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Evaluation of Heat Effects on Fentanyl Transdermal Delivery Systems Using *In Vitro* Permeation and *In Vitro* Release Methods

Qian Zhang^a, Michael Murawsky^a, Terri D. LaCount^a, Jinsong Hao^{b,c}, Priyanka Ghosh^d, Sam G. Raney^d, Gerald B. Kasting^a, S. Kevin Li^{a,*}

^aDivision of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267, USA

^bDepartment of Pharmaceutical Science and Research, School of Pharmacy, Marshall University, Huntington, WV 25755, USA

^cDepartment of Pharmaceutical Sciences, College of Pharmacy & Health Sciences, Campbell University, Buies Creek, NC 27506, USA

^dDivision of Therapeutic Performance, Office of Research and Standards, Office of Generic Drugs, U.S. Food and Drug Administration, Silver Spring, MD 20993, USA

Abstract

Experimental conditions that could impact the evaluation of heat effects on transdermal delivery systems (TDS) using an *in vitro* permeation test (IVPT) and *in vitro* release testing (IVRT) were examined. Fentanyl was the model TDS. IVPT was performed using Franz diffusion cell, heating lamp, and human skin with seven heat application regimens. IVRT setup was similar to IVPT, without using skin. Dissolution study was conducted in a modified dissolution chamber. The activation energy of skin permeation for fentanyl was determined using aqueous solution of fentanyl. In IVPT, the increase of temperature from 32° C to 42° C resulted in a 2-fold increase in flux for fentanyl TDS, consistent with the activation energy determined. The magnitude of flux increase was affected by the heat exposure onset time and duration: higher flux was observed when heat was applied earlier or following sustained heat application. Heat induced flux increases could not be observed when inadequate sampling time points were used, suggesting the importance of optimizing sampling time points. Drug release from TDS evaluated using IVRT was fast and the skin was the rate-limiting barrier for TDS fentanyl delivery under elevated temperature.

Keywords

Transdermal; Fentanyl; Transdermal delivery system (TDS); *In vitro* permeation test (IVPT); *In vitro* drug release testing (IVRT); Heat effect; Transport activation energy; Skin permeation

*Correspondence author. College of Pharmacy, University of Cincinnati, 231 Albert Sabin Way, MSB # 3005, Cincinnati, OH 45267-0514., kevin.li@uc.edu (S.K. Li).

Appendix A. Supplementary Data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.xphs.2020.07.013>.

Introduction

The application of external heat or exposure to elevated temperature can enhance drug delivery from transdermal delivery systems (TDS) either by increasing the permeability of the skin and peripheral blood flow and/or by accelerating the rate of drug release from the TDS.^{1–6} The enhancement in drug delivery due to heat application can lead to drug overdose.^{7–9} TDS products are usually designed to contain an excess amount of drug in order to sustain a constant drug delivery rate over the duration of TDS wear, but this inherent product design can also lead to a higher potential for unintended significant increase in drug concentration in the systemic circulation when the drug delivery rates from TDS are altered under the influence of elevated temperature. Therefore, many currently marketed TDS products have warning labels against heat exposure during product use.¹⁰

Numerous studies have been conducted to elucidate the effects of heat on the performance of dermatological products *in vivo* and *in vitro*.^{10–17} *In vitro* permeation test (IVPT) using *ex vivo* human skin is one of the commonly used models for the evaluation of heat effects on TDS.¹⁸ In these IVPT studies, the skin is usually maintained at a set temperature of around 32° C to mimic normal skin temperature (preferably measured at the surface of the skin) and the temperature is varied between 37 and 45° C to mimic elevated temperature conditions.^{19,20} However, significant differences exist within the study designs available in the literature with respect to the methods used for application of heat, duration of heat application, etc., and such differences in the experimental protocols used by different research groups make it difficult to utilize the existing datasets to compare the influence of heat on TDS. Recent studies have suggested that carefully designed IVPT studies can be used to compare the relative effects of heat on TDS and discriminate between differences in TDS design with different formulations.^{21,22}

In a previous study, the skin temperature range for the evaluation of TDS following heat exposure was suggested to be between 32° C and 42° C in IVPT studies and a heat application method using an external heating lamp was developed for IVPT to evaluate heat effects on TDS.²³ These study conditions were used to evaluate heat effects on nicotine TDS from two manufacturers.^{24,25} The results in the study showed ~2-fold (average) nicotine flux increase for both TDS when the skin surface temperature was increased from 32° C to 42° C. In addition to the experimental results, a computational heat and mass transport model was developed and employed to provide mechanistic insight into drug delivery from the TDS.²⁶ Model predictions for various heat application protocols were in general agreement with the corresponding experimental data in the IVPT study of nicotine TDS.^{25,26} The ~2-fold nicotine flux increase is also consistent with another study using nicotine TDS from the same two manufacturers and an IVPT method where the temperature of the Franz diffusion cell was modulated using a water-bath system.²² It is critical to understand the experimental factors that may influence the results obtained from these IVPT studies on different active pharmaceutical ingredients (API) with differences in physicochemical properties or different TDS containing the same API but with differences in formulation. The latter may facilitate the use of the IVPT method for evaluating heat effects of generic TDS products compared to the reference listed drug (RLD).

