Transdermal formulation of 4-benzylpiperidine for cocaine-use disorder

Sindhu S. Ganti[a], Hiep X. Nguyen[a], Kevin S. Murnane[a], Bruce E. Blough[b], Ajay K. Banga[a],*

[a] Department of Pharmaceutical Sciences, College of Pharmacy, Mercer University, Atlanta, GA, 30341, USA
[b] Center for Organic and Medicinal Chemistry, Research Triangle Institute, Research Triangle Park, NC, 27709, USA

ARTICLE INFO

ABSTRACT

Cocaine-use disorder is a major public health problem, yet there is no FDA approved treatment. The distinguished preclinical efficacy of 4-benzylpiperidine as substitute agonist for cocaine-use-disorder along with the therapeutic benefits of transdermal delivery, make it an excellent candidate for transdermal delivery. The purpose of this study was to investigate the *in vitro* transdermal delivery of 4-benzylpiperidine across dermatomed human skin. Mathematical models were used to calculate the theoretical and experimental drug percutaneous absorption. Gels were formulated with varying amount of gelling agent and subjected to rheological analysis. Franz cells were used to investigate the *in vitro* permeation. Transdermal permeation of 4-benzylpiperidine from propylene glycol solution (1, 10, 20 and 50 mg/mL) corresponded to 16%–31% delivery (49.45 ± 11.60, 258.47 ± 48.50, 600.26 ± 74.18, $1945.20 \pm 405.59\,\mu g/cm^2$). The average cumulative amount of drug delivered from gel formulation was $1824.90 \pm 425.12\,\mu g/cm^2$. Thixotropic test demonstrated 2% hydroxyl propyl cellulose based gel to have the highest structure recovery ratio. The calculated theoretical permeability coefficient and theoretical flux value ($32.637\,\mu g/cm^2/h$) predicted high percutaneous absorption. This was further validated by the experimentally determined permeability coefficients and flux values ($62.73 \pm 12.14\,\mu g/cm^2/h$), demonstrating proficient transdermal delivery of 4-benzylpiperidine.

1. Introduction

Cocaine-use disorder is a significant and insidious public health problem, with 1.5 million Americans reporting current cocaine use in 2014 [14]. Despite evidence for sustained prevalence, clinical harm, demand for treatment along with the decades of research, currently there are no FDA-approved pharmacotherapies to treat cocaine-use disorder [57–59]. Previous attempts to develop a medication for cocaine-use disorder have focused largely on substitute agonist approaches. Substitute-agonist therapies mimic key aspects of the abused drug to reduce craving and withdrawal and promote abstinence. The goal of such agonist-based pharmacotherapies is to use a medication that has similar pharmacological effects to that of the abused drug, while providing slower onset over abused drug to reduce abuse liability, and prolong the duration of action to promote compliance [12]. Research over the last decade has suggested that this substitute agonist-based strategy may be useful in treating cocaine-use disorders [15,47].

For other highly abused insidious drugs such as heroin, substitute agonist therapy as a maintenance strategy has been successful for addiction treatment [20,36]. FDA-approved substitute-agonist therapies for substance-use disorders include methadone, buprenorphine, varenicline, and transdermal and buccal formulations of nicotine. It was the relative success of these medications for treatment of substance-use that stimulated initial research on potential of agonist medications to treat cocaine dependence [32]. Strengths of this approach include the clinical success of these agents, better compliance, reduced withdrawal and craving, and excellent efficacy profiles in preclinical models. Weaknesses include the risk of toxic drug interactions during relapse and diversion for abuse. These weaknesses of conventional dosage forms can be mitigated through transdermal formulation of the substitute agonist. Transdermal formulation provides slow and sustained drug delivery. Slow drug onset can reduce abuse potential and long duration of action can reduce the frequency of required treatment leading to better compliance and reduce problematic neuroadaptations to the severe oscillations in drug levels that often occur with drug abuse. Further, transdermal formulation can be an abuse deterrent as it is harder and more time consuming to extract the effects of the drug over a pill or tablet [42].

Cocaine is a nonselective reuptake inhibitor of three monoamine transporters: dopamine, serotonin and norepinephrine [24] and [43].

The behavioral effects of cocaine associated with its abuse liability have been attributed primarily to its actions at the dopamine transporter (DAT) [44] which has been confirmed in rodent, nonhuman primate, and human studies. Positive correlations between the *in vitro* potency of cocaine analogs at DAT binding and their *in vivo* potency in producing locomotor-stimulant effects in rodents [7] and [21] as well as cocaine-like behavioral effects in squirrel monkeys [4,24,54] has been established.

4-benzylpiperidine is a phenethylamine substrate-based dopamine/norepinephrine (DA/NE) releaser. Although there are many targets for cocaine-use disorder that have been identified, substitute agonists that function as substrate-based DA/NE releasers have demonstrated promising efficacy in preclinical models and double-blind placebo controlled clinical trials ([32]; [12]). Researchers have previously demonstrated the efficacy of DA/NE selective substrate-based releasers [2,3,34,35] to decrease cocaine vs. food choice in nonhuman primates. This is a highly reproducible finding across DA/NE selective releasers, with phenmetrazine, phendimetrazine, and 4-benzylpiperidine all shown to be effective in cocaine vs. food choice during 7-day continuous treatment experiments [33]. 4-benzylpiperidine has shown to be effective in preclinical models as a substitute agonist for cocaine-use disorder but has a rapid onset of action, producing its peak effects within 10 min of administration, and a short duration of action of 10–30 min. The value of an agonist medication lies in its ability to target pharmacological receptors to produce effects for a long duration of time with slower onset; thereby reducing cravings for cocaine consumption while ensuring lower toxicity than produced by cocaine use. Transdermal drug delivery sustains the duration of action of 4-benzylpiperidine and promotes the agonist properties just mentioned. The small molecular weight (175), moderate lipophilicity (log P 2.924) and low melting point (6–7 °C) of 4-benzylpiperidine make it an excellent candidate for transdermal drug delivery [40]. Considering its distinguished preclinical efficacy in human-relevant animal models and the therapeutic benefits of transdermal delivery of substitute agonists for cocaine-use disorder, in the present study our aim was to investigate the *in vitro* transdermal delivery of 4-benzylpiperidine over dermatomed human skin. The study was further extended to include the formulation, rheological evaluation and transdermal delivery of a hydroxyl propyl cellulose based gel of 4-benzylpiperidine. This is the first of its kind study that reports the transdermal delivery of 4-benzylpiperidine over dermatomed human skin.

2. Materials and methods

2.1. Materials

4-Benzylpiperidine (99% purity) was obtained from Sigma Aldrich (St. Louis, MO, USA). Acetonitrile and Phosphate Buffered Saline (PBS) were purchased from Fisher Scientific (NJ, USA). Hydroxy propyl cellulose (HPC) (Klucel HF Pharm HPC) was procured from Ashland (Covington, KY, USA). EpiDerm™ skin irritation kit (OECD TG 439) was purchased from Mattek Corporation (Ashland, MA, U.S.A). De-ionized water was used to prepare all solutions required in this study and for HPLC analysis.

2.2. Methods

2.2.1. Permeability coefficient determination

Theoretical permeability coefficient was calculated using the Guy Potts Eq. (1);

$$\text{Log Kp} = -2.7 + 1.7*\text{log } P - 0.0061*\text{MW} \tag{1}$$

Where Kp is the permeability coefficient, P is the octanol-water partition coefficient and MW is the molecular weight [39]. Theoretical maximum drug flux (Jmax) across the skin was calculated from Kp × saturation solubility (intrinsic solubility) of drug in water [31].

The experimental permeability coefficient was calculated by using Eq. (2);

$$\text{Kp} = \text{J/C X A} \tag{2}$$

Where J is the flux at steady state (mg/h), Kp is the permeability coefficient (cm/h), C is the concentration in the donor ($\mu g/cm^3$), and A is the diffusion area (cm^2) of the drug, which in our study was constant ($0.64 \, cm^2$). The steady state flux (J) was determined from the slope of the linear portion of the average cumulative amount versus time plot. The time required to reach steady state (lag time) was determined by extrapolating the linear portion of permeation *vs.* the time curve to the time axis [9,13].

2.2.2. Formulation of 4-benzylpiperidine gels

4-benzylpiperidine gels were formulated using three different concentrations (1.5%, 2%, 4%) of gelling agent (hydroxyl propyl cellulose). 4-benzylpiperidine was initially dissolved in propylene glycol (PG) and then dissolved in water. Hydroxyl propyl cellulose powder was slowly added to the vortex of agitated water containing drug in PG at room temperature (< 35 °C). Addition of hydroxyl propyl cellulose powder was slow enough to not form lumps but was completed before any appreciable viscosity buildup was achieved in the solution. The rate of agitation was then reduced, but continued until a gel consistency was formed [18]. All gels contained 2 g of drug in 20 g of gel. The composition of the three gels is presented in Table 1.

