

RESEARCH ARTICLE



Microneedle-assisted transdermal delivery of Zolmitriptan: effect of microneedle geometry, *in vitro* permeation experiments, scaling analyses and numerical simulations

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ABSTRACT

Objective: The present study was aimed to investigate the effect of salient microneedle (MN) geometry parameters like length, density, shape and type on transdermal permeation enhancement of Zolmitriptan (ZMT).

Methods: Two types of MN devices *viz.* AdminPatch[®] arrays (ADM) (0.6, 0.9, 1.2 and 1.5 mm lengths) and laboratory fabricated polymeric MNs (PM) of 0.6 mm length were employed. In the case of PMs, arrays were applied thrice at different places within a 1.77 cm² skin area (PM-3) to maintain the MN density closer to 0.6 mm ADM. Scaling analyses was done using dimensionless parameters like concentration of ZMT (C_t/C_s), thickness (h/L) and surface area of the skin (Sa/L^2).

Results: Micro-injection molding technique was employed to fabricate PM. Histological studies revealed that the PM, owing to their geometry/design, formed wider and deeper microconduits when compared to ADM of similar length. Approximately 3.17- and 3.65-fold increase in ZMT flux values were observed with 1.5 mm ADM and PM-3 applications when compared to the passive studies. Good correlations were observed between different dimensionless parameters with scaling analyses. Numerical simulations, using MATLAB and COMSOL software, based on experimental data and histological images provided information regarding the ZMT skin distribution after MN application.

Discussion: Both from experimental studies and simulations, it was inferred that PM were more effective in enhancing the transdermal delivery of ZMT when compared to ADM.

Conclusions: The study suggests that MN application enhances the ZMT transdermal permeation and the geometrical parameters of MNs play an important role in the degree of such enhancement.

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Introduction

Migraine is a chronic, debilitating headache affecting ~19% of the population globally, with 2–3 times more incidence in women than in men. It is a neurovascular disorder whose pathophysiology is still not clearly understood. An attack/episode of migraine may last from 2 to 72 h, associated with characteristic symptoms like a pulsating and throbbing headache generally on one side of the head, nausea, vomiting, sensitivity to light, etc. that generally worsens with physical activity. Triptans are 5HT or serotonin receptor agonists that act specifically on migraine. These cause constriction of the dilated cranial blood vessels, which is thought to be the cause of migraine^{1,2}.

The first among this class of drugs is Sumatriptan, followed by newer ones *viz.* Zolmitriptan, Naratriptan, Rizatriptan, Eletriptan, Almotriptan, Avitriptan and Frovatriptan. Zolmitriptan (ZMT) was the second triptan available in USA and many countries, and was developed with the goal of creating a more bioavailable, longer half-life, more rapidly absorbed and centrally active oral tablet than Sumatriptan². ZMT is available as oral tablet, nasal spray and oral dispersible tablet formulations, with an optimum dose being 2.5 mg orally, with a maximum daily dose of 10 mg/day³. With all

three formulations, the bioavailability is between 40 and 48%. Nasal sprays of ZMT do offer a pharmacokinetic advantage of a rapid absorption across nasal mucosa, which results in a much faster onset of action. However, the overall therapeutic gain or efficacy is not improved significantly when compared to oral formulations. All these formulations of ZMT were reported to have a good chance of therapeutic failure with the first dose, requiring a second dose within 2 h to avoid a recurrence of the attack within 24 h^{3,4}.

An ideal delivery system for ZMT must improve the bioavailability and ensure a good therapeutic gain within 2 h, i.e. a faster onset of action and a low 24 h recurrence rate when compared to existing options, without compromising patient compliance and ease of handling. Transdermal delivery has gained a lot of interest as an alternative to oral and parenteral routes, and research is going on extensively to open this route for systemic administration of a vast array of drug molecules including large molecules like insulin, growth hormone, etc.⁵.

ZMT is a relatively hydrophilic molecule with a log *p* values of 1.6 and does not have ideal properties to permeate through skin and hence a suitable permeation enhancement technique should be employed to achieve clinically significant rates of drug permeation.

Only a few studies have reported on the transdermal delivery of ZMT, using chemical enhancers and iontophoresis as permeation enhancement techniques^{6–8}. Subedi et al., 2011⁷ studied the effect of different formulation variables like the specific types of pressure sensitive adhesives (PSA), solvent system, permeation enhancers, crystallization inhibitors, etc. on transdermal flux of ZMT and reported that permeation enhancers (polyoxyethylene alkyl ethers) resulted in a considerable increase in transdermal flux of ZMT, but induced ZMT crystallization⁷. Lui and Fang, 2015⁸, also developed an adhesive transdermal patch containing ZMT using DURO-TAK[®] 87–4098 (PSA) and 10% Azone as permeation enhancer at a drug load of 4% ZMT and reported that 88 ± 51 ng/mL ZMT was detected in systemic circulation within 15 min of administration of the patches *in vivo* using rabbit as an animal model, and concluded that further optimization can enable effective delivery of ZMT via a transdermal route⁸.

Extensive work in the literature emphasizes that microneedles (MN), which are minimally invasive systems that can overcome the barrier functions of skin and improve the permeation of drug molecules including macromolecules, could be used to effectively enhance the permeation of ZMT via a transdermal route^{9–14}. It was also reported that such enhancement is dependent on many aspects of MN design, such as type, shape of MNs, density, materials, etc. There is also an interplay of the effect of these variables and, as such, their effects on the permeation of a drug molecule are generally non-intuitive. As a result, their effects are best studied for specific case^{15,16}.

Consistent with this idea, the present study was designed using a systematic approach to investigate the efficiency of MNs in enhancing the transdermal permeation of ZMT and to explore the effect of MN geometrical parameters *viz.* type, MN length, design/shape and density on the extent of ZMT transdermal permeation enhancement. Two types of MN devices *viz.* commercially available AdminPatch[®] arrays (ADM) (0.6, 0.9, 1.2 and 1.5 mm length) and lab fabricated polymeric MN arrays (PM) (0.6 mm length) of different MN densities were employed for the same purpose (Figure 1). A number of different techniques such as etching, lithography and molding were discussed widely in the literature for the manufacture of MNs of different dimensions (100–1800 μ m in length), shapes with different materials (plastics, silicon, ceramics, metals, etc.). Injection molding offers several advantages in terms of ease of scalability for bulk manufacture, range of materials and good reproducibility¹⁷.

In this study, micro-injection molding was employed for fabrication of the PM using PEEK LT-3 (polyether ether ketone) as reported in Nair et al¹⁸.

Materials and methods

Materials

AdminPatch[®] MN arrays were purchased from AdminMed, Sunnyvale, CA. ZMT was obtained from Mylan Laboratories Limited, Hyderabad, India; sodium chloride, isopropyl alcohol, propylene glycol (PG) and 1% w/v Safranin solution from Loba Chemie, Mumbai, India; formic acid, acetonitrile, methanol and HPLC water from Merck Specialities Pvt. Ltd, Mumbai, India; haematoxylin and eosin stain from Sigma-Aldrich, Bengaluru, India. All the reagents and chemicals used in the study were of HPLC grade. Pig ear skin was obtained from local abattoirs.

Fabrication of PM

MN array components consisted of 25 conical needles, each 0.6 mm in length with a base diameter of 0.3 mm, in a 5×5 array over a 0.5×0.5 cm² area, supported by a circular substrate of diameter 17.5 mm and thickness 0.5 mm. Moulding of these components was performed using a Wittmann–Battenfeld Micro-Power 15 micro-injection molding machine as shown in Figure 2(A). This machine offers a high repeatability, accurate dosing, clean room facility and robotic component handling which is ideal for the bulk manufacture of MNs. The MN mold insert (Figure 2(B)) was made out of Stavax ESR (Bohler-Uddeholm Corporation, Elgin, IL). Sink electrical discharge machining (EDM) was used to create negative MN features into the insert and was outsourced to Isometric Tool & Design, Inc. (New Richmond, WI).

