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RESEARCH PAPER

Physicochemical Modulation of Skin Barrier by Microwave for Transdermal Drug Delivery

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ABSTRACT

Purpose To investigate mechanism of microwave enhancing drug permeation transdermally through its action on skin.

Methods Hydrophilic pectin-sulphanilamide films, with or without oleic acid (OA), were subjected to drug release and skin permeation studies. The skins were untreated or microwave-treated, and characterized by infrared spectroscopy, Raman spectroscopy, thermal, electron microscopy and histology techniques.

Results Skin treatment by microwave at 2450 MHz for 5 min promoted drug permeation from OA-free film without incurring skin damage. Skin treatment by microwave followed by film loaded with drug and OA resulted in permeation of all drug molecules that were released from film. Microwave exerted spacing of lipid architecture of stratum corneum into structureless domains which was unattainable by OA. It allowed OA to permeate stratum corneum and accumulate in dermis at a greater ease, and synergistically inducing lipid/keratin fluidization at hydrophobic C-H and hydrophilic O-H, N-H, C-O, C=O, C-N regimes of skin, and promoting drug permeation.

Conclusion The microwave technology is evidently feasible for use in promotion of drug permeation across the skin barrier. It represents a new approach in transdermal drug delivery.

KEYWORDS dermis · microwave · oleic acid · stratum corneum · transdermal drug delivery

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INTRODUCTION

Microwave, an electromagnetic wave, is characterized by frequencies between 300 MHz and 300 GHz equivalent to wavelengths of 1 m to 0.01 m (1). Principally, microwave is not a form of heat, but a form of energy which manifests as heat through its interaction with materials *via* ionic and particularly dipole rotation of the object molecules. Alternatively, microwave is found to interact with materials non-thermally where there is no marked rise in temperature at the site of interaction (2).

Over the past decades, microwave (900 to 2500 MHz) has been investigated for its potential applications in biomedical field. Local microwave hyperthermia (3,4) and external microwave hyperthermia (5) have been evaluated as a tool to treat superficial transitional bladder cell carcinoma, and locally advanced, unresectable or recurrent rectal cancer respectively, in combination with chemotherapy or external beam radiotherapy. In addition, transurethral microwave thermotherapy has emerged as an alternative method to surgery for treatment of benign prostatic hyperplasia (6). Microwave diathermy has also been used for pain relief, tendon extensibility improvement, muscle and joint stiffness reduction, as well as, stimulation of medically beneficial physiological responses through deep tissue heating (7). Low intensity continuous microwave, on the other hand, is found to be able to negate postoperative purulent wound predominantly caused by pyrogenic *Staphylococcus aureus* (8).

Lately, microwave is used to design controlled-release drug delivery systems *via* its ability to heat, elasticize, induce crosslink, coacervation or denaturation in polymeric matrix, and amorphize drug crystals (1,9). Microwave is also employed to increase localization of thermally responsive polymeric carrier of drugs at tissue levels and promote the activity of drugs, as well as, improve permeability and drug diffusion attributes across the blood–brain barrier *via* inducing hyperthermia at the biological interface (7,10,11).

Typically, a high intensity microwave at 80 to 975 W is employed to process pharmaceutical materials into controlled-release dosage forms (1). In the case of biological modification, microwave exposure limit of 1 mW/cm^2 or microwave power up to 70 W has been suggested (6,8). Modification of physiological attributes of biological objects *via* heating is commonly run using microwave of frequencies between 900 and 2450 MHz (3,4,7,11–13). A low frequency microwave at 915 MHz is advocated for deep tissue modulation, while microwave at a higher frequency of 2450 MHz is largely deemed suitable to interact with surfaces of biological objects due to its shorter wavelength (13).

In transdermal drug delivery, various methods have been employed to improve the skin permeability to drugs. These include hydration (14), stratum corneum removal (15), use of chemical penetration enhancers (16,17), external forces such as sonophoresis (18–21), electric current (iontophoresis) (22,23), transient high-voltage electrical pulses (electroporation) (24,25), microneedles (26,27), photomechanical wave (28), and pressurized gas (29). Lately, the promotion of transdermal drug delivery has been found achievable using microwave through prolonged interaction of skin with high intensity radiation directly, as proposed by Moghimi *et al.* (2010) (30). Unlike iontophoresis, electroporation and sonophoresis which use electrical energy or ultrasound energy with a therapeutic frequency of 1 to 3 MHz, the microwave is envisaged to mediate transdermal drug delivery through exerting thermal/non-thermal effects on skin as a result of interaction between electromagnetic waves and biological objects. Nonetheless, the associated mechanism of microwave effects on skin microstructure has yet to be investigated. This study aims to elucidate the possibility of promoting transdermal drug delivery using low intensity microwave and with short skin exposure duration, taking into consideration of practicality and safety. In addition, the mechanism of microwave in drug permeation enhancement through its action on skin tissue is elucidated, for the first time.

MATERIALS AND METHODS

Materials

Pectin (P, methoxy content=9.0%, galacturonic acid content=87.6%, Sigma, USA) was used as a matrix polymer of film with sulphanilamide (SN, Sigma, USA) as a model water-soluble hydrophilic drug, and oleic acid (OA, Fluka, Germany) as a chemical penetration enhancer. Pectin was selected for its possessed mucosa penetration and film forming properties (9). Sulphanilamide was used as hydrophilic drug molecules were known to have a low skin permeation capacity (19). Disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, sodium chloride, hydrochloric acid (Merck, Germany), as well as, sodium azide (Ajax Finechem, Australia) were used to prepare USP phosphate buffer solutions. Oil Red O Stain Kit (Scy-Tek Laboratories, USA), Harris haematoxylin (VWR International, UK) and eosin (Microm International, Germany) were used in histology examination.

Film Preparation

An accurately weighed amount of 2.5% w/w aqueous pectin solution, with or without SN and OA, was transferred into a teflon dish (internal diameter=3 cm). The theoretical content of pectin in each film was standardized at 37.5 mg (9). In the case of drug- and OA-loaded film, 5 mg SN and 3.75 mg OA were incorporated into the matrix respectively (SN=10.80%; OA=8.10%). The solution was subjected to hot air drying at 40.0±0.5°C for 6 h. The formed film was collected and further conditioned in a desiccator at 25±1°C and 50±5% relative humidity for at least 5 days prior to subsequent experiments to ensure complete drying. The thickness of film was measured at three different sample points using digital micrometer (Mitutoyo, Japan). At least triplicates were carried out for each formulation.

Drug Content

A total of five fractions from each film were subjected to drug content assay. An accurately weighed film fraction was dissolved in USP phosphate buffer solution pH 7.4 and analysed for SN using ultra-violet (UV) spectrophotometry technique at wavelengths of 260.1 nm (Cary 50 Conc, Varian Australia Pty Ltd, Australia) with limits of detection and quantification at 0.17 μg/ml and 0.52 μg/ml respectively. Blank film was used as a control. The drug content was defined as the percentage of drug embedded in a unit weight of film.

Drug Release Study

The cumulative drug release profiles of films were examined using the vertical diffusion cells with a programmable MicroettePlus Q-Pak™ autosampler (Hanson Research, USA). The receptor glass chamber was filled with 7 ml of air bubble-free phosphate buffer solution USP pH 5.5 in simulation of pH environment of skin where drug was first released. A Tuffryn® membrane (pore size=0.45 μm, HT-450, Pall Corp., USA) was placed over the receptor chamber with film sample located on the top surfaces of Tuffryn® membrane facing the donor compartment. The temperature of buffer solution was maintained at 32±2°C and the buffer solution was magnetically stirred at 400 rpm throughout the study. Five ml aliquot was withdrawn at various time

intervals for 24 h and subjected to UV spectrophotometric assay for SN. Fresh buffer solution was replaced at each interval to maintain the sink condition of receptor chamber. Blank film was used as a control. The percentage of drug release was calculated with respect to the drug content of film. The drug content was expressed as the percentage of drug encapsulated in a unit weight of film. Triplicates were carried out for each formulation and the results averaged.