2.2.3. Rheological assessment

The gels were subjected to rheological analysis using a rheometer (Rheoplus/32 V3.62, Anton Paar Germany GmbH, Germany) to assess the flow properties and structural stability to determine the gel composition with the optimal properties. This was achieved by performing rotational and oscillatory tests of the gels at 32 °C using a parallel-plate spindle (PP 25/S) with the diameter of 24.99 mm and a gap of 100 μm maintained between the plates. For all the tests, enough gel to cover the lower plate was applied, and when the parallel plate spindle touches the gel with zero gap the excess gel is wiped off. Between each gel assessment, the plate was cleaned with 75% ethanol and wiped dry [41,52].

2.2.3.1. Flow curves.
All the gels were subjected to shear at an increasing rate of 0–100 s^{-1} and the viscosity versus the resulting shear rate rheograms were plotted. The data obtained was then fit in to the Herschel–Bulkley model: $\tau = \tau y + K\gamma n$ by the rheoplus software in built in the rheometer. In the Herschel–Bulkley model, τ represents shear stress (Pa), τy the yield stress (Pa), K the flow consistency index (Pa.sn), γ the shear rate (0–100 s^{-1}) and n the flow behavior index [41]. The values of shear stress, yield stress, flow consistency index and flow behavior index were recorded for all the gels.

2.2.3.2. Amplitude sweep.
The gels were subjected to an increasing strain of 1–100% at a constant angular frequency (10 rad/s). The resulting storage modulus (G′) and loss modulus (G″) rheograms versus the increasing strain (1–100%) were plotted on logarithmic scale. The linear viscoelastic region (LVR) of the gels was recorded and used to choose the strain value to be applied for the subsequent frequency sweep and thixotropy oscillation tests.

Table 1
Compositions of 4-benzylpiperidine gels with varying amounts of hydroxyl propyl cellulose (HPC).

Ingredients	1.5% HPC gel (g)	2% HPC gel (g)	4% HPC gel (g)
HPC	0.3	0.4	0.8
Water	5	5	5
Drug	2	2	2
PG	12.7	12.6	12.2

2.2.3.3. Frequency sweep. The LVR obtained from the amplitude sweep test was used to set the constant strain (1% for the 1% gel and 5% for the 2% and 4% gels) for the gels in the frequency sweep test. Gels were exposed to an increasing angular frequency of 0.1–100 rad/s at the predetermined constant strain. Similar to the amplitude test curve, a logarithmic rheogram of G′ and G″ were plotted against the increasing frequency.

2.2.3.4. Thixotropy oscillation. Similar to frequency test, the LVR obtained from the amplitude sweep test was used to set the constant strain (1% for the 1% gel and 5% for the 2% and 4% gels) for the gels in the oscillatory thixotropic test. Periodic changes in shear rate of $10\,s^{-1}$ for $100\,s$ followed by $3000\,s^{-1}$ for $15\,s$ followed by at $10\,s^{-1}$ for $100\,s$ was applied on all the gels and the resulting structural recovery ratio was recorded. The test evaluates the effect of high shear on the structural breakdown and ability to rebuild upon structural breakdown behavior of the gels.

2.2.4. Skin preparation

Dermatomed human skin obtained from New York Firefighters skin bank (New York, USA) was stored at $-80\,°C$. Prior to *in vitro* permeation studies; dermatomed human skin was thawed in 10 mM PBS solution and cut into appropriately sized pieces. Skin used for all the experiments in our study was from the same donor with thickness of $683.47\ \pm\ 80.31\,\mu m$.

2.2.5. Skin electrical resistance and thickness measurement

Skin integrity of all dermatomed human skin samples used was measured prior *in vitro* permeation. Skin samples were clamped between a receptor compartment containing 5 mL 10 mM PBS and donor compartment containing 500 µL of 10 mM PBS of the vertical Franz diffusion cell setup and allowed a 15 min equilibration time. Equilibration was followed by assessing skin impedance by dipping the silver chloride electrode in the donor compartment and the silver electrode in the receptor compartment and applying a 100 mV voltage at 20 MHz and a load resistor (R_L) was connected in series with skin (V_S). The resulting voltage was measured by a 33220A function/arbitrary waveform generator (V_0) and read by a 34410A 61/2 digital multimeter (Agilent Technologies, Newark Element 14, Palatine, IL, USA). Skin resistance (R_S) was calculated using Eq. (3);

$$RS = V_S R_L / (V_O - V_S) \qquad (3)$$

Where, R_L and V_O were set at 100 kΩ and 100 mV, respectively [10].

Skin samples that had a resistance of $2\,k\Omega/cm^2$ or less were discarded and replaced with new skin sample. Dermatomed human skin (New York Fire Fighters, NY, USA) was tested for thickness using material thickness gauge (0-1in/0–25 mm, Electromatic Equipment Co., Inc. Cedarhurst, NY, USA).

2.2.6. In vitro permeation study

The transdermal permeation of 4-benzylpiperidine through human dermatomed skin was studied using *in vitro* vertical static Franz diffusion cells (PermeGear, Hellertown, PA, USA). Each Franz cell (n ≥ 3) comprised of a donor compartment into which the drug solution or gel was added, a receiver compartment with 5 mL of 10 mM PBS (pH 7.4) and human dermatomed skin of an effective surface area of $0.64\,cm^2$ was clamped between the two compartments. The superficial layer of the skin is exposed to the donor compartment while the dermal side of the skin faces the receptor compartment. The donor compartments were exposed to room temperature while the receiver compartments were maintained at $37\,\pm\,1\,°C$ and continuously stirred to maintain sink conditions. For testing the effect of concentration on permeation, a donor concentration of 100 µL of 1000 µg/ml, 10000 µg/ml, 20000 µg/ml, and 50000 µg/ml of 4-benzylpiperidine in PG were added in to donor compartment and samples were drawn at regular intervals from the receiver compartment over 24 h. *In vitro* permeation of 4-benzylpiperidine from gel formulation was performed similarly by adding 100 mg (100000 µg) of the gel containing 10 mg (10000 µg) of drug into the donor compartment and samples were withdrawn at regular intervals over 24 h. Amount of drug release from PG solution and gel formulation and permeated through the skin into the receiver compartment of the Franz cells was detected by Reverse Phase-High Performance Liquid Chromatography (RP-HPLC).

2.2.7. Skin irritation studies

Skin irritation test was performed with *in vitro* EpiDerm 3D human tissue model to predict the irritation potential of topically applied 4-benzylpiperidine in PG solution and the 4-benzylpiperidine incorporated gel formulation. The test was performed over three consecutive days with day one being the same day as the test kit was received. On day 1, the EpiDerm tissues in the kit were conditioned by incubation to release transport-stress related compounds and debris overnight. Post pre-incubation, tissues were topically exposed to 4-benzylpiperidine in PG solution for 60 min. Three tissue replicates were used per test chemical and for the positive control and negative control. The tissues were then thoroughly rinsed, blotted to remove the test chemical, transferred to fresh medium and incubated for 18 h. Incubation was followed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The assay was performed by transferring the tissues to 96-well plate containing MTT medium with a 3-h incubation time. Post incubation, the blue formazan salt formed by cellular mitochondria is extracted with isopropanol and the optical density of the extracted formazan was determined using a spectrophotometer at 570 nm. Similar protocol was followed to test the skin irritation potential of 4-benzylpiperidine incorporated gel formulation. Relative cell viability was calculated for each tissue as % of the mean of the negative control. The skin irritation potential of 4-benzylpiperidine in PG solution and 4-benzylpiperidine gel was predicted based on if the remaining relative cell viability was below 50% [26]; [28].

2.2.8. Quantitative analysis and method validation

Quantitative analysis of 4-benzylpiperidine was carried out on HPLC Waters 2695 Separation Module attached to a Waters 2996 photodiode array detector. A prodigy 5 u ODS (2) 150 × 4.60 mm 5-µm column was used at 25 °C. Samples (20 µL) was injected into the column at flow rate of 1.0 mL/min using mobile phase of acetonitrile and deionized water (0.05% v/v TFA) in gradient mode. Percentage of acetonitrile increased from 10% to 80% from 0 to 8 min, remained at 80% till 13 min, then decreased to 10% at 13.01 min and maintained at 10% until 15 min. Drug detection wavelength was 259 nm while the retention time was 5.5 min. The HPLC method was sensitive, accurate and reliable (Linear range of 0.5–350 µg/mL, R^2 = 0.999).