The polymer used for this study is PEEK (polyetheretherketone) LT-3 grade material, which is an FDA-approved semi-crystalline biomaterial manufactured by Invibio Inc., Lancashire, UK and is widely employed for medical use. The attractive characteristics of PEEK LT-3 for this application include its excellent processability, dimensional stability, mechanical strength, chemical resistance and resistance to gamma and ethylene oxide sterilization methods. To ensure the moisture content of the material was suitable for

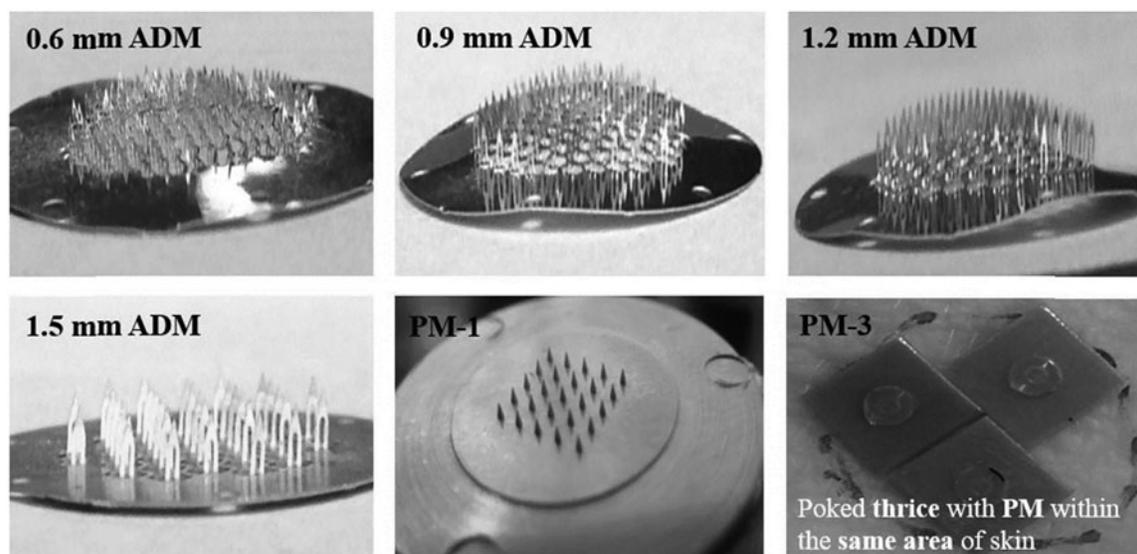


Figure 1. Different MNs used in the study.

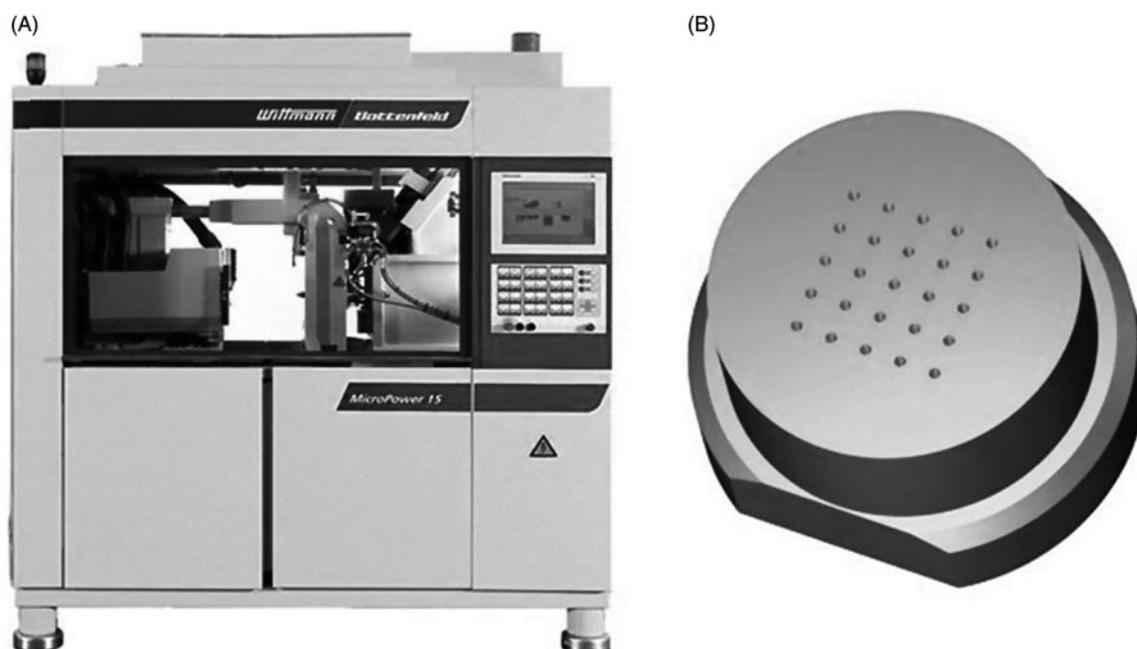


Figure 2. (A) Battenfeld micro-power 15 molding machine and (B) 3D drawing of the microneedle insert.

Table 1. Microinjection molding parameters.

Parameter	Value
Melt temperature (°C)	400
Maximum injection velocity (mm/s)	750
Clamping force (kN)	50
Holding pressure (bars)	600
Mould temperature (°C)	210

Table 2. Brightness switch settings used for the confocal measurements.

Position (μm)	Brightness
600	49
550	47.1
520	49.1
20	75.5
0	48.4

processing, the material was dried at a temperature of 150 °C for 4 h prior to molding.

Moulding trials were performed to optimize the process parameters based on measurement of the needle dimensions and substrate flatness. The optimized parameters for the molding of PM are shown Table 1. Once the process was stabilized, samples were collected for the subsequent experimental work.

MN dimensional evaluation

Scanning electron microscope

A Hitachi TM-3000 table-top Scanning Electron Microscope (Tokyo, Japan) was used for analysis of the MN insert and needle geometry. The Hitachi TM-300 has a magnification from 15–30,000 \times , sample chamber of 700 mm diameter and 50 mm thickness. It features 5 kV, 15 kV and analysis observation modes. Because of the high aspect ratio and low contrast of the MNs, analysis mode was used for imaging. The major advantage of using TM-3000 SEM was it works under low vacuum and does not need specimen coating.

Confocal laser microscope

It is very important to measure the tip radius and height of the MNs and the most common methods used are optical or electron microscopy. Because of its steep and complex structures, a 3D image analysis gives a better measurement of the needle geometry and quality control. In this study, an Olympus vertical

scanning laser confocal microscope LEXT OLS 4000 was used to accurately measure the tip radius and height of MN arrays.

The device offers a broad magnification range from 108 to 17,280 \times and the exact 3D reconstruction of the MNs. The confocal laser microscope LEXT scans the surfaces with a laser beam with the wavelength of 405 nm thus allowing submicron visualization of material and component surfaces with the resolution of down to 0.10 μm. Measurements were taken with the 20 \times lens using the wide range stitching feature with 20% overlap to produce a measurement area of 5 \times 5 mm. A five level brightness switch (Table 2) was enabled to accurately illuminate the specimen.

Atomic force microscope

The most important feature of the MN is that the tip has to be sharp in order to effectively pierce the skin. So as to confirm the measurements made from confocal laser microscope, AFM (MFP-3D Asylum Research, Santa Barbara, CA) was used to measure the tip radius of the MNs. All AFM scans were made using an MFP-3D scanner from Asylum Research. Silicon nitride cantilever tips (Applied Nano, Santa Clara, CA) with a tip radius of 15 nm and spring constant of 0.3 Nm were used. Images were interpreted using integrated MFP-3D™ Igor software (Asylum Research). Errors in the piezo-linearity were corrected for by using zero- and first-order flattening.

