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MICROWAVE: EFFECTS AND IMPLICATIONS IN TRANSDERMAL DRUG DELIVERY

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Abstract—This study investigated transdermal drug delivery mechanisms of pectin and pectin-oleic acid (OA) gels and their effects on skin barrier treated by microwave. Hydrophilic pectin-sulphanilamide gels, with or without OA penetration enhancer, 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. Unlike solid film, skin treatment by microwave at 2450 MHz demoted drug permeation especially from OA-rich pectin gel. The pectin-skin binding was facilitated by gel with freely soluble pectin molecules instead of solid film with entangled chains. It was promoted when microwave fluidized stratum corneum into structureless domains, or OA extracted endogenous lipid fraction and formed separate phases within intercellular lipid lamellae. This led to a remarkable decrease in transdermal drug permeation. Microwave-enhanced transdermal delivery must not be implemented with pectin gel. In skin treated by microwave, the penetration enhancer in gel can act as a permeation retardant.

1. INTRODUCTION

Transdermal drug delivery provides a controlled continuous delivery of drug molecules from the surface of skin, through its layers, and into the systemic circulation [1, 2]. It avoids enzymatic activity in gastrointestinal tract and hepatic portal system which can result in excessive drug degradation and loss of therapeutic

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effectiveness [3, 4]. Nevertheless, the skin permeation propensity of most drugs is practically low due to the impermeable nature of epidermis. Various approaches have been devised to improve the skin permeability to drugs. The current advances include microneedles [5–8], iontophoresis (electric current) [9, 10], ultrasound [11–13], photomechanical waves [14], electroporation (transient high-voltage electrical pulses) [15, 16] and pressurized air techniques [17, 18].

Microwave is an electromagnetic wave which has frequencies between 300 MHz and 300 GHz [19]. It receives a widespread application in food, microbiological, biomedical, analytical and pharmaceutical fields with respect to drying [20], sterilization [21, 22], hyperthermia treatment of bladder cell carcinoma, rectal cancer and benign prostatic hyperplasia [23–26], diathermia treatment for pain relief, tendon extensibility improvement, muscle and joint stiffness reduction [27], wound healing [28], endometrial ablation [29], breast tumor detection [30], nanostructure imaging [31], molecular profiling [32, 33] and controlled release pharmaceutical dosage form design [4, 34–40].

Recently, microwave finds its new application in transdermal drug delivery [41, 42]. The microwave is able to enhance drug permeation transdermally in a non-thermal mode with no significant temperature rise on skin. At 2450 MHz, the treatment of skin by microwave for 5 min at the irradiation intensity of $0.14\,\mathrm{mW/cm^2}$ is found to promote transdermal permeation of polar sulphanilamide from solid pectin film by an enhancement ratio of 1.25 to 2.94 [42]. The drug permeation is promoted by microwave through exerting spacing of lipid architecture of stratum corneum into structureless domains without incurring skin damage, and allowing permeation enhancer such as oleic acid to permeate stratum corneum and accumulate in dermis to synergistically induce skin lipid/protein fluidization for ease of drug diffusion.

Skin treatment by microwave followed by solid pectin film loaded with sulphanilamide and oleic acid result in permeation of all drug molecules that are released from film [42]. In the absence of either oleic acid or microwave, not all released drug is able to permeate across the skin within the given duration. The solid pectin film releases only $36.24 \pm 3.25\%$ of sulphanilamide in 24 h due to slow polymer hydration and viscous polymeric network is formed to retard the drug release. This restricts the amount of drug in film which can be permeated through skin. It is envisaged that the formulation of sulphanilamide in the form of pectin gel can provide a higher extent of drug release than that of solid pectin film. The drug release will not be a limiting factor of drug permeation. Concomitant application of microwave and oleic acid with pectin gel as drug carrier is hypothesized to be able

to further increase the degree of transdermal drug permeation. As such, this study aims to investigate transdermal drug delivery profiles of pectin and pectin-oleic acid gels and their effects on skin barrier treated by microwave. The influences of microwave and oleic acid on drug permeation property of pectin gel are elucidated with mechanisms of transdermal drug delivery evaluated against that of solid pectin film.