2.2.9. Data analysis

Data analysis was performed using Microsoft Excel 2016. Student's t-test was used for statistical analysis and p value of less than 0.05 was considered for significant difference between the test groups.

3. Results and discussion

3.1. Permeability coefficient

Permeability coefficient (Kp) is a measure of the transport of a molecule from a vehicle across skin. Theoretical permeability coefficient is a parameter describing the intrinsic, concentration-independent, ability of a molecule to cross the stratum corneum barrier. Permeability coefficient values of drug are increasingly important when its dermal delivery has not been previously investigated. To the best of our knowledge, percutaneous absorption studies or predictions for 4-benzylpiperidine has not been reported and this study is the first of its kind to report the same. Most mathematical models used to estimate drugs permeability coefficients recognize the importance of the drug

Table 2
Log P and molecular weight values from literature, spartan and chemicalize.

Parameter	Calculated spartan and chemicalize value	Literature value
Log P	2.5	2.9
Molecular weight	175	175

partitioning across skin lipids and the molecular size. The widely used Guy Potts equation accounts for the physiochemical properties of the drug such as log P and molecular weight. Since log P and molecular weight of a drug are constant, the calculated Kp of the drug will also remain constant unlike experimental Kp that varies with formulation and concentration of the drug [22]. The theoretical permeability coefficient of 4-benzylpiperidine calculated by Guy Potts equation using log P and molecular weight values from literature (Table 2.) was 20.3×10^{-3} cm/h. In this study, we used spartan and chemicalize software to calculate log P, molecular weight. Spartan and chemcalize is a molecular modelling and computational chemistry software. The molecular weight of the drug generated by spartan and chemicalize was same as the literature value but the log P values differed by 0.4. Using the same Guy Potts equation but replacing literature values with the values obtained by Spartan (Table 2.), the Kp was calculated as 10.1×10^{-3} cm/h. These calculated theoretical Kp values differ since the log P values differ. Both the calculated theoretical Kp values are high and hence predict high percutaneous absorption of the drug. This is not particularly remarkable since the drug is moderately lipophilic with a smaller molecular weight aids and these characteristics are considered ideal for transdermal delivery. In percutaneous absorption studies the efficiency of drug penetration of the drug is described by the permeability coefficient Kp. The theoretical Kp value based on the physiochemical properties of the drug gives an insight into its ability to passively permeate stratum corneum and epidermis. The ability to predict percutaneous penetration of a drug based on these physicochemical characteristics would potentially enhance dermal drug development by decreasing the need for excessive *in vitro* and *in vivo* animal and human skin penetration studies [51].

While Guy Potts equation helps predict the percutaneous absorption of a drug, from a therapeutic and toxicological perspective, the maximum flux of the drug (Jmax) is also of significance. It correlates directly with the maximum dose deliverable over a given period. The maximum flux at which a chemical can cross a unit area of skin, is theoretically achieved when it is maintained as a saturated solution on the surface. The combination of the drug's skin permeability coefficient and aqueous solubility of nonionic compounds will afford a straightforward method for assessing the percutaneous absorption potential of new compounds in terms of the maximum flux. Hence, Jmax employs the use of physiochemical properties of the drug and the intrinsic solubility of drug in water to predict the drug's percutaneous absorption. The predicted Jmax of 4-benzylpiperidine across the skin was calculated as 32.637 $\mu g/cm^2/h$. Water solubility (3.12 mg/mL) and Kp (10.4×10^{-3} cm/h) values were calculated using chemicalize (MarvinSketch: version 6.2.2, ChemAxon, Hungary, Europe). When skin permeation measurements are not available, this model can readily provide initial estimates of maximum transdermal flux and thereby predict the potential success of a drug for transdermal delivery. Concurring with the calculated theoretical permeability coefficient of the drug, the calculated maximum flux of the drug also indicates high percutaneous absorption, validating the drug to be an excellent transdermal candidate [31,39].

Early quantitative structure-activity relationship studies to predict skin permeation of chemicals demonstrate a linear correlation between hydrophobicity and permeability [45,49]. Patel and colleagues demonstrated the effects of hydrophobicity, molecular size and the hydrogen bonding capability of a molecule affect its ability to permeate skin [38]. In addition, Lipinski's Rule of Five proposed by Chris Lipinski

to predict the permeability of molecule across skin also supports the influence of hydrophobicity and molecular size on permeability [23]. Melting point of the permeant also plays an important role in determining its percutaneous absorption. As log P increases, the aqueous solubility decreases, and the lower the melting point, the higher the solubility. It is observed that compounds which diffuse through the skin most readily, are those having log P around 2 and a low melting point [11]. 4-benzylpiperidine is a liquid at room temperature with a low melting point of 6–7 °C (obtained from chemicalize). Compounds such as nicotine and nitroglycerine like 4-benzylpiperidine are liquids at room temperature, small molecular weight and lipophilic and have demonstrated to be successful transdermal candidates with commercially viability. As previously discussed, Guy and Potts's model and maximum flux models affirm the influence of hydrophilicity of the drug on its *in vitro* percutaneous absorption across excised human skin. All these theories and models authenticate that the permeants that are best absorbed through the skin are therefore small and moderately lipophilic. In our current investigation, 4-benzylpiperidine satisfies all the ideal criterions of ideal transdermal candidate and hence predicted to have high percutaneous absorption.

While theoretically calculated predictions are important and can provide an initial screening basis for compounds, they cannot replace experimental values [29]. Contrary to theoretical permeability coefficients, experimental K_p's for a drug from different formulations vary since it is dependent on the formulation and concentration of the drug. An apparent limitation of theoretical maximum flux is that the calculation is based on an aqueous formulation. The permeability coefficient will, of course, change with the nature of the formulation placed on the skin, as will the drug's solubility. From a theoretical standpoint, a saturated solution in any solvent should result in the same steady-state flux across the skin, because the chemical potential of the drug in any saturated solution is maximal. However, in practice, such equivalence is not always observed, because many solvents are themselves able to change the barrier properties of the skin and lead to flux rates that do not conform to simple thermodynamic arguments [17]. Therefore, it is important to calculate the experimental permeability coefficient of the drug formulations. The average Kp of the drug from 1000 μg/ml, 10000 μg/ml, 20000 μg/ml and 50000 μg/ml drug in PG solution was experimentally calculated as $3.0 \times 10^{-3} \pm 0.9 \times 10^{-3}$ cm/h, $2.0 \times 10^{-3} \pm 0.4 \times 10^{-3}$ cm/h, $2.0 \times 10^{-3} \pm 0.4 \times 10^{-3}$ and $2.7 \times 10^{-3} \pm 0.5 \times 10^{-3}$ cm/h respectively. Drug from gel showed an experimental permeability coefficient of $0.48 \times 10^{-3} \pm 0.09 \times 10^{-3}$ cm/h. The experimental values used to calculate the permeability coefficients are presented in Table 3. The experimentally determined permeability coefficients of the drug from PG solution were in a close range and the slight variance is due the difference in donor concentrations. Experimental Kp considers the concentration, vehicle and formulation aspects making it more realistic. In our study, the theoretically calculated Kp value of the drug was higher than the experimentally determined Kp (s). Experimental Kp of the drug from gel formulation is lower than that of the drug from PG solution and theoretical Kp. This observation is in line as gels provide a slower onset of action and sustained release of the drug over solutions due to the cross linking of polymers in gels.

3.2. Rheological evaluation results

The development of topical and transdermal pharmaceutical dosage forms involves several desirable qualities that influence patient acceptability and clinical efficacy of the product. These include optimal mechanical properties like ease of removal of product from the container, ease of spreading on the skin, good bioadhesion to ensure retention at the site of application, acceptable viscosity, drug release and drug absorption. Moreover, products designed for topical or transdermal use will be subjected to shearing forces (rubbing over skin and

Table 3

In vitro flux of 4-benzylpiperidine through cadaver human skin.