High-performance liquid chromatography method

Rapid-high high-performance liquid chromatography photodiode array (RP-HPLC-PDA) method was developed specifically for the

analysis of ZMT in the transdermal permeation samples. A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler and SPD-M20A PDA detector was used for analysis of the samples. Data acquisition was carried out using LC solutions software. Separation was achieved on an Agilent Eclipse column (150 × 4.6 mm; 5 μm). The mobile phase comprised of 0.02% v/v formic acid: methanol 75:25 (v/v) at a flow rate of 1 ml/min with an injection volume of 20 μL and the eluents were monitored at 221 nm. The developed method was validated as per the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines.

Solubility studies

The solubility of ZMT was studied in different vehicle combinations of PG, polyethylene glycol-400 (PEG) and saline (S) at 70:30, 80:20 and 90:10% v/v ratios. To each vehicle system, an excess amount of ZMT was added and vortexed for 1 min in order to obtain a saturated solution and the solutions were equilibrated at 37 °C in an orbital shaker for 24 h. After equilibration, the samples were centrifuged at 3000 rpm for 10 min and filtered through a nylon syringe filter (0.45 μm) and all the samples were appropriately diluted and analyzed by HPLC method.

Skin preparation

Pig ears were collected from the local abattoirs immediately after animals were killed by electric current. The ears were transported to the laboratory in a cooling box without previous treatment. In the laboratory, the pig ears were washed carefully with distilled water and the hair was removed from the external part of pig ear using an electrical hair clipper. Carefully the full-thickness skin from the external part of the pig ear was separated from underlying cartilage using a scalpel and excess fat underlying the skin was removed to a thickness of 1.2 mm was employed for the *in vitro* transdermal permeation studies. The dermis side was wiped with isopropyl alcohol to remove the residual adhering fat. Processed skin samples were individually wrapped in plastic bags without air entrapment and stored in a deep freezer at −20 °C until further use.

Application of MNs on skin samples

Prior to the *in vitro* skin permeation experiments, the skin samples were allowed to be brought to room temperature and then the skin surface was carefully washed with saline. Two types of MN devices *viz.* ADM (0.6, 0.9, 1.2 and 1.5 mm) and PM (0.6 mm) were used to poke the skin surface under thumb pressure. In the case of PM, both single (PM-1) and triple (PM-3) insertions at different places within a 1.77 cm² skin area (PM-3) were made in order to maintain the MN density closer to ADM of 0.6 mm length as shown in Figure 1. The MNs were periodically checked during this process for potential damage using a stereomicroscope.

Surface visualization and histological examination of the skin samples

To visually confirm the disruption of skin layers by the MNs, the arrays were pressed over the pig ear skin under thumb pressure and held for 1 min. Then, the skin was stained with safranin dye (1% w/v in water) and wiped with isopropanol cotton swabs for the identification of the microconduits formed. In the case of the

histological studies, the skin section samples with and without MN treatments were prepared after staining with hematoxylin and eosin for visualization of skin layers and to display a clear indentation by MN penetration. The sections were observed under a microscope (Olympus; Noida, India). The width and depth of microconduits formed were also calculated in triplicate ($n = 3$ skin samples for each MN) with the help of Toup View 3.2 Software (Irwin, CA). For control, skin samples without MN treatment were also prepared.

In vitro skin permeation studies

The *in vitro* transdermal permeation studies were performed using a vertical type Franz diffusion cell apparatus fitted with a water circulation system, a water heater and an eight-stage magnetic stirrer (Orchid Scientifics, Nasik, India). Franz diffusion cells with an effective diffusion area of 1.77 cm² and a receptor volume of ~14 ml were used. Saline was used as the receptor fluid. Pig ear skin was mounted between the donor and receptor cells with the stratum corneum (SC) facing towards the donor cell. The receptor medium was stirred for uniform drug distribution at a speed of 600 rpm throughout the experiment. Care was taken to prevent the entrapment of air bubbles at the interface of the skin (dermis) and the receptor solution. The surface of the skin was maintained at 32 °C using a circulating water bath. After equilibration, 500 μL of donor solutions containing excess amount of ZMT were applied on to the skin. Samples (500 μL) were withdrawn from the receptor fluid at 6-h increments up to 48 h and replaced with the fresh saline to maintain a constant volume. All the samples were stored at 4 °C, prior to the HPLC analysis.

The cumulative permeation profiles were plotted for the cumulative amount of drug permeated (nmol/cm²) as a function of time, for untreated and microneedle-treated skin. The flux values and the respective lag times were obtained from the slope and the X-intercept of the steady-state portion of the cumulative permeation profiles. Apparent permeability and diffusion coefficient values were computed from Fick's first law of diffusion:

$$\frac{1}{A} \left(\frac{dM}{dt} \right) = J_s = K_p \Delta C$$

J_s is the steady-state flux (nmol/cm²/h), M is the cumulative amount of drug permeating the skin (nmol/cm²), A is the area of the skin (1.77 cm²), K_p is the apparent permeability coefficient (cm/h) and ΔC is the difference in concentrations of ZMT in the donor and receiver. Sink conditions were maintained in the receiver throughout the experiment and hence ΔC was approximated to be the drug concentration in the donor compartment.

Enhancement ratios were also computed to evaluate the relative efficiency of different MNs on the ZMT skin permeation enhancement. The enhancement ratios were calculated as follows:

$$\text{Enhancement ratio} = \frac{\text{Cumulative amount or fFlux obtained after MN application}}{\text{Cumulative amount or fFlux obtained from passive studies}}$$

ZMT content in skin

After the completion of the permeation studies, skin samples were studied for drug disposition. The skin tissue exposed to the donor solution was cut with a scalpel and washed with filtered water

Table 3. Model parameters for dimensional scaling analyses of the data.

Parameter	Value
Duration for medication (t_m)	48 h
Surface area of skin exposed (S_a)	1.77 cm ²
Thickness of stratum corneum (h_{sc})	0.002 cm
Total thickness of membrane (distance to blood vessel) (h)	0.12 cm
Effective skin thickness (h_e)	Variable
Diffusion coefficient in viable skin (D)	Variable
Volume of fluid in receptor compartment (distribution) (V_d)	14 mL
Skin surface/donor concentration (C_s)	Saturated solution was charged as the donor solution
Microneedle length (L)	0.06, 0.09, 0.12 and 0.15 cm

and blotted with a paper towel in order to remove the drug which adhered to the surface. Then skin was minced with a scalpel, and placed in a pre-weighed vial. The drug was extracted from the skin by equilibrating with 5 ml of acetonitrile at 32 °C in an orbital shaker. The solutions were then analyzed by HPLC to determine the ZMT content.

Scaling analyses

Dimensionless correlations between the amount of ZMT permeated (C_t/C_s) and other variables such as the thickness (h/L) and the surface area (S_a/L^2) of the skin were made in order to gain insights into the overall phenomenon of ZMT transdermal permeation enhancement by MN application. The scaling analyses were carried out based on the principles of the Buckingham π theorem where it is defined that the dimensionless concentration of a drug, which permeates through skin, can be defined in terms of key non-dimensional parameters (e.g. MN lengths) using the procedures described in previous articles^{13,19}.

Equation 1 describes the relationship of all the model parameters used for such analyses (Table 3).

$$\frac{C_t}{C_s} = K \left[\frac{S_a L^4 K_e}{V_d h D} \right]^n \quad (1)$$

Where, "K" is a dimensionless constant and "n" is an unknown power; C_t and C_s are the amount of ZMT permeated at a given time "t" (48 h) and the amount of drug loaded in the donor compartment for diffusion (surface concentration on skin); S_a is the surface area of skin available for diffusion; L is the length of microneedles; K_e and V_d are the first-order elimination constant and the volume of receptor fluid; "h" is the thickness of skin and D is the diffusion coefficient of ZMT in skin.

