

## Research Article

Theme: Team Science and Education for Pharmaceuticals: the NIPTE Model

Guest Editors: Ajaz S. Hussain, Kenneth Morris, and Vadim J. Gurvich

# The Sensitivity of *In Vitro* Permeation Tests to Chemical Penetration Enhancer Concentration Changes in Fentanyl Transdermal Delivery Systems

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**Abstract.** Chemical penetration enhancers (CPEs) are frequently incorporated into transdermal delivery systems (TDSs) to improve drug delivery and to reduce the required drug load in formulations. However, the minimum detectable effect of formulation changes to CPE-containing TDSs using *in vitro* permeation tests (IVPT), a widely used method to characterize permeation of topically applied drug products, remains unclear. The objective of the current exploratory study was to investigate the sensitivity of IVPT in assessing permeation changes with CPE concentration modifications and subsequently the feasibility of IVPT's use for support of quality control related to relative CPE concentration variation in a given formulation. A series of drug-in-adhesive (DIA) fentanyl TDSs with different amounts of CPEs were prepared, and IVPT studies utilizing porcine and human skin were performed. Although IVPT could discern TDSs with different amounts of CPE by significant differences in flux profiles, maximum flux ( $J_{\max}$ ) values, and total permeation amounts, the magnitudes of the CPE increment needed to see such significant differences were very high (43–300%) indicating that IVPT may have limitations in detecting small changes in CPE amounts in some TDSs. Possible reasons for such limitations include formulation polymer and/or other excipients, type of CPE, variability associated with IVPT, skin type used, and disrupted stratum corneum (SC) barrier effects caused by CPEs.

**KEY WORDS:** chemical penetration enhancer (CPE); transdermal delivery system (TDS); *in vitro* permeation test (IVPT); fentanyl.

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**Abbreviation** API, active pharmaceutical ingredient; CPE, chemical penetration enhancer; DPG, dipropylene glycol; FDA, Food and Drug Administration; HPLC, high-performance liquid chromatography; IPM, isopropyl myristate; IVPT, *in vitro* permeation test;  $J_{\max}$ , maximum flux; LLOQ, Lower limit of quantification; OA, oleic acid; OLA, oleyl alcohol; PIB, polyisobutylene; PSA, pressure-sensitive adhesive; SC, stratum corneum; TDS, transdermal delivery system; TEWL, transepidermal water loss

## INTRODUCTION

The effects of chemical penetration enhancers (CPEs) are widely known, and their frequency of use in transdermal delivery systems (TDSs) has been increasing (1,2). Many of the marketed TDS products have CPEs in their formulations, such as isopropyl myristate (IPM) in the fentanyl TDS (Apotex Corp.), butylene glycol, oleic acid (OA), propylene glycol, and dipropylene glycol (DPG) in Vivelle® estradiol TDS (Novartis Pharmaceuticals Corporation), and ethanol, glyceryl monooleate, methyl laureate, and glycerin in Androderm® testosterone TDS (Actavis Pharma, Inc.). More than 300 chemicals have been studied for their ability to act as CPEs, and there have been increasing efforts in understanding their complex mechanisms of action (1–3). Some of the potential mechanisms of action include modifying the conformation of the stratum corneum (SC) intracellular keratin, affecting desmosomes to disrupt cohesion between corneocytes, modifying intercellular lipid domains, altering the solvent nature of SC, and enhancing thermodynamic activity of drug in the formulation (1,4,5).

The incorporation of CPEs in TDSs not only helps with drug delivery but also reduces the amount of required drug load in formulations. The high amount of drug load in TDSs has been a concern of regulatory agencies, as the high residual drug amount after the intended wear time can pose a safety risk to those who wear the product longer than indicated, abuse residual drug, or get unintentional exposure from discarded TDS products. Consequently, the U.S. Food and Drug Administration (FDA) published a Guidance for Industry, Residual Drug in Transdermal and Related Drug Delivery Systems, which encourages reducing residual drug by optimizing formulation, design, and components of the products, including the use of penetration enhancers (6).

Despite increased use of CPEs in TDSs, there is no well-defined understanding of how the incorporation of varying concentrations of CPEs is reflected in permeation results from *in vitro* test methods, such as the *in vitro* permeation test (IVPT), and thus what manufacturing flexibility may be permissible with respect to CPE content. In addition, there is no specific guideline available for testing TDS products that include CPEs. While IVPT is a widely used method to evaluate and compare permeation profiles of topically delivered drug products in pharmaceutical development, the effect of varying concentrations of CPEs on active pharmaceutical ingredient (API) penetration using IVPT remains ambiguous.