Kinetics of Drug Release

The mechanism of drug release was investigated by fitting the drug release data to Korsmeyer-Peppas dissolution model as expressed by

$$F = kt^n \quad (1)$$

where F is the amount of drug released at time t (min), k is the drug release rate constant incorporating the properties of polymeric system and drug, and n is the release exponent indicative of drug release mechanism. The n and k values were obtained from the plots of log$F$ against log$t$ and the goodness of fit of the drug release data was evaluated by linear regression. The value of n=0.5 represents Fickian diffusional (Case I) release, $0.5<n<1.0$ represents non-Fickian (Anomalous) release, n=1.0 indicates Case II (Zero order) release and $n>1.0$ indicates Super Case II release (31). Case II release refers to transport of drug solute *via* the dissolution of polymeric matrix due to relaxation of polymer chains, whereas Anomalous release refers to the summation of both drug diffusion and polymer dissolution controlled drug release. Super Case II release denotes drug dissolution which is controlled by polymer relaxation and is characterized by a sigmoidal release pattern.

FTIR Spectroscopy

2.5% w/w of film, with respect to potassium bromide (KBr) disc, was mixed with dry KBr (FTIR grade, Aldrich, Germany). The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Each disc was scanned at a resolution of 4 cm^{-1} over a wavenumber region of 450 to 4000 cm^{-1} using a FTIR spectrometer (Spectrum RX1 System, Perkin Elmer, USA). The characteristic peaks of IR transmission spectra were recorded. At least triplicates were carried out for each batch of sample and the results averaged.

Preparation of Skin

Healthy male *Sprague dawley* rats (Genetic Improvement and Farm Technologies Sdn Bhd, Malaysia), aged 3 months and weighed 200 to 250 g, were acclimatized for 7 days in individual housing under 12 h light/dark cycle with deionized water and standard pelletized food (GoldCoin Enterprise, Malaysia) given *ad libitum*. The ambient temperature was set at 25±2°C with relative humidity maintained at 55 ±5% in caging system.

The rats were sacrificed by cervical dislocation technique. Their ventral region of abdominal skin was then shaved using a sharp blade to remove hair and the full thickness skin was surgically removed. The subcutaneous fat was carefully detached from the skin. The defatted skin was subsequently cut into an appropriate size, cleansed with 0.9% w/v sodium chloride solution, wrapped in an aluminum foil and stored in a freezer at −80±1°C until use. The thickness of skin was measured at three different sample points using a caliper (Mitutoyo, Japan). Prior to use, all skin samples were thawed for 3 h at 25±2°C. All preparative processes were conducted in accordance to the university ethics regulations adapting the international guidelines (OECD Environment, Health and Safety) on the conduct of animal experimentation. The preparative process brought minimal changes to sample with reference to preliminary trials. When required, the epidermis of skin was isolated from skin sample through soaking in water at 60±2°C for 2 min, followed by gentle peeling using a pair of blunt-end forceps.

Microwave Treatment of Skin and Drug Permeation Study

A new diffusion cell was fabricated to accommodate the use of microwave probe in skin treatment and drug permeation study. It was made using borosilicate glass and was equipped with a domain for insertion of microwave probe above the donor compartment (Fig. 1). The receptor glass chamber of the diffusion cell was filled with 125 ml of air bubble-free USP phosphate buffer solution pH 7.4 (sink condition) containing 0.01% w/v sodium azide added to retard microbial growth in simulation of blood pH where drug reached after permeation. The skin (thickness=0.60±0.09 mm) was prehydrated with buffer during thawing and transferred to equilibrate with the receptor fluid for 1 h with its epidermis facing the donor compartment, followed by *in situ* microwave treatment of skin from the direction of epidermis using a contact mode vector network analyser system (R&S®ZVA, Rohde & Schwarz, Germany) where 1 mW microwave (0.14 mW/cm^2) was delivered at frequencies of 915, 2450 and 3985 MHz for 2.5 and 5 min. Immediately following microwave irradiation, a film sample was placed on the top surface of skin. The temperature of buffer solution was maintained at 37±2°C and the buffer solution was magnetically stirred at 650 rpm throughout the study. A higher stirring speed was used in case of microwave-enabled

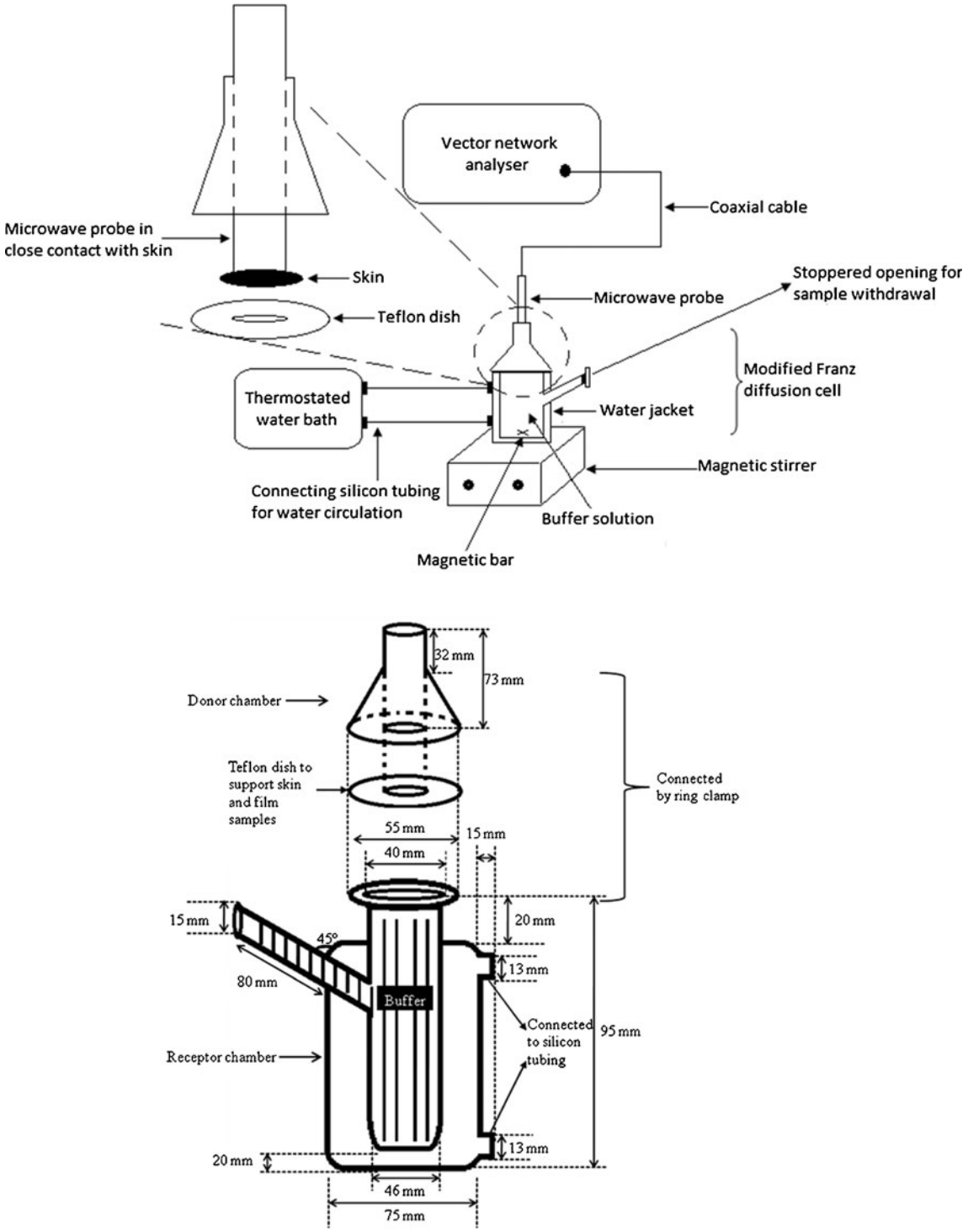

Fig. 1 Schematic diagram of microwave-enabled diffusion cell.

diffusion cell in order to ensure the buffer solution was homogeneously mixed and consistently in contact with the skin sample. Four ml aliquot was withdrawn at various time intervals for 24 h and the samples were subjected to high performance liquid chromatography (HPLC, Agilent Technologies 1200 Series, Germany) analysis for SN. Fresh buffer solution was replaced at each interval. At least triplicates were carried out for each formulation and the results averaged. The lag time, flux, permeability coefficient, enhancement ratio and extent of drug permeation were computed. When required, the isolated epidermis (thickness $=0.32 \pm 0.04$ mm) was used in replacement of skin sample as permeation barrier.

