A REVIEW ARTICLE: IN VITRO RELEASE TECHNIQUES FOR TOPICAL FORMULATIONS

Prakashkumar B. Modi1*, Nehal J. Shah1,2
1School of Pharmacy, RK University, Kasturbadham, Rajkot-360020, Gujarat, India.
2Pharmaceutical Chemistry, Indubhai Patel College of Pharmacy and Research Centre, Dharmaji-388430, Gujarat, India.

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ABSTRACT
An in vitro release testing (IVRT) plays an important role in several area during the topical product development. This review article represents different IVRT techniques such as Franz diffusion cells method, Enhancer cells method, Flow through cell method and Parallel artificial membrane permeability assay (PAMPA) and their application in topical formulation development. It can be used as an in vitro surrogate for in vivo performance that can guide formulation development, sameness study for bio-waiver and ascertain the need for bioequivalence tests in generic topical formulations. PAMPA can be utilized for evaluation of drug retention factor, permeability parameters and permeability coefficients during product development.

Corresponding author
Prakashkumar B. Modi
School of Pharmacy,
RK University, Kasturbadham,
Rajkot-360020, Gujarat, India.
pbm1980@gmail.com
+919000198312


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INTRODUCTION

Topically applied drug products fall into two general categories: those applied to achieve local action and those applied to achieve systemic effects after absorption through the skin into the blood circulation. Local action can occur at or on the surface of the application site (e.g., stratum corneum, ocular epithelium), in the underlying tissues (e.g., epidermis and or dermis) and on subcutaneous tissues (e.g., muscle or joint). Topically applied drug products include, but are not restricted to creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, solutions, and transdermal delivery systems (Patches). Historically, it has been challenging to carry out bioavailability/bioequivalence studies for semisolid drug product for the purpose of demonstrating the continued quality, efficacy and “sameness” of the product upon instituting certain changes in manufacturing process or substitution of excipients. Alternatively, in vitro tests such as determination of solubility, particle size, rate of release of the active ingredient and product homogeneity have been the main measures of product uniformity and quality equivalency. Among these, in vitro release testing (IVRT) of active ingredient has drawn much attention as a result of the issuance of the SUPAC-SS (Guidance for industry for nonsterile semisolid dosage forms). Many manufacturers of topical drugs have devoted significant resources to develop and validate IVRT during the drug product development process.

This review article represents in vitro release test importance, principle and factors which contribute in drug release (diffusion) mechanism. Article represent the use of different IVRT techniques in topical formulations development.

IVRT Principle

Human skin is natural barrier to outside agents. Drug substances administered through semisolid preparations (creams, ointments, and gels) must penetrate the layers of the skin to have benefit. An appropriate in vitro apparatus for release-rate testing of topical products will mimic skin permeation kinetics, including donor, membrane, and a receptor medium that is analyzed for drug concentration.

IVRT principle is to determine the diffusion of active ingredient from the semisolid matrix, across a membrane, into an appropriate medium, representing the clinical use of semisolid dosage form as close as possible.

Fick’s first law of diffusion:

\[ J = -D \frac{\partial c}{\partial x} \]

where

- \( J \) = rate of transfer per unit surface area (flux)
- \( c \) = the concentration of the diffusing substance
- \( x \) = distance the substance travels at right angles to the plane and
- \( D \) = the diffusion coefficient in \((\text{length})^2/(\text{time})\)

Fick’s second law of diffusion:

\[ \frac{\partial^2 c}{\partial t^2} = D \frac{\partial^2 c}{\partial x^2} \]

Cumulative drug release (\(\mu g/cm^2\)) of analyte is determined by following equation.

\[
Q = \frac{[C_{n}V + \sum_{i=1}^{n-1}CiS]}{A}
\]

Where,

- \( Q \) = Cumulative amount of drug released per surface area of membrane (\(\mu g/cm^2\))
- \( C_{n} \) = Concentration of drug determined at \(n^{th}\) sampling interval
- \( V \) = Volume of individual Franz diffusion cell
- \( n-1 \)
- \( \sum_{i=1}^{n-1}Ci = \) Sum of concentration of drug determined at sampling intervals 1 through \(n-1\)
- \( S \) = sampling volume
- \( A \) = Surface area of diffusion

Factors affecting the release of drug

a) Particle size of API
b) pH of API
c) Incorporation of API in semisolid matrix
d) Solubility of API in semisolid matrix
e) Viscosity
f) Spread ability
g) Overall pH
h) Moisture content of dosage form
i) Presence of emollients and penetration enhancers
j) Effect of excipients on release of API from matrix
k) Compatibility of excipients with API and with environmental agents such as moisture, gases, to affect release of API.
l) Manufacturing process
m) Manufacturing site.
IVRT Techniques