Our current study was designed to address whether IVPT is a feasible method to evaluate the magnitude of enhancement in API penetration when CPE concentrations vary. Specifically, IVPT was performed using fentanyl TDSs with varying amounts of CPEs to investigate whether IVPT results can discriminate the influence of different amounts of

CPEs in TDS formulations. In order to minimize the influence of other formulation factors (*i.e.* excipients) that could influence flux changes, drug-in-adhesive (DIA) TDSs with only three ingredients, API, pressure-sensitive adhesive (PSA), and CPE, were formulated, with varying amounts of CPE. IVPT experiments were performed using the prepared TDSs utilizing porcine and human skin.

## MATERIALS AND METHODS

### Preparation of Fentanyl TDS

Each component of the TDS, (fentanyl, adhesive, and CPE) was combined in a glass vial. The composition details in each formulation are listed in Table I. The mixture was stirred thoroughly to obtain a homogeneous mixture, sonicated for 20 min, and placed at room temperature overnight to remove any remaining air bubbles. The mixture was then extruded onto a release liner (ScotchPak™ 1022; St. Paul, MN) using a coater (ChemInstruments EZ-Coater EC-200; Fairfield, OH) and a clearance applicator (Gardco® AP-B5354; Pompano Beach, FL), resulting in a wet film with a thickness of 20 mil. Coated film was kept at room temperature under a fume hood for 15 min and then dried at 90°C for 15 min in a convection oven to remove any residual organic solvent from the film. Dried film was then laminated with a backing membrane (ScotchPak™ 1006; St. Paul, MN) using a laboratory laminator (ChemInstruments Benchtop Laboratory Laminator LL-100; Fairfield, OH). The prepared TDSs were stored in a heat-sealed foil pouch at room temperature until the IVPT experiment (storage duration did not exceed 24 h).

**Table I.** Weight Percent (%) of Matrix Mixture for Prepared Fentanyl TDS

	A	B	C	D	E
F1					
Fentanyl	2.5	2.5	2.5	2.5	2.5
CPE	0	12 (OA)	12 (OLA)	12 (DPG)	12 (IPM)
Acrylic PSA (DURO-TAK® 87-4098)	97.5	85.5	85.5	85.5	85.5
F2					
Fentanyl	2.5	2.5	2.5	2.5	2.5
CPE (OA)	0	10	12	14	17
Acrylic PSA (DURO-TAK® 87-4098)	97.5	87.5	85.5	83.5	80.5
F3					
Fentanyl	2.5	2.5	2.5	2.5	2.5
CPE (OA)	0	5	7	10	20
PIB PSA (DURO-TAK® 87-6908)	97.5	92.5	90.5	87.5	77.5
F4					
Fentanyl	2.5	2.5	2.5	2.5	2.5
CPE (IPM)	0	5	7	10	20
PIB PSA (DURO-TAK® 87-6908)	97.5	92.5	90.5	87.5	77.5
F5					
Fentanyl	2.5	2.5	2.5	2.5	2.5
CPE (IPM)	0	5	7	10	20
PIB PSA (DURO-TAK® 87-6908)	97.5	92.5	90.5	87.5	77.5
F6					
Fentanyl	2.5	2.5	2.5	2.5	2.5
CPE (IPM)	0	7	8	9	10
PIB PSA (DURO-TAK® 87-6908)	97.5	90.5	89.5	88.5	87.5

## Skin Preparation

Yucatan miniature pig skin (purchased from Sinclair Bio Resources, LLC; Auxvasse, MO) and human skin (obtained from the Cooperative Human Tissue Network after harvest with consent during abdominoplasty surgery) used for IVPT experiments were dermatomed to a thickness of  $250 \pm 50 \mu\text{m}$ . Details of skin preparation were reported in previous work (7).

## *In vitro* Permeation Test

IVPT experiments were conducted using a PermeGear® flow-through in-line diffusion system (Hellertown, PA). Diffusion cells with membrane supports with a permeation area of  $0.95 \text{ cm}^2$  were used. The receiver solution was 0.9% saline with 0.005% gentamicin at a flow rate of 0.8 mL/h. Each skin section was mounted onto the diffusion cell with receiver solution flowing underneath for at least 1 h and checked for integrity by a transepidermal water loss (TEWL) measurement (cyberDERM, Inc., Broomall, PA) prior to experiment initiation. TDSs were cut into circular discs, matching the permeation area of the skin in the diffusion cell and applied on top of the skin. Adhesion of the disc was ensured by first rubbing the disc onto the skin surface using a flat-bottom surface of a typical HPLC (high-performance liquid chromatography) vial and second by applying a piece of non-occlusive polypropylene knitted mesh (0.15 mm monofilament,  $3.0 \times 2.8 \text{ mm}$  pores, 47 GSM; SurgicalMesh™ Division of Textile Development Associates, Inc.) to cover the skin and TDS disc to prevent lift of the disc during the experiment. Diffusion samples were automatically collected into scintillation vials every 3 h up to 48 h using the fraction collector and analyzed by HPLC. All IVPT experiments were performed with 3–4 replicates per formulation.