HPLC

The ODS Hypersil column (150×4.6 mm; 5 μm; Thermo Electron Corporation, USA) was used with mobile phase consisted of water (A) and acetonitrile (B) at a gradient change of 10% B to 25% B in 5 min and a flow rate of 1 ml/min using sulphamerazine as the internal standard. The column compartment temperature and UV detector wavelength were kept at 50°C and 260 nm respectively. The volume of sample injection was 5 μl. The limits of detection and quantification were 0.10 μg/ml and 0.31 μg/ml respectively. The precision and accuracy of HPLC were 1.72% and 97.06% respectively.

Physicochemical Analysis of Skin

The skin was first pre-hydrated with buffer during thawing and transferred to equilibrate with receptor fluid in the diffusion cell for 1 h, followed by *in situ* microwave treatment from the direction of epidermis using a contact mode vector network analyser system (R&S®ZVA, Rohde & Schwarz, Germany) where 1 mW microwave was delivered at frequencies of 915, 2450 and 3985 MHz for 2.5 and 5 min. Excess moisture of skin was then removed through gently dabbing over a delicate tissue paper. The skin was subjected to attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), Raman spectroscopy, differential scanning calorimetry (DSC), histology and microscopy analysis. OA, at an amount corresponding to its load in a film, was applied onto the skin after its microwave treatment, when necessary.

ATR-FTIR Spectroscopy

The characteristic peaks of infrared transmission spectra were recorded by FTIR spectrometer (Spectrum 100, Perkin Elmer, USA) using MIRacle ATR accessory (PIKE Technologies, Madison, USA). The skin sample was placed onto the surface of zinc selenide crystal using a pressure clamp to ensure close contact and highest sampling sensitivity. Both epidermis and dermis of the same skin were characterized at a resolution of 4 cm^{-1} over a wavenumber region of 675 to 4000 cm^{-1} by adhering the respective skin surface onto zinc selenide crystal. At least triplicates were carried out for each sample and the results averaged.

Raman Spectroscopy

Raman spectra were collected using a Raman spectrometer (RamanStation 400 F, Perkin Elmer, USA) equipped with a fiber optic probe which provided a focused beam of maximum 100 mW laser radiation at 785 nm. Both epidermis and dermis of the same skin were characterized by mounting the probe at the respective focal spots of skin and laser scanned over a wavenumber of 400 to 3500 cm^{-1}. At least triplicates were carried out for each sample and the results averaged.

DSC

DSC thermograms were obtained using a differential scanning calorimeter (Pyris 6 DSC, Perkin Elmer, USA). Three mg of full thickness skin were crimped in a standard aluminium pan and heated from 30 to 100°C at a heating rate of 10°C/min under constant purging of nitrogen at 40 ml/min. The characteristic peak temperature and enthalpy values of endotherm were recorded. At least triplicates were carried out for each sample and the results averaged.

Scanning Electron Microscopy

The surface structure of stratum corneum was examined using SEM technique (JSM-6701F, Jeol, Japan). Prior to the analysis, the skin was cut into 3×3 mm and immediately immersed into 0.9% w/v of sodium chloride solution. The skin was then transferred into a solution containing 1% glutaraldehyde in 0.1 M cacodylate buffer at pH 7 for 5 min, followed by washing using distilled water for 5 min before subjecting to dehydration cycle using a series of ethanol washes: 70% ethanol for 5 min, 85% ethanol for 5 min, 95% ethanol for 5 min, and 100% ethanol for 10 min. The skin was subsequently fixed in the hexamethyldisilazane solution for 10 min and air-dried at room temperature for 2 h. The fixed skin was mounted onto stud with a carbon tape and platinum-coated before viewing directly using SEM at a magnification level of 1000×. Representative sections were photographed.

Stereomicroscopy

The color changes of skin was viewed directly under a stereomicroscope (MZ75, Leica, Germany) at a magnification level of 12.5×. Representative sections were photographed.

Histology

Full thickness skin was obtained through perpendicular skin sectioning at 90° to the surfaces of epidermis by means of a cryostat (CM1850UV-1-1, Leica, Germany) at a thickness of 5 μm. It then subjected to standard staining procedure using Oil Red O Stain Kit or hematoxylin-eosin system. The stained skin was viewed under a brightfield microscope (DM2000, Leica, Germany) at a magnification level of 100×. All experiments were completed within 24 h. Representative sections were photographed.

Thermal Effect of Microwave

106 μl of USP phosphate buffer solution pH 7.4 was transferred into a glass petri dish (internal diameter=3 cm) and subjected to microwave irradiation at the frequency of 2450 MHz for 2.5 and 5 min using a vector network analyser system (R&S®ZVA, Rohde & Schwarz, Germany). The temperature changes of the buffer solution were monitored *in situ* by using a non-contact mode infrared thermometer (OPTEX Thermo-Hunter, Japan). Similar experiments were conducted on rats (n=3). Subsequently, the liver, kidney and lung of these rats as well as a fresh batch of rats which were not treated by microwave were harvested and examined by histological studies using hematoxylin-eosin staining system. Triplicates were conducted and the results averaged.

Statistical Analysis

Results were expressed as a mean of at least three experiments with the corresponding standard deviation. Statistical data analysis was carried out using SPSS software version 16.0 and a statistically significant difference was denoted by $p<0.05$. Student's t-test and analysis of variance (ANOVA)/post hoc analysis by Tukey HSD test were employed when necessary.

RESULTS AND DISCUSSION

The preparation of pectin films carrying SN and OA by solvent-evaporation technique produced flat matrices with an average drug content of 10.75±0.17%. The P, P-OA, P-SN and P-SN-OA films had thicknesses of 0.040±0.006, 0.038±0.003, 0.054±0.008 and 0.054±0.006 mm respectively. The film thickness increased upon drug loading (Student's t-test: p=0.04) and was not affected by OA inclusion (Student's t-test: p=0.98).

Drug Release and Drug Permeation Studies

Drug release of P-SN and P-SN-OA films was characterized by a burst release phase owing to rapid migration of drug located at the surfaces of matrix into receptor fluid, followed by sustained-release of drug embedded in matrix core (Fig. 2a). Both P-SN and P-SN-OA films had their drug release *via* diffusion mechanism (Table 1). The release of drug was slower in P-SN-OA film than P-SN film (ANOVA: $p<0.05$). This was possibly due to drug release retardation by hydrophobic oleic acid embedded in P-SN-OA matrix. In addition, the oleic acid interacted with pectin *via* O-H moiety as suggested by the reduction in FTIR wavenumbers at 3190.5±11.1 cm^{-1} and 3596.1±5.0 cm^{-1} of P film subsequent to the incorporation of OA (Fig. 3d–e). It was also interacted with drug in matrix *via* C=O moiety, inferring from the translation of dual FTIR peaks of P-SN film at wavenumber between 1600 and 1750 cm^{-1} into a single band at 1715.8±4.5 cm^{-1} (Fig. 3f–g). The summative influence of oleic acid retarded drug release. Nevertheless, both P-SN and P-SN-OA films had comparable drug permeation profiles across the skin (drug permeation level at 24 h: P-SN=564.40±143.10 μg; P-SN-OA=638.60±185.74 μg) (Fig. 2b). This was aptly explained by the well known permeation enhancement property of oleic acid (32). In comparison to drug release, the skin permeation levels of drug from P-SN and P-SN-OA films were considerably low. Their drug permeation extent was less than 15% of drug content in a matrix over a period of 24 h (Fig. 2b).

