrated approach to qualify and validate an **IVRT** method for acyclovir cream 5% was performed.

- methodological, (individual parameters of IVRT)
- and other critical components of the test system

(apparatus and laboratory qualification, HPLC method validation and IVRT method validation)

IVRT apparatus qualification	IVRT method validation	Qualification of the laboratory	HPLC-UV method validation
 Capacity of the cells Diameter of the orifice Temperature of the receptor medium Temperature on the membrane surface Speed of the magnetic stirrer Dispensed sampling volume 	 Membrane inertness Receptor medium solubility Linearity Precision and reproducibility Sensitivity Specificity Selectivity Robustness Recovery 	 Intra-run variability Inter-run variability Product sameness testing 	 Selectivity and specificity Linearity Accuracy Precision and robustness Stability LOQ and LOD

IVRT & IVPT: Data Analysis

The amount of drug released from the sample at different time intervals is quantified and calculated (Q):

$$Q = \left\{C_n V + \sum_{i=1}^{n-1} C_i S\right\} / A$$

- Q : the cumulative amount of the drug released per surface area of the membrane (mcg/cm²)
- C_n : the concentration of the compound (μ g/mL) determined at nth sampling interval
- V : the volume of individual Franz diffusion cell
- C_i : the sum of concentrations of the compound (μ g/mL) determined at sampling intervals 1 to n-1
- S : the volume of sampling aliquot
- A : the surface area

IVRT

- The release rate is the slope of the line described by *Q* values versus per square root of the time.
- Non-parametrical statistical method used for the comparion of the slopes
- Apparent amount <30 %
- Acceptance limit: 75-133.33%

Ι ΥΡΤ

- The flux is the slope of the line described by Q values versus the time.
- Permeability coeficient (Kp) is calculated from donor drug concentration and calculated flux.
- Different statistical analysis used for the comparison of the slopes.

IVRT: Selection of membrane: filter interference

- Various synthetic membranes are used in IVRT to separate the donor and receptor compartment.
 - (e.g., cellulose acetate/nitrate/mixed ester, regenerated cellulose, or polytetrafluoroethylene)
- Synthetic membranes should
 - act as an inert support rather than barrier
 - provide least resistance to drug diffusion (*non-rate limiting*)
- The selection of appropriate membrane is crucial in the design of IVRT.
 - the membrane must be compatible with formulation and
 - no interference with the drug substance.



Compound	MW (g/mol)	Log P	Hydrogen acceptor group	Hydrogen donor group	Formulation Type
Acyclovir	225.21	-1.59	7	3	Cream
Ketoprofen	254.285	3.1	3	1	Gel
Flurbiprofen	244.261	4.16	2	1	Gel







Regenerated Cellulose Membrane

Mixed Cellulose Membrane

Cellulose Acetate Membrane

Kocabaş Ö, Kahraman E. and Güngör S. unpublished data



Kocabaş Ö, Kahraman E. and Güngör S. unpublished data

Acyclovir:

 The hydrogene bonds occur between hydroxyl and amine groups of the regenerated cellulose membrane and acceptor and donor groups in acyclovir. Therefore, the release of acyclovir across regenerated cellulose membrane was lower than those of other membranes.

Ketoprofen:

 The hydrogene bonds do not intensively form between the regenerated cellulose membrane and ketoprofen due to its less numbers of acceptor, donor groups. The release rate of ketoprofen across regenerated cellulose membrane is similar to those of other membranes.

Flurbiprofen:

 The hydrogene bonds could generate more strongly between hydroxyl groups in the regenerated cellulose membrane and, also fluorine atom in flurbiprofen in comparison with the oxygen atom of ketone in ketoprofen. This hydrogene bond is much more effective in compared to acceptor, donor groups of drug.



Regenerated Cellulose Membrane



Mixed Cellulose Membrane

Alclomethasone



Hydrogene acceptor group: 5 Hydrogene donor group: 3 MW: 521,047 g/mol Log P: 3.2

Kahraman E., Akdilek N. and Güngör S. unpublished data



□ Alclomethasone forms hydrogene bonds *via* only donor groups in the mixed membrane, whereas both of acceptor and donor groups in the drug are capable of formation of the hydrogen bonds, in the case of the regenerated cellulose membrane.

□ Thus, drug release through the regenerated cellulose membrane is lower than that of mixed cellulose membrane.

IVRT: Regulatory Perspectives

Scale Up and Post Approval Changes for Nonsterile Semisolid Dosage Forms, SUPAC- SS, 1997

- Initially, IVRT has been recommended by the FDA to assess the similarity of a topical semisolid product following post-approval changes.
- This guidance regulates the use of IVRT as evidence of product therapeutic and pharmaceutical sameness between the initially approved product and post-change product.

