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Strat-M® synthetic membrane: Permeability comparison to human cadaver skin

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ABSTRACT

The aim of this work was to investigate the correlation of permeation behavior of transdermal formulations through a novel synthetic membrane (Strat-M® EMD Millipore, MA) and human cadaver skin. Strat-M® membranes were designed with the intent to share similar structural and chemical characteristics found in the human skin however, omitting any biological behavior due to the absence of viable cells. Both human skin and the membrane display a layered structure with a very tight top layer. Additionally, the Strat-M® membrane contains a combination of lipids in a specific ratio similar to what is found in the human stratum corneum (SC). Formulations containing nicotine and a chemical penetration enhancer (CPE) were used for evaluating drug penetration to understand how each enhancer impacts the permeability of nicotine as a model compound. The permeability measurements of human cadaver skin and Strat-M® membrane were performed with Franz diffusion cell methods accompanied by HPLC analysis. A good correlation of the permeability data was obtained through human cadaver skin and Strat-M® membrane. Thus, Strat-M® has the potential to be used as a screening tool for evaluating topical/transdermal formulations through the human cadaver skin.

1. Introduction

Synthetic membranes for in vitro permeation studies were originally developed to be used as an alternative to using a human skin models (SUPAC, 1997). Determination of drug permeation of formulations using ex vivo human skin methods possesses several drawbacks which hinder reproducibility data of drug candidate screening including: variations of skin thickness from skin donors, diseased skin states, skin storage conditions, membrane preparation complexity, density of hair follicles, age of donor, and high laboratory costs (Mathes et al., 2014; Semlin et al., 2011). Some advantages of using a synthetic membrane are: controlled membrane thickness, faster membrane preparation time, low storage space, and relatively low cost. The human stratum corneum (SC) is commonly the rate limiting step for successful API (active pharmaceutical ingredient) delivery (Andrews et al., 2013; Baroni et al., 2012). It consists of 10–15 parallel layers of corneocytes embedded in an intercellular lipid matrix of mainly ceramides (50%), cholesterol (25%) and free fatty acids (15%), in a bricks and mortar arrangement (Trommer and Neubert, 2006). There are several techniques such as chemical enhancement, physical enhancement, and drug modification that have been employed to change the barrier properties of stratum corneum (Pathan and Setty, 2009). Among these using chemical penetration enhancers is the most widely used technique since these compounds can reversibly alter the stratum corneum's barrier function (Shah et al., 2012). Usually chemical enhancers act by lipid disruption and at acceptable concentrations they interact and affect the stratum corneum intercellular lipid domain or organization and make the stratum corenum more permeable (Vavrova et al., 2005). Understanding the physiochemical relationship of API/vehicle interactions through a membrane barrier is critical for selection of optimal formulation penetration enhancement efficacy (Flaten et al., 2015). In this regard, we report here on the development of nicotine solutions containing penetration enhancers to evaluate the permeability correlations of Strat-M® (EMD Millipore, MA) synthetic membrane with human cadaver skin. Nicotine is commonly used for nicotine replacement therapy (NRT) to encourage successful smoking cessation from tobacco products (Wadgave and Nagesh, 2016). Particular chemical properties such as: low molecular weight (162.23 g/mol), logP (1.2)

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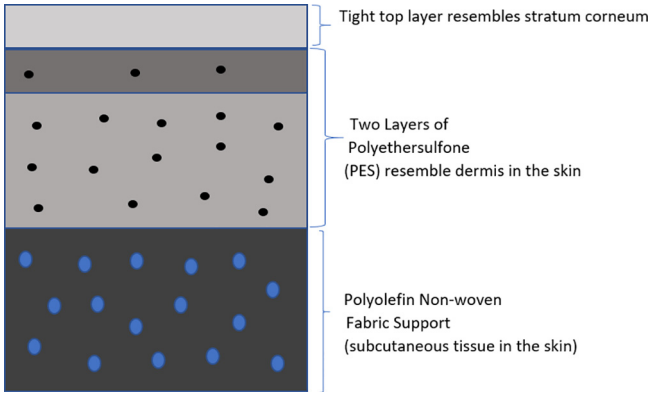

Fig. 1. Multilayered structure of Strat-M® membrane.

and its wide-spread use make this compound an ideal candidate for testing. It is commercially available as a TDDS (Transdermal Delivery System).

Most commonly used synthetic membranes models lack the biological composition of a highly structured stratum corneum, metabolic processes, and interactions of proteins found in the human epidermis. Strat-M® membranes were designed with the intent to share similar structural and chemical characteristics found in the human epidermis. The multiple layers of stratum corneum undergo a process called keratinization. In this process as the cells formed and migrated upwards to the skin surface from the basal layer stem cells the concentration of oxygen and nutrients decrease and the cells become flatter and accumulation of keratin and lipids occurs. Strat-M® membrane was engineered to mimic the layered structure and lipid chemistry of human skin (Fig. 1). The thickness of each Strat-M® membrane is approximately 300 μm; comprising a top layer supported by two layers of porous polyether sulfone (PES) on top of one single layer of polyolefin non-woven fabric support. Membrane layers are increasingly more porous and open and also increasingly larger in thickness to mimic different layers of human skin (epidermis, dermis and subcutaneous tissue). These multiple layers of the membrane create a morphology similar to that of human skin. Both human skin and the membrane display a layered structure with a very tight top layer. The porous membrane was treated with a proprietary blend of synthetic lipids. Skin contains various lipids, such as phospholipids and ceramides, which impart hydrophobic character to skin. This synthetic membrane contains a combination of lipids (ceramides, cholesterol, free fatty acids, and other components) in a specific ratio similar to what is found in the human SC.