Franz diffusion cell[2-7]

The Franz diffusion cell is the most popular technique for conducting in vitro release study. In vitro release rate comparison between pre-change and post-change products for approval of SUPAC related changes by using vertical diffusion cell procedure is require as per FDA’s guidance for industry on Scale Up and Post Approval Changes for Semisolid (SUPAC-SS) dosage forms. In such studies a receptor solution is placed into the receptor compartment, which is maintained at 32°C. An artificial support membrane is placed over the diffusion cell opening. The formulation is applied to the membrane (typically an “infinite” amount of formulation is applied), and the cell is capped (the surface of the formulation is usually left open to room air, not occluded). The cells are stirred with a magnetic stir bar, usually at a speed of 600 rpm. Samples are then withdrawn from the receptor solution at regular time intervals, typically over the course of 6-8 hours. Typical diagram of Franz diffusion cells is depicted in figure 1. It is made up of donor chamber and receptor chamber. Membrane is placed between donor chamber and receptor chamber. Sample is applied in donor chamber and over the membrane. Receptor chamber contains receptor medium, which is maintained at required temperature by heat circulation by water jacket.

![Figure 1 Typical diagram of Franz diffusion cell.](image)

Appropriate membrane and receptor medium selections are critical decisions in method development. Selection of the receptor medium depend on the solubility of drug substance and it may need to contain alcohol and surfactant for adequate solubility. Avoid air bubble formation at the interface with the membrane. Typically receptor medium such as phosphate buffer saline pH 7.40, phosphate buffer with different pH range, medium containing surfactants, hydro alcoholic medium etc. are preferable for the study. A synthetic membrane is used as an inert support membrane, which is placed between sample and receptor medium. Typically synthetic membranes such as Nylon, Teflon, Polysulfone, Cellulose etc. are preferable for the study. In vitro test may also possible without synthetic membrane depending on the nature of the drug product. The Franz cell has been used with and without synthetic membranes for some ointments, resulting in no difference in release rate results. The drug release characteristics usually follow the Higuchi model. As with transdermal products, the test temperature is typically set at 32°C to reflect the usual skin temperature. For vaginal creams, test temperature is set at 37°C.

For the product sameness, study can be conducted with 6 cells run each with reference and test product batches simultaneously. Each experiment can be run as follows:

```

T  R  T  
Cell 1 Cell 2 Cell 3

R  T  R  
Cell 4 Cell 5 Cell 6

```

Slopes are calculated for each cell based on cumulative amount release versus time. Slope ratio of T/R are calculated for all cells and ranked (from lower to higher value). 8th and 29th ranked slope ratios should fall between 75% and 133.33% indicate as product is equivalent.
Failure at first level triggers the second level of comparison, 4 additional runs of 6-cell each for reference and test products are carried out and slopes are computed. A total of 18 slopes for each batch is obtained and same T/R ratios are computed and ranked. Acceptance criteria for the sameness is 115th and 225th ranked ratios should fall within 75% and 133.33%.

Enhancer cells[8-10]

The enhancer cell technique is very similar to the paddle-over-disk method, where the enhancer cell rests in the bottom of a dissolution vessel, with a paddle stirring media above it (figure 2). The main difference in this technique, is the use of enhancer cell for in vitro release study with or without artificial membranes. The use of an artificial membrane can significantly reduce or eliminate problems associated with dissolution/dispersion of the formulation. One major drawback of this technique is the potential for the formulation to dissolve in the receptor medium, thus measuring dissolution rather than release. However, for formulations which do not dissolve/disperse in the receptor medium, this technique could be used to conduct in vitro release studies, using well-established, universal equipment. As with paddle-over-disk, this technique lends itself to full automation.

Figure 2 Typical diagram of Enhancer cells apparatus.

Inverted rotating cylinder:

Another technique which can be used is the inverted rotating cylinder (figure 3). This technique simply uses a machined, round cavity in a piece of Teflon or stainless steel as the holder of the drug product.

Figure 3 Typical diagram of Inverted rotating cylinder.

Once loaded with formulation, the cylinder can be lowered into a standard USP dissolution vessel, and rotated (typically at 50 rpm). This method does not use a membrane, and can only be used with formulation which do not dissolve or disperse in the receptor medium. This method has been shown to be quite useful for hydrophobic ointment bases containing solubilized drug[10].
Flow through Cell

The system consists of a reservoir containing the dissolution/release medium, a pump that forces the medium upwards through the vertically positioned flow-cell, and a water bath to control the temperature in the cell. It can be used for in vitro release study for topical formulations. The typical diagram of the instrument is depicted in figure 4.

![Flow through Cell Diagram](image)

Figure 4 Typical diagram of Flow through Cell.