IVRT: Regulatory Perspectives (SUPAC- SS, 1997)

- □ IVRT is recommended to satisfy that consistent product performance will be performed after Level 2 changes:
 - changes in the components or composition (between 5 and 10% of excipients, with the total additive effect of all excipients changes being no more than 10%)
 - changes in manufacturing process (such as the rate of mixing, the rate of cooling, operating speeds and holding times)
 - changes in manufacturing equipment (different design or principle)
 - changes in batch size equipment (Scale-up/scale-down of manufacture, more specifically changes in batch size beyond a factor of 10)
 - changes in manufacturing site
- IVRT can be used to requalify the product, if the ratio between the release rate for the post-change product over the release rate for the initially approved product falls within 75% to 133.3%.

IVRT: Regulatory Perspectives USP Chapter <1724> (2013)

Chapter <1724> -USP36/NF31, First Supplement Semisolid Drug Products-Performance Tests, 2013.

□ **IVRT** has been recognized as performance test by USP.

- Provides general information on assessment of in-vitro performance for semisolid drug products.
- Describes the apparatus to be used for performance test of topical dosage forms
- Describes details in developing IVRT with respect to the selection of key parameters

IVRT & IVPT: Regulatory Perspective Biowaiver option

- For the approval of a generic drug product, regulatory agencies mostly require clinical endpoint studies (*«gold standard»*) to validate the therapeutic equivalence of test product compared to referans product.
- A waiver from the clinical endpoint studies is generally granted for solutions. However, the waiver option is not applied to semisolid dosage forms.
- In recent years, FDA has published a few draft guidance in which the demonstration of IVRT and IVPT data has been recommended.

Topical Generic Products vs Reference (RLD)

Definition of Q1, Q2 & Q3



Potential differences in Q3 demonstrate the physical properties of a semisolid dosage forms such as **its rheological behavior** and **in vitro drug release pattern**.

The parameters affecting microstructure (Q3) of a topical product

- Appearance
- Physicochemical features of the formulation (such as pH, specific gravity)
- □ The characteristics of drug substance (such as its polymorphic form, globule size distribution, particle size and its distribution)
- Rheological behavior of the semisolid formulation
- Drug release characteristics of the formulation

The microstructure similarity of the dosage form can be demonstrated based on the data obtained from welldesigned IVRT method.

Workshop on

"Evaluation of Topical Drug Products-Current Challenges in Bioequivalence, Quality, and Novel Assessment Technologies" March 12–14, 2013, Rockville, Maryland, USA

- □ The workshop organized by Product Quality Research Institute (PQRI) and cosponsored by AAPS, EUFEPS, FIP and USP.
 - The Decision Tree Strawman of Topical Products for the assessment of bioequivalence was proposed.
 - The decision tree was based on the Q1, Q2, and Q3 evaluation of the generic and RLD products.
 - According to the proposal; If the generic and RLD products are qualitative (Q1) and quantitative equal (Q2), and Q3 equivalence can be shown by in vitro tests, such as IVPT and IVRT: clinical study may be waived.

Yacobi et al., Pharm.Res. (2014) 31: 837-846.

The Strawman Decision Tree of Topical Products for the Assessment of Bioequivalence



Miranda et al. European Journal of Pharmaceutical Sciences 122 (2018) 264–272.

Topical Drug Classification System- TCS

International Journal of Pharmaceutics 491 (2015) 21-25



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The Biopharmaceutics Classification System (BCS) for oral immediate release solid drug products has been very successful; its implementation in drug industry and regulatory approval has shown significant progress. This has been the case primarily because BCS was developed using sound scientific judgment. Following the success of BCS, we have considered the topical drug products for similar classification system based on sound scientific principles.

- TCS is proposed by V. Shah and his co-workers as a classification system to justify biowaiver along with in vitro drug release characteristics of topical drug products.
 - The classification is based on the qualitative (Q1) and quantitative composition (Q2), microstructure arrangements of matter (Q3) of a topical product and in vitro drug release (IVR).

Topical Drug Classification System- TCS

Topical drug products are classified into 4 classes.



Shah et al. International Journal of Pharmaceutics, 509 (2016) 35-40.