Strat-M® serves a purpose to be a cost-effective membrane for testing and optimizing pharmaceutical formulations with good reproducibility to increase confidence during early stage drug/formulation development. This synthetic membrane can be used for high throughput formulation screening during the early stages of formulation development to test for API's, personal care products, pesticides, cosmetic actives, and chemical warfare protective formulations. Furthermore, a need for high quality methods that help determine safety and bioequivalence for formulations including those containing CPE's are sought by regulatory agencies to speed up the long approval times needed in order for generic drug clearance and approvals (Lionberger, 2008). Bioequivalence studies are typically conducted using human cadaver skin or animal models. Unfortunately these models experience a number of drawbacks that make them not very suitable for development including: complex sample preparation, strict sample storage requirements, biohazard issues and expensive study costs (Godin and Touitou, 2007).

The objective of the present study was to compare different formulations containing various enhancers regarding their ability to enhance or reduce the delivery of nicotine through human cadaver skin and to correlate that to Strat-M® synthetic membrane to examine the usefulness of this membrane as a convenient screening tool to investigate topically applied formulations and TDDS.

2. Materials and methods

2.1. Materials

Polysorbate 80 (Tween 80), eucalyptol, *N*-methyl-2-pyrrolidone (NMP), propylene glycol, sodium phosphate monobasic were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Nicotine was purchased from Alfa Aesar (Haverhill, MA, USA) and Laurocapram (Azone) was purchased from BOC Sciences (Shirley, NY, USA). Phosphate-buffered saline tablets (PBS, pH 7.4) was purchased from MP Biomedicals, LLC (Solon, OH, USA) and o-Phosphoric Acid 85% was purchased from Fisher Scientific (Hampton, NH, USA). Dermatomed human cadaver skin from the posterior torso of three different donors were obtained from New York Firefighter Skin Bank (NY, USA). Strat-M®, high-performance liquid chromatography (HPLC) grade water and acetonitrile was a gift from EMD Millipore (Danvers, MA, USA).

2.2. Preparation of formulations

Five formulations were prepared (10 mL) containing 1% nicotine, with or without 5% of enhancer (Azone, tween 80, eucalyptol, or *N*-methyl-2-pyrrolidone) in propylene glycol.

2.3. In vitro skin permeation test (IVPT) studies

Each of the five formulations (as described above) were applied to dermatomed human cadaver skin with the dermal side in contact with filtered PBS (pH 7.4) and Strat-M® membrane with the shiny side in contact with the donor compartment, both mounted on Franz diffusion cells with a donor area of 0.64 cm^2 and a receptor volume of 5.0 mL (Permegear Inc., Hellertown, PA). Dermatomed human cadaver skin samples (~500 μm) from the posterior torso of three different donors (2 white males at the age of 68, 45 and one white female at the age of 34) obtained from New York Firefighters Skin Bank (New York, NY) were used for skin permeation study. Prior to using the skin, the samples were slowly thawed, cut into appropriate pieces and then soaked in filtered PBS (pH 7.4) for 15 min. Strat-M® membrane (EMD Millipore, MA, USA) does not require any pretreatment, thus was used immediately after removing from the packaging. The skin and the Strat-M® membrane was not occluded in the Franz cells and the receptor compartment of each cell was filled with filtered PBS (pH 7.4) and maintained at 37 °C under synchronous continuous stirring using magnetic stirrers at 600 rpm. The diffusion cells were allowed to equilibrate at 37 °C for 15 min. Then at time zero 200-μL of formulation was added to the donor compartment of each Franz diffusion cell using a positive displacement pipette set and the dose was spread through the surface with the tip of pipette. At each time point (1, 2, 3, 4, 5, 6, 7 and 8 h) 300μL of receptor were withdrawn from the sampling port. At the end of 8 h, all receptor samples were analyzed using a valid HPLC method described below.

2.4. High-performance liquid chromatography (HPLC)

Nicotine was quantified using a validated HPLC method and an Agilent 1100 series HPLC (Agilent Technologies, CA, USA) coupled with UV (259 nm) and a diode array detector (DAD). A mobile phase of 65% sodium phosphate buffer (adjusted to pH 3.2 with 85% orthophosphoric acid) and 35% acetonitrile was pumped at a flow rate of 1.0 mL/min through a Phenomenex Luna® 5 μm C18(2) 100 Å Column 250 × 4.6 mm (ambient temperature). The retention time for nicotine was 2.5 min. The method was linear at a concentration range 4–500 μg/mL with R^2 of 1. The limit of quantification is 1.2 μg/mL and the limit