2. MATERIALS AND METHODS

2.1. Materials

Pectin (P, methoxy content = 9.0%, galacturonic acid content = 87.6%, molecular weight = 2.04×10^6 Da, Sigma, USA) was used as matrix polymer of gel and film. Sulphanilamide (SN, Sigma, USA) was selected as a model water-soluble hydrophilic drug with oleic acid (OA, Fluka, Germany) as permeation enhancer. Pectin was selected for it possessed mucosa penetration and film forming properties [4]. Sulphanilamide was used as hydrophilic drug molecules were known to have a low skin permeation capacity [17]. Disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, sodium chloride, hydrochloric acid (Merck, Germany), as well as, sodium azide (Ajax Finechem, Australia) were used to prepare United States Pharmacopoeia (USP) phosphate buffer solutions. Oil Red O Stain Kit (ScyTek Laboratories, USA), Harris haematoxylin (VWR International, UK) and eosin (Microm International, Germany) were used in histology examination.

2.2. Gel and Film Preparation

Five %w/w pectin gel, with or without SN and OA, was prepared by dissolving an accurately weighed amount of pectin in deionised water through continuous stirring at 25 ± 1°C for 15 h. The gel was standardized to contain 37.5 mg pectin with 5 mg SN and 3.75 mg OA in 0.75 g drug- and OA-loaded gels respectively. At least triplicates were carried out for each formulation.

The pectin film was similarly prepared with an accurately weighed amount of 2.5 %w/w aqueous pectin solution, with or without SN and OA, being transferred into a teflon dish (internal diameter = 3 cm). 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). The film was standardized to contain

the same amount of pectin, SN and OA as 0.75 g gel. At least triplicates were carried out for each formulation.

2.3. Drug Content

An accurately weighed gel or film was dissolved in USP phosphate buffer solution pH 7.4 and analysed for SN using ultra-violet (UV) spectrophotometry technique at the wavelength 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 sample was used as a control. The drug content was defined as the percentage of drug embedded in a unit weight of gel or film. Five replicates were carried out and the results averaged.

2.4. Drug Release Study

The cumulative drug release profiles of gel and film were examined using the vertical diffusion cells with a programmable MicroettePlus Q-Pak^{TM} 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 gel or 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 in simulation of skin temperature 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 gel or film was used as a control. The percentage of drug release was calculated with respect to the drug content of gel or film. The drug content was expressed as the percentage of drug encapsulated in a unit weight of gel or film. Triplicates were carried out for each formulation and the results averaged.

The mechanism of drug release was investigated by fitting the drug release data into Weibull function as expressed by

$$F = 1 - \exp\left(-at^b\right) \tag{1}$$

where F is the drug fraction released at time t, and a and b are constants [43]. b, as a shape parameter, is characterized as exponential ($b = 1$), sigmoidal ($b > 1$) or parabolic ($b < 1$).

2.5. 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 deionised water and standard pelletized food (GoldCoin Enterprise, Malaysia) given *ad libitum*. The ambient temperature was set at $25 \pm 2^{\circ}$C with relative humidity maintained at $55 \pm 5\%$ 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 $-80 \pm 1^{\circ}$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 $25 \pm 2^{\circ}$C. All preparative processes (animal living condition, animal sacrifice and skin isolation) were approved and conducted in accordance to the institutional 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. Full thickness skin was used in experiments as drug permeation enhancement property of microwave mediated through its interaction with epidermis and dermis in a collective manner [42]. Comparative outcomes were obtained from studies using the rat skin.

2.6. 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 [42]. It was made using borosilicate glass and was equipped with a domain for insertion of microwave probe above the donor compartment. 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. USP phosphate buffer solution was used in simulation of blood where drug reached after permeation. The skin (thickness $= 0.60 \pm 0.09$ mm) was pre-hydrated 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 analyzer system (R & S®ZVA, Rohde & Schwarz, Germany), where 1 mW microwave (0.14 mW/cm^2) was delivered at frequency of 2450 MHz for 5 min. Immediately following microwave irradiation, a gel or film sample was placed on the top surface of skin. The temperature of buffer solution was maintained at 37±2°C in simulation of blood temperature and the buffer solution was magnetically stirred at 650 rpm throughout the study. A higher stirring speed was used in case of microwave-enabled diffusion cell in order to ensure the buffer solution was homogeneously mixed and consistently in contact with the test 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.