Microwave Treatment of Skin and Drug Permeation Study

On the note that the skin permeation extent of drug from P-SN and P-SN-OA films was low, subsequent experiments then examined the effects of microwave on skin and their influences on drug permeation profiles of P-SN and P-SN-OA films. Treatment of skin by microwave at 2450 MHz for 2.5 min increased drug permeation from P-SN film at the late permeation phase (Fig. 2b). Extending the microwave treatment duration of skin from 2.5 min to 5 min further promoted the extent of drug permeation from the same matrix (ANOVA: p=0.008). The percentage drug permeation of P-SN film at 24 h increased from 11.29±2.86% in case of using untreated skin to 22.18±1.86% when skin treated by microwave at 2450 MHz for 5 min was utilized as permeation barrier. However, microwave treatment of skin longer than 5 min *viz.* 10 min was deemed inappropriate as charring of skin was evidently shown by its color changes from whitish to brownish (Fig. 4). The skin was similarly charred when microwave of a lower frequency at 915 MHz was applied for a short duration of 5 min. This could be due to microwave was delivered at a frequency close to the resonant frequency of a biological object (900 MHz) (33), thereby generating intense vibration of skin components and heat-burn effects in a short irradiation time. Such observation was supported by a study of Engin *et al.* (1995) where radiation treatment at 915 MHz was found to induce erythema, edema and ulceration of skin readily (34). Using a higher frequency at 3985 MHz, the microwave did not annihilate the skin structure but it was neither effective to promote drug permeation of P-SN film (Fig. 2b).

Experimentally, microwave of frequency 2450 MHz was useful as drug permeation aid in transdermal delivery. With prior treatment of skin by microwave at 2450 MHz for 5 min, the drug permeation was remarkably raised by the availability of OA from P-SN-OA film (Fig. 2b). The

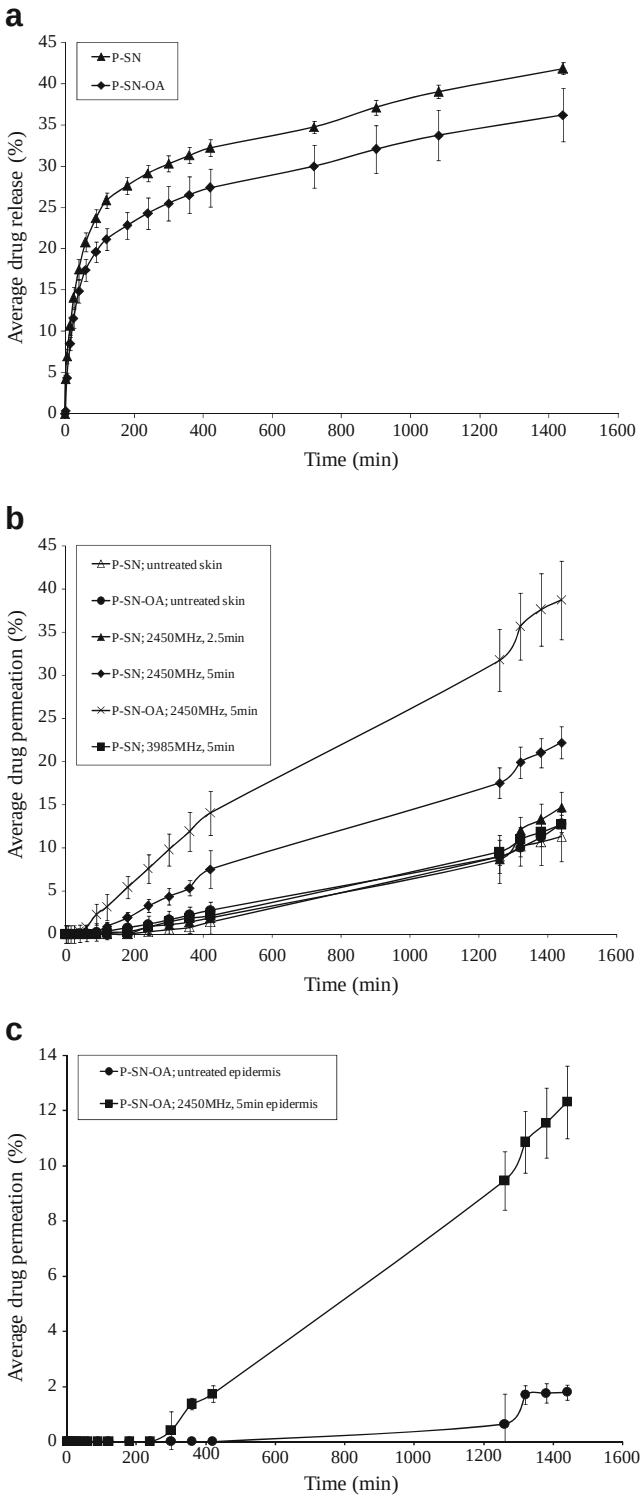

Fig. 2 Drug release profiles of (**a**) P-SN and P-SN-OA films, and drug permeation profiles of (**b**) P-SN and P-SN-OA films through skin treated by microwave under various irradiation conditions and (**c**) P-SN-OA films through epidermis treated by microwave at 2450 MHz for 5 min.

percentage drug permeation of P-SN-OA film at 24 h was 38.69±4.54% (Percentage drug released from P-SN-OA film at 24 h=36.24±3.25%) (Fig. 2a and b). Combining skin treatment by microwave and formulation of P-SN film with OA, the degree of drug permeation could meet the extent of drug release at prolonged application phase. In the absence of either OA or microwave, not all released drug was able to permeate across the skin within the given duration. Microwave and oleic acid provided a synergistic influence in enabling drug permeation through skin. This was supported by the corresponding reduction in lag time, and increase in flux, permeability coefficient, enhancement ratio and permeation extent of drug at 24 h (Table 1). The level of drug permeation was remarkably greater than that of drug alone which exhibited a permeability coefficient value at $4.86\times10^{-7}\pm3.21\times10^{-7}$ cm h^{-1}.

Mechanisms of Drug Permeation

The skin samples employed in the present study constituted of epidermis and dermis (Fig. 5a). The stratum corneum of epidermis has a heterogeneous structure which is made of dead keratin-rich corneocytes embedded in a lamellar lipid matrix (32,35). The extracellular lipids of corneocytes are arranged in multiple lamellar structures as one of its components, ceramide, can provide polar moieties required for such arrangement and are capable of forming extensive hydrogen bonding essential for structural stabilization (36). Both keratin-rich corneocytes and lipid matrix are responsible for the impermeable attribute of skin (37). In our preliminary trials, the diffusion of model drug SN was however impeded by both epidermis and dermis. On the note that microwave is a radiation with relatively long wavelengths, drug permeation mechanism was thus established with reference to the effects of microwave on both epidermis and dermis, and in relation to the collective consequence on transdermal drug diffusion.