Using Equation 1, the correlations between the dimensionless ZMT concentration (C_t/C_s) against the dimensionless parameters of the study (h/L and S_a/L^2) have been established considering that all other variables remain unchanged.

Numerical simulations of experimental studies

A set of well-defined numerical simulations were carried out in order to gain insights into the specific effects of MN shape, dimensions, force of insertion of MN, etc. on the overall permeation enhancement of ZMT, and also to obtain the information about ZMT distribution within the skin layers during permeation, which are otherwise difficult to obtain directly from the experimental data. The simulations were carried out using MATLAB (MathWorks, Natick, MA) and COMSOL Multiphysics (COMSOL Multiphysics Pvt.

Ltd., Stockholm, Sweden) programs. The MATLAB program was used to process the histological sections images of skin treated with MNs and these processed images were further coupled with the experimental permeation parameter, e.g. passive diffusion coefficient and imported into COMSOL simulator software^{13,20}.

Statistical analysis of the data

Results of the experimental data were subjected to statistical analysis by one-way analysis of variance (ANOVA) [using Fisher's least significance difference (LSD) *post hoc* test] using SYSTAT 13 software (Systat Software Inc., San Jose, CA). Results with a p values of $<.05$ were considered to have statistically significant variance. Mean of replicate measurements ($n = 3$) with corresponding standard deviation (SD) was used to represent the data and to plot the graphs.

Results

Fabrication and characterization of PM

The actual dimensions of the PM were determined using 3D Laser Scanning Confocal Microscopy (LSCM) (Figure 3(A)), atomic force microscopy (AFM) (Figure 3(B)) and scanning electron microscopy (SEM) (Figure 3(C)). The measurement data suggests an average microneedle form which was $556 \pm 30 \mu\text{m}$ in height with a tip radius of $32 \pm 8 \mu\text{m}$, a base diameter of $300 \mu\text{m}$ and MN interspacing of ~ 1 mm at the base.

For better comparison and understanding of the difference in the shape/design between PM and ADM devices, 3D confocal images of the 0.6 mm ADM were recorded and are shown in Figure 3(D). Also, the difference in base width among the devices can be clearly observed from the Figure 1. The various geometry parameters of both types of MN devices are given in Table 4.

Analytical method

A rapid and sensitive HPLC-PDA method was developed for the selective quantification of ZMT in transdermal permeation studies. Under the conditions, ZMT eluted at 2.8 min (Figure 4(A)) with good peak shape. Also, the specificity of the method to ZMT was demonstrated by the UV spectrum and the peak purity index curves (Figure 4(B,C)). The method was validated as per ICH guidelines and complied with all the requirements.

Solubility studies

Solubility studies for ZMT were performed with a view to select an appropriate donor vehicle for conducting *in vitro* skin permeation studies. PG:S and PEG:S combinations at 70:30, 80:20 and 90:10% v/v were studied for ZMT solubility. Solubility data of ZMT is shown in Figure 5.

Surface visualization and histological examination of skin samples

In order to visually confirm the disruption of skin layers by MN insertion, the arrays were pressed over the pig ear skin under thumb pressure and held for 1 min. Following removal of the arrays, the skin samples were stained with safranin (1% w/v). The digital photographs of MN-treated skin are shown in Figure 6.

Histological images of skin treated with MNs are shown in Figure 7. From the images, the SC disruption and the formation of

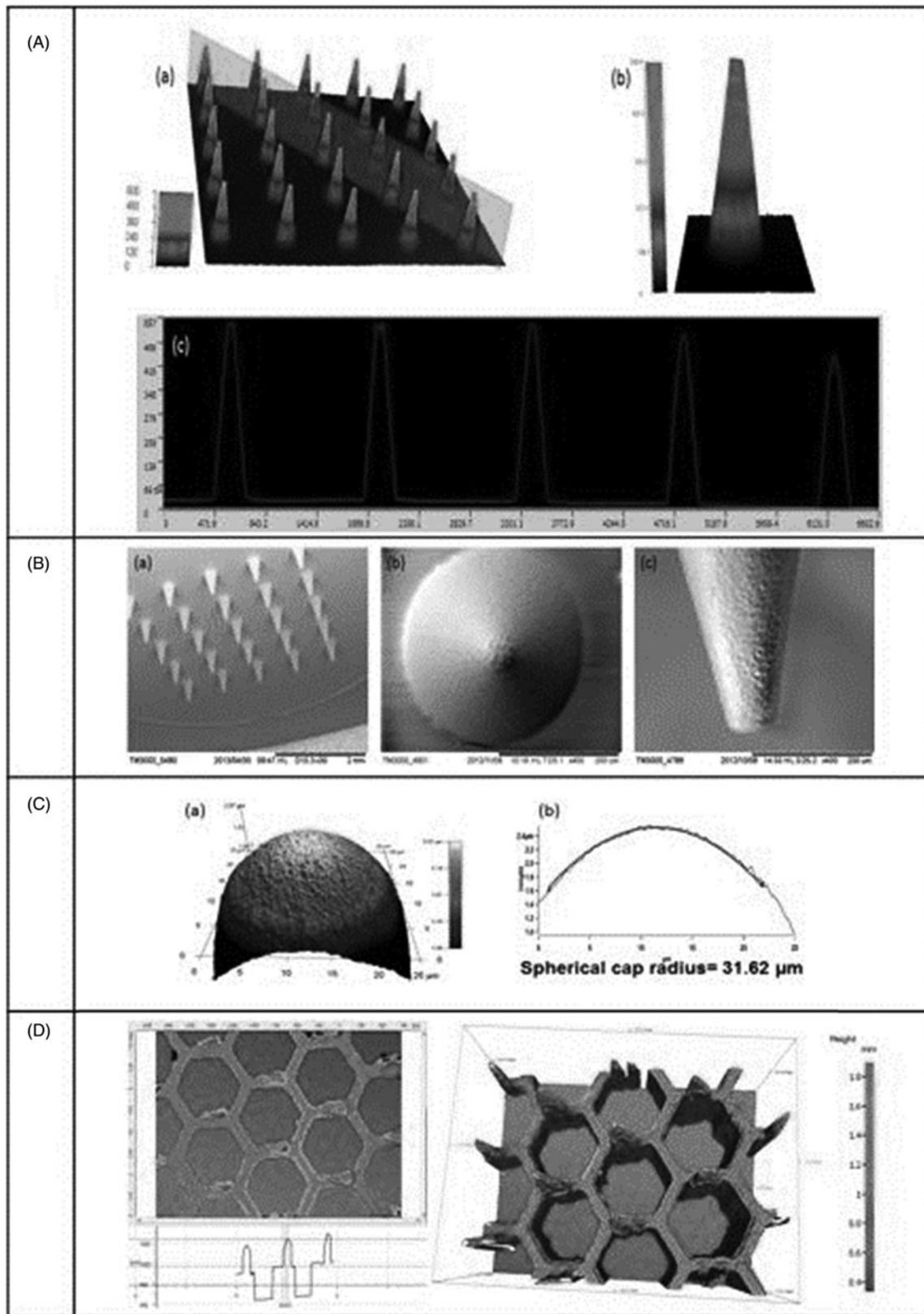
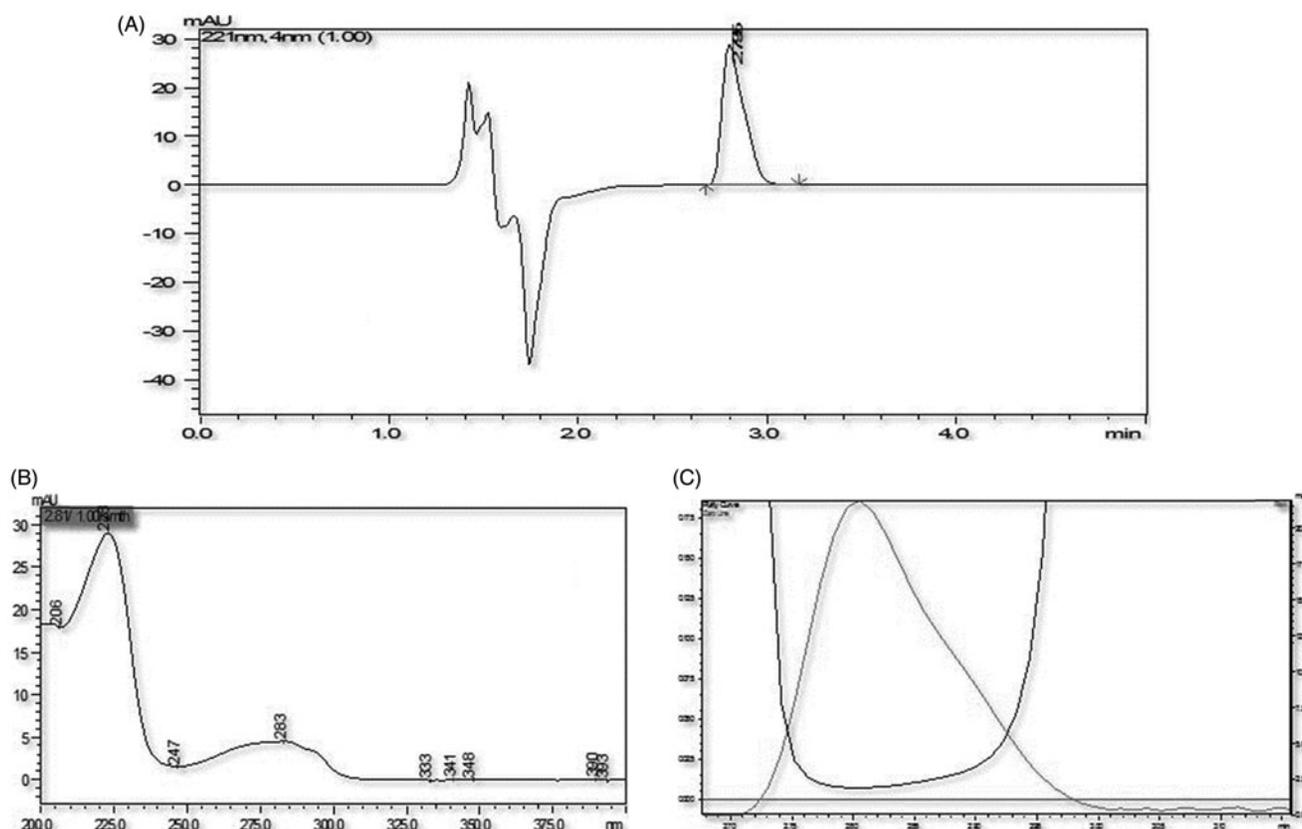
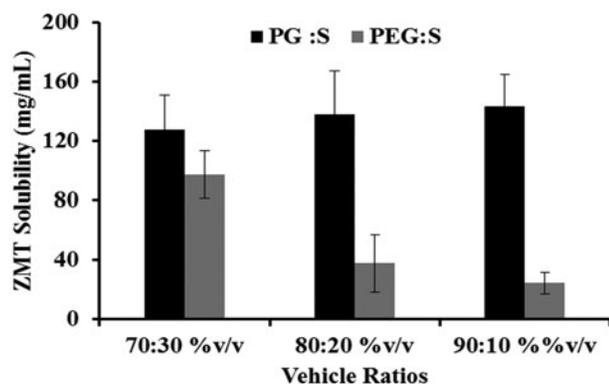


