White Paper

Title: **Diffusion Method Development Guidelines**

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Purpose

The purpose of this document is to provide the reader with guidelines on how to approach method development for diffusion cell systems. This document assumes that the reader is already familiar with the basic principles of diffusion and diffusion testing.

Definition(s)

In Vitro Permeation Test (IVPT) is a test designed to mimic biological conditions. It is primarily used for product development.

In Vitro Release Test (IVRT) is a test designed to determine product quality. It is primarily used for quality control of semisolids.

IVPT and IVRT

IVPTs and IVRTs serve very different purposes. The IVPT helps determine how the product will behave when applied. The IVRT is a quality control tool that monitors batch to batch uniformity.

IVPTs typically use natural membranes with a finite dose exposed to the atmosphere. The receptor media is biologically relevant. Open top cell tops or patch tops are recommended for IVPT testing.

Generally, IVPTs should have good in vitro/in vivo correlation (IVIVC). However, due to the use of natural membranes, significant variability is likely.

IVRT methods are developed to detect differences from batch to batch. IVRTs typically run with synthetic membranes because the focus of the test is on reproducibility. An organic water mix of receptor media is often used, and occluded (standard) cell tops are recommended for this type of receptor media. The results usually have little or no IVIVC, which results in limited use for product development. However, a sufficient IVRT method will show when any change in product occurs that may affect performance.

Equipment Selection

Method development also involves determining the best equipment to use for testing. Each apparatus type has its own advantages and disadvantages. If performance targets are available for the test, then those may be used to help determine which type of apparatus may be best suited.

Vertical Diffusion Cells (VDCs):

Advantages

- More flexibility during R&D because of the variety of cell tops. Novel membranes may be used as well as differing donor amounts.
    - Patch Top: Allows for dosage to be exposed to the atmosphere. Good for patches and IVPT testing.
    - Iontophoresis Top: Allows for iontophoresis methods that pass an electrical current through the membrane.
    - Open Top Cell Top: Allows for dosages ranging from 1-6 mL in volume.
    - Nail Holder Cell Top: Allows the use of finger or toe nails as membranes, as well as corneas for ophthalmic applications.
- Ideal for material or methods with lower release rates because of their smaller volumes.
- May reduce validation requirements because they are more established in the industry.

Disadvantages

- Sink conditions may be difficult to achieve because small cell volume may not be suitable for higher release rate products or methods.
- Heavy operator dependence leads to human error and mistakes. Additional operator training is usually required in order to be proficient with this instrument.

- May be difficult to automate certain methods.
    - 4 mL diffusion cells are recommended for manual methods only. The tubing rinse volume requirements may affect the sample if it exceeds the volume amount that can be removed from the diffusion cell without affecting the sample.
    - 7 mL or 12 mL diffusions cells are better suited for automated because their internal volume is large enough to allow for rinsing of the collector lines. **Figure 1** illustrates replacement media (the red dye) traveling through a 7 mL diffusion cell.

Figure 1: 7 mL diffusion cell with 2.0 mL of replacement media (red).

Cell Volume	**Maximum Volume Replaced without Dilution of Sample** (Rinse Vol. + Sample Vol.)
4 mL	2.0 mL
7 mL	2.5 mL
12 mL	5.0 mL

Table 1: Typical amount of replacement solution that can be used with vertical diffusion cells.

Immersion Cells

Advantages

- Plug and play compatibility with existing dissolution testers. No need to purchase additional equipment.
- Larger amount of receptor media allows for better sink conditions, even with materials and methods that have a high release rate. Receptor media volumes exceeding 100 mL are possible.
- Less operator training required because starting the test and sampling are very similar to dissolution resting procedures.

Disadvantages

- Immersion cell methods may require extra validation because the apparatus is not widely used in the industry.
- Minimum receptor media volume is 50 mL. For materials with a low release rate, this may create problems with detecting the API in solution.
- Donor chamber is restricted to 530 µL. For most cases, this should be an infinite dose (but not always).

- Limited flexibility for R&D. The immersion cells are primarily a QC tool designed to work with synthetic membranes with a 25 mm diameter.

Questions to Ask

While the method is still in the development process, some basic questions based on the targeted performance of the material will help in choosing the appropriate apparatus.