Epidermis

Microwave is known to preferentially interact with polar materials (1). The treatment of skin by microwave had shown to reduce its strength of hydrogen bonding possibly through interaction of microwave with keratin and/or polar moieties of ceramide and other lipid materials in stratum corneum. This was indicated by FTIR findings of untreated skin where its wavenumber corresponding to O-H and/or N-H at 3347.07±5.69 cm^{-1} increased in skin samples treated by microwave (Fig. 6Aa–d). Analysis of Raman spectra, on the other hand, showed that the peak in association with C-H moiety of skin at 2913.22±6.82 cm^{-1} subsided upon microwave irradiation of the tissue (Fig. 7Aa–d). The polar fractions of lipid had been converted from ordered to disordered state. This fluidized the alkyl chain packing and created spaces within the lipid bilayer thereby promoting drug permeation.

Table I Derivative Data from Drug Permeation Study of P-SN and P-SN-OA Films Using Untreated Skin or Skin Treated by Microwave as the Permeation Barrier

Film type	Microwave condition	Lag time (min)	Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Permeability coefficient ($\times 10^{-5}$ cm h^{-1})	Enhancement ratio	Extent of drug permeation at 24 h (%)
Full thickness skin						
P-SN	Untreated	299.9 ± 34.8	3.62 ± 1.23	2.79 ± 0.13	-	11.29 ± 2.86
P-SN	2450 MHz 2.5 min	223.8 ± 0.90	4.57 ± 0.72	3.49 ± 0.55	1.25	14.68 ± 1.82
P-SN	2450 MHz 5 min	37.6 ± 43.2	6.50 ± 0.59	4.97 ± 0.45	1.78	22.18 ± 1.86
P-SN	3985 MHz 5 min	166.5 ± 33.0	3.98 ± 1.21	3.04 ± 0.92	1.09	12.69 ± 3.63
P-SN-OA	Untreated	96.4 ± 30.4	3.92 ± 1.07	3.00 ± 0.81	1.07	12.77 ± 3.71
P-SN-OA	2450 MHz 5 min	2.07 ± 3.6	10.73 ± 1.10	8.19 ± 0.84	2.94	38.69 ± 4.54
Epidermis						
P-SN-OA	Untreated	507.8 ± 50.6	0.09 ± 0.06	0.07 ± 0.04	-	1.79 ± 0.27
P-SN-OA	2450 MHz 5 min	201.7 ± 53.4	4.74 ± 0.56	3.62 ± 0.43	51.7	12.33 ± 1.32

Drug content=0.71 mg/cm^2; oleic acid content=0.53 mg/cm^2; surface area of release and permeation=7.07 cm^2

Extent of drug release at 24 h - P-SN film: 2.094 ± 0.037 mg; P-SN-OA film: 1.812 ± 0.162 mg

Mechanism of drug release - P-SN film: n=0.332 ± 0.022, r=0.975 ± 0.003; P-SN-OA film: n=0.524 ± 0.060, r=0.876 ± 0.030

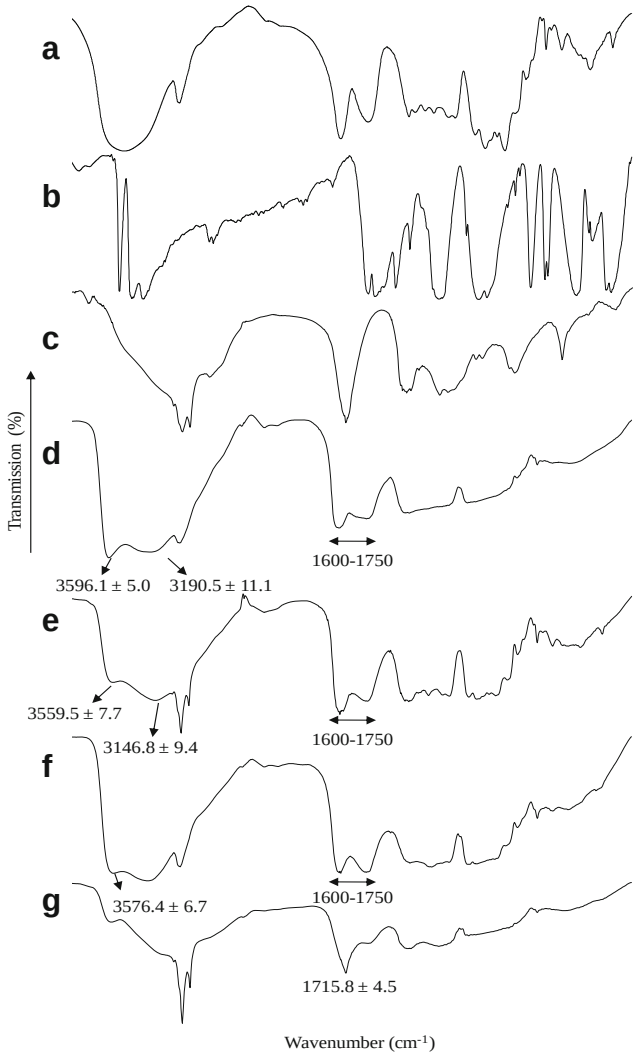

Fig. 3 FTIR spectra of (**a**) P, (**b**) SN, (**c**) OA, (**d**) P film, (**e**) P-OA film, (**f**) P-SN film and (**g**) P-SN-OA film.

Oleic acid is an unsaturated fatty acid with a *cis* double bond at C9 induces a kink in its hydrocarbon chain (32,35,37). Owing to the bent *cis* configuration, oleic acid has been reported to be able to disturb the highly ordered intercellular lipid packing of predominantly hydrophobic saturated chains and increase the fluidity of lipid membrane in skin tissue for drug permeation (32,35,37,38). In the present investigation, the introduction of oleic acid onto the epidermis of skin was found to induce changes in both hydrophobic as well as hydrophilic regimes of lipid and/or keratin domains. FTIR study indicated that the wavenumber of peaks at 3347.07 ± 5.69 cm^{-1} and 1548.73 ± 0.60 cm^{-1} ascribing O-H and N-H/C-N amide II respectively of lipid and/or keratin increased with skin treated by oleic acid (Fig. 6Aa and e). Similarly, Raman analysis was characterized by diminishing peaks related to C-H, C=O and C-O moieties of lipid and/or protein of untreated skin at wavenumbers of 2913.22 ± 6.82 cm^{-1} and between 1300 and 1900 cm^{-1} (Fig. 7Aa and e). The oleic acid could have exerted its fluidizing action on polar head groups of ceramide as a result of spacing of alkyl chain organization (36). Using oleic acid, a greater extent of skin permeability was attainable following fluidization of both polar and non-polar regimes. As a result, the extent of drug permeation in the present investigation was comparable in cases of P-SN and P-SN-OA films though the drug release propensity of P-SN-OA was significantly lower than P-SN matrix (Fig. 2a and b).

The permeability of skin barrier and the transdermal permeation propensity of drug can be further promoted *via* treating the skin by both microwave and oleic acid (Figs. 2b, 6Aa, e and f, 7Aa, e and f). The treatment of skin by microwave and oleic acid fluidized the intercellular lipid of epidermis. In combination with microwave, the late addition of oleic acid might extract a fraction of endogenous lipid and form separate phases within the intercellular lipid

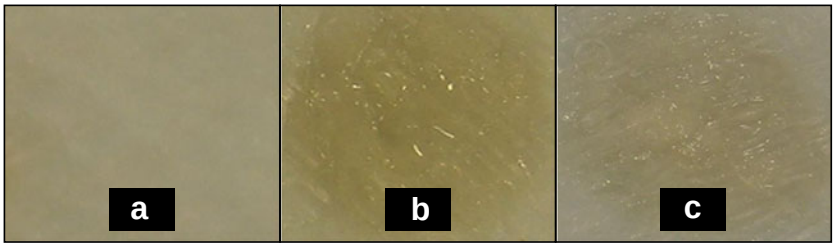

Fig. 4 Microscopic pictographs of (**a**) untreated skin, skin treated by microwave at (**b**) 915 MHz for 5 min and (**c**) 2450 MHz for 10 min.

lamellae (37–40). This resulted in permeability defects and a further rise in interfacial area between phases which translated to a remarkable delivery of hydrophilic SN transdermally.