Different types of cells are available for testing tablets, powders, suppositories, hard and soft-gelatin capsules, implants, semisolids and suppositories. For orally administered solid dosage forms, two different cells are described: the large cell (22.6 mm i.d) and the small cell (12 mm i.d.) that provide approximate volumes of 19 ml and 8 ml respectively for dissolution. Usually the bottom cone of the cell is filled with small glass beads (about 1 mm diameter) and one (about 5 mm diameter) is positioned at the apex to prevent material from descending into the inlet tubing. Different amounts of small glass beads can be used according to the experimental setup. The sample can be placed upon a holder but also can be placed on or within the glass-bead bed. For dispersed systems (i.e. suspensions, powders), mixing of the sample within the glass-bead bed has been reported.

The flow-through cell apparatus can operate in two different modes: 1) as an open system with fresh solvent from the reservoir continuously passing through the cell 2) as closed system where a fixed volume of liquid is recycled. The open system is selected for samples that require high volume of media (i.e. low solubility compounds), and the closed system is selected when a low volume of medium is required.

A filter is positioned at the inner top of the cell to retain undissolved material. Usually glass fiber filters are used (single or combination of different pore sizes). The use of glass wool is sometimes suggested for dosage forms with insoluble and sticky particles. Appropriate selection of the filter is required for efficient filtration and to avoid backpressure created by filter resistance.

Advantages:
1) Medium and flow rate can be changed within a single run.
2) Sink conditions can be maintained, due to the continuous flow of fresh medium, when the system operates in the open-loop configuration. This feature is important for the study of poorly soluble drugs.
3) Study of samples with low drug loading is feasible when the system operates in the closed-loop configuration, as small volume of medium can be used.
4) Different sample types and dosage forms can be studied.
5) Release from dosage forms over extended periods can be studied, as this set up eliminates the evaporation issue that can be observed with other apparatus.
6) The hydrodynamics inside the cell are not affected by media change and sampling, as can occur in traditional closed systems.

Parallel Artificial Membrane Permeability Assay (PAMPA)

The skin PAMPA system includes STIRWELL™ plates with a proprietary filter supporting an artificial membrane which simulates skin. Buffers, test compounds, hydration solutions and ancillary components are also supplied. Schematic view of PAMPA is depicted in figure 5, where sample solution is placed in top plate.

![Parallel Artificial Membrane Permeability Assay](image)
Skin PAMPA allows rapid screening of large numbers of drugs and preformulations at low cost. It incorporates both trans and paracellular permeation routes establishing good correlation with human skin penetration. This in vitro technique is used for screening of components or formulations based on permeability/penetration. It is used for determination of retention factor, permeability parameters and permeability co-efficient for large number of drugs.

Some of the corticosteroid standards evaluated for retention factor (R), permeation parameters $C_A(t)/C_D(0)$ and permeability coefficients ($\log P_e$) by PAMPA test with membrane containing 70% silicone oil and 30% isopropyl myristate and values are reported in table 1.

Table 1: Values of retention factor, permeation parameters and permeability coefficient by PAMPA test.

<table>
<thead>
<tr>
<th>Corticosteroids</th>
<th>R(%)</th>
<th>$C_A(t)/C_D(0)$</th>
<th>$\log P_e$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluocinolone acetonide</td>
<td>&lt;1</td>
<td>6.7 ± 1.2</td>
<td>-5.26 ± 0.15</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>4.2 ± 2.0</td>
<td>1.3 ± 0.8</td>
<td>-5.99 ± 0.10</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>4.7 ± 2.3</td>
<td>10.8 ± 1.5</td>
<td>-5.01 ± 0.17</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>1.5 ± 0.9</td>
<td>0.8 ± 0.5</td>
<td>-6.23 ± 0.09</td>
</tr>
<tr>
<td>Hydrocortisone acetate</td>
<td>&lt;1</td>
<td>10.0 ± 1.7</td>
<td>-5.07 ± 0.19</td>
</tr>
<tr>
<td>Mometasone furoate</td>
<td>81.0 ± 2.9</td>
<td>7.7 ± 1.5</td>
<td>-4.10 ± 0.15</td>
</tr>
</tbody>
</table>

Advantages of PAMPA:
1) correlate the data with human skin
2) available for ready to use
3) stable and cost effective
4) disposable, no washing.

CONCLUSION
This review article described In vitro release techniques such as Franz diffusion cell, Enhancer cell assembly, Inverted rotating cylinder, Flow through cells and PAMPA. Depending on the applicability and study requirements, these techniques can be utilized at different stages of the topical product development as screening tool, for product sameness study etc. PAMPA can be utilized for evaluation of drug retention factor, permeation parameters and permeability coefficient with different solvent system during the product development.

REFERENCES