Transdermal fentanyl is an analgesic and is currently used for the palliative treatment of moderate to severe chronic cancer and nonmalignant pain.^{27–30} Fentanyl TDS was selected as the model drug product in the present study due to the following reasons. First, fentanyl TDS is one of the most widely used TDS in the United States and the effects of heat on fentanyl TDS have been extensively reported in the literature. Second, there are currently seven approved therapeutically equivalent fentanyl TDS products.³¹ Although these TDS products are designed to deliver the same nominal amount of fentanyl, allowable differences exist in their product designs with respect to the types of excipients/inactive ingredients used to achieve the nominal delivery rate, the shape and size of the drug products, the amount of fentanyl in the TDS and consequently the residual fentanyl that remains in the TDS after the wear period. Therefore, the choice of fentanyl as a model product allows us to evaluate critical parameters in the IVPT setup by comparing the relative enhancement in drug delivery following exposure to heat from products that are expected to deliver the same amount of drug nominally under normal temperature conditions. In this study we chose to use three different fentanyl TDS products, the RLD and two approved generic TDS products.

Using fentanyl as the model drug, the objectives of the present study were to (a) determine the IVPT experimental conditions that could impact the evaluation of heat effects on TDS, particularly, the protocol to identify the “worst case scenario” of heat effect on TDS, (b) investigate the mechanisms of enhanced drug delivery from TDS under elevated temperature such as the effects on drug permeation across the skin and drug release from TDS using IVPT and *in vitro* drug release testing (IVRT), respectively, (c) identify the experimental factors in the heating-lamp IVPT and IVRT methods that could influence the results and the comparison of heat effects between RLD and generic TDS products, and (d) examine other factors such as activation energy to predict heat effect on TDS. IVPT studies using Franz diffusion cell and skin with varied heat application protocols, IVRT using Franz diffusion cell and filter membrane, dissolution study using a modified dissolution chamber (solution in a vial), and side-by-side diffusion cell permeation study were performed. Table 1 summarizes the approaches used in the present study.

Experimental

Materials

Fentanyl matrix TDS from three different manufacturers were investigated. The reference TDS was Duragesic® Fentanyl Transdermal System, 50 µg/h (Janssen, Titusville, NJ) manufactured by Alza Corp (Mountain View, CA) (designated as TDS-Z), National Drug Code (NDC) 50458-0092-05, lot# 1304820P1, 1411167P1, and 1509761P1. The generic TDS were Fentanyl Transdermal System, 50 µg/h, from Apotex Corp (Weston, FL) manufactured by Aveva Drug Delivery Systems (Walgreens, Deerfield, IL) (designated as TDS-V), NDC 60505-7007-02, lot# 41632, 43495, and 44728 and Fentanyl Transdermal System, 50 µg/h, manufactured by Mylan Pharmaceuticals (Canonsburg, PA) (designated as TDS-M), NDC 00378-9122-98, lot# 6E0113, 614202, and 614340. Fentanyl citrate reference standard was purchased from USP (Rockville, MD). Fentanyl hydrochloride was obtained from Mallinckrodt Pharmaceuticals (St. Louis, MO). Silicone gel was purchased

from ACE Hardware Corp. (Oak Brook, IL). Powdered food color, 5523 red, was from Apollo Mold Co., Inc. (Tulsa, OK). Membrane filters (MF membrane, 0.45 μ m nitrocellulose, HAWP) was obtained from Merck Millipore Ltd (Cork, Ireland). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Pharmaco-AAPER (Shelbyville, KY). HPLC grade glacial acetic acid was obtained from EMD Chemicals (Gibbstown, NJ). Phosphate buffered saline (PBS) tablets and ammonium acetate were purchased from MP Biomedicals, LLC (Solon, OH). PBS consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride at pH 7.4 was prepared by PBS tablets and deionized water. Sodium azide (NaN_3) was obtained from Acros Organics (Morris Plains, NJ) and was added to PBS as a preservative at 0.02% (w/v).

Preparation of Human Skin

Excised split-thickness human cadaver skin from posterior torso of males aged between 45 and 67 years were obtained from the New York Firefighters Skin Bank (New York, NY) and stored at -80°C . To prepare the skin before the experiment, the skin was thawed in PBS at room temperature according to the skin bank package instructions. Unused skin was patted dry with Kimwipe, wrapped in aluminum foil and stored in a freezer at -20°C for later use. The use of human tissues was approved by the Institutional Review Board (IRB) at the University of Cincinnati, Cincinnati, OH.

In Vitro Permeation Test (IVPT) for Fentanyl TDS: General Setup

The split thickness skin (4 skin donors, 3–4 skin samples as replicates of each skin donor) was cut into appropriate sizes and mounted on Franz diffusion cells (diffusion area of around 0.7 cm^2). The receptor compartment was filled to capacity with PBS ($\sim 6.5\text{ mL}$), and the donor compartment was open to ambient laboratory environment. The Franz diffusion cells were then placed in adjustable holders in a concentric configuration²³ on a stirring plate such that the receptor solution in contact with the underside of the dermis was stirred magnetically at approximately 700 RPM. The integrity of the skin in the diffusion cells was checked using both skin electrical resistance and transepidermal water loss (TEWL) before the application of TDS in the donor chamber. The skin resistance and TEWL prescreening methods have been described previously.³²

IVPT: Heat Application Using Heating Lamp

Heat was applied on the top of TDS using a heating lamp (HL-1, Physitemp Instruments) as described previously with some modifications.^{23,24} During heat application, the heating lamp was controlled by a proportional-integral-derivative (PID) controller (TCAT-2, Physitemp Instruments). Temperatures in Franz diffusion cells were measured using temperature probes (e.g., Ultrafine IT-Series Flexible Microprobe, IT-24P, Physitemp Instruments) and the measurements were recorded by data logger and computer software (Thermes USB-Temperature Data Acquisition System and DasyLab-Lite Software; Physitemp Instruments). TDS was cut into a number of small circular pieces with a diameter of 0.86 cm (0.58 cm^2 area) using a cork borer. After removing the release liner, a temperature probe (IT-24P) was attached to the bottom surface of the circular TDS. The TDS was then placed on the skin in the donor chamber with the probe at the TDS/skin

