Received: 25 September 2021 | Accepted: 9 March 2022

DOI: 10.1111/srt.13154

ORIGINAL ARTICLE

WILEY

Flow-through versus static in vitro percutaneous penetration at 50 years: Possible relevance for bioequivalence

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Abstract

Objective: Compare the relevance of flow-through versus static diffusion cells data as relates to bioequivalence.

Methods: Search was conducted on PubMed and Google Scholar. Keywords utilized: static cells, flow-through cells, percutaneous permeation, percutaneous absorption, dermal absorption, and types of permeation.

Results: Fifteen articles were identified with no consistent significant differences between flow-through and static diffusion cells identified; any differences could exist for two main reasons. (1) Sampling time differences and (2) physical chemistry (lipophilic vs hydrophilic) of the penetrant examined.

Conclusion: Even though there was no consistent significant difference observed, labs have generally adapted to the method they regularly use, which is usually stated in their respective articles. Well-designed multicentered prospective comparative experiments should clarify potential advantages and disadvantages for each. For flow-through systems, the flow rate that most approximates to comparable in vivo data for animals and humans may be preferable.

KEYWORDS
diffusion, flow-through, Franz cell, in vitro percutaneous absorption, permeation, static

1 | INTRODUCTION

Oh et al.,[1] while discussing regulatory/scientific aspects of in vitro permeation, summarized the status of static versus flow-through in vitro skin permeation methodology; this manuscript details the published data on the sameness or differences thereof.

In World War II England, Traeger utilized static diffusion cells to initiate and eventually popularize in vitro percutaneous experiments—with the main aim of understanding chemical warfare agent percutaneous penetration, toxicity, and decontamination.

Post World War II, Francis Marzulli, of the United States Food and Drug Administration, worked with Traeger and developed/published on the advantages of flow-through system.[2]

On Marzulli's retirement, Robert Bronaugh continued Marzulli's FDA laboratory and published extensively on in vitro penetration, utilizing the flow-through system. In the 1960s, Bronaugh summarized his comparative data on static versus flow-through systems.[3]

In 1969, William Crutcher compared data on flow-through versus static systems and concluded that flow-through systems could yield higher penetration rates with higher perfusates.[4] In the intervening 51 years, four additional data sets have become available.

Scientists often prefer to use the static cell to measure permeation due to the simpler procedure, but with the introduction of flow-through systems, the question remains: is there a significant difference between the two? We strive to answer this for several reasons. Finding the differences or similarities between both methods will help

TABLE 1 Amount of various compounds permeated through static and flow-through cells

Compound (Author, Reference #)	Static	Flow-Through	Statistical Significance
Saline[a] (Crutcher, [4])	0.2 ml	0.1 ml	N/A
Water[b] (Bronaugh, [3])	4.4 ± 0.2 (5)	4.3 ± 0.4 (5)	N/S
Cortisone[b] (Bronaugh, [3])	6.3 ± 0.8 (8)	8.5 ± 0.9 (5)	N/S
Benzoic acid[b] (Bronaugh, [3])	48.6 ± 3.8 (6)	45.9 ± 7.6 (5)	N/S
Tritiated water[a] (Clowes, [5])	11.8 ± 0.8 (40)	7.1 ± 0.6 (12)	0.000
Tritiated water[b] (Clowes, [5])	23.0 ± 1.7 (15)	19.8 ± 1.0 (9)	0.000
Mannitol[a] (Clowes, [5])	4.0 ± 0.6 (15)	8.7 ± 2.0 (10)	0.000
Mannitol[b] (Clowes, [5])	0.3 ± 0.1 (18)	0.4 ± 0.1 (12)	0.012
Acetamidophenol[b] (Hughes, [6])	15.4 ± 6.5	27.8 ± 12.8	0.049
Propionylamidophenol[b] (Hughes, [6])	20.9 ± 23.8	19.1 ± 6.8	N/S
Phenol[b] (Hughes, [6])	97.6 ± 0.5	95.4 ± 1.6	0.007
Cyanophenol[b] (Hughes, [6])	75.4 ± 6.2	87.3 ± 5.4	0.002
Nitrophenol[b] (Hughes, [6])	70.0 ± 3.7	75.1 ± 7.3	N/S
Chlorophenol[b] (Hughes, [6])	87.4 ± 2.2	90.5 ± 2.2	0.019
Iodophenol[b] (Hughes, [6])	80.4 ± 12.0	73.3 ± 16.0	N/S
Pentyloxyphenol[b] (Hughes, [6])	73.3 ± 8.7	71.2 ± 9.3	N/S
Heptyloxyphenol[b] (Hughes, [6])	65.3 ± 12.6	45.9 ± 17.5	0.037
Acetaminophen[c] (Waters, [7])	88.9%	84.9%	N/A
Caffeine[c] (Waters, [7])	98.2%	98.9%	N/A
Carbamazepine[c] (Waters, [7])	70.6%	69.7%	N/A
Cimetidine[c] (Waters, [7])	57.2%	57.1%	N/A
Diclofenac[c] (Waters, [7])	85.5%	88.0%	N/A
Fenoprofen[c] (Waters, [7])	92.4%	95.9%	N/A
Fluconazole[c] (Waters, [7])	93.8%	92.8%	N/A
Flurbiprofen[c] (Waters, [7])	91.8%	93.7%	N/A
Fosinopril[c] (Waters, [7])	34.7%	35.7%	N/A
Gemfibrozil[c] (Waters, [7])	90.6%	92.7%	N/A
Haloperidol[c] (Waters, 7)	62.8%	49.7%	N/A
Ibuprofen[c] (Waters, [7])	92.0%	94.1%	N/A
Indomethacin[c] (Waters, [7])	86.4%	95.4%	N/A
Ketoprofen[c] (Waters, [7])	89.7%	83.7%	N/A
Leflunomide[c] (Waters, [7])	86.1%	89.1%	N/A
Lidocaine[c] (Waters, [7])	86.7%	88.1%	N/A
Linezolid[c] (Waters, [7])	87.4%	91.2%	N/A
Meloxicam[c] (Waters, [7])	77.6%	76.6%	N/A
Moexipril[c] (Waters, [7])	26.6%	37.8%	N/A
Naproxen[c] (Waters, [7])	91.9%	95.1%	N/A
Phenylbutazone[c] (Waters, [7])	94.4%	97.5%	N/A
Piroxicam[c] (Waters, [7])	80.7%	82.7%	N/A
Quinine[c] (Waters, [7])	93.9%	96.7%	N/A
Theophylline[c] (Waters, [7])	97.9%	98.4%	N/A
Zolmitriptan[c] (Waters, [7])	70.9%	68.0%	N/A

