

Studying In Vitro Skin Penetration and Drug Release to Optimize Dermatological Formulations

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In vitro percutaneous absorption system containing Franz static diffusion cells and drive console.

In vitro skin penetration and drug-release studies are **the simplest and most cost-effective methods** for characterizing a drug's skin absorption and penetration profiles.

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Have you ever wondered why your promising topical drug failed in a clinical study when all the pharmacology data pointed toward success? Was the drug really ineffective, or did the formulation fail to deliver the drug?

You may never know without drug delivery data from studies characterizing drug release, skin absorption, and skin penetration following the topical application. In vitro skin penetration studies are conducted during topical formulation development to identify and select promising formulations. These formulations provide optimal drug release and drug deposition into the desired skin layer (stratum corneum, epidermis, or dermis). Failure to conduct these studies could lead to deceptive toxicology results and unsuccessful clinical studies—not because of drug activity, but because of formulation characteristics.

In vitro skin penetration studies are conducted to measure the rate and extent of compound transport across the skin and the extent of compound retention on and in the skin. Depending on the drug, the dosage form, the excipients, penetration enhancers, and other formulation variables, differences greater than 20-fold in drug deposition—penetration have been observed among rationally designed formulations tested with the same drug and concentration.

A formulation optimized for skin deposition and drug penetration offers the advantage of less potential irritation (if the drug is irritating), lower drug cost (by using a lower concentration), and maximum opportunity for clinical efficacy. In short, in vitro skin penetration studies are

necessary for selecting the dermatologic formulation that will progress to the clinic.

The first step in drug delivery following topical application is the release of drug from a formulation. The rate of release depends on drug thermodynamic activity in the formulation and is measured using diffusion cell systems similar to those used in the in vitro skin penetration studies. A low drug-release rate generally corresponds to low bioavailability following topical application. Typically, a low drug concentration formula with high drug solubility will retain drug on the skin surface and have a low drug-release rate. Therefore, characterization of drug release from a formulation can provide valuable information about formulation strategy and selection.

Another use of drug-release studies is in regulatory scale-up and postapproval change (SUPAC) testing to verify that no change exists in product quality or performance after a manufacturing process modification. A key component of a Level 2 or 3 change is in vitro drug-release testing for pre- and postchange formulations.

Equipment

Both static and flow-through diffusion cell systems are made by several manufacturers. With its manual collection of receptor fluid samples, the Franz static diffusion cell system (with its large skin area and static receptor compartment) was found to be most appropriate for characterizing skin deposition and penetration of drugs from formulations with very low drug-permeation rates.

With the appropriate substitution of a membrane for skin and the selection of re-



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Sampling of receptor solution for assay from an *in vitro* percutaneous absorption system using Franz static diffusion cells.

ceptor solution, the Franz static diffusion cell system and the Hanson Research (Chatsworth, CA) automated diffusion cell system can be used for drug-release studies. The Bronaugh flow-through diffusion cell system offers the advantage of continuous perfusion of the underside of the skin (the dermis) with fresh receptor fluid to maintain sink conditions for drug diffusion. The system also provides automated receptor-fluid sample collection at specified time periods. The Bronaugh flow-through diffusion cell system is the most suitable for high-volume characterization of multiple formulations.

Skin source

The source of skin in an *in vitro* skin penetration study is critical. Results from a skin penetration study are only as good as the skin used. Human skin is preferred over animal skin because of large differences in drug deposition and penetration across species.

Using dermatomed human skin obtained from patients who underwent elective surgery is recommended as opposed to using cadaver skin. To control variation in skin penetration, one should use skin from a single donor and the same anatomical site in a given study. Such use allows for comparable results among the various formu-

lations tested and eliminates the variability that occurs when multiple donors or skin from various anatomical sites are used.

In vitro test methods

For a typical skin-penetration study, test formulations are applied to excised skin mounted on either the Franz static or Bronaugh flow-through diffusion cell systems. Skin should be exposed to the drug a maximum of 24 h because of deterioration of skin integrity with time. Receptor-fluid samples are collected at regular intervals over the exposure period for each cell. Several methods can be used to remove the residual formulation from the skin surface following the designated exposure period: washing, wiping, tape-stripping, and combinations thereof. After the removal of the residual dose from the skin surface, the epidermis is separated from the dermis by physical methods. Diffusion-cell washes, skin surface material removal samples, epidermis-, dermis-, and receptor-fluid samples are then analyzed for drug content. A greater number of formulations can be characterized in a study using the Bronaugh flow-through diffusion cell system because of the automated receptor-fluid collection and generally smaller amount of skin used per cell compared with the static diffusion cell systems.

In vitro drug-release studies are simplified skin-penetration studies. After the application of the test formulations to the membrane mounted on the Franz static diffusion cell system, receptor-fluid samples are collected at defined intervals of drug exposure and are subsequently analyzed for drug content.

Analyzing samples

For *in vitro* skin penetration studies, the skin retention of a drug and drug penetration can be assessed by the use of either radiolabeled drugs (usually carbon-14 or tritium labeled) or by the use of such analytical techniques as high performance liquid chromatography coupled with sensitive detectors. The use of radiolabeled drugs in the formulations to be characterized is often preferred because of the high detection sensitivity afforded, especially if drug-penetration levels are very low because of the drug's properties or its concentration in the formulation.

Alternatively, drug levels in the skin and receptor fluid can be measured using sensitive analytical techniques and equipment. With *in vitro* drug release testing, conventional analytical test methods are typically used.

Using the results

These studies provide information for selecting the dosage form and formulation for the optimal delivery of topical therapeutic products. Formulation objectives with respect to the drug delivery profile depend on the intended use of a topical product. For sunscreens, antifungals, and keratolytic products, enhanced drug de-



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Dosing procedure for *in vitro* percutaneous absorption studies: determining formulation dose by weight.

livery and retention in the stratum corneum (the outer layer of skin) is desired. Conversely, topical products that are intended to modify the physiology of the skin require drug deposition in and often through the lower layers of the skin (viable epidermis and dermis). Topical products usually require a delicate balance of drug deposition on and in the skin along with skin penetration to optimize clinical efficacy and safety. Comparative *in vitro* drug release and skin penetration profiles of candidate formulations provide important data in the complex process of selecting a formulation to progress to the clinic. **PT**