interface.²⁴ A circular piece of plastic mesh (area of 1.8 cm², Saint-Gobain ADFORS, Grand Island, NY) was placed on top of the TDS to ensure contact between TDS and the skin. The temperature of the receptor chamber was controlled by a water jacket connected to a circulating water-bath and monitored with a temperature probe (IT-24P). The temperature of the receptor chamber was adjusted to the temperature (around 34 °C) that would provide skin surface temperature of 32 °C before heat application and in the control experiment. Skin temperature generally reached the target temperature of 42 °C and achieved steady state within 30 min. Skin that encountered temperatures outside the temperature range of 40.5–44.4 °C for more than 30 min at any point in the experiment was excluded from the study. Skin surface temperature was monitored continuously with the temperature probe throughout the duration of the experiment. In addition, an infrared (IR) thermal camera (FLIR-E63900, FLIR Systems, Sweden) was used to check for temperature uniformity of the TDS in the diffusion cells at the end of the experiments. Representative temperature profiles and an IR image of the TDS in the IVPT study are shown in Figs. S1 and S2 (in Supplemental Materials). In the present study, the IR thermal camera appeared to be less reliable in its ability to measure the absolute value of temperature at the TDS surface than the temperature probe because different material surfaces could have different emissivities³³ and therefore the camera was used only as the supplemental method for checking temperature uniformity of the TDS. Fig. 1 shows the temperatures on the surface of the TDS obtained from the IR thermal camera and temperature probes in a calibration study when TDS were placed on a temperature-controlled surface (a glass water-bath). The temperatures were monitored by one temperature probe attached on the backing of the TDS and another probe at the interface of the TDS and temperature-controlled surface. During this study, the IR thermal camera consistently showed lower temperature values for TDS-V compared to the other TDS under the same temperature conditions. As a result, the temperature probes were used as the main method for monitoring TDS temperatures during the present IVPT and IVRT studies. Baseline reference at 32 °C (Protocol 1) and seven heat application regimens at 42 °C from 11 to 12 h, 18–19 h, 6–72 h, 24–72 h, or 48–72 h, multiple heat of 6–7 h, 18–19 h, and 48–72 h, and entire duration from 0 to 72 h (Protocols 2–8) were examined in the present study to identify the condition that represents the “worst case scenario” for evaluation of heat effects on fentanyl TDS. The protocols are summarized in Table 1.

IVPT: Sample Collection and Drug Extraction

At each sampling time point, an aliquot of 0.5 mL of receptor solution was removed and stored in a glass vial at 4 °C in a refrigerator for subsequent analysis. Meanwhile, 0.5 mL of PBS was added back into the receptor compartment to maintain its volume. After the last sampling, TDS was removed and transferred to a glass vial filled with 5 mL mixture of acetonitrile: water (50: 50) under stirring at room temperature for extraction. Fentanyl remaining in the TDS was extracted for two days to quantify the amount of drug remaining in the TDS. All samples were stored at 4 °C until analysis (usually assayed within one week).

In Vitro Drug Release Testing (IVRT)

Drug release experiments were performed in the Franz diffusion cells using a filter membrane (Millipore membrane) similar to those of *in vitro* drug release experiments in USP performance test using vertical diffusion cell for topical drug products.³⁴ The filter membrane was equilibrated in PBS overnight before commencing the experiment. Using the method described in the IVPT study, two temperature conditions were evaluated in the IVRT study: 72 h heat at 42 °C and the control without heat at 32 °C.

Dissolution Test

The *in vitro* drug release experiment was performed in 10 mL PBS in a glass vial instead of the Franz diffusion cell. Specifically, the fentanyl TDS (at least $n = 3$ for each TDS) were cut into small circular pieces with a diameter of 0.86 cm (0.58 cm² area) with a cork borer. A small amount of silicone gel was applied to the center of the backing layer of fentanyl TDS. The TDS was then attached to the glass wall of a scintillation vial by the silicone gel. After the gel was cured overnight, the release liner of fentanyl TDS was removed, and 10 mL PBS were added into each vial. The vials were then kept in water-baths at 32 °C and 42 °C, respectively, and were stirred magnetically at approximately 700 RPM. The temperatures of the water-baths were monitored by thermometers over the entire duration of the experiments. Aliquot samples of 0.5 mL were taken at 2, 4, 6, 8, 10, and 24 h and the same volume of PBS was added into the scintillation vial immediately after each sample withdrawal to maintain a constant volume. The extraction of the remaining fentanyl in TDS at the end of the dissolution study was conducted using the same procedure as described in section “IVPT: Sample Collection and Drug Extraction.”

Side-By-Side Diffusion Cell: Skin Permeation Without TDS

In vitro skin permeation experiments were conducted in side-by-side diffusion cells (effective area ~0.7 cm²)²⁴ to determine the activation energy of skin permeation of fentanyl without TDS. Split-thickness skin (4 skin donors, 2 skin samples as replicates of each skin donor, total of 8 skin samples) was cut into small square pieces of 1.5 cm × 1.5 cm and equilibrated in a Petri dish containing PBS. The skin together with a rubber gasket was then mounted in the diffusion cell with the dermis facing the receptor chamber. 2 mL PBS were added into the receptor and donor chamber, respectively. The diffusion cells were placed in a circulating water-bath at 32 ± 1 °C and continuously stirred with a stirring plate of 700 RPM. Skin integrity was checked by measuring skin resistance before the permeation experiments with the same procedure as described in the IVPT study. After the skin integrity test, the solution in the donor chamber was removed and replaced with 2 mL fentanyl drug solution. The donor drug solution was prepared by dissolving fentanyl hydrochloride in PBS to obtain 0.5 mg/mL solution and the pH was 7.3 measured by a pH meter (Oakton PC 700, Oakton Instruments, Vernon Hills, Illinois). At specified time points (0, 4, 6, 8, 10, and 12 h), 10 µL aliquots was taken from the donor chamber and 0.5 mL sample was withdrawn from the receptor chamber and replaced with 0.5 mL PBS. The collected samples were assayed by HPLC, and the same experiment was performed at 42 ± 1 °C following the same procedures as described above. The steady state fluxes (calculated using the diffusion cell area of 0.7 cm²) and permeability coefficients of skin for fentanyl were determined.

