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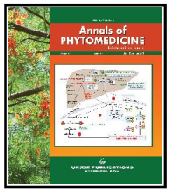

Review Article : Open Access

A review of *in vitro* release test for method development and validation of semi-solid dosage forms

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Abstract

The *in vitro* release test (IVRT) is a widely used method that can be employed to evaluate the performance attributes of a semisolid topical dosage form, which includes creams, gels and ointments. Additionally, IVRT has been utilized in bioequivalency studies after post-approval modifications to a product, have been indicated, as well as in the detection of formulation changes. The combined effects of multiple physiochemical properties, such as particle or droplet size, viscosity, matter microstructure arrangement, and dosage form aggregation state, can be reflected by the *in vitro* release test (IVRT). The purpose of developing and validating this method was to ensure its robustness, accuracy, selectivity, reproducibility, precision, and reliability. To effectively evaluate the similarity between topical products and to verify that the applied method has the required discriminatory power to detect differences between products, should such differences exist, the IVRT method was demonstrated using a novel approach. A state-of-the-art method for developing and assessing topical formulations is an automated IVRT diffusion system. Additionally, the possible advantages are examined.

1. Introduction

A technique for developing and assessing semi-solid dosage forms, such as gels, ointments, and creams, is the *in vitro* release test (IVRT). Therefore, a straightforward, dependable, and repeatable technique is required to determine the characteristics of release of a topical semi-solid dosage form using a diffusion cell. The IVRT method is used to validate and check the accuracy and precision, also known as repeatability and reproducibility.

According to the FDA's SUPAC-SS guidance, the cumulative impact of several physical and chemical parameters, such as the rheological characteristics of the dosage form and the solubility and particle size of the active ingredients, can be represented by an *in vitro* release rate. For semisolid products, IVR testing is therefore a useful test to assess the sameness of a product under particular expansion and post-approval modifications. Understanding the physiochemical properties and efficacy of topical preparations is also beneficial. An effective IVR testing methodology must replicate the kinetics of skin penetration, taking into account the drug concentration analysis of the donor, membrane, and receptor medium (Klein *et al.,* 2018). The most popular IVRT method can be applied with receptor solution, applied formulation, synthetic membrane, or human cadaver skin. Its open chamber design is reminiscent of the franz diffusion cell system. Additionally, IVRT is a USP compendial method used for semisolid dosage form performance testing and a very helpful tool in product development.

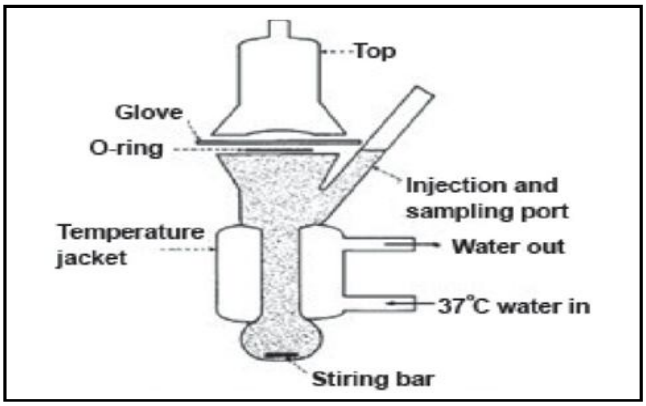

Figure 1: Schematic of franz cell diffusion model.

For IVRT examinations, the industry standard is the franz diffusion cell shown in Figure 1. The vertical cell is composed of two chambers: a receptor chamber below that contains a receptor medium and a donor chamber above that contains the test product. A synthetic membrane (made of nylon, polycarbonate, polysulfone, polytetrafluoroethylene, cellulose acetate/nitrate/mixed ester, *etc.*) divides the two chambers. The membrane separates the donor compartment, which contains the test product, from the receptor compartment, which is filled with collecting medium. PBS, or phosphate-buffered saline, is the recommended collection medium, however, it may not always be sufficient for an efficient IVRT technique.