Regulatory Perspectives: Application of IVRT&IVPT data on Assessment of Bioequivalence

- FDA Product-Specific Guidances for Generic Drug Development released for topical products in recent years.
- FDA published a few draft guidance for some topical products including *«acyclovir cream»*, *«dapsone gel»* and *«ivermectin cream» «benzyl alcohol»* have been to compare the bioequivalence of generics and RLD.
 - It has been proposed that the combination of IVRT and IVPT using an *ex vivo* human skin model can be used with providing other supplementary specifications.

IVRT & IVPT: Biowaiver options for Topical Semisolid Dosage Forms-FDA Draft Guidances

Draft Guidance on Acyclovir

This draft guidance, v Administration (FD and is not binding or	vhen finalized, will repres	ent the current thinking Draft Guida	of the Food and Drug nce on Ivermectin
requirements of the a the Office of Generia	This draft guidance, who Administration (FDA and is not binding on	en finalized, will repres	ent the current thinking of the Food and Drug Draft Guidance on Dapsone
	requirements of the ar the Office of Generic	This draft guidance, w Administration (FDA	hen finalized, will represent the current thinking of the Food and Drug Draft Guidance on Benzyl Alcohol
		requirements of the a the Office of Generic	This draft guidance, once finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind the FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the Office of Generic Drugs.

Compound	Strength	Dosage Form	1 st Option	Date
Acyclovir	5.0%	Ointment	IVRT	2016
		Cream		2010
Ivermectin	1.0%	Cream	IVRT/IVPT+PKE	2016
	5.0%	Gel	IVRT/IVPT+PKE	2016
Dapsone	7.5%	Gel	IVRT/IVPT+PKE	2017
	10.0%	Cream	IVRT	2017
Benzyl alcohol	5.0 %	Lotion	IVRT	2017

IVRT & IVPT: Biowaiver options for Topical Semisolid Dosage Forms- FDA Draft Guidances

- □ It is asked to demonstrate that Generic formulation (test) must provide Q1/Q2 criteria
 - the test and RLD formulations are qualitatively and quantitatively the same
- The test and RLD formulation must have the same physicochemical characteristics
- The test and RLD must exhibit the same drug release rate and permeaton profile obtained from a validated IVRT and IVPT methods.
- The test and RLD products are bioequivalent based upon an acceptable in vitro pharmacokinetic (PK) study with one lot each of the test and RLD products (dapson, ivermectine).
- □ Additional requirements that need to be presented:
 - Comparison of physical and structural similarity for the test and RLD products should include the detailed physicochemical characterizations of test and RLD products.
 - The tests have to been performed in a minimum of three batches of the test and three batches of RLD.

IVRT & IVPT: Biowaiver options for Topical Semisolid Dosage Forms- FDA Draft Guidances

□ Physicochemical characterizations for test and RLD products:

- Assessment of appearance
- Analysis of the polymorphic form of the drug substance in the topical product.
- Analysis of particle size distribution and crystal habit with representative microscopic images at multiple magnifications.
- Analysis of the rheological behavior which may be characterized using a rheometer that is appropriate for monitoring the non-Newtonian flow behavior of semi-solid dosage forms.
 - A complete flow curve of shear stress (or viscosity) vs. shear rate should consist of multiple data points across the range of attainable shear rates, until low or high shear plateaus are identified.
 - Yield stress values should be reported if the material tested exhibits plastic flow behavior.

IVRT & IVPT: Biowaiver options for Topical Semisolid Dosage Forms- FDA Draft Guidances

- Although, clinical endpoint studies must be carried out to prove the therapeutic equivalence, the draft guidance on micronized tretinoin gel (0.1% and 0.04%) indicates the importance of IVPT using dermatomed human skin to support pharmaceutical equivalence of the product.
- ❑ When the systemic absorption is observed following topical application, PK endpoint studies could be used to prove the bioequivalence of the topical products, however, the quantification of drug substances is limited due to the low concentrations.
- □ In addition to the PK evaluation, **IVRT** and **IVPT** should be also carried out (*dapson and ivermectine*).

EMA: Concept paper on the development of a guideline on quality and equivalence of topical products, 2014



2 December 2014 EMA/CHMP/QWP/558185/2014 Committee for Medicinal Products for Human use (CHMP)

Concept paper on the development of a guideline on quality and equivalence of topical products

EMA published a **concept paper** (EMA/CHMP/QWP/558185/2014)

Clinical trials are in principle necessary to demonstrate therapeutic equivalence, but other models may be used, if adequately validated.

In many cases, these other models have exhibited poor accuracy, sensitivity, reproducibility, in vitro in vivo correlation and have been unable to provide convincing evidence to predict the therapeutic equivalence.