Activation energy was calculated by the ratio of the permeability coefficients at 32 °C and 42 °C and the Arrhenius relationship.²⁴

HPLC Analysis

Fentanyl concentrations were quantified using a HPLC analytical method modified from USP and Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc., Addison, IL) consisting of two pumps (LC-20 AT), a variable wavelength UV absorbance detector (SPD-20A), and an autoinjector (SIL-20A) at room temperature. A Microsorb-MV100-5 C18 column was used (15 cm × 4.6 mm, 4.6 μm, Varian, Lake Forest, CA). The mobile phase was 4 vol of ammonium acetate solution (1 in 100) and 6 vol of a mixture of methanol: acetonitrile: glacial acetic acid (400: 200: 0.6). The pH of the mobile phase was adjusted to 6.6 ± 0.1 with glacial acetic acid if necessary. The flow rate was 2.0 mL/min, and the injection volume was 25 μL. The detection wavelength was 230 nm. Fentanyl USP standard was used in the first set of experiments to verify the assay method. Standard solutions of 0.5–50 μg/mL fentanyl were prepared in PBS to construct the calibration curve.

Data and Statistical Analyses

In the IVPT, IVRT, and dissolution studies, cumulative amounts of fentanyl delivered into the receptor chamber were determined. Fluxes were calculated using the slope of each sampling time interval in the cumulative amount vs. time plot and the contact area of the TDS sample (TDS area of 0.58 cm²). To compare the effects of heat on the generic and RLD drug products, the rates of fentanyl delivery were calculated using the fluxes measured in the IVPT experiments normalized to the area of the TDS (50 μg/h TDS: TDS-Z, 23 cm²; TDS-M, 13 cm²; and TDS-V, 21 cm²). The delivery rates were plotted against the mid-points of the sampling time intervals. In the side-by-side diffusion cell study, the steady-state flux was calculated using the slope of the linear region in the cumulative amount vs. time plot and the diffusional area of the diffusion cell. In the IVPT study, the averages of the data of the skin samples from each skin donor were calculated, and the mean ± SEM of these skin donor average values were determined and presented. In the IVRT and dissolution studies, the mean ± SEM of the data were determined and presented. Student's t-test was performed using Microsoft Excel (Redmond, Washington). ANOVA with post tests were performed using GraphPad InStat (GraphPad Software, La Jolla, CA). Data from all skin samples, not the average values of the skin samples for each skin donor, were used in the statistical analyses (to capture all data and variability). In the statistical tests, differences were considered statistically significant at $p < 0.05$.

Results

Heat Effects on Skin Permeation In Vitro

Fig. 2 shows the drug delivery rate profiles of the fentanyl products in IVPT under the seven heating protocol conditions (42 °C from 11 to 12 h, 18–19 h, 6–72 h, 24–72 h, 48–72 h, multiple heat of 6–7 h, 18–19 h, and 48–72 h, and 0–72 h; Protocols 2–8) and the no heat control (32 °C; Protocol 1). For the 0–72 h no heat control, the rates of fentanyl delivery from the three TDS are within the range of the nominal drug delivery rate listed on the product label of 50 μg/h (Fig. 2a). When heat was applied over the entire 72 h application

(Fig. 2h), significantly higher fentanyl permeation was observed across skin at 42 °C than that at 32 °C in the first 20 h ($p < 0.05$, ANOVA). The delivery rates then decreased to the label value of 50 µg/h around 40–50 h during heat application.

Fig. 2d–2f show the delivery rates when the TDS was exposed to elevated temperature of 42 °C from 6 to 72 h, 24–72 h, and 48–72 h, respectively. Fentanyl delivery rates increased when heat was applied at 6 h, 24 h, or 48 h and then gradually decreased over time. Higher delivery rates were observed when heat was applied earlier either at 6 h or 24 h compared to at 48 h during the wear period of the product. Fig. 2b and c shows the delivery rate profiles when the heat was applied between 11–12 h and 18–19 h, respectively. Similar to the protocols with longer heat application, higher delivery rates were observed at the elevated temperature in these 1-h heating protocols. Also, in these two heating protocols, the TDS was removed at 19 h. No appreciable flux was observed 6 h after TDS removal.

In the multiple heating protocol, heat was first applied for 1 h from 6 to 7 h and then 18–19 h followed by heat application at 48 h until the completion of the experiment. Significant heat-induced flux increases were observed when heat was applied from 18 to 19 h and 48–72 h, respectively, for TDS-M as shown in Fig. 2g ($p < 0.05$, ANOVA).

In general, an average of ~2-fold increase in flux was observed at the elevated temperature for the studied fentanyl TDS. In addition, TDS-V generally showed lower delivery rates compared to TDS-Z and TDS-M, but this difference was not statistically significant except when heat was applied at or after 24 h (heating protocols of 24–72 h, 48–72 h, and multiple heat; Fig. 2e–2g, respectively) ($p < 0.05$, ANOVA).