2.7. 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.

2.8. 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 analyzer system (R & S®ZVA, Rohde & Schwarz, Germany), where 1 mW microwave was delivered at the frequency of 2450 MHz for 5 min. Excess moisture of skin was then removed through gentle 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 scanning electron microscopy (SEM) analysis. Film or gel was applied onto the skin after its microwave treatment, when necessary. The physicochemical changes of this skin were examined after 4 h of

standing with buffer solution maintained at $37 \pm 2°C$ and magnetically stirred at 650 rpm, where the time interval corresponded to phases of changes in drug permeation as a function of gel/film formulation.

2.9. 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) (Fig. 1). 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 $4000\,cm^{-1}$ with an acquisition time of 1.5 min by adhering the respective skin surface onto zinc selenide crystal. At least triplicates were carried out for each sample and the results averaged.

2.10. Raman Spectroscopy

Raman spectra were collected using a Raman spectrometer (RamanStation 400F, Perkin Elmer, USA) equipped with a fiber optic probe which provided a focused beam of maximum 100 mW laser radiation at 785 nm (Fig. 1). 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 $3500\,cm^{-1}$ with an acquisition time of 5 s. At least triplicates were carried out for each sample and the results averaged.

2.11. DSC

DSC thermograms were obtained using a differential scanning calorimeter (Pyris 6 DSC, Perkin Elmer, USA) (Fig. 1). 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.

2.12. SEM

The surface structure of stratum corneum was examined using SEM technique (Quanta 450 FEG ESEM, FEI, Holland). Prior to the analysis, the skin was cut into 3×3 mm and mounted onto stud with