The assaying of successively obtained samples of the receptor medium facilitates the observation of drug diffusion across the

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membrane from the semisolid product. An aliquot of medium is taken out of the receptor compartment at predetermined intervals, usually using HPLC, to analyse the drug content. After every sampling, the receptor compartment is refilled with the new medium. To guarantee that an IVR testing method is trustworthy and repeatable, it is essential to establish a robust, repeatable IVR Testing method that is sensitive enough to reveal variations in the physicochemical and rheological properties of the formulation and stress conditions. However, there are not many well-established techniques to ensure that data released *in vitro* is accurate, consistent and repeatable.

USP general hapter <1724> requirements for semisolid preparations are fulfilled through IVR testing. Tests of performance for semisolid pharmaceuticals. The diffusion cell is a stable and reproducible technique for measuring the release of medication from semisolid dosage forms (Miranda *et al*., 2019).

2. IVRT principle and theory

When released from the semisolid formulation, molecules diffuse from an area of higher concentration to an area of lower concentration. Since the amount of drug released per unit area (μg/cm^2) is proportional to the square root of time, plotting the average cumulative release against the square root of time should produce a straight line (as required by SUPAC-SS2). The slope of this line is used to calculate flux amount released/cm^2/h. The drug's release is examined over a six-hour period, which is the usual application time for a topical product (Olejnik *et al*., 2012).

Semisolid dosage forms release drugs according to a simplified version of the Higuchi equation (Equation 1).

Equation 1

Q= 2C0. (Dt/n)1/2

where

Q = Amount of drug released per unit area of application

C = Initial concentration of drug

D = diffusion coefficient of drug t = time

Since human skin acts as a natural barrier against external substances, medication administered *via* semisolid forms, like gels, ointments and creams, needs to penetrate the skin to be effective (Higuchi, 1962).

3. Factors affecting the analysis of the sample in IVRT

The following are some of the factors affecting sample analysis:

3.1 Temperature

An *in vitro* system's temperature must be regulated to minimise changes in the experiment's development and maintain the target temperature. The analysis of the samples will be impacted by temperature fluctuations. The highest temperature that a skin surface was able to tolerate *in vivo* was approximately 42-43°C. As temperatures got higher, or as TDS thermal resistance increased both *in vivo* and *in vitro*, the temperature differential between the skin and TDS surface grew. As the temperature dropped, so did the lag time.

3.2 Volume of samples

The collected sample's volume has an impact on the analysis of the samples; if all the samples are obtained from various cells and have various volumes, the flux rate will also vary.

3.3 Sampling time

Plotting actual linearity requires at least six sampling time points. If, the samples were not collected promptly, the total amount of compounds found within the membrane after a given exposure period, the total quantity of compounds that cross the membrane over an extended sampling period and the total quantity of compounds that cross the membrane in a specified period will not be examined. There will be no determination of the flux rate.

3.4 Flow rate of receptor solution

One important consideration is the volume of receptor solution pumped into the receptor compartment of the cell. By altering the receptor compartment's volume and flow rate, several analyses of the samples can be carried out. The volume pumped through the cell in a particular amount of time needs to be substantially larger than the receptor compartment's volume to maintain sink conditions (Himanshu Gupta and Patil, 2020).

3.5 Amount of applied dose

Sample analysis is greatly impacted by the dosage administered; a rise in drug concentration also results in a rise in flux (Zhang and Yu, 2017; Mounika and Hymavathi, 2021).

3.6 Occluded donor chamber

If, air exposure occurs during the experiment and the donor chamber is not blocked, the release rate may change (Khatana *et al*., 2023).