Dermis

As a long wavelength radiation, microwave was deemed to be able to reach skin tissue beyond epidermis. The dermis of skin underwent similar physicochemical changes as epidermis under the influences of microwave with and without the use of oleic acid (Figs. 6 and 7). The dermis of untreated skin was constituted of *trans* lipid which was characterized by a Raman wavenumber of 2897.46 cm^{-1} instead of 2913.22 cm^{-1} in epidermis where the majority of *gauche* lipid was available (Fig. 7Aa and Ba). Unlike *gauche* lipid, the spectrum feature of *trans* lipid was not detectable by means of FTIR technique (Fig. 6Aa and Ba) for this is a less

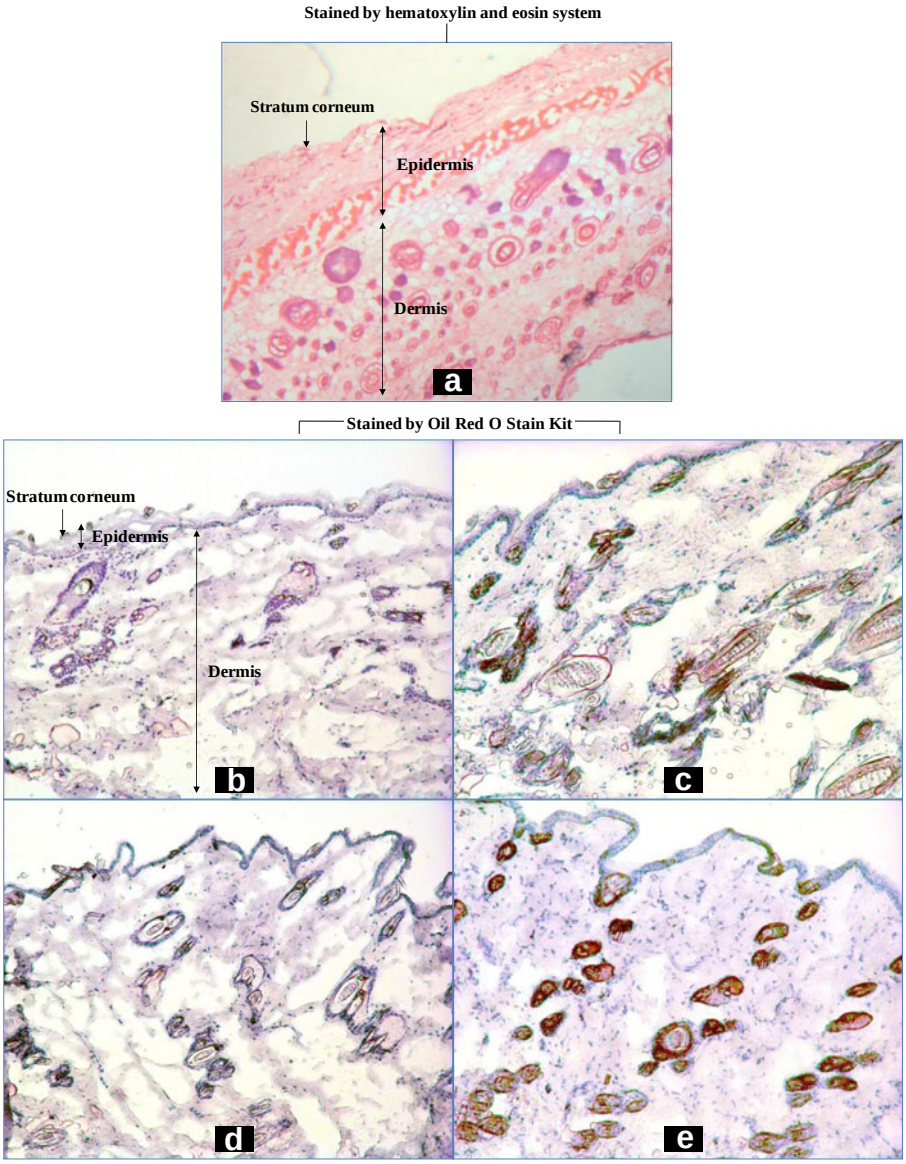

Fig. 5 Cross-sectional histology of (**a**) untreated skin stained by hematoxylin and eosin system, (**b**) untreated skin, (**c**) skin treated by microwave at 2450 MHz for 5 min, (**d**) skin treated by OA and (**e**) skin treated by microwave at 2450 MHz for 5 min followed by OA, stained by Oil Red O Stain Kit.

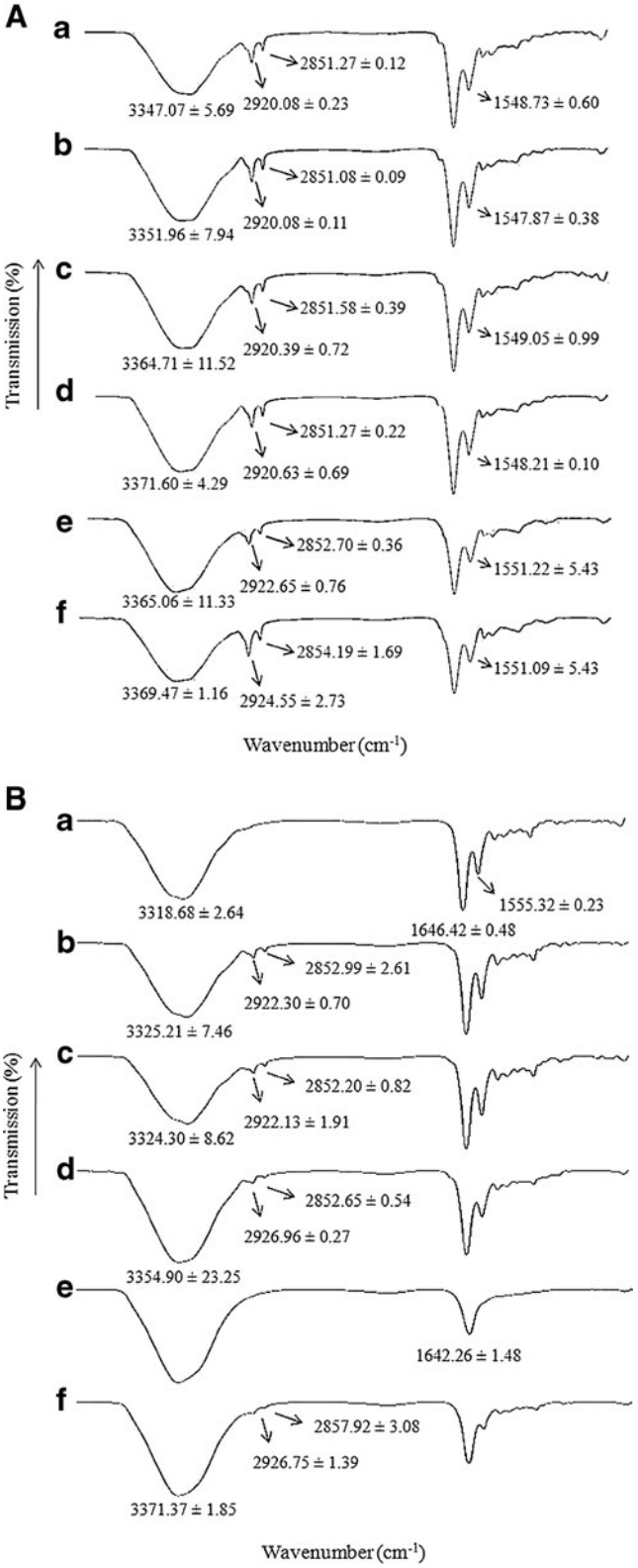

Fig. 6 ATR-FTIR spectra of (**A**) Epidermis: (**a**) untreated skin, skin treated by microwave at (**b**) 2450 MHz for 2.5 min, (**c**) 2450 MHz for 5 min and (**d**) 3985 MHz for 5 min, (**e**) skin treated by OA and (**f**) skin treated by microwave at 2450 MHz for 5 min followed by OA. (**B**) Dermis: (**a**) untreated skin, skin treated by microwave at (**b**) 2450 MHz for 2.5 min, (**c**) 2450 MHz for 5 min and (**d**) 3985 MHz for 5 min, (**e**) skin treated by OA and (**f**) skin treated by microwave at 2450 MHz for 5 min followed by OA.