## Extraction of Fentanyl from TDS

After IVPT experiments, each TDS disc was removed from the skin and analyzed to quantify residual amount of fentanyl. The TDS disc was cut into small pieces and added to a 15-mL centrifuge tube. Unused TDS discs ( $n = 3$  per formulation) were also analyzed in the same manner to determine the amount of fentanyl content in an unused TDS disc and to evaluate uniformity of fentanyl content in different sections of the prepared fentanyl TDS. The extraction solvent, 10 mL of methyl tertiary butyl ether per TDS disc, was added to each tube. The tube was then capped, covered with Parafilm®, and sonicated for 10 min. After sonication, they were shaken at 200 rpm for 24 h, centrifuged at  $20,800 \times g$ , and an aliquot of supernatant was used for HPLC analysis.

## HPLC Analysis of Samples

All samples were analyzed and quantified for fentanyl using a previously reported HPLC method (7). The concentration of fentanyl standards ranged from 0.05 to  $25 \mu\text{g/mL}$ , with a lower limit of quantification (LLOQ) of  $0.05 \mu\text{g/mL}$ . The IVPT samples were diluted with acetonitrile in 7:3 ratio ( $v/v$ ) prior to HPLC analysis. Standards were prepared in 0.9% saline:acetonitrile (7:3,  $v/v$ ) and analyzed with each set of IVPT samples. An aliquot of the extracted fentanyl samples from the TDS discs was evaporated under a stream of nitrogen gas and

reconstituted in methanol:10 mM sodium 1-heptane sulfonate (pH 2.5) (1:1,  $v/v$ ) with a 10-fold dilution. All extraction samples from the TDS disc were analyzed with a set of standards prepared in methanol:10 mM sodium 1-heptane sulfonate (pH 2.5) (1:1,  $v/v$ ).

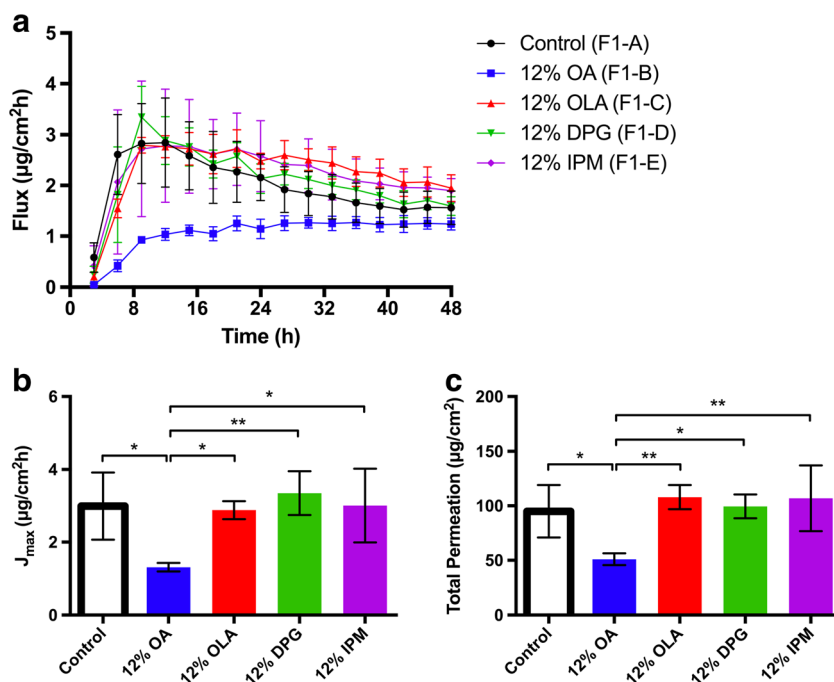
## Data Analysis and Statistical Analysis

All of the experimental results are reported as mean  $\pm$  SD, with  $n = 3$ –4 replicates. Samples with a calculated concentration below LLOQ ( $0.05 \mu\text{g/mL}$ ) after HPLC analysis were treated as zero. Statistical analyses were performed using GraphPad Prism® software. A one-way ANOVA test followed by Tukey's *post-hoc* multiple pairwise comparisons was used to compare prepared TDS formulations. Differences were considered to be significant when  $p \leq 0.05$ , and significant differences were indicated as follows: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ ; \*\*\*\* $p \leq 0.0001$ .

## RESULTS AND DISCUSSION

The current exploratory study investigated whether IVPT experiments utilizing *ex vivo* skin can detect small changes in the amount of CPE in a DIA fentanyl TDS. Since there are several factors other than a CPE in TDS formulations that contribute to flux changes, this approach involved formulating TDSs with only three components: the API (fentanyl), PSA, and a CPE.

