

OPTIMISING IN VITRO TESTING FOR TRANSDERMAL AND TOPICAL DRUG PRODUCTS

In vitro testing is an effective, cost-efficient way of assessing the performance and quality of drug products delivered via the skin. This article by Tony Copley, Managing Director, Copley Scientific, examines the regulatory framework for transdermal and topical products and the most popular techniques for quantifying their release characteristics.

Whether for topical action, or as a means of introducing systemically acting drugs into the body, the skin is an important route of administration for a wide range of pharmaceutical products.

Topical products are intended for localised action and offer immediate relief for dermatological conditions, while transdermal drug products (TDPs) are typically designed to release an active ingredient through the skin into the bloodstream, over a prolonged period. This crucial difference directly impacts the *in vitro* test methods of relevance to each product type, which have been substantially refined over recent years.

This article reviews the regulatory framework associated with testing both TDPs and topicals, most specifically semisolids, and the test methods described in the relatively recently revised United States Pharmacopeia (USP) Chapter <1724>.¹ A key focus is the equipment used for *in vitro* drug release testing and the factors that must be controlled to ensure the successful, relevant measurements required for regulatory compliance.

WHY USE THE SKIN FOR DRUG DELIVERY?

Topical formulations for the localised treatment of skin conditions enable the delivery of relatively high concentrations of drug directly to the site of action, with minimal risk of systemic exposure and accompanying side effects. Such products include foams, sprays or aerosols, and principally semisolids, a classification that encompasses gels, ointments, pastes, suspensions and lotions, and is a key focus here. All topical products are easy to use and have a high degree of patient acceptance/compliance. Furthermore, semisolids are

often formulated to deliver a moisturising effect, which as well as offering immediate patient relief, can enhance efficacy.

Like topical products, TDPs share the attraction of high patient acceptability, but there are additional inherent benefits compared with alternatives such as oral or injected drug delivery. Transdermal drug delivery avoids first-pass metabolism in the gastrointestinal tract, which can damage the efficacy of certain drugs, and enables steady, controlled release over a prolonged period. As a result, TDPs – typically patches – are commonly used for the sustained delivery of, for example, hormones and treatments for smoking cessation.

Skin is a highly efficient barrier against the outside environment. Therefore, ensuring that a transdermally delivered drug reaches the intended site of action – whether that involves penetrating the outer part of the epidermis or reaching the bloodstream – is a defining challenge.

Physical and chemical properties, including liposolubility, molecular weight and electronic structure, have a big effect on the penetration of molecules through skin and influence which drug molecules can be most successfully formulated for transdermal delivery. Because skin is generally non-polar in nature, non-polar carriers with low molecular weight can penetrate it more easily; small lipophilic molecules tend to be optimal drug candidates.

PRODUCT & PERFORMANCE TESTING

There are two categories of test routinely specified for drug products – those for product quality and those which quantify product performance. Quality tests assess general physical attributes, which for a TDP include measurements of tack and

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adhesion. Product performance tests, in contrast, focus specifically on the release of the pharmacologically active substance from the formulation matrix – a patch in the case of a TDP, for example, or an ointment or cream in the case of a topical semisolid.

In vitro performance testing strategies for topical semisolids and TDPs are in many ways analogous to dissolution testing for oral formulations, but the additional barrier to diffusion presented by the skin adds a layer of complexity. Methods for testing transdermal drug delivery have consequently been expanded beyond the simple measurement of dissolution rate across a solid-liquid interface to include the kinetics of membrane transfer.

The European Medicines Agency (EMA) does not currently offer specific guidance for semisolids, and there are no specific European Pharmacopoeia (Ph Eur) chapters. However, product quality testing for semisolids is included in USP Chapter <3>,² which outlines the need to measure properties such as apparent viscosity and product uniformity over the assigned shelf life.

These methods are complemented by those in USP Chapter <1724>¹ for performance testing via the measurement of drug release, with three different apparatuses specified – Vertical Diffusion (or Franz) Cell (VDC), Immersion Cell and Flow-Through Cell (USP Apparatus 4). There is no US FDA Guidance associated with new submissions for semisolids, but guidance for scale-up and post-approval changes similarly references the Franz Cell.³

The EMA offers comprehensive guidance for TDPs, referencing both performance and product quality tests.⁴ Guidance for performance testing indicates that the release characteristics of the active substance should be tested with a suitable dissolution method as specified in the Ph Eur.⁵

Proposed techniques based on modified tablet dissolution testing procedures include the Paddle over Disk, Rotating Cylinder and Reciprocating Holder methods. Compendial methods for TDPs in the USP include those specified in USP Chapter <3>² for product quality and in USP Chapter <724>⁶ for product performance. This latter chapter, like the Ph Eur, references the Paddle over Disk and Rotating Cylinder methods.