The percentage of fentanyl remaining in TDS for each heat application protocol was determined using the data from the extraction studies at the end of the IVPT experiments and the total drug content in the TDS products per the values listed in the product labels, and the data are presented in Table 2. The table also presents the average total drug recovery in the IVPT experiments, which was determined using the data in the IVPT and extraction studies and the total TDS drug content. The average total recovery of fentanyl in TDS-Z, TDS-V, and TDS-M were $110 \pm 2\%$, $95 \pm 1\%$ and $105 \pm 2\%$, respectively. The higher percent of fentanyl remaining in TDS in Protocols 2 and 3 ($p < 0.05$, ANOVA) was likely due to the earlier removal of TDS from the skin at 19 h in those protocols compared to the 72 h protocols. Lower percent of fentanyl was found to remain in the TDS in the protocols that were exposed to sustained heat compared to that of the no heat control ($p < 0.05$, ANOVA, except for TDS-Z) and the result supports the hypothesis that drug depletion is a primary cause of the decreasing flux observed after 20 h during the sustained heat application protocols. Overall, the percent remaining data show a trend aligned with heat exposure duration and TDS application of the protocols. The different percent of fentanyl remaining in TDS-Z, TDS-V, and TDS-M at the end of the application period (after 72-h drug delivery) is consistent with the expectation because although the three drug products are designed to deliver fentanyl at the same rate, they have different drug loads, and as a result, there are significant differences in the amounts of fentanyl remaining in the TDS at the end of the wear period.

Effect of Sampling Frequency and Delayed Heat Effect

For the 11–12 h heating protocol (Protocol 2), the initial sampling time points were designed to be 2 h, 4 h, 6 h, 8 h, 10 h, 11 h, 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h, which had limited temporal resolution after the termination of heat at 12 h. However, unlike TDS-V and TDS-M, TDS-Z did not show a heat effect when it was initially tested with the 11–12 h heating protocol without sampling point at 13 h (data not shown). This could be attributed to a delayed heat effect on TDS-Z, which was not captured in the original study design.

Therefore, the 11–12 h heat experiment was repeated with TDS-Z and a modified sampling protocol that added 13 h and 14 h sampling points after heat termination at 12 h. The repeated experiments with TDS-Z demonstrated comparable heat effects with TDS-V and TDS-M except with the delay (Fig. 2b). A similar result was also observed with TDS-Z when heat was applied at 18–19 h (Protocol 3) without sampling points at 20 h and 21 h (Fig. 3); initial sampling time points were 2 h, 4 h, 6 h, 8 h, 10 h, 17 h, 18 h, 19 h, 24 h, 36 h, 48 h, 60 h, and 72 h. When the experiments were repeated with additional sampling time points at 20 h and 21 h to capture the fluxes at 19.5 h and 20.5 h, a heat effect was observed. Together, these results indicate that a delayed heat effect was observed with TDS-Z when short duration 1-h heat was applied. This observation suggests that the sampling time points should be carefully chosen during experimental design to adequately capture the effect of heat on drug delivery from TDS.

Larger heat effect was found when heat was applied for a longer span of time (vs. short heat duration), e.g., for TDS-Z. Fig. 4 illustrates this effect by comparing the results of the 1-h heat application at 6–7 h in the multiple heating protocol (Protocol 7) and the sustained heat application in the 6–72 h heating protocol (Protocol 4). Higher maximum delivery rates were observed when heat was applied under the sustained heat application ($p < 0.05$, Student's *t*-test), in which the flux continued to increase at 7 h and afterwards (compared to the highest delivery rates in the 6–7 h heating phase of the multiple heating protocol).

Side-By-Side Diffusion Cell Experiment

Skin permeation experiments without TDS in side-by-side diffusion cells were conducted at 32 °C and 42 °C using aqueous solution of fentanyl to determine the activation energy of fentanyl permeation across the skin. The permeability coefficients calculated from the steady state fluxes of fentanyl in PBS at 32 °C and 42 °C were 0.014 ± 0.002 cm/h and 0.031 ± 0.003 cm/h, respectively (Mean $\pm$ SEM, $n = 4$ skin donors; 2 skin replicates for each skin donor, total of 8 skin samples). The 10 °C increase from 32 °C to 42 °C increased the skin permeability of fentanyl by approximately 2 $\times$. Using the permeability coefficients and Arrhenius relationship, the experimentally determined activation energy (E_a) for fentanyl skin permeation was 61.5 kJ/mol. The increase in flux at 42 °C (or the corresponding E_a value) of fentanyl in aqueous solution is consistent with the result in the IVPT study using fentanyl TDS. In addition, this E_a value is consistent with the normal range of activation energy for skin permeation reported in the literature for permeants of similar molecular sizes and lipophilicities.³⁵

In Vitro Drug Release (IVRT) Study

IVRT study was conducted in Franz diffusion cells using filter membrane at 32 °C and 42 °C for the fentanyl TDS. Heat was applied over the entire duration for the 42 °C experiments. Fig. 5 (square symbols) shows the fentanyl flux profiles across the filter membrane at the elevated temperature compared to those of the 32 °C control. As shown in the figure, all fentanyl TDS have a fast drug releasing mechanism, with initial flux values (in the first 3 h) ~4–20 times higher than those in the IVPT and with most of the drug released within the first 24 h. Unexpectedly, drug release at 42 °C was not faster than that at 32 °C for TDS-Z and TDS-V. These results contradict the skin permeation results in the IVPT study. It is therefore hypothesized that when the membrane was not the major permeation barrier (filter membrane used in this study instead of skin), the unstirred ABL³⁶ in the Franz diffusion cell could be the rate-limiting barrier,^{37,38} and the influence of heat on the ABL became a significant factor.²³ Particularly, an increase in the effective thickness of the ABL due to the heat flux from the heating lamp could decrease the overall drug release rate from TDS into the receptor chamber. The dissolution study and dye diffusion experiments were performed to test this hypothesis.