The first set of TDS formulations (F1-A through E; Table I) was prepared in order to investigate the effect of different types of CPEs in fentanyl TDSs. Acrylic PSA was chosen from the three most commonly used PSAs (acrylic, PIB, and silicone) (8) due to its compatibility with various CPEs at a reasonable level based on exploratory formulation trials (data not shown). The F1 TDSs were formulated with 2.5% of fentanyl based in acrylic PSA: one control TDS without a CPE and four TDSs, each with the same percentage, 12%, of a different CPE. When an IVPT experiment was performed on porcine skin using these five formulations, the resulting flux profiles of fentanyl were very similar for all, except formulation F1-B, containing OA (Fig. 1a). Mean maximum flux ( $J_{\text{max}}$ ) and total permeation amount over 48 h from F1-B were significantly lower compared to the other four formulations (Fig. 1b,c). Despite a relatively high level (12%) of CPE incorporated in F1 TDSs, the other three TDSs, containing OLA, DPG, and IPM, did not show significantly different flux profiles compared to the control TDS. Interestingly, the addition of OA in the TDS decreased permeation of fentanyl through the skin, instead of enhancing it. This is likely due to the increased solubility of fentanyl in the adhesive matrix upon addition of OA, hence decreasing the thermodynamic activity and making the fentanyl diffusion rate slower from the adhesive layer. Such decreased flux due to addition of OA is in line with findings of Mehdizadeh *et al.* (9). They reported that the enhancing effect of OA reached a plateau at 10%, and steady-state flux decreased when OA concentration was increased further to 15%. Since some CPEs, such as Azone, are known to be more effective at low concentrations compared to those at high concentrations (1), it is assumed

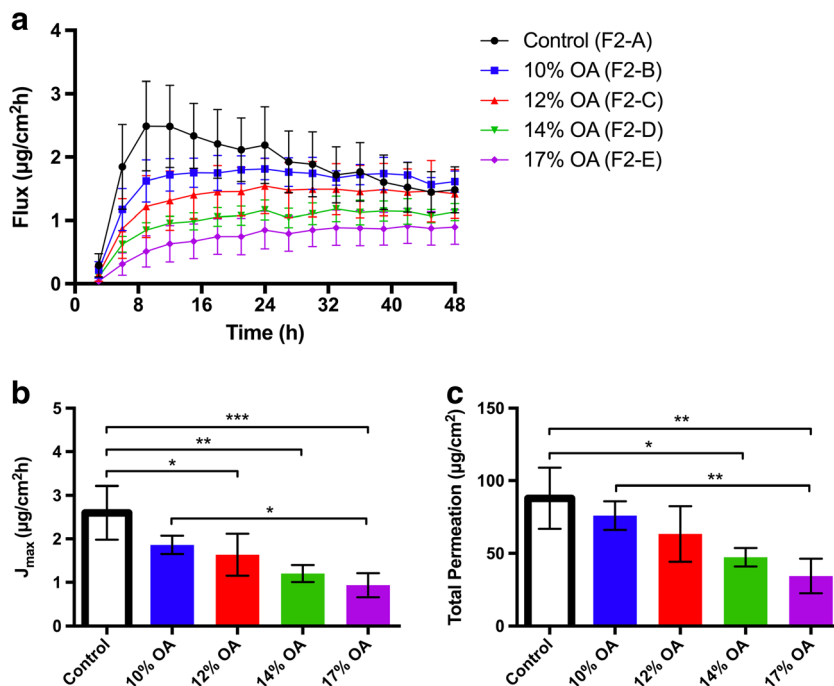


**Fig. 1.** a Permeation profiles, b comparison of  $J_{\text{max}}$ , and c comparison of total permeation amount from fentanyl TDSs containing different CPEs at the level of 12% in acrylic PSA (mean  $\pm$  SD;  $n = 4$  skin pieces from a single porcine skin donor)

that OA at lower concentrations and/or with formulation optimization would increase the flux of fentanyl.

Next, the effect of OA in acrylic PSA was explored further by formulating fentanyl TDSs with different amounts of OA (F2-A through E; Table I). The resulting flux profiles of fentanyl through porcine skin from the five formulations containing 0, 10, 12, 14, and 17% of OA in acrylic PSA are

shown in Fig. 2a. The higher amount of OA in TDSs resulted in a lower flux profile of fentanyl, with the control TDS containing no CPE exhibiting the highest flux profile among the five formulations. The  $J_{\text{max}}$  observed from the control TDS, F2-A, was significantly higher at  $2.6 \mu\text{g}/\text{cm}^2 \text{h}$ , compared to  $J_{\text{max}}$  from F2-C, D, and E, containing 12, 14, and 17% of OA, respectively (Fig. 2b). The total permeation



**Fig. 2.** a Permeation profiles, b comparison of  $J_{\text{max}}$ , and c comparison of total permeation amount from fentanyl TDSs containing different amounts of OA in acrylic PSA (mean  $\pm$  SD;  $n = 4$  skin pieces from a single porcine skin donor)

amount of fentanyl over 48 h was significantly higher from the control TDS without OA (F2-A) compared to either F2-D or F2-E, containing 14 and 17% of OA, respectively (Fig. 2c). The statistically significant difference in IVPT results among the four TDSs containing different levels of OA could only be seen when the amount of OA increased by 70% (10% OA to 17% OA) (Fig. 2b,c).