IN VITRO DRUG RELEASE TESTING

By quantifying product performance, *in vitro* drug release testing enables the assessment of different formulations/products, along with evaluation of the impact of process changes and QC. The adequate characterisation of drug release from the dosage form requires the generation of a release profile with values being determined as a function of time. The often complex composition and release mechanisms of semisolids and TDPs require multi-point release tests to characterise the drug product robustly and to test for batch-to-batch and shelf-life consistency.

Experimental conditions must be discriminating enough to detect

manufacturing variables that may affect product performance, as well as reflecting the physiological conditions at the site of drug administration, and are directly influenced by differences between the dosage forms. Semisolids are typically hydrocarbon-based systems or oil-in-water emulsions, incorporating additional ingredients such as emulsifiers, stabilisers, pH buffers and preservatives. In contrast, TDPs are administered via physical devices that may incorporate multiple polymeric membranes and layered matrices. The test methods and apparatuses specified are designed to produce meaningful and relevant data for these quite different physical forms.

TDPs

Of the three compendial methods specified for testing TDPs, Paddle over Disk is increasingly preferred on account of its simplicity. This is described as Method 5 in USP <724> and Method 1 in Ph Eur Chapter 2.9.4. The Rotating Cylinder method shares the advantage of specification in both the USP and Ph Eur (Method 6 and Method 3, respectively) and consequently is the most widely used alternative. The Reciprocating Cylinder method is rarely used and, therefore, is not covered in further detail here.

Paddle over Disk

Paddle over Disk (Figure 1) is a modified version of the standard Method 2 (Paddle Method) pharmacopoeial specification for dissolution testing. It makes use of standard dissolution testing apparatus – together

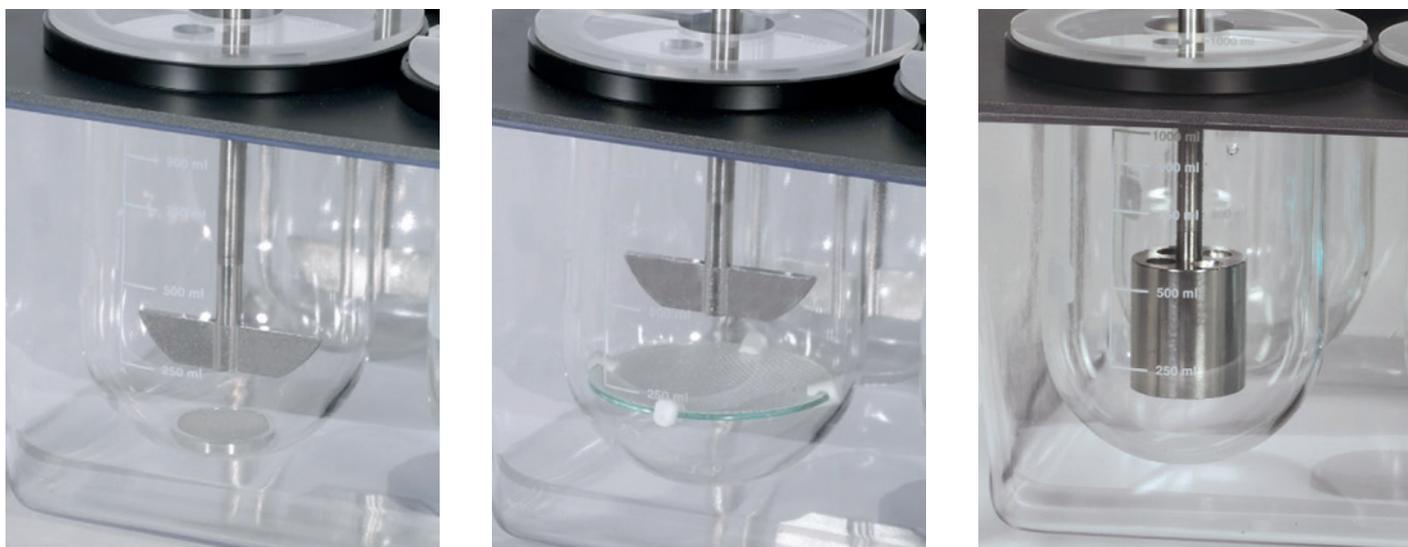


Figure 1: Paddle over Disk is the more popular performance test method for TDPs (images left and centre) but the Rotating Cylinder (image on the right) method also enjoys significant use.