Figure 3. (A) 3D confocal image of (a) front view of the PEEK MN array, (b) single MN of PEEK and (c) line measurement of the PM array; (B) SEM image of (a) MN array of PEEK, (b) top view of the needle and (c) single MN of PEEK; (C) AFM image of the PEEK MN tip and (b) line graph showing the spherical cap radius of the needle; and (D) 3D confocal image of 0.6 mm ADM showing its design parameters.

Table 4. Different geometry parameters of MNs used in the study.

Parameter	ADM ^a				PM
	0.6	0.9	1.2	1.5	
Length (μm)	600	900	1200	1500	560 ± 30
Number of MNs (1.77 cm ²)	187	85	41	31	25
Shape	Flat (2D)				Conical (3D)
Thickness of each MN (μm) 78 (uniform till tip) ^b					300 (~30 at tip)
Thickness of array base (μm)					300
Material	Medical grade stainless steel (SS 316L)				PEEK LT-3 (polyether ether ketone)

^aValues are from manufacturer²¹.

^bThe width and thickness of the MN and the base varies with length.

Figure 4. (A) RIZ chromatogram; (B) UV spectrum; (C) Peak purity index.

Figure 5. Solubility data of ZMT in PG:S and PEG:S solvent systems.

microconduits across skin layers was clearly evident. The average penetration depth (vertical) ($n=3$) of the MNs was found to be 180.26 ± 30.39 , 302.22 ± 22.28 , 400.85 ± 20.83 , 478.48 ± 67.72 and $338.20 \pm 22.66 \mu\text{m}$ for ADM (0.6, 0.9, 1.2 and 1.5 mm) and PM, respectively.

In vitro permeation studies

The comparative *in vitro* pig ear skin permeation profiles of ZMT without and with MN application were shown in Figure 8. Various ZMT permeation parameters viz. cumulative amount permeated at the end of 48 h, steady-state flux, lag time, permeability and diffusion coefficients without and with microneedle treatment were given in Table 5.

Scaling analyses

Using scaling analyses, correlations were developed between the dimensionless parameters, namely, the amount of ZMT permeated (C_t/C_s) and skin thickness (h/L) and surface area of skin (S_a/L^2) in order to derive a better understanding on the effect of specific geometry parameters of MN, like length (L), on overall permeation enhancement by MNs of same design (ADM) (Figure 9(A,B), respectively)^{13,19}. Equations 2 and 3 describe such relationships among the considered parameters within the given range.

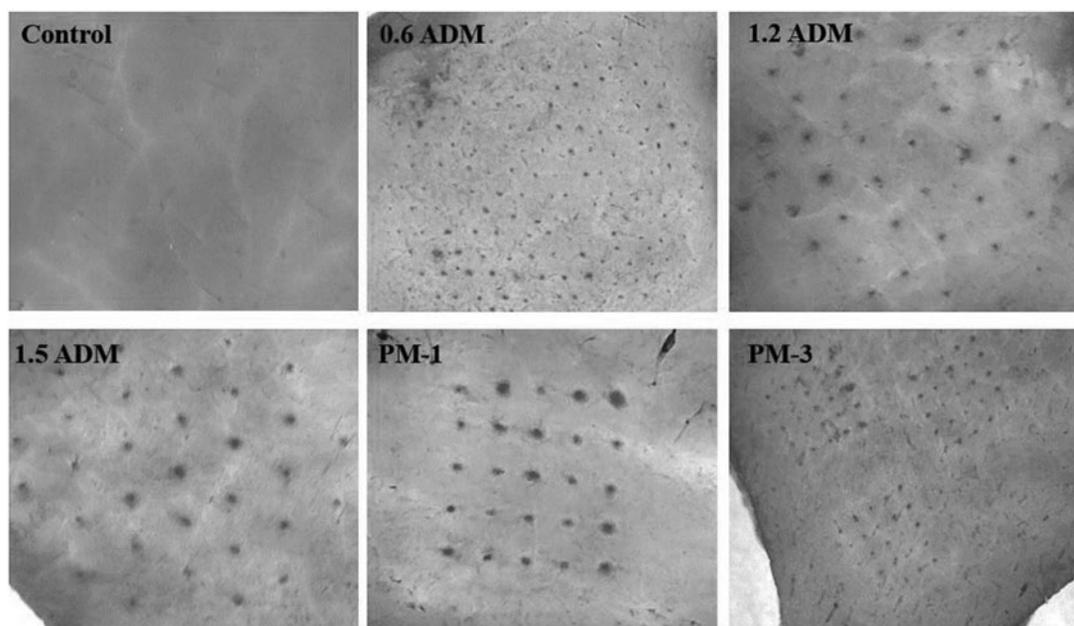


Figure 6. Surface images of stained skin without and with MN treatment.