4. IVR process description

The donor chamber is coated with a thick layer of the semisolid product, which is then in contact with a medium within a reservoir (*i.e.,* receptor chamber) as part of the process.When the drug material diffuses into the reservoir from the formulation through an inert, highly permeable support membrane, the reservoir acts as a receptor (Raghavi *et al.,* 2023; Akella Anuradha *et al*., 2023). Following that, samples are taken out of the receptor chamber at predetermined intervals. The cumulative amount released is plotted for every cell, along with the amount of drug released (μg/cm^2) at each sampling time (Klein *et al*., 2010).

5. IVRT method development

For IVRT to be successful, there must be sufficient drug transport from a test material across a membrane and into a receiving medium. The goal of identifying the best experimental parameters is to choose the right membrane, receiving medium and sampling schedule by taking into account the physiochemical properties of an API. The industry's increased focus on the development of semisolid formulations has ruled the current demand for more dependable and repeatable IVRT methods and prompted further research into how the technique can be further optimized (Fan *et al*., 2007; Divya Singh and Sanjeev Singh, 2021).

Since one of IVRT's objectives is to serve as a replacement for *in vivo* testing, PBS is usually the preferred option. Therefore, it is preferable to use the medium that most closely mimics the relevant physiological

fluid. Surfactants, liposomal preparations, alcohols, and other substances can also be utilized to improve the API's solubility in the receiving medium (Zhang *et al.,* 2004).

5.1 Developing an IVRT method generally involves the following steps:

i. The initial step in creating a receptor medium that keeps sink conditions and avoids saturation is soluble screening. Sink conditions occur when the receptor medium "exceeds 10% of Cs (drug solubility in the releasing matrix) at the end of the test" and has a relatively "high capacity to dissolve or carry away the drug." An "appropriate receptor medium, such as an aqueous buffer for water-soluble drugs or a hydroalcoholic medium for sparingly water-soluble drugs" may be used as a reasonable starting point, according to the SUPACSS FDA guidance.

ii. Select a membrane with minimal drug binding, no leachable, no rate-limiting effects on drug release and repeatability. Synthetic membranes come in a variety of options, with pore size, thickness and hydrophobicity all being controllably variable. Water makes up a large portion of many topical products, so hydrophilic/ hydrophilized synthetic membranes are commonly utilized.

iii. Select an appropriate testing apparatus. The franz diffusion cell assembly, which is most frequently used to test semi-solid topical dosage forms (USP <1724>).

iv. Number of samples: Since three-cell systems have been employed, a minimum of six cells (SUPAC-SS) is advised for characterizing the release rate (profile) of an API from semisolid products.

v. The receptor medium's temperature is typically adjusted to 32 ± 1°C to mimic the skin's natural surface temperature. The recommended temperature for vaginal drug products is 37 ± 1°C (Tiffner *et al.,* 2021).

vi. Sampling application

A finite or pseudo-infinite dose will be administered to the test system's donor compartment, dependent upon the methodology employed to construe the release date, for the following reasons:

- A pseudo-infinite dose is preferable to a finite dose.
- It simplifies diffusion kinetics and lessens variability caused by minute mass variations in finite dosing.
- Maintains the "30% rule".
- Facilitates the administration of the dose.

vii. Selecting time points to assess the drug release profile from the product (SUPAC-SS advises using at least five-time points).

viii. A suitable, sensitive and validated analytical method will be used to analyse the samples and ascertain the amount and concentration of drug released.

ix. Further parameters that are assessed include the temperature of the receptor solution, the method of formulation addition, the speed at which the sample is stirred and the volume of the sample.

Utilising this method requires knowledgeable analytical scientists with the ability to create and validate (LC-MS) or (HPLC) (Olejnik *et al*., 2012).