Figure 2: A Model B VDC in an open (left) and closed (right) configuration.



Figure 3: VDC designs such as Model C (right) eliminate the cell leakage/back diffusion issues associated with the double port arrangement of the Model A design (left).

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with a disc assembly designed to hold the TDP at the bottom of the test vessel. The standard disc comprises a 35 mm sieve with a pore size of 125 μm mounted in a 41.2 mm diameter stainless steel holder, and is suitable for patches up to a maximum of 16 mm in outside diameter.

The patch for testing is mounted on the disc with its release side uppermost.

A second, larger system comprising a 90 mm watch glass-patch-PTFE, is available for larger diameter patches and is often preferred since experimental investigations indicate that this gives results almost identical to those from other more complex apparatus.

Whichever size is used, the disc assembly is placed at the bottom of the vessel parallel to the lower edge of the paddle. Paddle height should be 25 mm from the surface of the disc assembly and a paddle speed of 50 or 100 rpm is typically selected. The dissolution vessel is filled with preheated, degassed media with a pH of 5-6 and held at 32°C to simulate *in vivo* skin conditions. Samples of dissolution media are extracted for assay at regular intervals over an appropriate test time to generate a release rate profile for the patch.

Rotating Cylinder

The Rotating Cylinder method is closely similar to Paddle over Disk but replaces the disc basket assembly with a cylinder stirring element (Figure 1). In this method, the transdermal patch is attached to the exterior of the cylinder using an appropriate adhesive, with a cylinder extension available for testing larger patches. Testing then proceeds in a strictly analogous way as with USP Method 5.

SEMISOLIDS

USP <1724> specifies measurement of both the total amount of drug released and the release rate for semisolid performance characterisation. Of the apparatuses specified, the VDC is rapidly emerging as the preferred choice because of its simplicity and reproducibility, although the Immersion Cell remains in widespread use. The less widely deployed Flow-Through Cell (USP Apparatus 4) is not covered here in detail, though, when used in combination with an automatic fraction collector, has claimed advantages, especially for testing formulations with rapid permeation characteristics.⁷

The Vertical Diffusion Cell

Three different designs of VDC are included in USP <1724>, but the basic principle of operation is the same in each case. In practice, each design can be assembled with either an open or closed cell top. In the open configuration, a sample is held in a donor chamber as shown in Figure 2, and is separated from the receptor media by an artificial membrane (or skin) designed to act as a conduit for diffusion to take place.

An open configuration offers the flexibility to test with just a minimal smear of semisolid, with a full chamber, or with an infinite reservoir, depending on test requirements. A closed configuration consists of a “three-part sandwich”, comprising a support disc, a PTFE sample chamber ring in which the sample is initially placed and the artificial membrane (or skin).

This sample sandwich is placed onto the cell such that the membrane is bathed in the receptor medium. The cell top is occluded to prevent the ingress of air and hence minimise back diffusion from sampling, whilst also providing a sample of defined volume.

Testing is typically conducted over a period of six hours, during which samples of receptor medium are extracted

periodically for assay, with an equal top up of fresh medium keeping the sample bathed in the receptor medium. The resulting data generates a time-dependent release profile where release is proportional to the square root of time, i.e. the profile should be a straight line with a gradient representing the release rate.

USP <1724> references three different VDC models with ports designed for sample withdrawal and media replacement (Figure 3). The receptor chamber of Model A has two ports while those of Models B and C have just a single port. In the case of Model A, the sample is withdrawn from the upper port by forcing replacement media into the cell via the lower port.

Although this design is well-suited to automation, it is also associated with cell leakage and/or back diffusion because of the upward pressure that operation exerts on the sample holding assembly. The simpler design of Models B and C largely eliminates these problems and is increasingly preferred. These latter two cells differ only in terms of size, with Model C enabling higher volume testing where this is helpful because of the drug concentration/dosage form concerned.

For all cell designs a magnetic stirring bar ensures a homogeneous temperature distribution and adequate mixing of the cell contents. Test temperatures are normally 32°C to reflect usual skin temperature (37°C for vaginal preparations), and may be maintained using a water-jacketed design and appropriate water circulation system. However, a more modern and efficient approach is to use a compact heated block, such as the HDT 1000 Vertical Diffusion Cell Test System from Copley Scientific, which holds ten VDCs and eliminates the excessive tubing associated with jacketed designs (Figure 4).