These correlations were determined for given thickness and surface area of skin while the MN length varies (0.6, 0.9, 1.2 and 1.5 mm).

$$\frac{C_t}{C_s} = 5.52 * 10^{-3} \left[\frac{h}{L} \right]^{-0.624} \quad \text{for } 0.8 \leq \frac{h}{L} \leq 2 \quad (2)$$

$$\frac{C_t}{C_s} = 2.47 * 10^{-2} \left[\frac{S_a}{L^2} \right]^{-0.312} \quad \text{for } 78.5 \leq \frac{S_a}{L^2} \leq 491.5 \quad (3)$$

Good correlation was observed ($R^2 > 0.83$) between the dimensionless parameters of the study (Figure 9).

Numerical simulations of experimental studies

The numerical simulations were carried out using the procedures discussed in previous articles based on *in vitro* passive diffusion coefficient values, coupled with histological section images (of corresponding MNs)^{13,20}. Different stages in processing the histological section images by MATLAB and COMSOL programs for simulations are shown in Figure 10. The numerical simulations were able to provide information regarding the skin distribution of ZMT after applying the MNs at any time point and depth (Figure 10(D)).

Discussion

The dimensions of the PM were found to be consistent and repeatable with good tip shape, confirming the complete filling of the PEEK into the MN insert cavity under the maintained processing conditions (Table 1) and the technique used is reliable for the bulk manufacture of PMs. Compression tests of PM on a steel plate using Bose Electroforce 3100 instrument with a 225 N load cell and Wintest[®] software (Bose, Eden Prairie, MN) revealed that the PMs were able to withstand compression forces of up to 8 N¹⁸.

A rapid and sensitive HPLC-PDA method was developed for the selective quantification of ZMT in transdermal permeation studies. The method complied with all the requirements of ICH guidelines

and was successfully employed for the quantitative estimation of ZMT in various samples throughout the study.

Solubility of ZMT was relatively higher in PG:S when compared with PEG:S combinations ($p > .05$) (Figure 5). However, a significant difference was not observed among PG:S vehicle combinations ($p > .05$). PG:S at 70:30% v/v was selected for the ZMT *in vitro* skin permeation studies.

Surface visualization of MN-treated skin showed clear distinctions in the number of the microconduits formed due to the differences in length and density of MNs in the ADM and PM devices (Figure 6). Skin layer disruption and the formation of microconduits was clearly evident from histological section images (Figure 7). The penetration depth ~25–35% of the original needle length for ADM and 55–60% for the PM MN. Even though the length of the MNs differs in ADM, the percentage of MN penetration is almost same, which is an indication of uniformity in thumb pressure under which MNs were applied at different times.

With the ADM devices, as the length of the MNs increased the penetration depth also increased. However, the microconduits were found to be wider and deeper with PM when compared to ADM of similar lengths i.e. 0.6 mm. These differences in the efficiency of creating microconduits in skin layers between the two types of MN devices (ADM and PM) may be attributed to the differences in geometry parameters like shape, design and type of fabricating material. Regarding the shape/design, the PMs were conical (3D) in shape, and the microconduits formed by PM were wider, while the microconduits formed using ADM looked merely like a cut on the skin (2D) as the arrays of ADM are low in thickness (2D) (Figure 3). Furthermore, owing to the sturdy and thick base (when compared to ADM) that supports the solid PM (Figure 1), the effective resistance (because of the viscoelastic nature) of the skin during application of arrays may be less for PM, while the ADM might have experienced greater resistance which is because of their thin base and array design²¹, resulting in a relatively shallow penetration with similar MN lengths (0.6 and 0.9 mm ADM).

Significant enhancement in ZMT permeation was observed after the application of MNs into the skin ($p < .05$) when compared to passive permeation studies (Figure 8). A 1.83, 1.91, 2.35 and 3.17-fold increase in the cumulative amount (48 h) of ZMT

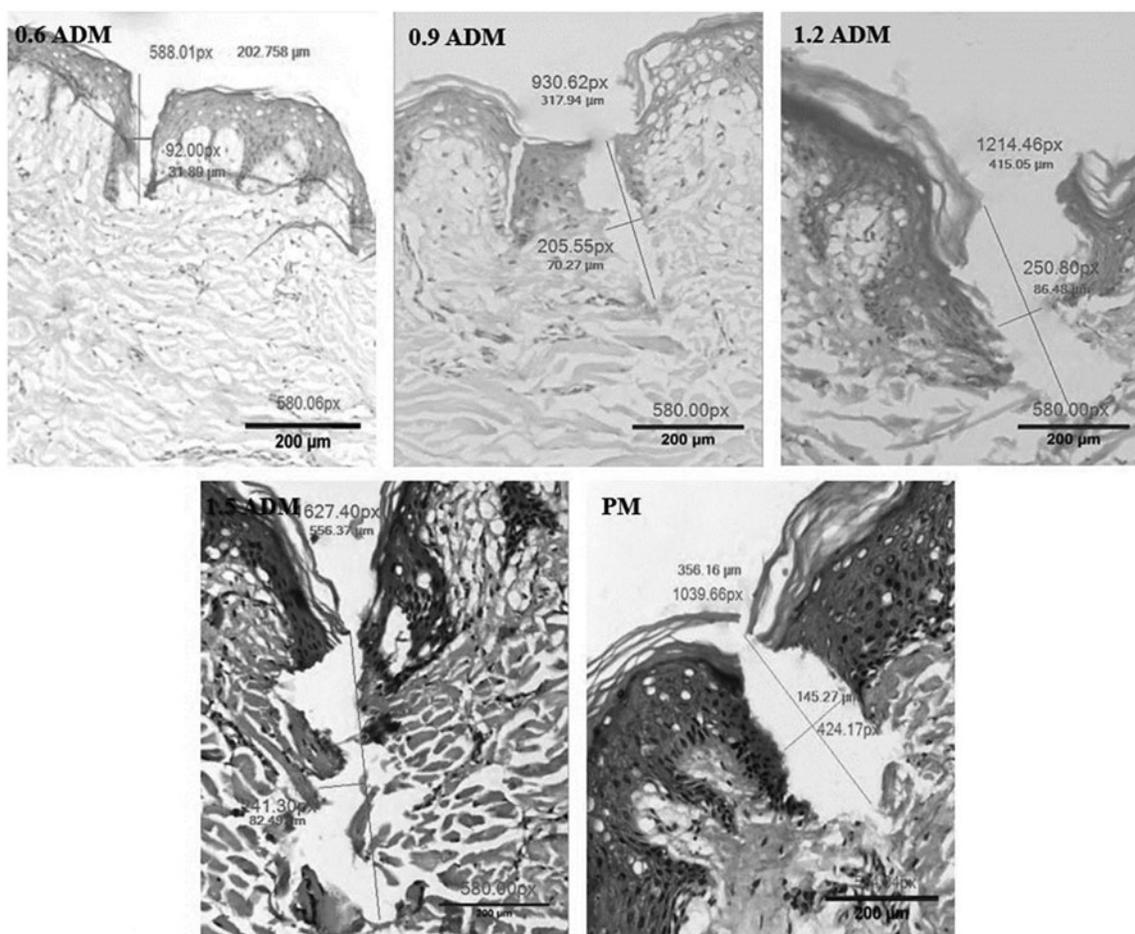


Figure 7. Penetration depth calculation of ADM and PM in skin (ToupView® screenshot).

permeated was observed with 0.6, 0.9, 1.2 and 1.5 mm ADM treatments, respectively, when compared to the passive permeation amount. A similar trend was observed with other permeation parameters like permeability and diffusion coefficient values (Table 5). Significant reduction in the lag time was observed after the application of the 1.5 mm ADM when compared to that with the passive data and other ADM lengths ($p < .05$). However, there is no statistically significant difference between the 0.9 and 1.2 mm ADM treatments in terms of lag time ($p > .05$).