Formulation	Concentration in donor (µg/cm^3)	Area (cm^2)	Steady state flux, J (µg/sq.cm/h)	Average flux ± SD (µg/cm^2/h)	Average lag time ± SD (hours)
1 mg/mL (1000 µg/ml) (Drug in PG solution)	100	0.64	2.3, 1.65, 2.52, 1.21 (n = 4)	1.92 ± 0.6	1.44 ± 0.26
10 mg/mL (10000 µg/ml) (Drug in PG solution)	1000	0.64	14.71, 11.66, 9.45, 15.02 (n = 4)	12.71 ± 2.65	2.10 ± 2.17
20 mg/mL (20000 µg/ml) (Drug in PG solution)	2000	0.64	30.03, 24.76, 28.43, 19.15 (n = 4)	25.60 ± 4.83	3.55 ± 0.73
50 mg/mL (50000 µg/ml) (Drug in PG solution)	5000	0.64	90.35, 80.80, 65.24, 106.97 (n = 4)	85.84 ± 17.48	2.26 ± 2.07
2 g (20000 µg) in 20 g (ug) (Drug in gel)	8300	0.64	62.27, 69.64, 39.65, 61.69, 71.51, 71.60 (n = 6)	62.73 ± 12.14	2.94 ± 0.56

flexing processes of skin) that are oscillatory in nature. Therefore, it is imperative to examine the effects of such oscillatory forces on the product rheology, and hence, ultimately on the clinical performance of the formulation [64].

The present investigation characterizes the mechanical, rheological and adhesive properties of gels prepared from HPC by rheological evaluation. Rotational and oscillatory rheometric evaluations are used to quantify the mechanical and textural properties of the gels, and the effect of oscillatory stresses similar to those experienced under physiological conditions, on their structural properties. The choice of gelling agent used in the present study for the formulation of gels was HPC. Gels formulated with HPC as the gelling agent; do not require neutralization with a base like in case of carbopol gels and can be formulated without application of heat [52].

3.2.1. Flow curves

As shown in Fig. 1A, the increase in concentration of gelling agent (HPC) from 1.5% to 2% and 4% resulted in an increase in viscosity of the gel. Furthermore, viscosity of each gel decreased with an increase in

the shear rate applied. This test is essential to demonstrate the spreadability of a gel upon application. Ideally the gel should have adequate viscosity such that it can be smoothly applied and spread over skin without sliding off the skin under minor gravitational force. Therefore, it is imperative to choose the appropriate concentration of gelling agent.

3.2.2. Amplitude sweep

Amplitude sweep provided information about the storage modulus (G′) and loss modulus (G″) of gels under a range of strains applied. G′ was greater than G″ for all the gels indicating the gels have more of a solid-like nature (Fig. 1B). Gels are semi-solid preparation hence these results are supportive for gel formulation. Additionally, the gap between G′ and G″ curve increased with the increase in concentration of gelling agent demonstrating the role of HPC content in determining the solidity of the gel formulation. Furthermore, as shown in Fig. 1B, there were cross section of G′ and G″ with this the study range of strain. The cross section indicated the point that the gel structure broke down from solid to liquid. The breaking point of gel structure was delayed from
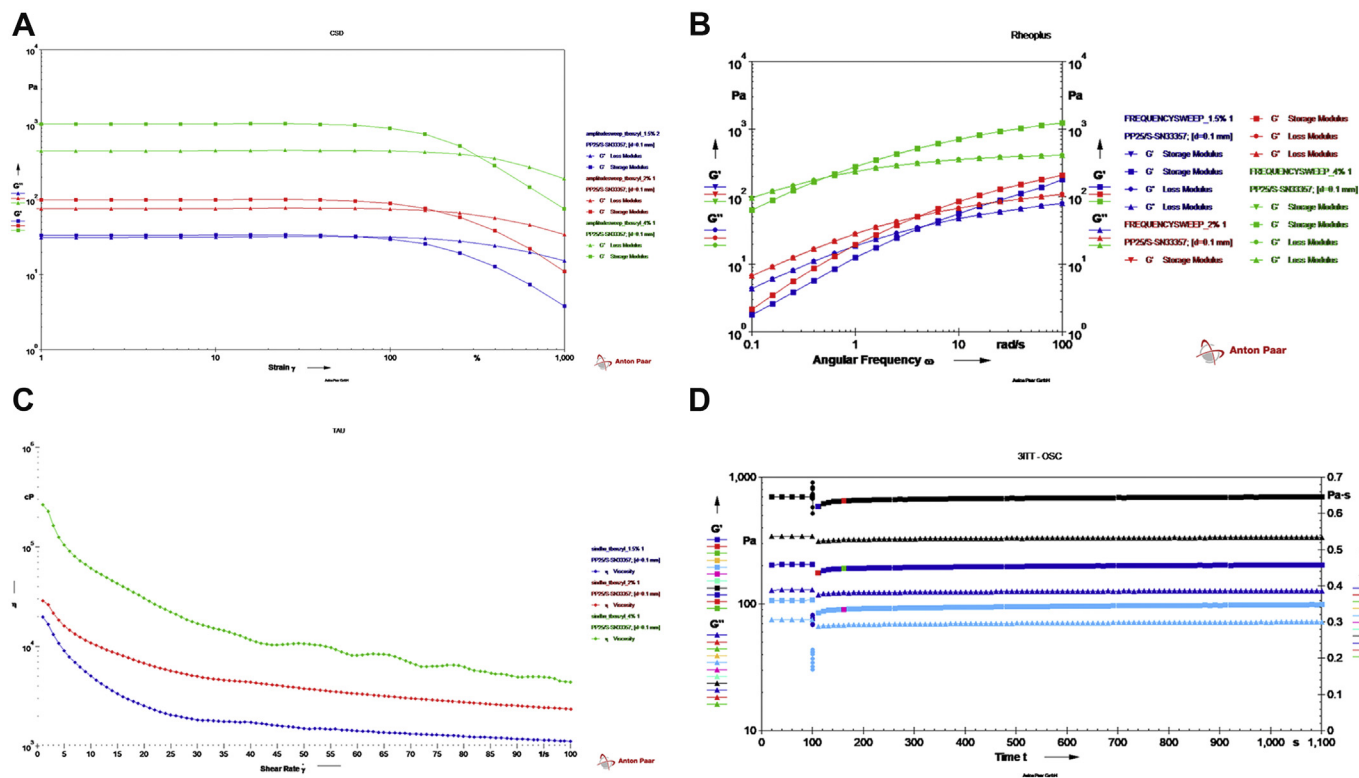

Fig. 1. Comparative rheograms of varying concetrations of HPC (gelling agent) based 4-benzylpiperidine gels corresponding to the following rheological tests: (**A**) Flow curves (**B**) Amplitude sweep (**C**) Frequency sweep (**D**) Thixotropy oscillation.

1.5% to 2% and 4% gel to notify that the higher gel concentration, the more stable the gel structure.

3.2.3. Frequency sweep

A cross over point (indicates the frequency at which the gel breaks) between storage modulus and loss modulus at lower frequency of 0.6 rad/s was observed for 4% gel formulation in comparison to 2% and 1.5% gel formulation which showed a cross over point at 4 rad/s, indicating 4% gel formulation is less stable. The storage modulus and loss modulus for 2% gel formulation were higher than that of 1.5% gel formulation indicating better structural stability of 2% gel formulation as shown in Fig. 1C.

3.2.4. Thixotropy test

The gel formulations were subjected to variable shear rates for different time intervals and the change in viscosity with respect to time was recorded and represented as shown in the graph below. The highest structure recovery ratio of 107.01% for 2% gel formulation was observed in comaprison to 4% gel formulation that had a recovery ratio of 79.092% and 98.807% for 1.5% gel formulation. Therefore, 2% gel formulation had better ability to rebuild its structure in comparison to 4% and 1.5% gel formulations (Fig. 1D).

3.3. HPLC method validation

The HPLC method was specific for 4-benzylpiperidine whose peak was unaffected by the background noise as well as other interferences on the chromatogram. The linearity of the method was studied to investigate the relationship between the drug concentration and the peak area. The linearity study was conducted with a total of 15 calibration standards: 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 150, 200, 250, 300, 350 µg/mL. The drug concentration (x) could be reliably predicted from the peak area (y) by the following linear regression equation Eq. (4).

$$y = 1485.6x + 2019.9 \quad (R2 = 0.999) \tag{4}$$

The method provided a good correlation between peak area and drug concentration in the linear range of 0.1–350 µg/mL. The limit of detection of the assay method was 0.30 µg/mL while the limit of quantification was 0.90 µg/mL. The intra-day mean accuracy was 104.48% for 2.5 µg/mL, 101.43% for 25 µg/mL, and 100.18% for 250 µg/mL (n = 6) while the intra-day precision was 4.66% for 2.5 µg/mL, 0.64% for 25 µg/mL, and 0.16% for 250 µg/mL (n = 6). We also studied the inter-day accuracy and precision of 4-benzylpiperidine analysis and reported a similar trend—the higher the drug concentration, the lower the variability. The inter-day accuracy was reported: 102.03% for 2.5 µg/mL, 101.31% for 25 µg/mL, and 100.19% for 250 µg/mL (n = 12). Meanwhile, the inter-day precision was 5.02% for 2.5 µg/mL, 0.87% for 25 µg/mL, and 0.17% for 250 µg/mL (n = 12). This HPLC method was validated to be specific, accurate, and reliable tool to measure the drug concentration in the collected samples.