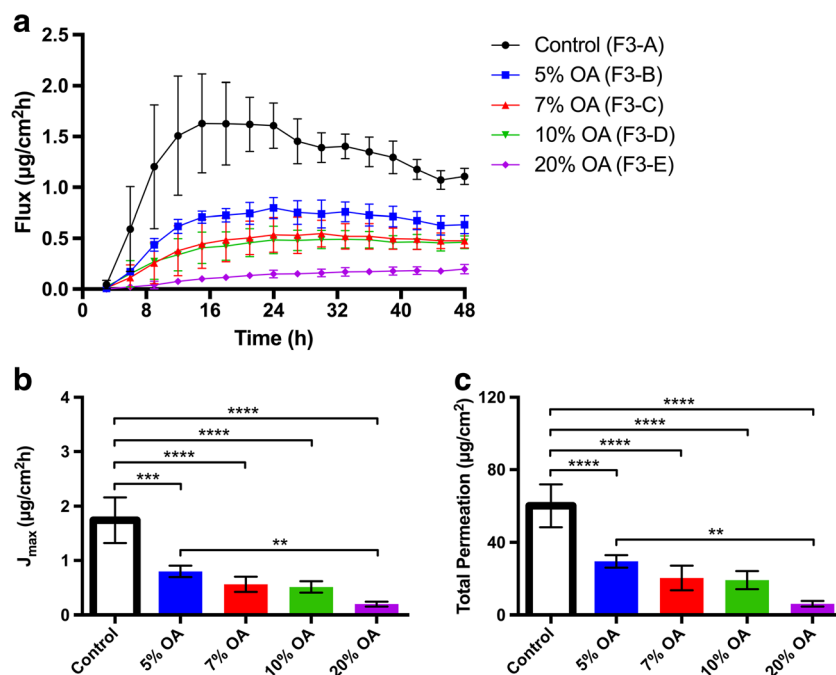
The next series of fentanyl TDS formulations, F3, were prepared in PIB PSA with differing levels of OA (0, 5, 7, 10, 20%; Table 1). The fentanyl flux profiles from these F3 TDSs were similar to those from the F2 TDSs prepared in acrylic PSA. The higher amount of OA in PIB PSA resulted in a lower flux profile of fentanyl (Fig. 3a). However, it seems that a greater change in the amount of OA in PIB PSA is needed in order to see a significant change in IVPT results. Significant differences in  $J_{\max}$  and total permeation amount were observed when there was a 300% change in the amount of OA (5 to 20% OA; Fig. 3b,c), compared to a 70% change in acrylic PSA (Fig. 2b,c). The control TDS without OA, F3-A, showed significantly higher  $J_{\max}$  and total permeation amount over 48 h, compared to the other four TDSs, F3-B, C, D, and E, containing 5, 7, 10, and 20% OA, respectively (Fig. 3b,c). These PSA-CPE differences occur when CPEs have different partition coefficients in the PSA.

Another CPE, IPM, in PIB PSA fentanyl TDSs was investigated in the F4 TDS formulations. Five F4 TDSs were prepared with various levels (0, 5, 7, 10, 20%) of IPM (Table 1). Flux profiles from these five TDSs, after performing an IVPT experiment on porcine skin, showed that the higher amount of IPM resulted in a higher flux of fentanyl (Fig. 4a), unlike previous formulations (F2 and F3). However, permeation differences among the five TDSs were less defined, as compared to differences among F2 formulations (OA in acrylic PSA; Fig. 2) or F3 formulations (OA in PIB PSA; Fig.