1. **How much material is needed for an infinite dose?**

   A pseudo infinite dose is required for IVRT methods in order to ensure that diffusion occurs in one direction (into the receptor media). Typically, this would mean that over the course of a diffusion test, not more than 30% of the API is released into solution.

Cell Top Type	Approximate Volume of Donor Chamber
Standard Occluded Cell Top	265 µL
Immersion Donor Chamber	530 µL
Open Top Cell Top	1-6 mL

Table 2: Approximate volumes of apparatus

2. **How much media is required for sink conditions?**

   If a material has a very high release rate, then attaining sink conditions in 6-10 mL of media may be difficult. Specific media requirements may not be determined, but if the product is projected to have a high release rate, a larger volume of receptor media will make it easier to attain the sink conditions.

3. **Will the equipment ever be used for different purposes?**

   If the equipment is in an R&D environment, it is beneficial to have access to different cell tops to allow for method development for vastly different products.

For environments that are already heavily geared toward dissolution testing, there may be significant cost savings when using the immersion cell. The immersion cell can be used with existing dissolution testers. Often, users require minimum retraining in order to adapt to immersion cell methods.

4. **Is there a desire to automate the method?**

   Automating diffusion methods requires special considerations.

   For vertical diffusion cells, the rinse volume requirements of the collector must be balanced against the total receptor media inside the diffusion cell. Generally only 7 mL or 12 mL diffusion cells are recommended for use with automated systems. The 4 mL diffusion cell volume is too small to allow for rinsing the collector tubing without the replacement media leaving the cell.

   Automating immersion cell systems is relatively easier. However, users must ensure that there is enough media in the vessel to keep the tip of the probe submerged. This may require running the test with a higher volume of media in order to ensure the media level does not drop below the probe during sampling. Media replace and/or return to vessel configurations are strongly recommended for automated immersion cell systems.

IVRT Method Development

To simplify method development, it is recommended to keep the following items the same for each method as often as possible:

- **Dosage Amount:** Standard occluded cell tops and immersion cells have a fixed volume for the donor chamber; consequently, using the same amount of material each time is relatively easy. When using open cell tops, the amount of material should be controlled to ensure uniformity.
- **Stirring Rate:** For most vertical diffusion cell methods, a stirring rate of 600 rpm should be sufficient. For immersion cell methods, 100 rpm method is sufficient. An

excessively high stirring rate may result in a change at the membrane and receptor media interface, which may affect diffusion. If the stirring rate is too low, the material in the receptor chamber may not be homogenous.

- **Sampling Amount:** For manually sampled vertical diffusion cell systems, a rinse volume of approximately 0.5 mL and a collection volume of approximately 0.5 mL is recommended. Due to rinse volume requirements of the MicroettePlus, it is recommended that a rinse volume of 2.0 mL is used with a sample collection volume of 0.3 to 0.5 mL.

  NOTE: In order to avoid dilution of samples, ensure that the total volume of the diffusion cell is taken into account. See Table 1 for more information.

The following items should be varied when attempting to develop an IVRT method.

- **Sample Points:** This will depend largely on the amount of material tested. A good sampling schedule will ensure that the first sample occurs after the diffusion cell has reached a steady state of diffusion (after the lag time), and the last sample occurs before 30% API in the sample is depleted. A typical test is approximately 6 hours long.

  NOTE: A minimum of 5 sample points is required for IVRT testing. This helps prove a steady state of diffusion at the time of the measurements.

- **Receptor Media:** A receptor solution should be determined based on the following:
  - Ability to be used with the HPLC method.
  - Solubility of API in the receptor solution. At a minimum, sink conditions must be maintained, and the receptor solution must be able to accommodate more than the amount of material released at the last sample point. Ideally, the receptor solution should be able to dissolve 10x the amount of material released during the test.

For example, if 2 mg of product was released at the end of the test, the receptor media in the cell should be capable of holding a minimum of 2.1 mg. It would be ideal to have receptor media capable of dissolving 20 mg.

- Typical solvents that can be used are acetonitrile, ethanol, methanol, and isopropanol mixed with water. Typical ratios should not exceed 80/20. Solvents should be chosen based on the API solubility and chemistry.