3.4. In vitro permeation study

Transdermal permeation of 4-benzylpiperidine from PG solution (1 mg/mL, 10 mg/mL, 20 mg/mL and 50 mg/mL) over 24 h through human dermatomed skin was high (49.45 ± 11.60, 258.47 ± 48.50, 600.26 ± 74.18, 1945.20 ± 405.59 µg/cm^2) amounting to 16%–31% delivery as shown in Fig. 2A.

The excellent passive transdermal permeation of 4-benzylpiperidine observed can be attributed to its ideal properties that aid in penetration of the stratum corneum. The low molecular mass, optimal oil in water partition coefficient, low melting point and high solubility of the drug in the solvent (PG), all contribute to its transdermal permeation. The calculated theoretical permeability coefficient and theoretical flux also predicted high percutaneous absorption. This was further validated by the experimentally determined permeability coefficients and steady state flux values.

The average flux of the drug increased linearly with increase in concentration as shown in Fig. 3A and the values are presented in Table 2. Flux is the amount of drug that permeates per square centimeter of skin (*in vitro* dermatomed human skin) per hour. The theoretical maximum flux calculated is calculated based on the drug's aqueous solubility and since the drug has low aqueous solubility in comparison to its solubility in PG, the predicted J_{max} is lower than the experimental flux with the maximum tested concentration. Based on our results, the flux of the drug can be altered by changing the concentration of drug. Therefore, we can calculate the dose required to treat cocaine dependence based on the flux required. Since there are no approved treatments for cocaine-use disorders on the market and no human trials on the use of 4-benzylpiperidine for treatment, the dose required cannot be accurately estimated at this stage. Since 4-benzylpiperidine demonstrates good transdermal permeation and the flux is linear, the required dose is attainable.

Thixotropic test demonstrated the 2% HPC based gel to have the highest structure recovery ratio of 107.01% compared to 4% HPC based gel formulation and 1.5% HPC based gel formulation that showed a recovery ratio of 79.1% and 98.8% respectively. Hence the 2% HPC based gel was found to have better structural stability and was used for the *in vitro* permeation studies. Franz diffusion cells (n = 6) were used to study the *in vitro* transdermal permeation of 4-benzylpiperidine from a HPC based gel formulation and the permeation profile is as shown in Fig. 2B. The average cumulative amount of drug delivered across human dermatomed skin after 24 h was 1824.90 ± 425.12 µg/cm^2, and this corresponded to 18% delivery.

The average flux of the drug from the gel over time showed rapid delivery within the first 2 h from 0.00 ± 0.00 (0 h) to 203.92 ± 62.46 (1 h) and 270.48 ± 55.53 µg/sq.cm/h (2 h) before a sudden decrease at 4 h (110.13 ± 22.53 µg/sq. cm/h) and provided sustained release until the end of the study (24 h). The value of the substitute agonist lies in its ability to prolong its effects on the pharmacological receptors so the need for the drug of dependence is reduced and since 4-benzylpiperidine in its preclinical models has shown to have a short duration of action, our goal in the present study was to increase the value of agonist treatment by prolonging the duration of action. Gels have the rheological properties that will give the drug a long residence time at the site of application and release the drug from the gels polymer matrix at a controlled and sustained mode [56]. As demonstrated by the permeation profile in Fig. 2B and flux profile in Fig. 3B, 4-benzylpiperidine in gel formulation provides the much needed sustained release over 24 h.

In our study, we incorporated 4-benzylpiperidine into a HPC based gel formulation. Gels are popular in dermatology because they are transparent, easily water washable, greaseless, thixotropic, easily spreadable, suitable for the incorporation of lipophilic compounds or insoluble solids, and present good rheological properties to increase residence time of gel on skin. Among the gelling agents extensively used in the pharmaceutical industry are cellulose derivatives such as methylcellulose (MC), carboxymethylcellulose (CMC), hydroxylpropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC). The large availability, nontoxicity, and the low cost of cellulose derivatives make them the first choice for the preparation of pharmaceutical gels. Among these HPC is a water soluble cellulose derivative with remarkable combination of properties: solubility in cold or hot polar organic solvents forming a smooth, clear, colloidal solution; surface activity; aqueous thickening and stabilizing properties [56].

3.5. Skin irritation study

For 4-benzylpiperidine in PG solution (1000 µg/mL), the relative cell viability for each tissue as % mean of the negative control was found to be 3.8 ± 0.50% as shown in Fig. 4A. According to the EpiDerm model test for skin irritation, if the remaining relative cell viability is below 50% then the tested compound (4-benzylpiperidine in
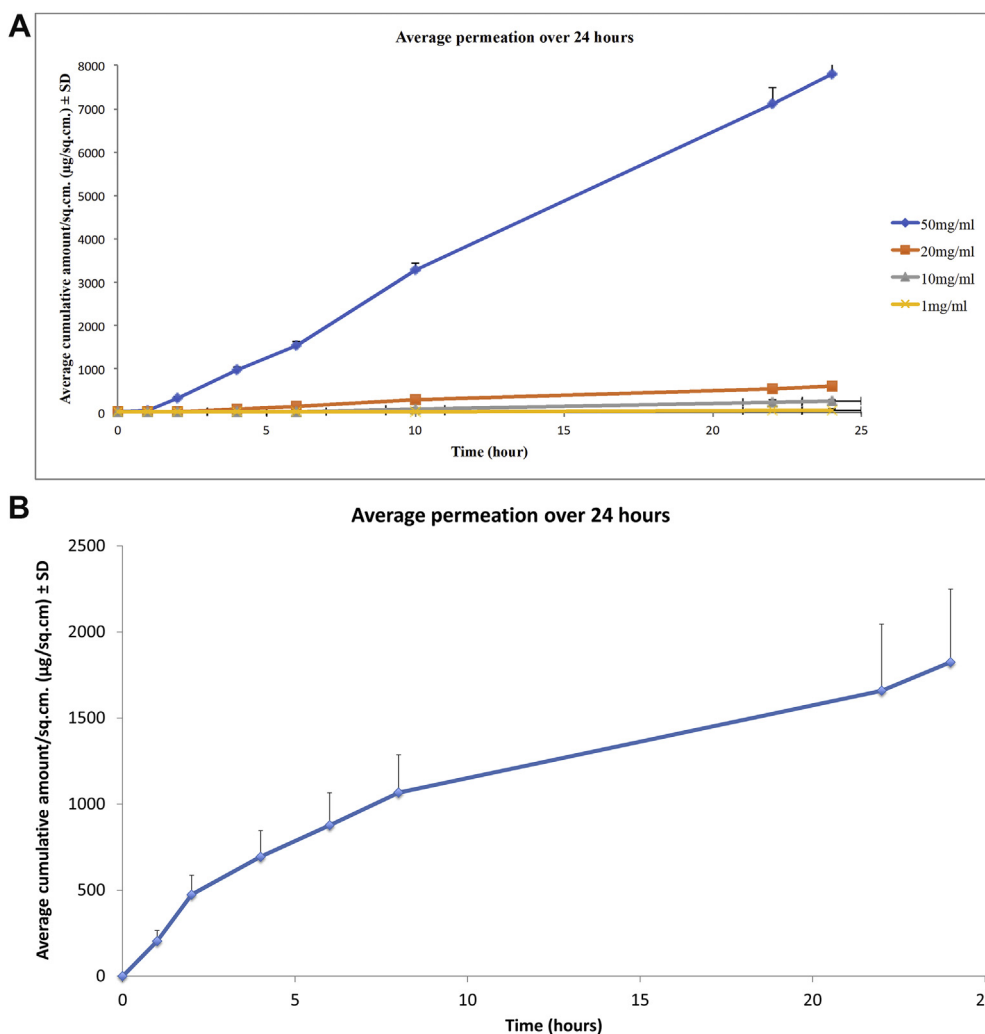
Fig. 2. Graphical representation of the *in vitro* permeation profile of 4-benzylpiperidine across dermatomed human cadaver skin: **(A)** *In vitro* permeation of varying concentrations of 4-benzylpiperidine from propylene solution (1 mg/mL, 10 mg/mL, 20 mg/mL and 50 mg/mL) **(B)** *In vitro* permeation of 4-benzylpiperidine from 2% HPC based gel.

PG solution) is said to cause potential skin irritation. 4-benzylpiperidine gel (final 2% gel) formulation demonstrated a relative cell viability of 11.7 ± 7.91% as shown in Fig. 4B. Hence the gel formulation of 4-benzylpiperidine is also considered to cause potential irritation to skin. In terms of relative cell viability, the gel formulation was found to be less irritant over the drug in PG solution. Although both the formulations tested were deemed as potential irritants, the results show that decreasing drug concentration and flux can reduce skin irritation.