The ZMT flux values were found to be in the order of $1.5 > 1.2 > 0.9 \geq 0.6 \text{ mm} > \text{passive}$ with ADM treatments. A 2.26-fold increase in ZMT flux was observed with the 1.5 mm when compared to passive studies. However, there is no statistically significant difference between the 0.6 and 0.9 mm treatments ($p > .05$). It was observed that the enhancement of ZMT permeation was only marginal with the application of the 0.6 and 0.9 mm ADM when compared to passive studies (Figure 8). This may be due to the fact that the skin disruption/penetration caused by 0.6 and 0.9 mm ADM was less, as supported by the histological section images (Figure 7). Moreover, the ZMT permeation was high with the 1.5 mm ADM when compared to 0.6, 0.9 and 1.2 mm even though the density of the MNs is low, which is possibly due to longer needle lengths which, in turn, resulted in deeper skin penetration.

With the PM application, ~1.37- and 3.65-fold increase in the cumulative amount (48 h) of ZMT permeated was observed with the PM-1 and PM-3 treatments, respectively, when compared to passive permeation amounts. The lag times were found to be

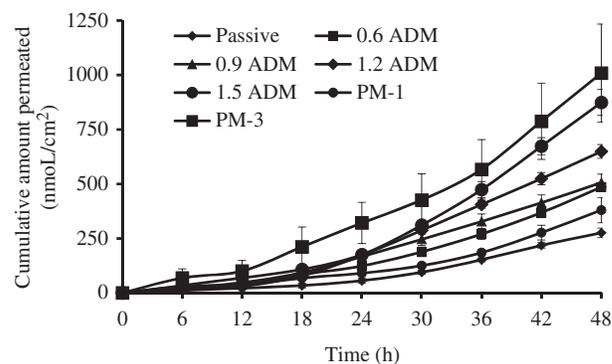


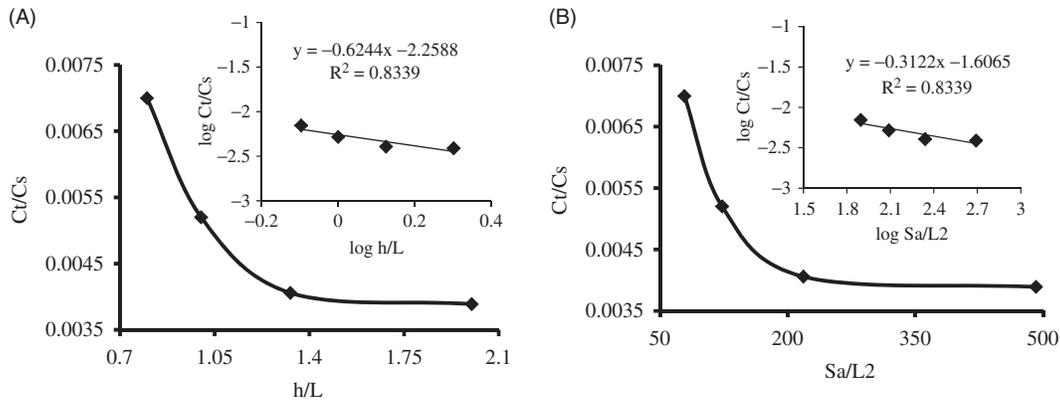
Figure 8. Comparative *in vitro* skin permeation profiles of ZMT from passive and MN treatments.

significantly lower for PM-3 when compared to passive and PM-1 treatments ($p < .05$). The ZMT flux values were found to be in the order of $\text{PM-3} > \text{PM-1} > \text{passive}$ treatments. A 1.25- and 2.96-fold increase in ZMT flux was observed with the PM-1 and PM-3, respectively, when compared to passive studies. A similar trend was observed with other ZMT permeation parameters like permeability and diffusion coefficient values (Table 5) with both the PM treatments.

Even though, the skin penetration by PM device (with 0.6 mm array) was significantly greater when compared to 0.6 and 0.9 mm ADM (Figure 7), the overall permeation enhancement in terms of flux, cumulative amount permeated etc. achieved with PM-1 was

Table 5. Permeation parameters of ZMT without and with MN treatments.

Skin treatment	Permeation parameters				
	Steady-state flux (nmol/cm ² /h)	Lag time (h)	Permeability coefficient ($\times 10^{-5}$) (cm/h)	Diffusion coefficient ($\times 10^{-10}$) (cm ² /s)	Skin content (μ g/g)
Passive	10.138 \pm 0.803	20.83 \pm 0.63	4.593 \pm 0.364	15.309 \pm 1.212	1627.67 \pm 164.23
0.6 ADM	15.019 \pm 0.164	17.66 \pm 1.37	6.797 \pm 0.074	22.658 \pm 0.248	1772.13 \pm 205.88
0.9 ADM	14.847 \pm 0.398	14.04 \pm 1.71	6.725 \pm 0.180	22.418 \pm 0.601	1800.12 \pm 302.82
1.2 ADM	19.168 \pm 1.311	14.52 \pm 0.97	8.683 \pm 0.594	28.943 \pm 1.979	2630.95 \pm 129.19
1.5 ADM	22.872 \pm 1.666	13.20 \pm 0.39	10.361 \pm 0.755	34.536 \pm 2.515	2832.67 \pm 367.25
PM-1	12.621 \pm 2.225	20.36 \pm 1.45	5.712 \pm 1.007	19.041 \pm 3.356	2124.60 \pm 121.57
PM-3	30.095 \pm 4.754	16.46 \pm 1.85	13.621 \pm 2.152	45.403 \pm 7.172	1899.31 \pm 152.33

**Figure 9.** Scaling relationship of different dimensionless groups for permeation of RIZ: (A) effects of dimensionless length of MNs and (B) effects of dimensionless surface area for diffusion.

found to be closer to those of 0.6 and 0.9 mm ADM ($p > .05$). This may be because the needle density of 0.6 and 0.9 mm ADM was greater when compared to PM-1 (Table 4), which may have compensated the variation in the extent of skin penetration among these MNs, and thus no significant variation in overall permeation enhancement was observed among these three MNs ($p > .05$).

In the case of the PM, a single application of array didn't cover the 1.77 cm² surface area of the skin and hence in order to maintain the MN density closer to the ADM of 0.6 mm length, the arrays were applied three times at different places within a 1.77 cm² skin area (PM-3) as shown in Figure 1. As the needle density was increased with PM-3, the ZMT transdermal permeation increased markedly and was similar to that obtained with 1.5 mm ADM ($p > .05$).

Moreover, it is intriguing to note although the needle density of PM-3 (75 MNs) was significantly greater when compared to the 1.5 mm ADM (31 MNs), the lag times were found to be significantly shorter with 1.5 mm ADM ($p < .05$), which may be because of deeper (vertical) penetration into skin. Even though the depth of the penetration was significantly lower with PM when compared to 1.5 mm ADM, the comparable permeation enhancement by PM-3 is may be because of higher needle density as stated earlier and wider microconduits formed as evidenced by histological section images (Figure 7).