Dissolution Study

The first experiment to test the ABL hypothesis was to compare TDS drug release in a modified dissolution chamber (solution in a vial) at 32 °C and 42 °C without using the filter membrane and Franz diffusion cell (IVRT study). The flux values between the IVRT and dissolution studies were also compared. As shown in Fig. 5 (circle symbols) at the initial time point, for all three TDS products, faster drug release (~1.5–3 times fluxes) from the TDS was observed in the vials compared to drug release in the Franz diffusion cells with the filter membrane ($p < 0.05$, Student's t-test). In addition, higher drug release was observed at the initial time point at 42 °C compared to 32 °C in the vials (approximately 1.2-, 1.4-, and 1.6-fold increases of fluxes for TDS-Z, TDS-V, and TDS-M, respectively) ($p < 0.05$, Student's t-test).

Dye Diffusion Experiments with Heating Lamp and Water-Bath Heating as the Heat Application Methods

Another experiment to test the ABL hypothesis was to examine the ABL by monitoring the diffusion of a liquid dye across the filter membrane in the Franz diffusion cell at 32 °C and 42 °C (see Supplemental Materials). In this experiment, the permeation of the dye into the receptor in the diffusion cell at 32 °C was observed within 30 min after the dye application. When the filter membrane was at 42 °C under the heating lamp, the diffusion of the dye was hindered in the region just beneath the membrane (Fig. S3 in Supplemental Materials) with no visible dye in the receptor for up to 1 h. The removal of heat from the 42 °C diffusion cell resulted in dye diffusion into the receptor within minutes as the diffusion cell cooled to 32 °C, demonstrating the heat dependency of the observation. The result suggests the presence of a stronger ABL barrier at the fluid/membrane interface underneath the membrane in the 42 °C diffusion cell (or an increase in the effective thickness of the ABL at 42 °C). This is consistent with the results in the fentanyl IVRT study. The increase in ABL at the elevated temperature could be attributed to the direction of the heat flux in the study, which was

applied from the top of the diffusion cell. The heating lamp caused a higher temperature and lower water density at the top of the receptor chamber and reduced the effect of convective mass transfer of the stirring in the receptor chamber near the receptor/membrane interface.

To further examine this hypothesis, the same dye diffusion experiment was conducted using the water-bath as the heat application method (see Supplemental Materials). In this experiment, dye diffusion in the 32 °C diffusion cell was slower than that in the 42 °C diffusion cell; dye permeation into the receptor was observed within 15 min after the dye application in the 42 °C diffusion cell with significantly stronger color intensity than that of the 32 °C diffusion cell (Fig. S4 in Supplemental Materials). This observation is consistent with higher aqueous diffusion coefficient of the dye at 42 °C than that at 32 °C. Together with the results in the dye diffusion study of the heating lamp, these results support the hypothesis of heat effect on the ABL: poorer mixing at the receptor/membrane interface in the receptor chamber at the elevated temperature when heat was applied from the top of the diffusion cell using a heating lamp.

Discussion

An adequately designed IVPT study evaluating heat effects on transdermal fentanyl products can provide useful information on the comparative assessment of drug delivery from TDS products following exposure to heat. The present study examined the factors that may influence the outcome of an IVPT study conducted using the heating-lamp method to evaluate heat effect on TDS. First, it was found that temperature control of TDS using IR thermal measurements could be affected by the material of the TDS backing layer, and an alternative method may be needed to adequately monitor the temperature at the surface of the skin during the experiments. Second, the IVPT results indicate that maximum heat-induced flux was usually observed when heat was applied earlier or for a sustained period for fentanyl TDS (Fig. 2). For example, for two of the three TDS tested, the maximum peak flux was observed when sustained heat was applied at 6 h, approximately the time at which maximum flux was reached for the baseline temperature condition. The clinical significance of this finding is that patients may be at greater risks of undesired increases in systemic fentanyl exposure when heat is applied earlier during the wear period following administration of TDS or when heat is applied for longer durations, e.g., overnight application of a heating pad. Third, good temporal resolution of sampling timepoints is needed to identify the differences in heat effects among fentanyl TDS when heat is applied for a relatively short duration. In general, similar trends of drug delivery profiles were observed for the three fentanyl TDS evaluated in this study. However, when heat was applied for 1 h in the 11–12 h or 18–19 h protocol, TDS-Z showed a delayed heat effect (Fig. 3), which was not the case for TDS-V and TDS-M. Overall, the results in the present study demonstrate similar trends in drug delivery profiles for TDS-Z, TDS-V, and TDS-M with and without heat application, but differences exist with regard to specific heat effects that were observed among these three TDS products. For example, lower delivery rates were observed for TDS-V than those of TDS-Z and TDS-M when heat was applied at 24 h and after (Fig. 2). The observed differences could be due to the differences in TDS formulations and designs, and these differences in TDS performance could only be detected with better temporal resolution (i.e., more sampling points) during and after heat application.