The EpiDermTM SIT *in vitro* model used in the current study was developed and designed to predict skin irritation potential of test compounds in the context of identification and classification of skin irritation hazard according to the European Union classification system. The test consists of a reconstructed human epidermis (RhE) and is based on the topical exposure of the test chemical to RhE followed by measurement of cell viability. Cell viability is quantitatively measured by the spectroscopic detection of blue formazan salt. The blue salt is formed by the dehydrogenase conversion of MTT [(3–4,5-dimethyl thiazole 2-yl) 2,5-diphenyltetrazoliumbromide], present in cell mitochondria. The percentage reduction of cell viability of tissues exposed to the test chemicals in comparison to negative controls (treated with water) is used to predict the skin irritation potential [30].

In the present study, the active ingredient: 4-benzylpiperidine may be the main contributor to cause potential skin irritation. The excipients involved in the transdermal formulation including water, PG and HPC

all have well known skin tolerability [63]. The material safety data sheets (MSDS) of 4-benzylpiperidine suggest it causes inflammation of the skin on contact in some persons and can accentuate any pre-existing dermatitis condition. Additionally, the MSDS indicate the skin irritation can be due to the pyridine or pyridine derivative aspects of the drug structure [40]. Previous studies have shown dissociation constant (pKa) of the drug can affect the physiological pH of the skin and lead to cutaneous irritation. Dissociation constants less than 4 or greater than 8 are known to be potentially irritant due to the changes caused in pH of skin [1,5,6,25,50]. 4-benzylpiperidine has a pka of 10.35 (calculated by chemicalize) and this can explain the changes caused on the skin that lead to potential irritation. Nicotine has a pka of 8.5, nitroglycerine has a pka of −5.6 and selegiline has a pka of 8.67 [40]. According to the above discussion and their pka values, these drugs can cause potential skin irritation and have reported to cause minor irritation, yet these drugs are widely accepted on the market for transdermal delivery without major reports of skin irritation [19,27,37,55].

Incorporating the potentially irritant drug into a gel formulation can reduce the exposure of concentrated drug on directly on the skin thereby minimizing irritation as seen in this study. The gel formulation protects the skin against the direct contact of 4-benzylpiperidine, making the formulation more practical and tolerable. Furthermore, it has been reported that hydrogels reduce skin irritation by absorbing moisture from the skin's surface [16]. Based on the results
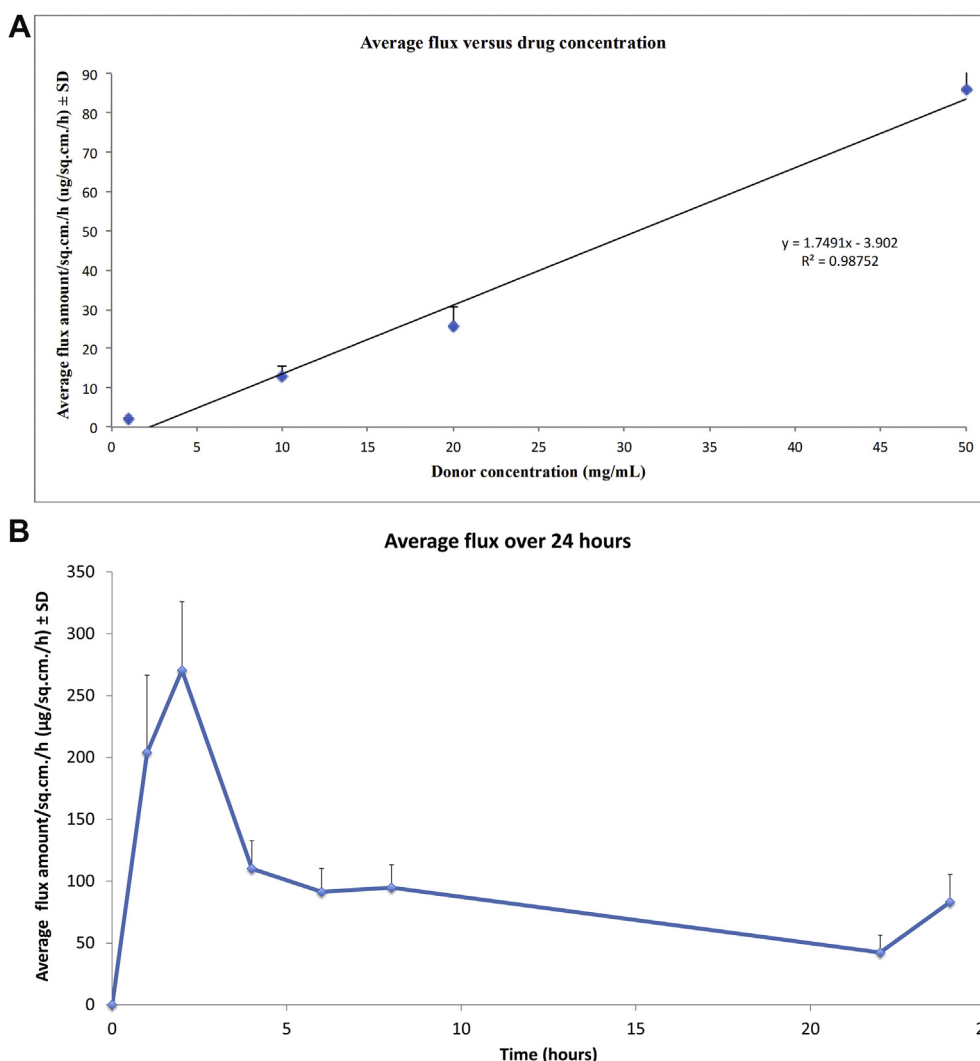
Fig. 3. Graphical representation of average flux of 4-benzylpiperidine across human dermatomed skin: **(A)** Average flux of 4-benzylpiperidine versus the donor concentration of drug in PG solution (100 μL of 1 mg/mL, 10 mg/mL, 20 mg/mL and 50 mg/mL). **(B)** Average flux of 4-benxylpiperidine from a 2% HPC based gel formulation.

demonstrated by Wester et al., [8] and Schäfer-Korting et al., it can be concluded that controlled-release systems like gels can reduce the potential irritation induced by topically applied drugs [8,48,62]. Hence based on our results though 4-benzylpiperidine was found to be a potential skin irritant with the Epiderm model, further testing is required to deem 4-benzylpiperidine determine the acceptability over skin.

4. Conclusion

Although cocaine dependence is a critical public health problem with decades of research to find a suitable pharmacological intervention, there are still no FDA-approved pharmacotherapies. Research has suggested and demonstrated the efficacy of substitute agonist-based strategies to treat cocaine-use disorders. 4-benzylpiperidine is one such substitute agonist that has shown promise in the preclinical human-relevant animal models but its short duration of action reduces its effectiveness as a substitute agonist. Consequently, the goal of our research was to prolong the duration of action of 4-benzylpiperidine by investigating the *in vitro* transdermal delivery across dermatomed human skin. The research included the formulation, rheological evaluation and transdermal delivery of a hydroxyl propyl cellulose based gel of 4-benzylpiperidine.

Theoretical and experimental permeability coefficient calculations

and the theoretical and experimental flux predictions suggested high percutaneous absorption of 4-benzylpiperidine. Transdermal permeation of varying concentrations of 4-benzylpiperidine from PG solution corresponded to 16 %–31% delivery. Thixotropic test demonstrated the 2% HPC based gel to have the best structural stability and was used for all *in vitro* permeation studies. 18% of 4-benzylpiperidine was delivered from the gel formulation across dermatomed human skin over 24 h. The excellent passive transdermal permeation of 4-benzylpiperidine demonstrated in our study was in concordance with the predicted percutaneous absorption and can be attributed to its ideal physiochemical properties that aid in penetration of the stratum corneum. 4-benzylpiperidine in propylene glycol and gel formulation was found to be potentially irritant to skin.