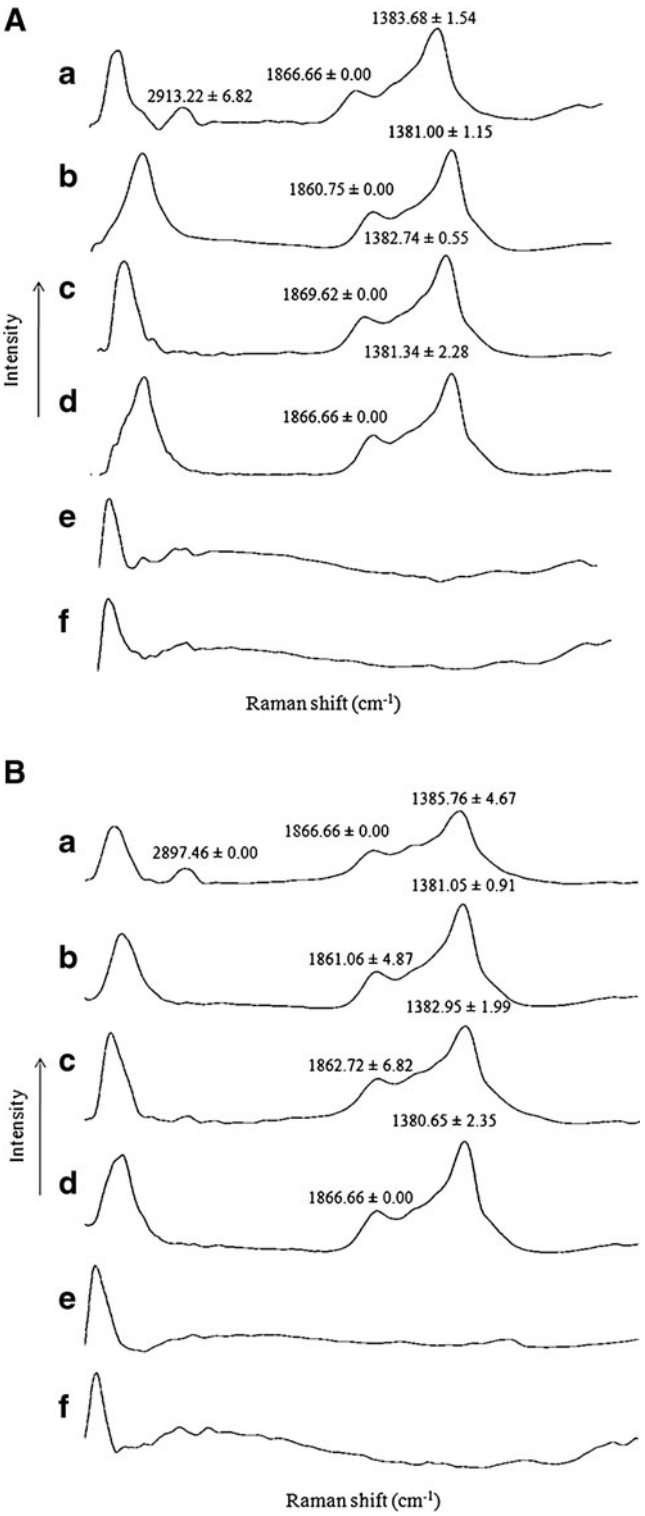

Fig. 7 Raman spectra of (**A**) Epidermis: (**a**) untreated skin, skin treated by microwave at (**b**) 2450 MHz for 2.5 min, (**c**) 2450 MHz for 5 min and (**d**) 3985 MHz for 5 min, (**e**) skin treated by OA and (**f**) skin treated by microwave at 2450 MHz for 5 min followed by OA. (**B**) Dermis: (**a**) untreated skin, skin treated by microwave at (**b**) 2450 MHz for 2.5 min, (**c**) 2450 MHz for 5 min and (**d**) 3985 MHz for 5 min, (**e**) skin treated by OA and (**f**) skin treated by microwave at 2450 MHz for 5 min followed by OA.

sensitive mode to elucidate non-polar bonds in comparison to Raman spectroscopy (41).

With reference to findings of epidermis, the lipid fluidity at dermis was probably also promoted by microwave through transforming the *trans* lipid to *gauche* conformers as suggested by the emergence of peaks between 2800 and 2900 cm^{-1} in FTIR spectra (Fig. 6Ba–d). It was increased by oleic acid through forming membrane with fluidized lateral chain packing (42), as denoted by a translation of dual FTIR peaks of untreated skin at 1555.32±0.23 cm^{-1} and 1646.42±0.48 cm^{-1} into a single peak at 1642.26± 1.48 cm^{-1} (Fig. 6Ba and e). The migration of oleic acid from surfaces of skin into dermis was not at all impeded as shown by FTIR and Raman studies (Figs. 6 and 7). Given that the skin was treated by microwave at 2450 MHz for 5 min, a higher penetration propensity of oleic acid into skin was found without incurring excessive damage onto the epidermis of skin (Fig. 5b–e). A higher concentration of oleic acid was identified at the immediate surrounding of sebaceous glands in skin treated by microwave, possibly due to its affinity towards the hydrophobic sebum (red-stained spots). The treatment of skin by microwave promoted oleic acid permeation. With the availability of a high fraction of oleic acid in core tissue of skin, microwave-enhanced transdermal delivery of hydrophilic SN can thus be further facilitated.

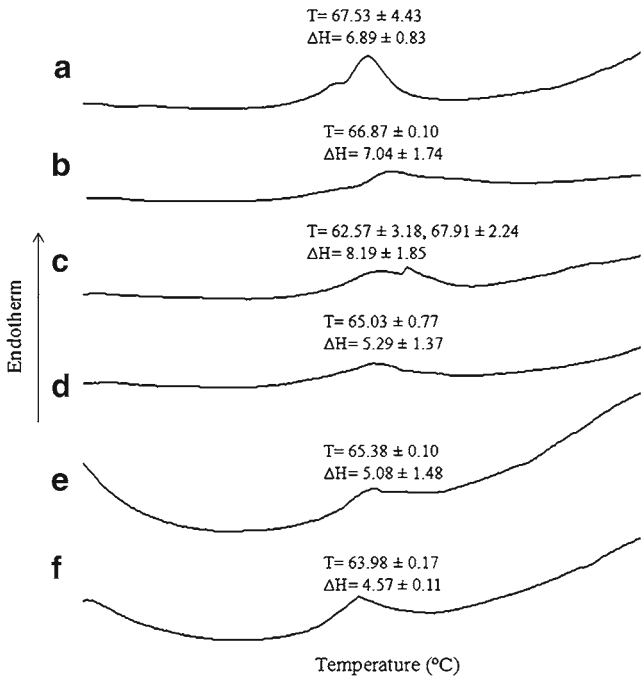

Fig. 8 DSC thermograms of (**a**) untreated skin, skin treated by microwave at (**b**) 2450 MHz for 2.5 min, (**c**) 2450 MHz for 5 min and (**d**) 3985 MHz for 5 min, (**e**) skin treated by OA and (**f**) skin treated by microwave at 2450 MHz for 5 min followed by OA.