- **Membrane:** A synthetic membrane should be chosen based on the following:
  - Pore size and the product viscosity. Typically a 0.45 µm pore size is sufficient, but more viscous materials may require a larger pore size in order to ensure diffusion is not restricted.
  - Hydrophobic versus hydrophilic materials. Typically a hydrophobic API would use a hydrophilic membrane, and a hydrophilic API would use a hydrophobic membrane. This reduces the chance of the API binding to the membrane.
  - Inertness (does not bind to the API). An ideal membrane would have no effect on diffusion results. However, a membrane that has no effect on diffusion results is rare, so a membrane with a minimal effect must be used.
  - Consistent commercial availability. Membranes that are difficult to obtain may cause delays in testing.
  - Chemical compatibility with the receptor media.

A clear stepwise process should be followed when developing the method. Below is an example process for developing a method. It is recommended to perform each set in triplicate, and test at least 3 different receptor media and 3 different membranes.

1. **Ensure the HPLC method is valid.**

   The method may need to be adjusted as validation continues, but an HPLC method that delivers consistent results is required to evaluate a diffusion method.

2. **Determine the solubility of the API in potential receptor media.**

   This can be done by using the desired receptor media to create standards. An ideal receptor media will dissolve approximately 10x the expected amount released.

3. **Run tests using a basic membrane with different receptor media.**

   a. The membrane should have basic compatibility with the media and API. It will be optimized in a later step.

   b. Review the results to determine the best media to use. The media should provide results that are high enough to ensure sensitivity, but not so high that the dosage has more than 30% of the API depleted during the test.

4. **Evaluate different membranes for binding of API.**

   This can be performed by filtering a standard of the API and ensuring that the filtered standard maintains the same concentration as the unfiltered standard.

5. **Run tests with different membranes using the ideal receptor media.**

   a. Ideally, membranes should not restrict diffusion; however, some restriction is likely. Consequently, the best membrane to use will restrict diffusion the least.

   b. Review the results of the tests using different membranes. The best membrane will show consistent results while providing minimal restriction to the diffusion process.

6. **Ensure the method is capable of detecting different concentrations of API.**

   a. Prepare the material at different concentrations and determine that the method is capable of detecting the differences. Use the best membrane and media that is determined through the process.

   b. Ideally, the differences in the slopes will be proportional to the concentration of the API.

7. **Ensure the method is robust.**
   - **a.** Different users should perform the test and still obtain similar results.
   - **b.** Different instruments (of the same type) should deliver similar results.
   - **c.** Intentionally adjust some conditions, such as temperature, to determine the impact they will have on the method.

Determining What Data is Acceptable

When users review the data, they should ensure that basic criteria are met:

1. No more than 30% of the API has been released.

   To some extent, this can be accomplished by adjustment of the sample points and diffusion test length. However, in many cases, a test of at least 5 hours is recommended in order to ensure that diffusion occurred at a steady state.

2. The data when graphed against $\sqrt{t}$ is linear.

   Typically an R^2 value greater than 0.90 is acceptable.

3. The slopes are fairly consistent.
   a. Less than 15% CV is typically acceptable.
   b. Less than 5% CV is very good.

4. There is no significant lag with the samples.

   Lag can typically be handled by adjusting the sample points.