In conclusion, our preliminary results are promising and demonstrate the proficiency of transdermal route for the passive delivery of 4-benzylpiperidine. Further clinical studies and skin irritation studies are required to investigate transdermal delivery of therapeutically relevant doses of 4-benzylpiperidine to treat cocaine-use disorders and to substantiate the tolerability of these doses over skin.
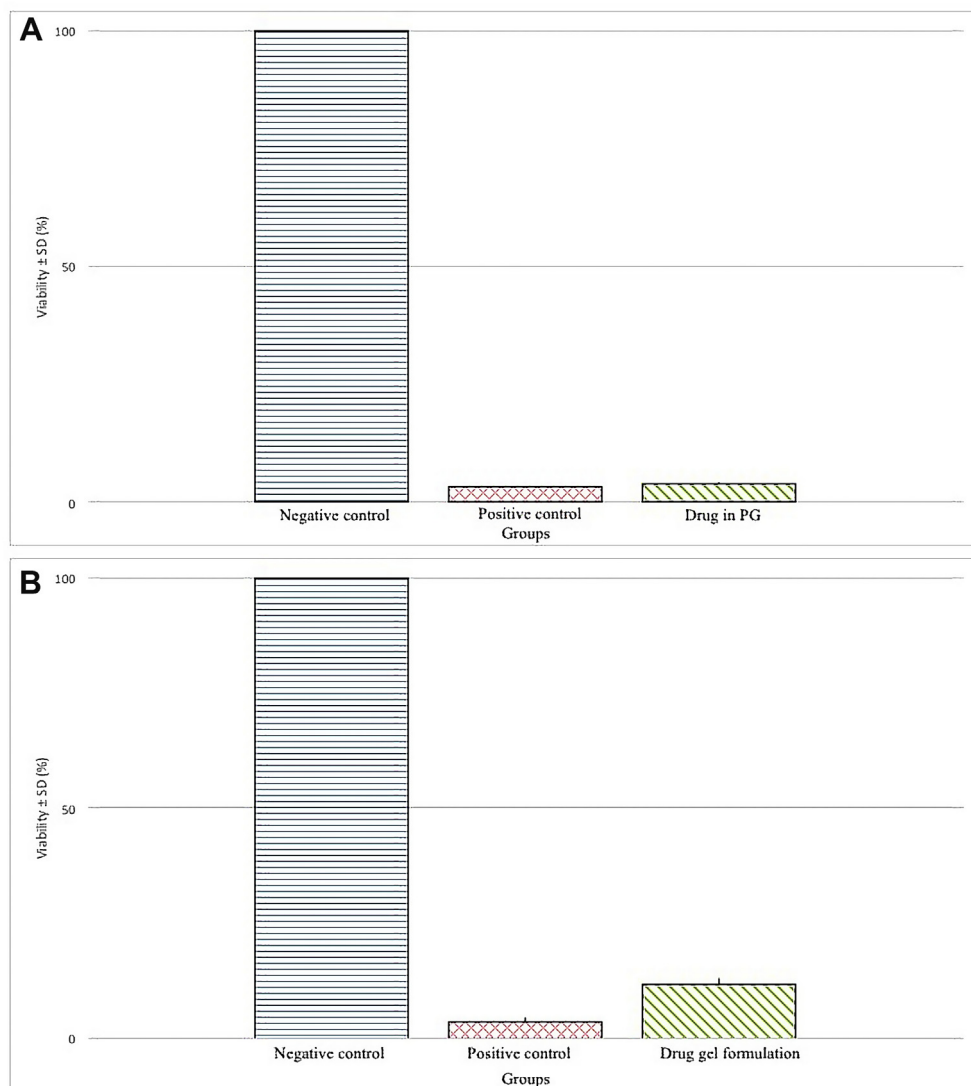
Fig. 4. The percentage relative tissue viability in *in vitro* skin irritation test of 4-benzylpiperidine compared to negative and positive controls: **(A)** The percentage relative tissue viability of 4-benzylpiperidine in PG solution (30 μL of 50 mg/mL drug in PG solution) **(B)** The percentage relative tissue viability of 4-benzylpiperidine gel (30 μL of the HPC based gel).

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgement

Appendix A. Supplementary data

Supplementary data related to this article can be found at https:// doi.org/10.1016/j.jddst.2018.07.012.

References

[1] P.H. Andersen, A. Nangia, P. Bjerring, H.I. Maibach, Chemical and pharmacologic skin irritation in man, Contact Dermatitis 25 (1991) 283–289.

[2] M.L. Banks, B.E. Blough, T.R. Fennell, R.W. Snyder, S.S. Negus, Effects of phendimetrazine treatment on cocaine vs food choice and extended-access cocaine consumption in rhesus monkeys, Neuropsychopharmacology 38 (2013) 2698–2707.

[3] M.L. Banks, B.E. Blough, S.S. Negus, Effects of monoamine releasers with varying selectivity for releasing dopamine/norepinephrine versus serotonin on choice between cocaine and food in rhesus monkeys, Behav. Pharmacol. 22 (2011) 824–836.

[4] J. Bergman, B.K. Madras, S.E. Johnson, R.D. Spealman, Effects of cocaine and related drugs in nonhuman primates. III. Self-administration by squirrel monkeys, J. Pharmacol. Exp. Therapeut. 251 (1989) 150–155.

[5] B.W.D. Berner, R.J. Steffens, G.C. Mazzenga, R. Hinz, R.H. Guy, H.I. Maibach, The relationship between pka and skin irritation for a series of basic penetrants in man, Fund. Appl. Toxicol. 15 (1990) 760–766.

[6] B. Berner, D.R. Wilson, R.H. Guy, G.C. Mazzenga, F.H. Clarke, H.I. Maibach, The relationship of pka and acute skin irritation in man, Pharm. Res. (N. Y.) 5 (1988) 660–663.

[7] E.J. Cline, U. Scheffel, J.W. Boja, F.I. Carroll, J.L. Katz, M.J. Kuhar, Behavioral effects of novel cocaine analogs: a comparison with in vivo receptor binding potency, J. Pharmacol. Exp. Therapeut. 260 (1992) 1174–1179.

[8] J. de Leeuw, H.C. de Vijlder, P. Bjerring, H.A.M. Neumann, Liposomes in dermatology today, J Eur Acad Dermatol. 23 (2009) 505–516.

[9] S. Dumitriu, V.I. Popa, Polymeric Biomaterials: Medicinal and Pharmaceutical Applications vol. 2, CRC Press, 2003.

[10] S.S. Ganti, A.K. Banga, Non-ablative fractional laser to facilitate transdermal

delivery, J. Pharmaceut. Sci. 105 (2016) 3324–3332.

[11] Ghafourian, E.G. Samaras, J.D. Brooks, J.E. Riviere, Modelling the effect of mixture components on permeation through skin T, Int. J. Pharm. 398 (2010) 28–32.

[12] J. Grabowski, J. Shearer, J. Merrill, S.S. Negus, Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence, Addict. Behav. 29 (2004) 1439–1464.

[13] M. Gujjar, A.K. Banga, Vehicle influence on permeation through intact and compromised skin, Int. J. Pharm. 472 (2014) 362–368.

[14] S.L. Hedden, J. Kennet, R. Lipari, G. Medley, P. Tice, E.A.P. Copello, L.A. Kroutil, Behavioral Health Trends in the United States: Results from the 2014 National Survey on Drug Use and Health. Report Was Prepared for the Substance Abuse and Mental Health Services Administration (SAMHSA) by RTI International under Contract No. HHSS283201300001C with SAMHSA, U.S. Department of Health and Human Services (HHS), 2014.

[15] D.V. Herin, C.R. Rush, J. Grabowski, Agonist-like pharmacotherapy for stimulant dependence: preclinical, human laboratory, and clinical studies, Ann. N. Y. Acad. Sci. 1187 (2010) 76–100.

[16] J.F. Hurkmans, H.E. Bodde, L.M. Driel, H.V. Doorne, H.E. Junginger, Skin irritation caused by transdermal drug delivery systems during long-term (5 days) application, Br. J. Dermatol. 112 (1985) 461–467.

[17] Kelmac medical, Permeability coefficients, http://www.kelmacmedical.us/permeability-coefficients/iii-estimation-of-permeability-coefficient-from-simple-physicochemical-parameters.html, (2016), Accessed date: 6 February 2018.

[18] Klucel hydroxypropylcellulose, Physical and chemical properties, http://www.ashland.com/file_source/Ashland/Product/Documents/Pharmaceutical/PC_11229_Klucel_HPC.pdf, (2018), Accessed date: 6 February 2018.

[19] S.C. Kolli, G. Chadha, J. Xiao, L.D. Parsons, R.J. Babu, Transdermal iontophoretic delivery of selegiline hydrochloride, in vitro, J. Drug Target. 18 (2010) 657–664.

[20] M.J. Kreek, K.S. LaForge, E. Butelman, Pharmacotherapy of addictions, Nat. Rev. Drug Discov. 1 (2002) 710–726.

[21] M.J. Kuhar, Neurotransmitter transporters as drug targets: recent research with a focus on the dopamine transporter, Pharmacologist 35 (1993) 28–33.