Both microwave and oleic acid had their ability to improve skin permeability and drug diffusion indicated. Using microwave at 3985 MHz, the level of drug permeation was nevertheless low despite the skin was markedly fluidized as suggested by FTIR and Raman studies (Figs. 2b, 6 and 7). Examination of the entire skin samples by DSC analysis indicated that a marked reduction in melting peak temperature of endotherm at 67.53±4.43°C of the untreated skin took place only after subjecting it to microwave treatment at 2450 MHz for a period of 5 min (Fig. 8). The treatment of skin by microwave at 2450 MHz for 5 min enabled a redistribution of skin surface components in a different mode which was unattainable when microwave was operated at a higher frequency of 3985 MHz (Fig. 9). The microwave irradiating at 2450 MHz could exert spacing of lipid architecture of the skin in a random manner with no build-up of specific structured domains as suggested by the conversion of skin surfaces into a smooth texture. It could probably aid to reduce the barrier of drug transport which formed between the structured domains.

The skin lipid fluidization ability of microwave at 2450 MHz was not possibly ascribed to its thermal effect mediating *via* water molecules or on skin of rats. Examination of temperature of buffer solution and rat skin irradiating with microwave indicated that there was a marginal rise in temperature of liquid and rat skin samples treated by microwave at 2450 MHz for 5 min (buffer solution - untreated: 22.50± 0.55°C; 2450 MHz 5 min: 26.20±1.17°C; rat skin - untreated: 30.30±0.50°C; 2450 MHz 5 min: 31.30±0.50°C) (Student's *t*-test: $p>0.30$). Further treatment of rat skin for an additional period of 5 min only resulted in a skin temperature of 31.80±0.40°C. Indeed, a high skin temperature rise by 7° C in sonophoresis has not been found to govern transdermal drug permeation (43). The microwave-induced transdermal drug delivery can be non-thermal in origin (30).

Future Perspectives

Microwave represents a recent approach in the arena of transdermal drug delivery, in comparison to electrical methods such as iontophoresis and electroporation which have undergone development for the past few decades and 17 years ago respectively (44–49). Similar to electrical methods, safety and clinical studies are imperative in order to translate the use of microwave into real practice. An early attempt in our laboratory indicated that *in vivo* exposure of skin to microwave did not lead to changes in histological profiles of internal organs of rats such as liver, kidney and lung (Fig. 10). It was possible that the penetration capacity of microwave into internal organs was negligible under the present experimental conditions or a long-term evaluation was required. Skin irritation or burn, hair damage and discomfort sensation are some drawbacks of electrical

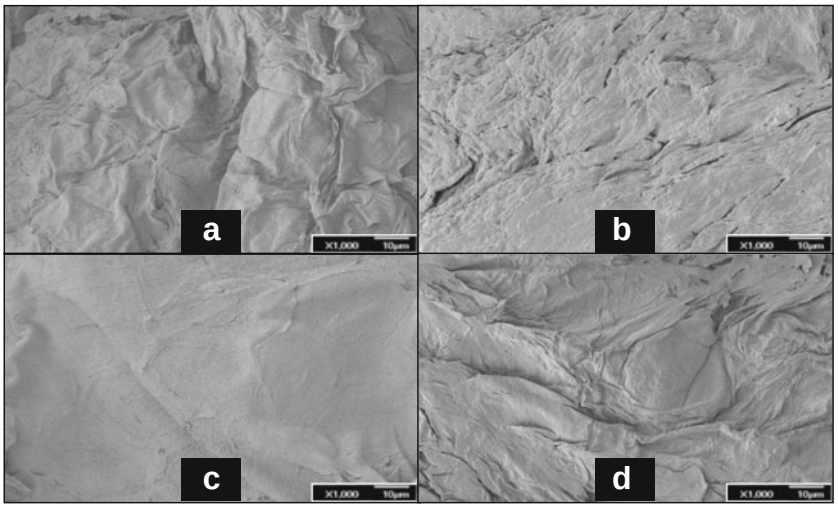

Fig. 9 SEM micrographs on surface morphology of epidermis: (**a**) untreated skin, skin treated by microwave at (**b**) 2450 MHz for 2.5 min, (**c**) 2450 MHz for 5 min and (**d**) 3985 MHz for 5 min.

methods which need rectification today (46,48,49). Microwave, as a relatively new technique, is too expected to be tested for the same adverse effects and the reversibility of these biomedical consequences.

Combination techniques have long been advocated in transdermal drug delivery. In the present investigation, the concurrent use of microwave and chemical penetration enhancer promotes transdermal drug delivery to a remarkable extent. In a study using epidermis as permeation barrier, a large enhancement in drug permeation was noted (Fig. 2b and c; Table 1) though direct comparison of permeation data between that of full thickness skin and epidermis could not be made due to differences in skin preparative condition and thickness of skin undergoing microwave treatment. The microwave may be combined with electrical methods in future applications as sole iontophoresis only enhances drug permeation largely *via* transappendageal pathway instead of lipid domain disorganization, and effectiveness of magnetophoresis is limited by maximum rotational energy that can be transferred to the cell membrane (44,47,48). The electrical methods transport drugs across the skin interface at μg levels (45,50–54). The present microwave set-up aids transdermal drug permeation at mg scale. Its joint application with electrical methods is envisaged to open a new door in skin drug delivery.

CONCLUSION

Skin treatment by microwave at 2450 MHz for 5 min promoted transdermal drug permeation from OA-free film without incurring skin damage when compared to microwave at a lower or higher frequency, for a shorter or longer duration. Treatment of skin by microwave followed by drug loaded film carrying OA further increased drug permeation propensity. Microwave exerted disorder of lipid framework

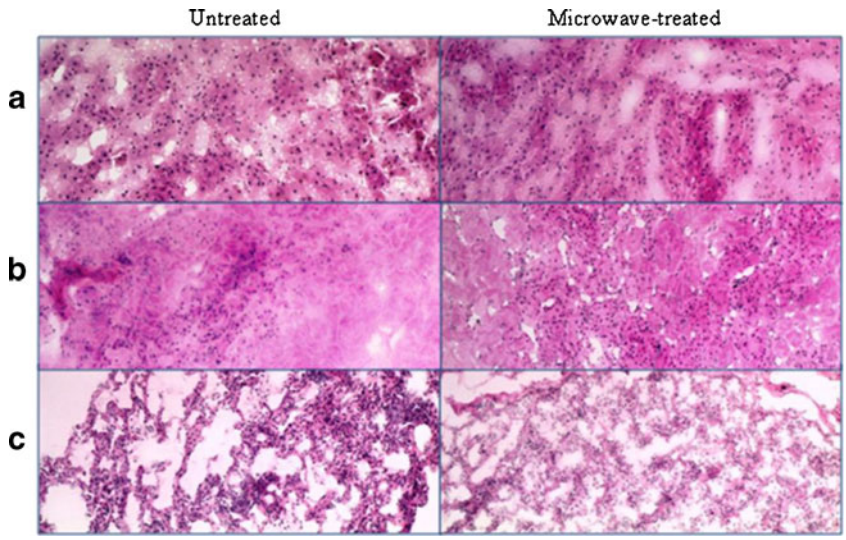

Fig. 10 Cross-sectional histology of (**a**) liver, (**b**) kidney and (**c**) lung of rats treated by microwave *in vivo* at 2450 MHz for 5 min.

of stratum corneum into structureless domains. It resulted in a higher cumulative OA concentration in skin and synergistically induced lipid/keratin fluidization at both stratum corneum and dermis thereby promoting drug permeation.

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