Heat effect on *in vitro* drug release from fentanyl TDS (without skin) was also investigated in the dissolution study and IVRT to delineate the rate-determining process of transdermal delivery from fentanyl TDS. In the dissolution study, the trend of decreasing fluxes over time and the lack of a significant heat effect at the later time points could be attributed to drug depletion in the TDS. The results from the drug release studies suggest that fentanyl is released under a fast drug releasing mechanism for the fentanyl TDS and the skin is the rate-limiting barrier, which primarily controls the enhancement in drug delivery under elevated temperature during the IVPT studies. It is interesting to note that the IVRT results are likely compromised due to heat effect on the ABL in the Franz diffusion cell. In the absence of the skin as the rate-limiting barrier for the permeation of fentanyl as is the case during IVRT studies using a filter membrane and when drug release from TDS is not the rate-determining factor (e.g., absence of an internal rate-controlling membrane), the ABL becomes the rate-limiting barrier during drug release from TDS in the diffusion cell. In the current IVRT study, heat application by a heating lamp resulted in a decrease in drug release from TDS at 42 °C compared to that at 32 °C in the diffusion cell due to the increase in the effective thickness of the ABL at 42 °C when heat is applied from the top of the diffusion cell. The results in the present study suggest that the ABL is the major barrier associated with the Franz diffusion cell when a highly permeable filter membrane is used in the IVRT study and when heat application is provided from the top of the diffusion cell. For the IVPT study in which the skin is the rate-limiting barrier for drug permeation, where the resistance of drug diffusion in the ABL is several orders of magnitudes smaller than that of skin, the effect of ABL on drug permeation becomes negligible. Hence, the effect of elevated temperature on ABL or the effect of ABL in general could be a significant factor in IVRT studies but not in IVPT studies.

Conclusion

Factors that influence the evaluation of heat effects using the heating-lamp IVPT and IVRT methods were investigated using fentanyl TDS. First, the temperature monitored by IR thermal measurements could be different from the actual temperature at the surface of the TDS, possibly due to the different emissivities of TDS surfaces. Hence, an alternative method or calibration of the IR thermal measurement device may be necessary to adequately capture the temperature at the surface of the TDS for certain TDS products. Second, fentanyl permeation across human skin *in vitro* was 2x faster at 42 °C than that of 32 °C, which is consistent with the activation energy determined in the skin permeation study in PBS without the TDS. Third, the dissolution study indicated a fast drug releasing mechanism for all the TDS that were evaluated, with higher drug release observed at the initial time points in the study under the influence of the elevated temperature. Together, the dissolution, IVRT, and dye diffusion studies revealed that the ABL could be a rate-limiting barrier to the release of fentanyl from the TDS in the Franz diffusion cell with a filter membrane when heat was applied from the top of the diffusion cell using the heating lamp. Additional optimization of the IVRT setup may be necessary to mitigate the influence of the ABL. Fourth, during the IVPT protocols, higher skin flux was observed when heat was applied earlier or with sustained heat application on the TDS, suggesting that early and sustained heating protocols could be used to identify the maximum heat effect on TDS. Lastly, sampling points

immediately after the termination of heat application are important to identify the delayed heat effect of TDS-Z after short duration heat exposure. These results reveal a number of factors that should be considered in IVPT and IVRT studies for the evaluations of TDS under the influence of elevated temperature. For IVPT, considerations should be given to the instrumentation such as temperature microprobe vs. IR thermometer, the temporal resolution such as the number and time interval of receptor sampling, and the time and duration of heat application to identify the maximum flux in the “worst case scenario” of heat effect on transdermal delivery. For IVRT, the additional factors of ABL and direction of heat flux from heating lamp vs. water-bath should be considered. The data from the heating-lamp method of Franz diffusion cell and filter membrane suggest that this IVRT model may not be suitable for heat-effect evaluation of drug release from TDS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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	32°C temperature controlled surface			42°C temperature controlled surface		
TDS	Temperature, probe at TDS/surface	Temperature, probe at TDS backing surface	Temperature, IR camera	Temperature, probe at TDS/surface	Temperature, probe at TDS backing surface	Temperature, IR camera
TDS-Z	32.7	32.5	32.4	42.7	42.3	43.8
TDS-V	32.7	32.6	28.2	42.6	42.0	37.4
TDS-M	32.5	32.6	32.6	42.7	42.5	43.4

Fig. 1.

Comparison of the thermal images at 32 °C (left image) and 42 °C (right image) for TDS-Z (Sp1), TDS-V (Sp2) and TDS-M (Sp3) in a calibration study. The table shows the temperatures measured by the temperature probes and IR thermal camera. The temperature images of TDS (circular shapes) were obtained from the IR thermal camera. A circulating water-bath was used to control the temperature on a surface and the three TDS were adhered on this temperature-controlled surface with one temperature probe at the TDS/surface interface and another temperature probe at the surface of the TDS backing. The temperature measurements from the probes and IR thermal camera in the calibration study are presented in the table (average of at least two measurements). Unlike TDS-Z and TDS-M, which have a transparent appearance on the TDS backing, TDS-V has a flesh color on its backing layer. The lower temperatures on the IR thermal camera could be due to lower emissivity from the colored backing of TDS-V.

Fig. 2. Profiles of fentanyl delivery rates for TDS-Z (diamonds), TDS-V (triangles) and TDS-M (squares) under (a) control condition at 32 °C (Protocol 1) and the conditions of heat application for (b) 11–12 h; (c) 18–19 h; (d) 6–72 h; (e) 24–72 h; (f) 48–72 h; (g) 6–7, 18–19 and 48–72 h; and (h) 0–72 h at 42 °C (Protocols 2–8). Mean $\pm$ SEM, $n = 4$ skin donors (3–4 skin samples as replicates of each skin donor). Arrows indicate the time when TDS was removed.

Fig. 3.

Effect of sampling interval on the fentanyl delivery rate for TDS-Z when heat was applied from 18 to 19 h (Protocol 3). Open diamonds represent the initial 72 h study results without sampling time points at 20 and 21 h. Solid diamonds represent the results of the repeated study including additional sampling time points at 20 and 21 h to capture the fluxes at 19.5 h and 20.5 h. The repeat experiment was only conducted for the duration of 24 h instead of 72 h because no appreciable flux was observed after 24 h when the TDS was removed at 19 h as shown in the initial study. Mean $\pm$ SEM, $n = 4$ skin donors (3 and 2–3 skin samples as replicates of each skin donor for the protocols with and without 20 and 21-h sampling time points, respectively).