[22] Kupczewska-Dobecka, M. Jakubowski, S. Czerczak, Calculating the dermal flux of chemicals with OELs based on their molecular structure: an attempt to assign the skin notation, Environ. Toxicol. Pharmacol. 30 (2010) 95–102.

[23] Lipinski's rule of five, Analysis of the content of SAR databases, http://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/lipinskis-rule-of-five, (2013), Accessed date: 6 February 2018.

[24] B.K. Madras, M.A. Fahey, J. Bergman, D.R. Canfield, R.D. Spealman, Effects of cocaine and related drugs in nonhuman primates. I. [3H]cocaine binding sites in caudate-putamen, J. Pharmacol. Exp. Therapeut. 251 (1989) 131–141.

[25] A. Mangia, P.H. Andersen, B. Berner, H.I. Maibach, High Dissociation Constants (pKa) of Basic Permeants Are Associated with in Vivo Skin Irritation in Man Contact Derm vol. 34, (1996), pp. 237–242.

[26] MatTek, In vitro EpiDermTM skin irritation test (EPI-200-SIT), MatTek corporation. For use with MatTek Corporation's reconstructed human epidermal model EpiDerm™ (EPI-200-SIT), https://www.mattek.com/wp-content/uploads/EPI-200-SIT-Skin-Irritation-Test-Protocol-MK-24-007-0023.pdf, (2018), Accessed date: 6 February 2018.

[27] MedlinePlus, Selegiline transdermal patch, https://medlineplus.gov/druginfo/meds/a607003.html, (2016), Accessed date: 6 February 2018.

[28] N. Morikawa, T. Kitagawa, K. Tomihata, Assessment of the *in Vitro* Skin Irritation of Chemicals Using the Vitrolife-SkinTM Human Skin Model Japanese Society for Alternatives to Animal Experiments vol. 14, (2007), pp. 417–423.

[29] G.P. Moss, D.R. Gullick, S.C. Wilkinson, Predictive Methods in Percutaneous Absorption, Springer-Verlag, Berlin Heidelberg, 2015.

[30] M. Murphy, A.J. Carmichael, Transdermal drug delivery systems and skin sensitivity reactions: incidence and management, Am. J. Clin. Dermatol. 1 (2000) 361–368.

[31] M. Milewskia, L. Audra, A.L. Stinchcomb, Estimation of maximum transdermal flux of nonionized xenobiotics from basic physicochemical determinants, Mol. Pharm. 9 (2012) 2111–2120.

[32] S.S. Negus, J. Henningfield, Agonist medications for the treatment of cocaine use disorder, Neuropsychopharmacology 40 (2015) 1815–1825.

[33] S.S. Negus, M.H. Baumann, R.B. Rothman, N.K. Mello, B.E. Blough, Selective suppression of cocaine- versus food-maintained responding by monoamine releasers in rhesus monkeys: benzylpiperazine, (+)phenmetrazine, and 4-benzylpiperidine, J. Pharmacol. Exp. Therapeut. 329 (2009) 272–281.

[34] S.S. Negus, N.K. Mello, Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a second-order schedule in rhesus monkeys, Drug Alcohol Depend. 70 (2003) 39–52.

[35] S.S. Negus, N.K. Mello, Effects of chronic d-amphetamine treatment on cocaine- and food-maintained responding under a progressive-ratio schedule in rhesus monkeys, Psychopharmacology (Berlin) 167 (2003) 324–332.

[36] M. Nyswander, The Drug Addict as a Patient, Grune and Stratton, New York, 1965.

[37] K.J. Palmer, M.M. Buckley, D. Faulds, Transdermal Nicotine a Review of its Pharmacodynamic and Pharmacokinetic Properties and Therapeutic Efficacy as an Aid to Smoking Cessation.Drugs vol. 44, (1992), pp. 498–529.

[38] H. Patel, T.W. ten Berge, M.T.D. Cronin, Quantitative structure-activity relationships (QSARs) for the prediction of skin permeation of exogenous chemicals, Chemosphere 48 (2015) 603–613.

[39] R.O. Potts, R.H. Guy, Predicting skin permeability, Pharmaceut. Res. 9 (1992) 663–669.

[40] Pubchem, Compound, summary- 4 benzylpiperidine, https://pubchem.ncbi.nlm.nih.gov/compound/4-Benzylpiperidine, (2018), Accessed date: 6 February 2018.

[41] A. Puri, H.X. Nguyen, A.K. Banga, Microneedle-mediated intradermal delivery of epigallocatechin-3-gallate, Int. J. Cosmet. Sci. 38 (2016) 512–523.

[42] A. Puri, K.S. Murnane, B.E. Blough, A.K. Banga, Effects of chemical and physical enhancement techniques on transdermal delivery of 3-fluoroamphetamine hydrochloride, Int. J. Pharm. 528 (2017) 452–462.

[43] M.E.A. Reith, B.E. Meisler, H. Sershen, A. Lajtha, Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior, Biochem. Pharmacol. 35 (1986) 1123–1129.

[44] M.C. Ritz, R.J. Lamb, S.R. Goldberg, M.J. Kuhar, Cocaine receptors on dopamine transporters are related to self-administration of cocaine, Science 237 (1987) 1219–1223.

[45] M.S. Roberts, R.A. Anderson, J. Swarbrick, Permeability of human epidermis to phenolic compounds, J. Pharm. Pharmacol. 29 (1977) 677–683.

[47] R.B. Rothman, B.E. Blough, W.L. Woolverton, K.G. Anderson, S.S. Negus, N.K. Mello, Development of a rationally designed, low abuse potential, biogenic amine releaser that suppresses cocaine self-administration, J. Pharmacol. Exp. Therapeut. 313 (2005) 1361–1369.

[48] Schä, M. fer-Korting, H.C. Korting, E. Ponce-Pöschl, Liposomal tretinoin for uncomplicated acne vulgaris, Clin. Invest. 72 (1994) 1086–1091.

[49] R.J. Scheuplein, I.H. Blank, Permeability of the skin, Physiol. Rev. 51 (1971) 702–747.

[50] M.H. Schmid-Wendtner, H.C. Korting, The pH of the skin surface and its impact on the barrier function, Skin Pharmacol. Physiol. 19 (2006) 296–302.

[51] J. Shen, L. Kromidas, T. Schultz, S. Bhatia, An in silico skin absorption model for fragrance materials, Food Chem. Toxicol. 74 (2014) 164–176.

[52] A. Sivaraman, S.S. Ganti, H. Nguyen, B. Gudrun, A. Wieber, L. Dieter, A.K. Banga, Development and evaluation of a polyvinyl alcohol based topical gel, J. Drug Deliv. Sci. Technol. 39 (2017) 210–216.

[54] R.D. Spealman, B.K. Madras, J. Bergman, Effects of cocaine and related drugs in nonhuman primates. II. Stimulant effects on scheduled-controlled behavior, J. Pharmacol. Exp. Therapeut. 251 (1989) 142–149.

[55] L. Vaillant, S. Biette, L. Machet, T. Constans, C. Monpère, Skin acceptance of transcutaneous nitroglycerin patches: a prospective study of 33 patients, Contact Dermatitis 23 (1990) 142–145.

[56] L. Vlaia, G. Coneac, I. Olariu, V. Vlaia, D. Lupuleasa, Cellulose-derivatives-based hydrogels as vehicles for dermal and transdermal drug delivery, emerging concepts, in: S.B. Majee (Ed.), Analysis and Applications of Hydrogels, Intech, 2016.

[57] F.J. Vocci, N.M. Appel, Approaches to the development of medications for the treatment of methamphetamine dependence, Addiction 102 (2007) 96–106.

[58] F.J. Vocci, J. Acri, A. Elkashef, Medication development for addictive disorders: the state of the science, Am. J. Psychiatr. 162 (2005) 1432–1440.

[59] N.D. Volkow, T.K. Li, Drug addiction: the neurobiology of behaviour gone awry, Nat. Rev. Neurosci. 5 (2004) 963–970.

[62] R.C. Wester, R. Patel, S. Nacht, J. Leyden, J. Melendres, H. Maibach, Controlled release of benzoyl peroxide from a porous microsphere polymeric system can reduce topical irritancy, J. Am. Acad. Dermatol. 24 (1991) 720–726.

[63] A. Williams, Pharmaceutical solvents as vehicles for topical dosage forms, in: P. Augustijns, M.E. Brewster (Eds.), Solvent Systems and Their Selection in Pharmaceutics and Biopharmaceutics, Springer, New York, 2007, pp. 403–426.

[64] D.S. Jones, D. Woolfson, A.F. Brown, Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers, Int. J. Phar. 15 (1997) 223–233.