Since data can be compromised by air collection at the membrane affecting diffusion, accessories such as the Vacuum Deaerating Apparatus Model VDA from Copley Scientific – which degasses the receptor medium ahead of testing – can also be helpful in streamlining and improving the accuracy of routine testing.

Immersion Cell

An Immersion Cell can be used to test semisolids with the conventional USP Apparatus 2 for dissolution testing and a small volume conversion kit. Adjustment tools enable the user to vary the volume of the reservoir within the cell.



Figure 4: Using a compact heating block for testing is an efficient approach and eliminates the 'spaghetti' of tubing associated with water jacketed designs.



Figure 5: An immersion cell (above) is used with the conventional USP Apparatus 2 fitted with a small volume conversion kit, as shown on the right.

Figure 5 shows the key components of the PTFE Immersion Cell, which include:

- The cell body, a variable volume compartment that holds the sample
- The 25 mm diameter membrane or skin sample
- A washer to hold the membrane in contact with the sample
- A retaining ring, which secures the membrane to the cell body.

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Testing is carried out in an analogous way as with the VDC, over a comparable period of time. Once the sample has been loaded and assembly is complete, the entire cell is immersed in receptor medium at the bottom of the dissolution tester vessel. Samples of receptor medium are then extracted periodically for assay to generate a release profile, with preheated, degassed medium added to top up as required.

REPRESENTATIVE TESTING: FACTORS INFLUENCING THE SUCCESS OF TESTING SEMISOLIDS

The *in vitro* drug release testing of TDPs is well documented, whilst that of semisolids less so. *In vitro* release testing does not directly model the behaviour of a product, and is therefore not a complete substitute for bioavailability or clinical studies. However, steps can be taken to enhance its relevance. Apparatus choice and test temperature are important factors but there are a number of other issues involved in testing semisolids to consider.

Choice of Dissolution/Receptor Medium

While diffusion is a spontaneous and irreversible process, it is influenced by the balance of intermolecular forces between the solvate and solute. Certain radicals, water and sodium chloride all affect the dissolution characteristics of a drug molecule in a potential receptor/dissolution medium, with the maxim “like dissolves

like” providing a good starting point for selection, along with the need to reflect the physiology of the skin. The pH of the medium is usually adjusted to lie in the range 5-6 for this reason.⁸

Dissolution rate depends on the degree of under-saturation in the liquid solvent film immediately adjacent to the solid solute, so it is also important to select a medium with high drug solubility. Low viscosity is also beneficial since this enhances the permeability of the membrane within the test set-up, reducing the resistance it presents to diffusion. Finally, it is essential that the chosen medium does not impact the integrity of the product or the membrane. It should be compatible with any polymers present and, in the case of semisolids, immiscible with the formulation.

Maintenance of Sink Conditions

An underlying premise of drug release testing is that it is measured under “sink conditions”¹ to minimise the influence of the experimental set-up on the relevance of the data. Sink conditions means that the concentration of drug dissolved in the receptor or dissolution medium is maintained at such a low level, relative to the concentration in the product itself, that it does not inhibit the diffusional process. This requirement may influence test apparatus choice in extreme cases, or more simply require a modification to the amount of sample used.

Membrane Choice

Membrane choice is an important aspect of semisolids testing, since the membrane acts as a support for the sample. Chosen membranes should be chemically inert to both the receptor and the product, and wet easily since complete wetting is essential to eliminate air from the pores of the membrane and enable an accurate measurement of diffusion. In addition, it is important to select a membrane with high permeability, such that the rate-limiting process is diffusion of the drug product from the formulation.¹

Synthetic membranes in widespread use are made from materials such as cellulose acetate, polycarbonate, nylon, polysulfone and Teflon, but newer transdermal test materials such as Strat-M can offer important advantages. Strat-M delivers data that are highly predictive of diffusion in human skin while avoiding the wetting, lot-to-lot variability, safety and storage limitations associated with real skin.

CONCLUSION

In vitro release testing procedures for both TDPs and topical products are now well-defined, robust and reproducible. Furthermore, relative to costly and time-consuming *in vivo* methods, *in vitro* drug release testing methods are simple, economical and more open to automation.

Understanding how apparatus works and the factors that affect the resulting data is key to the successful application of the specified performance tests for semisolids and TDPs from development through to QC.

Such understanding underpins the valuable use of *in vitro* testing to demonstrate batch-to-batch uniformity, to support the demonstration of *in vivo* bioequivalence or to assess the impact of post-approval changes to excipients, batch size or manufacturing processes.

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