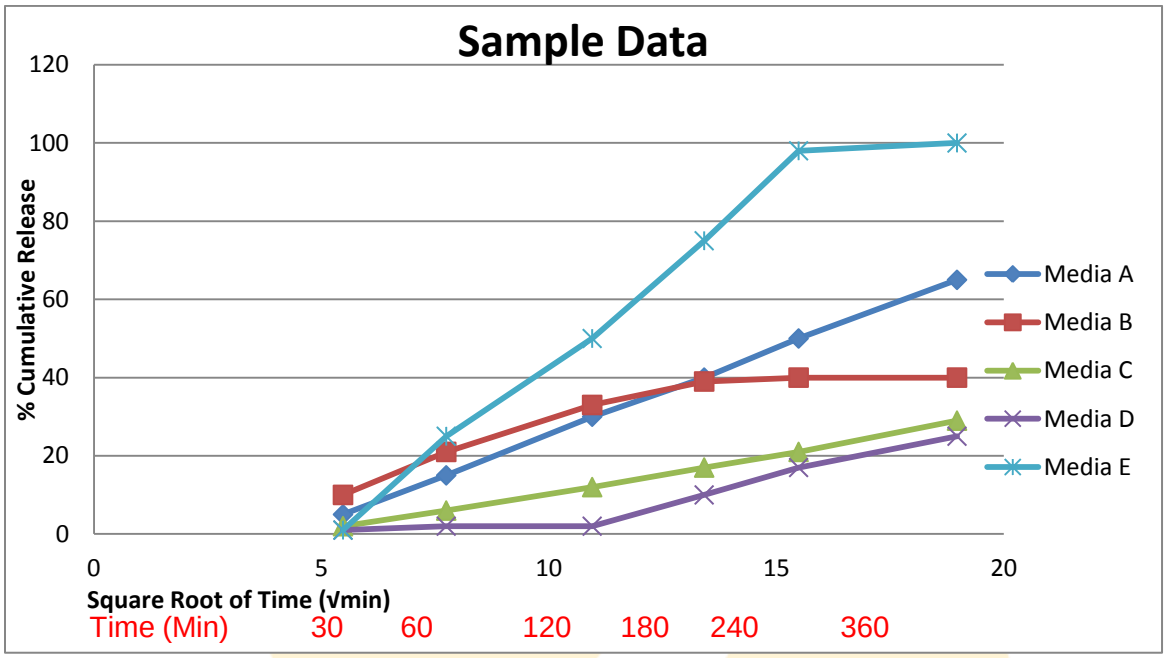

Figure 2 : Extreme Examples of Diffusion Data

Figure 2 demonstrates some extreme examples to look for:

Media A: The results are linear, which is very good. However, the release rate is too high; more than 60% of the API was released.

Media B: Since the results flatten before 100%, there were likely issues with the sink conditions for this test.

Media C: This is linear, and the total amount released is below 30%. This is an ideal candidate for the final media.

Media D: Media D is an extreme example of lag, because the material failed to release for more than 2 hours. Typically, this only applies to very early time points, such as 30 minutes or earlier, before a steady state has been established.

Media E: As the results level off near 100% released, the entire dosage was depleted. This is unacceptable for further testing. Either the dosage amount must be increased significantly or a different media type should be selected.

When Is a Method Acceptable?

What makes a method acceptable is ultimately up to the user. There are a number of factors that should be evaluated to justify whether a method is acceptable or not. Proper method development involves balancing opposing forces (such as release rate and discrimination versus dosage depletion and robustness); the user must decide what is most important based on the available data.

- Linearity (R^2): This is the linearity of the slope obtained over all the samples of the test run. Typically, R^2 values below 0.90 are unacceptable.
- Variability (%CV): This is the amount of variability of the slopes, from cell to cell in a single test, as well as from test to test. Typically, a %CV above 15% is unacceptable for an IVRT.
- Sensitivity: This is the ability of the method to detect changes in concentration of the API.
- Specificity: This is the ability of the method to show a proportional change in the release rate based on a change in the amount of API.
- Selectivity: This the ability of the method to determine if something is inequivalent. Results will be evaluated against the method used in the FDA SUPAC-SS.
- Dose depletion: This is the amount of API that leaves the sample. It is generally recommended that no more than 30% of the API is released. If more than 30% of the API is released, then the method may not be robust.

- Robustness: This is how the method reacts to deliberate changes in the testing apparatus, such as temperature, stirring speed, receptor solution mixture, and dosage volume.

Additional References

The following materials are recommended for additional reading:

- FDA SUPAC-SS Guidance (1997)
- USP General Chapter <3>, <1092>, <1724>

Technical Support

If additional technical support is required, please contact Hanson Research at www.hansonresearch.com/tsr.htm or email techsupport@hansonresearch.com.

Disclaimer

1. This document only provides a *suggested* starting point for those new to diffusion method development. This does not cover every possibility or condition.
2. The guidelines provided herein may not be suitable for all products.
3. Hanson Research assumes no responsibility for any issues relating to this document that may arise with regulatory agencies. Hanson Research recommends all readers to follow GMP/GLP and regulatory requirements.