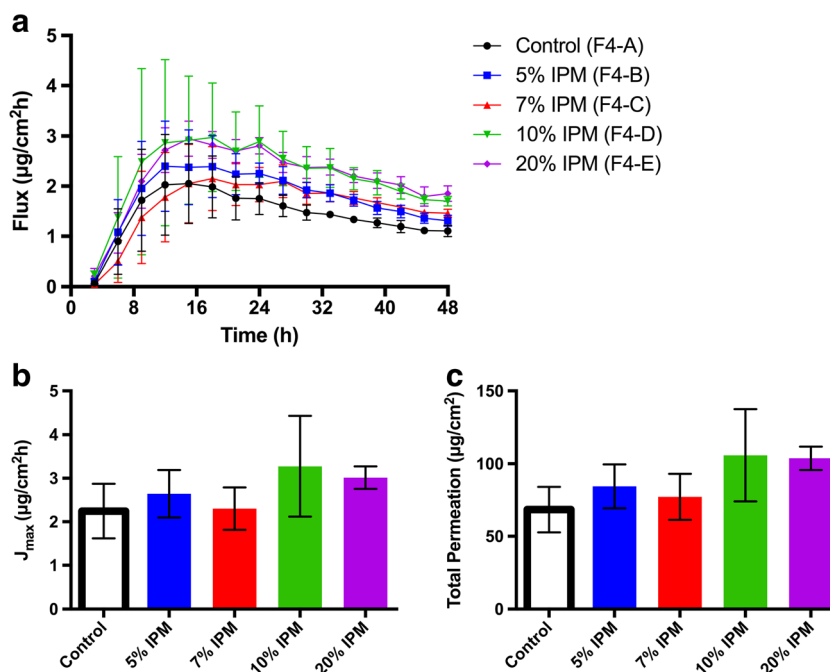
3). Consequently, there were no significant differences among the five F4 TDSs in terms of  $J_{\max}$  and total permeation amount over 48 h (Fig. 4b,c).

The four F5 TDSs were prepared similarly to the F4 TDSs (Table 1) to further explore the effect of IPM on IVPT utilizing human skin, instead of the porcine skin. The trend seen from the flux profiles of these four F5 TDSs was similar to the results seen from the F4 TDSs in Fig. 4; the higher amount of IPM resulted in a higher flux profile with small differences among TDSs (Fig. 5a). No statistically significant differences in  $J_{\max}$  values were observed among the four TDSs (Fig. 5b). The total permeation amount over 48 h was significantly higher from F5-D, containing 10% of IPM, compared to that of F5-A, without a CPE (Fig. 5c).

The IPM in PIB PSA for the fentanyl TDS was further explored on human skin by preparing five F6 formulations with a smaller variation in the amount of IPM (Table 1). The F6-E TDS containing 10% of IPM resulted in the highest flux level, while the F6-A TDS containing 0% of IPM resulted in the lowest flux level among the five F6 TDSs (Fig. 6a). In addition, IVPT results showed a significant difference when the amount of IPM increased by 43% (from 7 to 10%), in terms of  $J_{\max}$  and total permeation amount (Fig. 6b,c). This difference observed on human skin was absent when similar F4 TDSs were tested on porcine skin (Fig. 4). Although our current exploratory results are not comprehensive, the findings suggest that human skin may be more sensitive to permeation changes due to changes in CPE levels in TDSs. This might be due to a slightly better barrier function and, thus, a slightly higher resistance of human SC, as compared to porcine SC. Since IPM's permeation enhancement effect is mainly due to its partitioning into the lipid domains of SC and disrupting the lamellar state of order (2,10), the denser lipid organization in human SC (11) might provide a greater



**Fig. 3.** a Permeation profiles, b comparison of  $J_{\max}$ , and c comparison of total permeation amount from fentanyl TDSs containing different amounts of OA in PIB PSA (mean  $\pm$  SD;  $n = 4$  skin pieces from a single porcine skin donor)

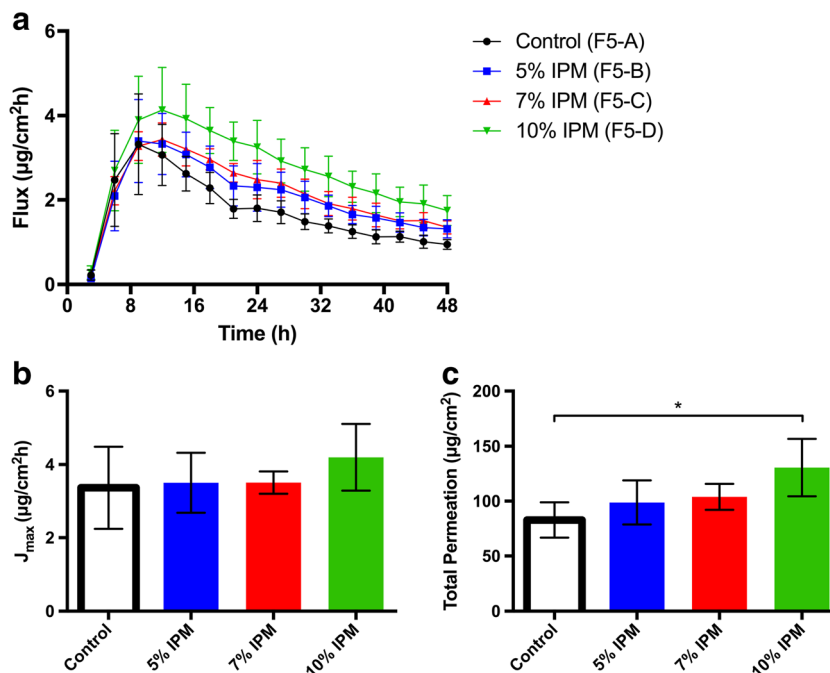


**Fig. 4.** **a** Permeation profiles, **b** comparison of  $J_{\text{max}}$ , and **c** comparison of total permeation amount from fentanyl TDSs containing different amounts of IPM in PIB PSA (mean  $\pm$  SD;  $n = 4$  skin pieces from a single porcine skin donor)