EMA: Concept paper on the development of a guideline on quality and equivalence of topical products, 2014

The extended concept of pharmaceutical equivalence combined with additional measures of equivalence:

- □ The quality data with the relevant reference medicinal product, including
 - qualitative and quantitative composition
 - microstructure
 - physical properties
 - product performance and administration
- □ The comparative data need to be representative, the test methods appropriate and validated, and
- □ Equivalence acceptance criteria should be adequate.
 - however, critical evaluation has to be considered: (as stated in the EMA concept paper),
 - drugs with narrow therapeutic index,
 - drugs with significant side effects and with drugs that require additional safety requirements

IVRT & IVPT: European assessment public reports

- Epiduo Gel (Adapalene 0.1% and Benzoyl peroxide 2.5%) (2007):
- **IVPT** data is presented as a part of the clinical development phase in new drug product application.
- In addition to PK data and clinical endpoint data, IVPT performed to prove the absence of significant differences between the dermal absorption of both drugs when administered in combination or as single product.

Tapin Cream (Lidocaine 25 mg/g and Prilocaine 25 mg/g) (2009):

- IVP across human skin of Tapin cream and the reference product (Emla) cream was compared. The accumulated amount of both drug substances (prilocaine and lidocaine) in the skin layers (*stratum corneum, epidermis and dermiş*) was also measured following in IVPT.
- The stability of the test product was also demonstrated with IVPT study in which the metabolite of prilocaine was added into the formulation and, it was shown that the accumulation of degradation product in the skin was not significantly different than that of the reference product.
- In addition, a pivotal, comparative clinical study was also performed.

IVRT & IVPT: European assessment public reports

- Diclofenac-Ratiopharm 30 mg/g Gel Diclofenac AbZ30 mg/g Gel. (2018):
- In addition to new clinical data, IVRT and IVPT data were also submitted.



Pharmaceutical equivalence

According concept paper on the development of a guideline on "quality and equivalence of topical products (EMA/CHMP/QWP/558185/2014)", pharmaceutical equivalence tests have been carried out comparing quality data of diclofenac sodium 30 mg/g gel with the relevant reference medicinal products, including qualitative and quantitative composition, microstructure, physical properties, product performance, and administration. The tests were performed with the EU product "Solaraze 3% gel" and also with the US product "Solaraze 3% gel".

Diclofenae sodium 30 mg/g gel and the EU reference product "Solaraze 3% gel" are considered to be pharmaceutically equivalent since:

- The pharmaceutical form is the same.
- The active substance content is the same.
- All the excipients used in both products are qualitatively same and quantitatively similar.
- Quality characteristics are essentially the same. In particular, the applicant has provided compared in-vitro diffusion studies showing that the diffusion profiles of the generic and EU reference product are comparable. In addition, the submitted in-vitro skin permeation study shows a similar cumulative permeation profile between reference and EU reference product.
 - Administration is the same.

In addition, the applicant submitted a supportive therapeutic equivalence study investigating the efficacy and safety of Diclofenac sodium 30 mg/g gel in comparison with placebo and with the US reference product (see Section III.3).

IVRT & IVPT: EMA Approach

- Compare to the FDA approach, IVRT and IVPT data without clinical studies for bioequivalence assessment of topical products in European countries has not been used to grant biowaiver.
- On the other hand, IVRT and IVPT has been accepted to evaluate the bioequivalence of topical products with other supplementary clinical studies.



- IVRT and IVPT has been considered a valuable tool in development of topical semisolid formulation.
 - to examine the effect of formulation components on the release of drug substance from different topical vehicles
 - to monitor the release rate and diffusion of drug products from semisolid dosage forms
 - to provide a scientific rationale for formulation selection.
- □ IVRT is used to assess the effect of following Level 2 , scale up and post-approval changes (SUPAC-SS).
- □ IVRT is also accepted as a robust test for quality assessment of topical semisolid dosage forms (USP <1724>)
- □ **IVRT** and **IVPT** have been added into the FDA draft guidances to demonstrate BE of a few generic topical products.
- EMA concept paper also states as a tool IVRT and IVPT on topical drug development.

Conclusion

- Although understandable some limitations of both test, IVRT and IVPT are considered as robust tests to determine the topical product performance that contribute the efficacy (*bioavailability*) of the drug substances in the skin.
- As mentioned by Shah and co-workers, "the well-established and time honored scientific principles" that emphasize IVRT and IVPT methods, assist the application of these in vitro methods for the bioequivalence assessment of topical products.
- In recent years, the addition of IVRT and IVPT into the FDA draft guidances to validate bioequivalence of a few generic topical product, further boosts their application in future.

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Thank you for your attention!

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