The enhancement in ZMT permeation was found to be in the order of 1.5 mm ADM \geq PM-3 $>$ 1.2 mm ADM $>$ 0.9 mm ADM \geq 0.6 mm ADM \geq PM-1 $>$ passive. Even though no correlation of ZMT skin content was observed with different MN treatments, significantly higher amounts of ZMT were found to be distributed in skin layers at the end of 48 h with MN-treated studies and is an indication of potential ZMT skin deposition.

Patel et al. (2009)⁶, investigated the effect of iontophoresis on transdermal permeation of ZMT both *in vitro* and *in vivo*.

The cumulative amount of ZMT permeated was found to be $\sim 263.7 \pm 92.7 \mu\text{g}/\text{cm}^2$, *in vitro*, at the end of 6 h with continuous application of current (0.25 mA/cm²). However, the preliminary studies carried out in the present investigation showed a similar result (cumulative amount permeated) at the end of 48 h with 1.5 mm ADM and PM-3 applications (251.08 ± 17.07 and $289.85 \pm 64.58 \mu\text{g}/\text{cm}^2$, respectively).

Moreover, iontophoresis was reported to be associated with itching, redness and skin irritation and tingling sensation, making iontophoresis less patient compliant²². The MN application, especially the poke and patch technique discussed in this investigation, is painless and easy to administer when compared to iontophoresis, thus alleviating any issues regarding patient compliance.

Overall, further enhancement in the cumulative amount permeated and other permeation parameters like steady-state flux of ZMT may be achieved by further optimizing the MN geometry, especially of the PM in terms of design, MN length and density, and other parameters of the study like drug loading, donor vehicle, formulation, etc. thus resulting in more rapid and effective ZMT delivery.

Dimensionless correlations were developed for ADM MNs (same type but differing in length) using scaling analyses. Good correlations were observed between the dimensionless parameters (C_t/C_s versus h/L and C_t/C_s versus S_a/L^2) (Figure 9). These correlations can be used to predict the amount of ZMT permeated with high accuracy for other MN lengths in the range of 0.6–1.5 mm with similar design as ADM.

From the results of numerical simulations, the flux was represented by white arrows in Figure 10(D) showing obvious increment at the upper layers of the skin which is caused by MN penetration. This increment may be extrapolated to a higher drug accumulation rate at the blood stream as well as shorter lag time for the diffusion to reach its steady state after the application of

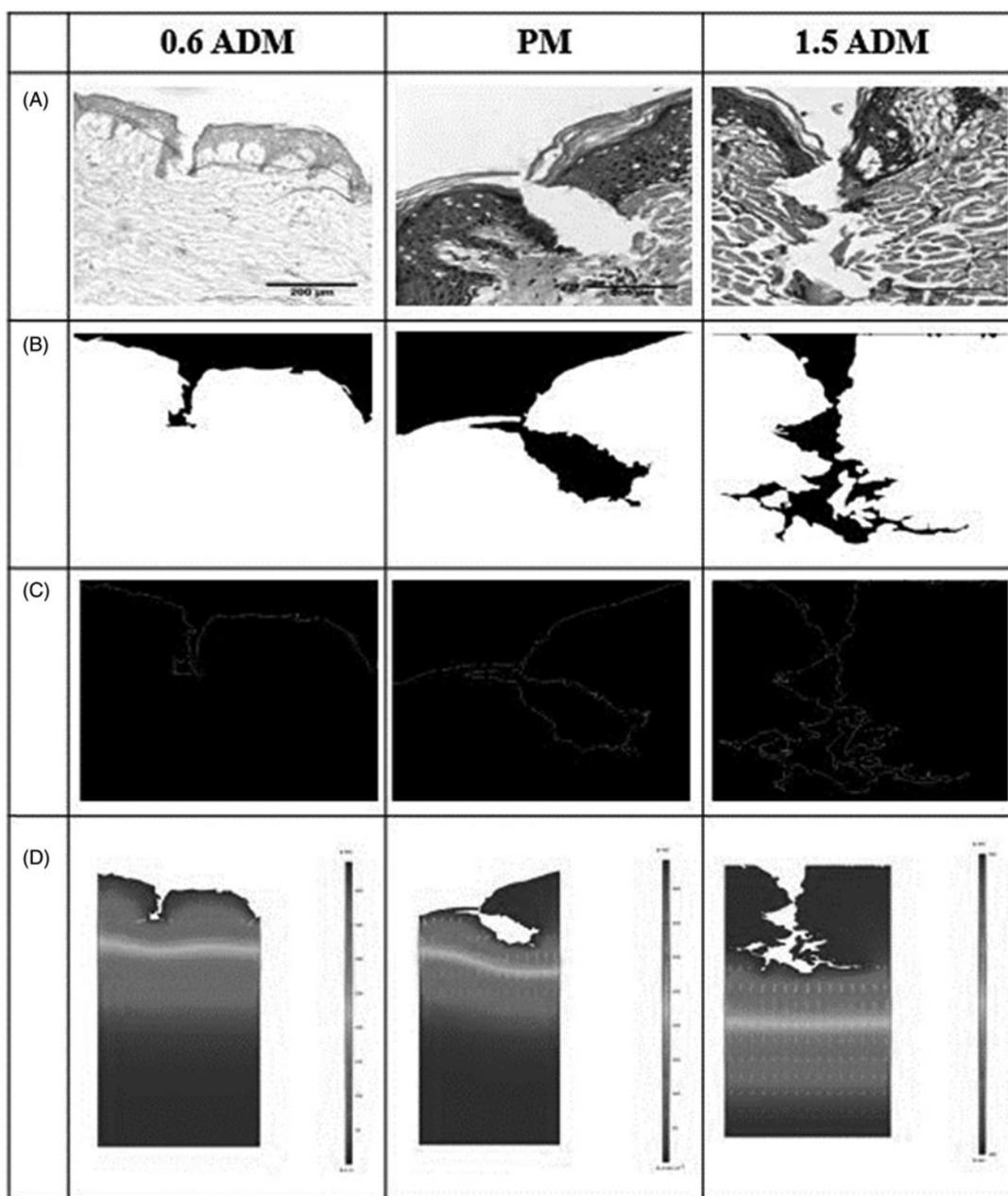


Figure 10. (A) The histological section images of skin. (B,C) Images treated and processed with MATLAB program. (D) Images simulated in COMSOL.

MNs. Based on the simulation results, the diffusion profile of ZMT showed significant permeation increment with 1.5 mm ADM and PM when compared to other MNs in the study. Furthermore, the advantage of greater penetration by PM can be further magnified by increasing the MN density, as in the case of PM-3.

These simulations can be of great research value as they may be used to predict the degree of transdermal permeation enhancement of ZMT with other (new) dimensions and designs of MNs using the respective histological section images and the passive diffusion coefficient data.

Conclusions

Two types of MN devices (ADM and PM) were employed in this investigation to derive a greater understanding on the effects of various geometry parameters specifically on the transdermal permeation of ZMT by MN application. It was observed that the

application of MNs significantly enhances the transdermal permeation of ZMT across pig ear skin. In addition, the type/shape, density and more importantly, the length of MNs (depth of penetration into skin) were found to play a crucial role in the overall permeation enhancement of ZMT using this technique. It may be inferred that the transdermal delivery of ZMT, at clinically significant levels and in a painless and non-invasive manner, may be possible using MN applications. This investigation can form a basis for further studies (*in vivo*) and for optimization of various MN parameters to achieve successful delivery of ZMT via MN-assisted transdermal delivery systems.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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