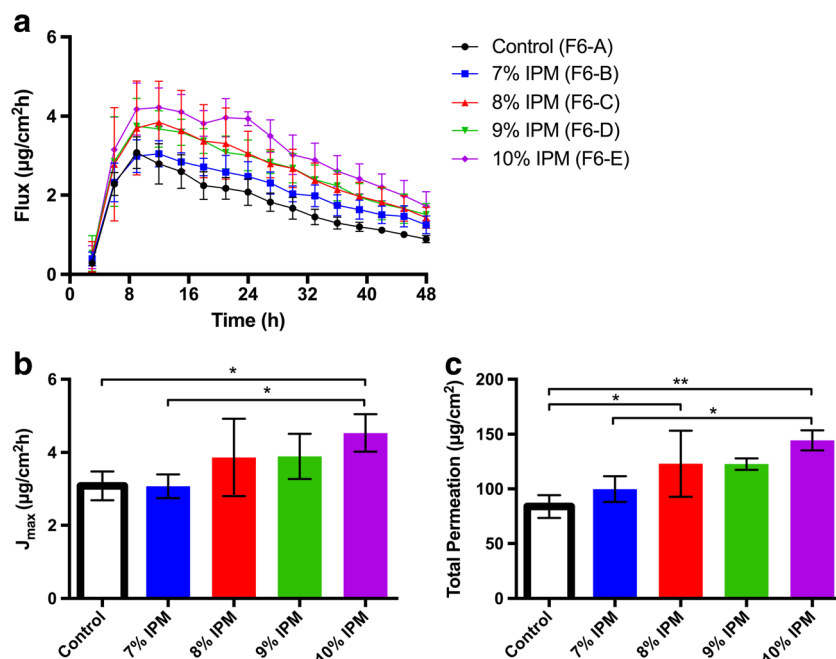
sensitivity to discern different levels of IPM in formulations. The greater sensitivity to small variations in transdermal formulations on human skin compared to porcine skin was also reported by Femenía-Font *et al.*, where the transdermal flux of sumatriptan with and without ethanol or PEG 600 was compared on porcine and human skin (12).

In addition, fentanyl flux profiles through porcine skin and human skin obtained from two different donors were

compared, from fentanyl TDSs prepared in PIB with 0 (control), 7, and 10% of IPM (Fig. 7). Flux values were generally higher through human skin than porcine skin, especially during the initial 24 h of the IVPT experiment. Although porcine skin is generally regarded to have a slightly higher percutaneous permeability compared to human skin (13), it may depend on the compounds being tested: 16 out of 76 chemicals were found to permeate



**Fig. 5.** **a** Permeation profiles, **b** comparison of  $J_{\text{max}}$ , and **c** comparison of total permeation amount from fentanyl TDSs containing different amounts of IPM in PIB PSA (mean  $\pm$  SD;  $n = 4$  skin pieces from Human Donor 1)



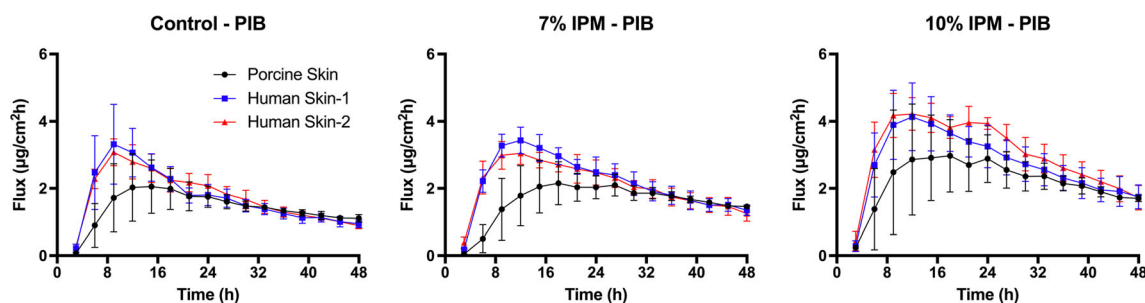
**Fig. 6.** **a** Permeation profiles, **b** comparison of  $J_{\text{max}}$ , and **c** comparison of total permeation amount from fentanyl TDSs containing different amounts of IPM in PIB PSA (mean  $\pm$  SD;  $n = 3-4$  skin pieces from Human Donor 2)

through the human skin at a higher rate compared to the porcine skin (14). The effect of CPEs on formulations and their impact on permeability through human *versus* porcine skin remain to be investigated further. The IVPT results on human skin obtained from two different donors were comparable for all three TDSs (Fig. 7).