Data presented in milliliters, mean ± standard deviation, and % permeated.
Number of determinations are shown in parentheses for data if provided.
[a]Permeated through human skin.
[b]Permeated through rat skin.
[c]Permeated through human intestine.
For statistical significance, $p < 0.05$.
If significant, p-value is shown - calculated by a two-sample T-test.
N/A, not applicable; N/S, not significant.

understand which method most accurately represents human skin in vivo and which may be most appropriate for bioequivalence evaluations.

2 | MATERIALS AND METHODS

We utilized the online databases PubMed and Google Scholar with the following search terms: static cells, flow-through cells, percutaneous permeation, percutaneous absorption, dermal absorption, types of permeation, and the Dermatology Research Library of the University of California Medical School.

3 | RESULTS

Table 1 summarizes 5 of the data sets, 3 of which provided comparative data: both Bronaugh[3] and Clowes[5] determined that there was no significant difference. However, only 1 data set, Hughes,[6] found a significant difference for 5 phenol cells and no significant difference for the remaining 4 phenols.

Hughes stated that his results, an anomaly in the common trend the other publications reported, varied based on the phenol used. At times, the flow-through or static cell was favorable, while at others, the difference was minimal. For example, the difference between the phenols and chlorophenol absorption was less than 4%. On the other hand, the difference between cyano-, heptyloxy-, and acetamidophenol was 14%, 30%, and 55%, respectively.

4 | DISCUSSION

There is often an insignificant difference in results between flow-through diffusion cells and static diffusion cells but with exceptions for two main reasons. 1) Time differences in sample collection. As stated in the introduction, the only previous comparative database was compiled by Crutcher,[4] who concluded that the differences were minimal. 2) The chemicals themselves. Highly water-soluble chemicals might compare favorably with the static system to the flow-through, whereas highly lipophilic might not. This could have affected the published results.

A limitation of the data is that most of the observers did not provide what was in the skin itself which might have changed with increasing flow rates. Publication bias cannot be ruled out.

IVPT is a standard methodology for bioequivalence of topical drugs. Yet, the details of how the assay is performed in terms of static versus flow-through are not as clear—and specifically, is there a preferred flow-through rate that will be discriminating between the innovator and generic drug for these studies?

The FDA and other regulatory bodies have simplified determining whether a change in manufacturing is sufficiently minimal to not require additional in vivo absorption studies and/or additional analytic chemistry and/or toxicologic investigations. This guidance is abbreviated SUPAC.[8] On August 18-20, 2021, the FDA hosted a three-day workshop on IVPT and IVRT methods.[9] These bridging studies provide efficiency in manufacturing, another motivation for valid IVPT studies.

5 | CONCLUSION

Taken together, the data show no clear consistent difference concerning results when using flow-through diffusion versus static diffusion cells. It is clear, however, why scientists and laboratories have adapted to one type of cell instead of using both. As the field has developed, several corporations have customized their own systems, making comparisons more complex. However, due to the varying results depend on the physical chemistry of the test compounds, determining the optimal conditions that most resemble in vivo human data should benefit from a wider selection of chemicals with varying physicochemical properties. Further, requirements for determining bioequivalence may or may not be identical to other needs, such as closely replicating in vivo data.[9]

AUTHOR CONTRIBUTIONS

K.A. and H.M. were responsible for all aspects.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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How to cite this article: Ajjarapu K, Maibach HI. Flow-through versus static in vitro percutaneous penetration at 50 years: Possible relevance for bioequivalence. Skin Res Technol. 2022;28:540–543. https://doi.org/10.1111/srt.13154