Fig. 4.

Effect of heat application duration on the fentanyl delivery rate for TDS-Z. Two heat application protocols were compared: 6–7 h heat from the multiple heating protocol (open symbols, Protocol 7) and 6–72 h heat (closed symbols, Protocol 4). Mean $\pm$ SEM, $n = 4$ skin donors (3–4 skin samples as replicates of each skin donor).

Fig. 5.

Drug release profiles at 32 °C (blue open symbols) and 42 °C (red closed symbols) in the IVRT study using filter membrane in the Franz diffusion cell (squares) and the dissolution study in the vial (circles) for (a) TDS-Z, (b) TDS-V, and (c) TDS-M. The secondary y-axis shows the delivery rates corresponding to the fluxes of the TDS products (e.g., $140 \mu\text{g}/\text{cm}^2/\text{h} \approx 3200 \mu\text{g}/\text{h}$ for TDS-Z) for comparison to the data in Fig. 2. The durations of the IVRT and dissolution studies were 72 h and 24 h, respectively. Data after 24 h are not presented in the figure because drug release after 24 h was negligible compared to those in the first 24 h (see Fig. S5, Supplemental Materials). Mean $\pm$ SEM (5–6 replicates for each temperature condition in the IVRT study, and 3–4 replicates for each temperature condition in the dissolution study). Some error bars are small and overlap with the symbols.

Table 1

Summary of Approaches to Investigate the Effects of Heat on TDS.

Approach	Experiment	Purpose
In <i>vitro</i> permeation test (IVPT)	<ul style="list-style-type: none"> Fentanyl TDS, seven heating protocols and control (no heat), Franz diffusion cell, skin, different sampling protocols 	<ul style="list-style-type: none"> Identify the IVPT experimental conditions for assessing heat effects on TDS and skin permeation of fentanyl
	<ul style="list-style-type: none"> Protocol 1: no heat (32 °C); Protocols 2 and 3: heat for 1 h at 11–12 h and 18–19 h, respectively, with TDS removal at 19 h; Protocol 4: heat from 6 to 72 h; Protocol 5: heat from 24 to 72 h; Protocol 6: heat from 48 to 72 h; Protocol 7: heat from 6 to 7 h, 18–19 h, and then 48–72 h; Protocol 8: heat from 0 to 72 h 	<ul style="list-style-type: none"> Protocol 1: baseline control condition; Protocols 2 and 3: short heat exposure effect mimicking those in a recent study²¹; Protocol 4: sustained heat exposure when the flux begins to reach its peak in the baseline profile (6 h time point); Protocol 5: sustained heat exposure after the flux begins to decrease from its peak in the baseline profile (24 h time point); Protocol 6: heat effect after a significant portion of the drug has been depleted from the TDS; Protocol 7: multiple heat application scenarios for the comparison of the short heat exposure between 6 and 7 h and sustained heat exposure from 6 to 72 h (vs. Protocol 4) and the effect of prior heat exposure at 6–7 h on subsequent heat exposures at 18–19 h (vs. Protocol 3) and heat exposures at 6–7 h and 18–19 h on subsequent exposure at 48–72 h (vs. Protocol 6); Protocol 8: sustained heat effect at the start of TDS application (including the lag time phase) for comparison to other heat exposures
In <i>vitro</i> release testing (IVRT)	<ul style="list-style-type: none"> Fentanyl TDS, one heating protocol and control (heat from 0 to 72 h and no heat), Franz diffusion cell, filter membrane 	<ul style="list-style-type: none"> Examine heat effects on drug release from TDS in Franz diffusion cell using IVRT
	<ul style="list-style-type: none"> Dye solution, one heating protocol and control, Franz diffusion cell, filter membrane 	<ul style="list-style-type: none"> Verify the effect of aqueous boundary layer (ABL) in Franz diffusion cell
Dissolution test	<ul style="list-style-type: none"> Fentanyl TDS, one heating protocol and control (heat from 0 to 24 h and no heat), 20-mL vial 	<ul style="list-style-type: none"> Examine heat effects on drug release from TDS with minimal ABL in a vial as dissolution chamber (vs. Franz diffusion cell)
Steady-state permeability test	<ul style="list-style-type: none"> Fentanyl solution, 32 °C and 42 °C for 12 h, side-by-side diffusion cell, skin 	<ul style="list-style-type: none"> Determine skin permeability for fentanyl and calculate activation energy

Table 2

Percent of Fentanyl Remaining in TDS After Skin Permeation Experiments and Percent Recovery in the Experiments.

Percent Remaining in TDS After Each Protocol ^a								Percent Recovery ^b
1	2	3	4	5	6	7	8	Average, All Protocols
no Heat	11–12 h	18–19 h	6–72 h	24–72 h	48–72 h	multiple Heat	0–72 h	
TDS-Z	66 ± 10	98 ± 5	94 ± 3	48 ± 7	60 ± 4	61 ± 8	58 ± 4	58 ± 10
TDS-V	52 ± 5	76 ± 3	74 ± 3	35 ± 5	35 ± 8	37 ± 7	35 ± 6	32 ± 7
TDS-M	46 ± 9	87 ± 6	91 ± 2	22 ± 6	16 ± 10	26 ± 14	27 ± 10	26 ± 8
								105 ± 2

^aMean ± SEM, n = 4 skin donors (3–4 skin replicates for each skin donor).

^bMean ± SEM, n = 8 protocols. Percent recovery was based on total drug content in the TDS according to the drug product labels. Percent recovery does not include fentanyl in skin; the amounts of fentanyl remaining in the skin were assumed to be small at the end of the experiments and were not determined during the extraction analysis.