The results from the current study suggest that it might be difficult to utilize IVPT to detect small changes in the amount of CPEs included in TDS formulations. Although significant differences in terms of  $J_{\text{max}}$  and total permeation amount were found in a few comparisons of TDSs containing different levels of CPEs, the magnitudes of CPE increment in order to see such differences were very high (43, 70, 300%). IVPT experiments with human skin reflect much of the inherent clinical variability associated with skin permeation. The physiological intersubject and intrasubject variability of human skin donor pieces used in an IVPT experiment provide most of the experimental standard deviation, rather than the fixed IVPT experimental parameters (15). In

addition, at maximum passive flux enhancement due to CPEs, the greatest decrease in the skin's barrier function is expected, which also results in higher variability of the flux values. Hence, it will be difficult to observe a statistically significant difference from a time point where high enhancement effect of CPEs is expected. In short, a small change of CPE amount in TDSs that results in a flux change to a lesser extent than the inherent IVPT variation will be difficult to detect with IVPT, as well as in the clinic. IVPT can detect significant flux changes that are not detectable in human studies, thereby often making IVPT a more sensitive method of flux change differentiation than a clinical trial (16,17). Although the IVPT method can detect flux changes, it is unable to detect human study effects like skin irritation and sensitization. Skin irritation and sensitization changes may be significant when one changes the amount of CPE in a TDS.

In order to assure that differences (or lack thereof) of flux profiles observed from IVPT were due to the differences in CPE amounts and not fentanyl amounts, three replicates of



**Fig. 7.** Comparisons of fentanyl permeation through porcine skin and human skin obtained from two donors, from TDSs containing 0, 7, or 10% IPM in PIB PSA (mean  $\pm$  SD;  $n = 4$  skin pieces)

**Table II.** Mean Amount (( $\mu\text{g}$ )  $\pm$  SD) (%CV) of Fentanyl Extracted from the Prepared TDSs (3 Replicates of 0.95 cm<sup>2</sup> Discs per Formulation)

	A	B	C	D	E
F1 <sup>a</sup>	218 $\pm$ 12 (6)	216 $\pm$ 11 (5)	209 $\pm$ 6 (3)	228 $\pm$ 12 (5)	205 $\pm$ 9 (4)
F2 <sup>a</sup>	233 $\pm$ 7 (3)	235 $\pm$ 11 (2)	248 $\pm$ 28 (11)	244 $\pm$ 6 (2)	241 $\pm$ 4 (2)
F3 <sup>a</sup>	212 $\pm$ 3 (1)	228 $\pm$ 23 (10)	221 $\pm$ 14 (6)	218 $\pm$ 9 (4)	246 $\pm$ 32 (13)
F4 <sup>a</sup>	226 $\pm$ 12 (5)	218 $\pm$ 20 (9)	230 $\pm$ 20 (9)	243 $\pm$ 24 (10)	243 $\pm$ 15 (6)
F5 <sup>a</sup>	217 $\pm$ 20 (9)	232 $\pm$ 6 (3)	223 $\pm$ 3 (1)	220 $\pm$ 7 (3)	–
F6 <sup>a</sup>	259 $\pm$ 21 (8)	246 $\pm$ 11 (5)	251 $\pm$ 7 (3)	246 $\pm$ 6 (2)	255 $\pm$ 7 (3)

<sup>a</sup> No statistically significant difference ( $p > 0.05$ ) in fentanyl content was found per each set of formulations (one-way ANOVA test followed by Tukey's *post-hoc* analysis)

0.95 cm<sup>2</sup> discs per formulation were analyzed to quantify the amount of fentanyl in unused discs. These discs were punched out from different areas of the prepared TDS film, in the same way as the discs used for IVPT experiments were punched out, to assure uniformity and homogeneity of the adhesive mixture. There was no statistically significant difference in fentanyl content among the four or five TDSs (A through D or E) per each set of formulations (Table II).

It must be acknowledged that the scope of our current study was limited to a single drug molecule, fentanyl, with three-component TDS formulations with minimal optimization. In addition, since it was an exploratory study, data was obtained from only a small number of replicates and experiment series without rigorous power calculations. Given the inherent variability associated with skin addressed previously, conducting multiple repetitive experiments based on rigorous power calculations was beyond the scope of the current study. While our limited data from the current study indicated that IVPT may not be an appropriate method to use for discerning small differences in CPE amounts incorporated in TDSs, it might be more sensitive for different drug molecules, excipients, and CPE combinations.

## CONCLUSIONS

The results from this exploratory study indicated that IVPT studies may have limitations in characterizing and discriminating varying amounts of CPEs in a DIA fentanyl TDS. Further studies with different drug molecules, excipients, CPE combinations, and additional skin donors are needed in order to fully understand the limitation and/or possibility of utilizing IVPT in quality control of TDSs containing CPEs.

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