5.2 Method parameters

- **Product dosage amount:** To achieve steady-state kinetics, use a pseudo-infinite dose.
- **Stirring rate:** A high rate of stirring could alter the interface between the receptor media and the membrane, which could impact diffusion. The medication in the receptor solution might not be uniform, if it is too low.
- **Sampling times**: The first sample should be taken during the steady state and before excessive drug depletion and the last sample should be taken after the diffusion cell has reached a steady state of diffusion (after the lag time).
- **Apparatus**: The IVRT apparatus is the franz cell, or vertical diffusion cell (Subhamalar *et al.,* 2023).
- **Membrane inertness**: The inertness of the membrane refers to its low drug binding, free resistance to diffusion and chemical compatibility with the receptor solution.
- **Receptor solution**: The drug's stability and solubility in the receptor solution (Dandamudi, 2017).

5.3 Method validation

For many years, batch-to-batch uniformity testing, product certification and validation have been accomplished with IVRT. It has been utilised more recently to improve formulations while developing new products (Vimal Raj and Sumithra, 2023).

The IVRT method's validation ensures that the test procedure can show the way drug release rates alter in response to modifications in formulation composition, batch or ingredient sources and/or manufacturing process. Many parameters are evaluated during the IVRT method validation process, such as accuracy, mass balance, dose depletion, precision (repeatability, intermediate precision, robustness) and recovery. The selection of validation parameters ought to be predicated on the needs of the product (Sesto Cabral *et al*., 2015).

5.4 *In vitro* release rate comparison test

To ensure an unbiased comparison test, the sample position inside the blank Franz cells should be randomly assigned. The Wilcoxon Rank sum/Mann-Whitney rank test is used to determine an appropriate 90% confidence interval (CI), which forms the basis of the analysis and entails a non-parametric assessment of release rate by statistical mean (Kanfer isadore, *et al.,* 2017).

5.5 Application of IVRT

- Evaluation of the product's regularity in its appearance of scale-up and post-approval modifications**.**
- Optimization of product performance (*i.e.,* release profile) during formulation development.
- Assessment of product stability/batch-to-batch uniformity QC test.
- Initial screening of the *in vivo* performance of lead candidates before proceeding with clinical endpoint *in vitro* permeation studies (Wellington *et al*., 2023).

5.6 Benefits of IVRT

An easy-to-use tool for assessing topical drug products' *in vitro* release profiles is the validated IVRT method. For regulatory filings, the technique can be used to guarantee both optimal thermodynamic activity and consistent batch-to-batch performance and quality over the formulation's shelf-life. The method can also be used to determine whether registration batches produced at various manufacturing locations or using various manufacturing processes are identical (Bashaw *et al.,* 2014).

Following obtaining regulatory approval, the IVRT method can help support site and other product changes while guaranteeing the quality of ongoing production.

The method ensures regulatory agencies can receive the necessary information while optimizing formulation development and contributing time and cost efficiencies. It does this by providing predictive estimates regarding a drug's *in vitro* performance. The ability to regulate the experiment's conditions in a way that is not feasible when utilizing human subjects is another important benefit of conducting research *in vitro*. This makes it possible for laboratory procedures to become more standardized (Shah *et al.,* 2022, Hymavathi, 2021).

6. Conclusion

IVRT is an effective method for comparing the underlying sameness in product quality characteristics and characterizing this rate of API release. IVRT is useful in developing and optimizing formulations while letting you provide complete quality control too. It helps in monitoring the production quality with time. IVRT is becoming more and more valuable in determining how well a developed formulation will work to deliver a drug to or across biological tissue. It is becoming more and more popular as a tool for product specification and development.

Since IVRT is likely to become a regulatory requirement in a few years, as IVRT is likely to become a regulatory requirement in a few years. They are used as a quality control tool to track batch-to-batch consistency and the product shelf-life programme in late development and the post-marketing phase. The primary benefits of this approach stem from its exceptional sensitivity and discriminating ability, which can frequently capture the physicochemical variations of topical semisolid pharmaceutical products. The bioequivalence of a product can be demonstrated through *in vitro* release testing in comparison to a pre-approved product. While vertical diffusion cells were previously used for this testing, automation is now being used to increase throughput.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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