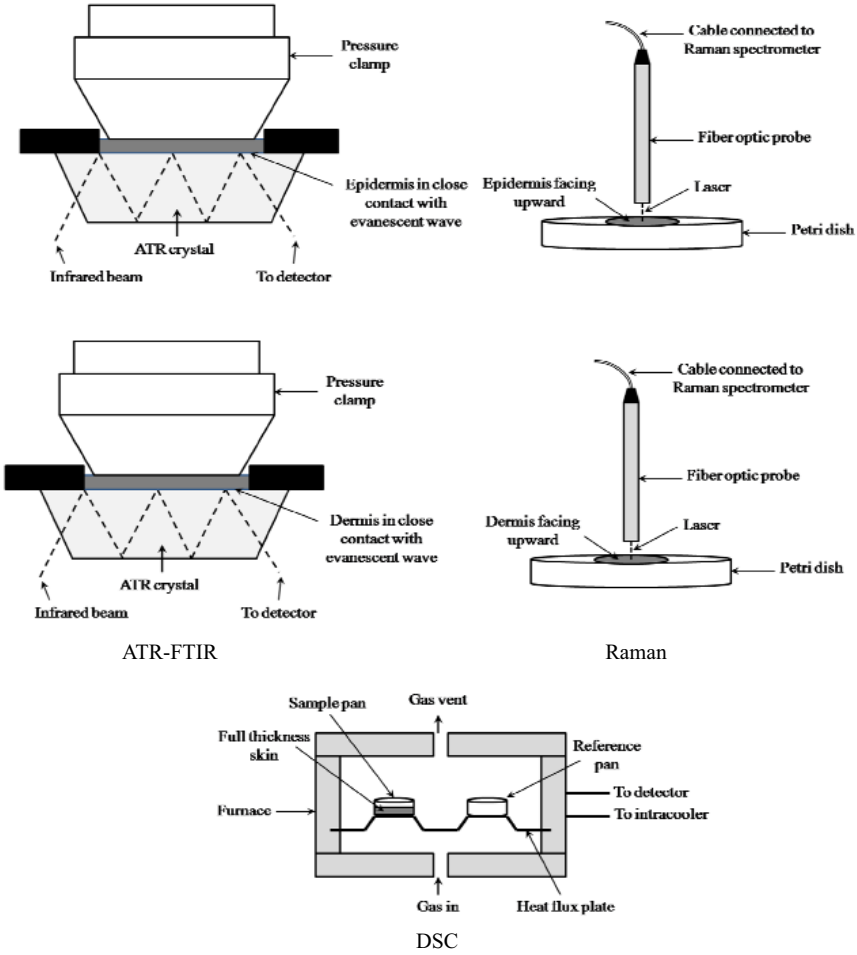


Figure 1. Schematic diagrams of ATR-FTIR spectroscopy, Raman spectroscopy and DSC experimental set up.

a carbon tape before viewing directly using SEM at a magnification level of 1000×. Representative sections were photographed.

2.13. 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.

2.14. 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.

3. RESULTS AND DISCUSSION

3.1. Drug Release Study

Liquid gel and solid film of the same pectin, drug and oleic acid contents were prepared. Drug release of gel and film 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. 2). The drug release propensity was higher from gel than film as solid film needed to undergo hydration to disentangle the network of polymeric matrix prior releasing the drug embedded in core. Both P-SN and P-SN-OA gels exhibited a comparable drug release profile (ANOVA: $p = 0.481$) with near complete release of all drug content at 24 h of dissolution. The release of drug was slower in P-SN-OA film than P-SN film (ANOVA: $p < 0.05$).

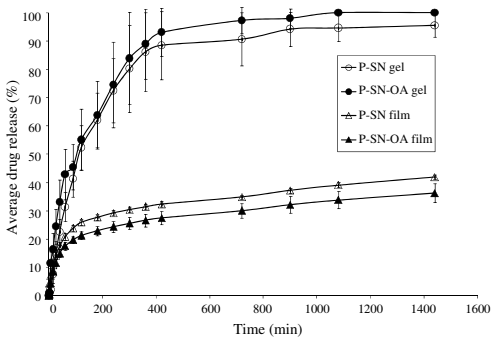


Figure 2. Drug release profiles of P-SN-OA gels and films.

This was due to interaction of oleic acid with pectin and drug via O-H and C=O moieties thereby hindering drug release [42]. The drug release kinetics of gel followed a combination mechanism constituting of diffusion and matrix erosion (P-SN gel: $b = 0.916$, $r^2 = 0.959$; P-SN-OA gel: $b = 0.745$, $r^2 = 0.983$), whereas drug in film released primarily via diffusion through a more ordered matrix structure (P-SN film: $b = 0.341$, $r^2 = 0.958$; P-SN-OA film: $b = 0.370$, $r^2 = 0.936$).

3.2. Microwave Treatment of Skin and Drug Permeation Study

A higher drug release propensity of gel than film was translated to a correspondingly higher drug permeation propensity of gel (drug permeation level at 24 h: P-SN gel = 3148.69 ± 83.22 μg; P-SN-OA gel = 1077.67 ± 137.24 μg) than film (drug permeation level at 24 h: P-SN film = 564.40 ± 143.10 μg; P-SN-OA film = 638.60 ± 185.74 μg) (ANOVA: $p = 0.000$) (Fig. 3(a)). The drug permeation propensity of P-SN gel was higher than that of pectin-free drug solution (drug permeation level at 24 h: SN solution = 672.71 ± 377.61 μg). This was possibly due to mucosal adhesive and penetration attribute of pectin which facilitated drug transport across the skin [42]. Nevertheless, not all drug released from this gel permeated the skin barrier. More than 30% of released drug were not able to diffuse through the skin and permeated into receptor fluid (Figs. 2 and 3(a)). The drug permeation propensity of P-SN film was found to be comparable to that of solution (Fig. 3(a)). Given that an excess amount of drug had released at the interface between film and skin as inferred from drug release study, the findings implied that freely soluble pectin molecules were required to promote transdermal drug permeation. The pectin in film was not readily available to promote transdermal drug permeation.

The microwave and oleic acid were reported to have a synergistic effect in promotion of transdermal drug delivery from solid pectin film [42] (Fig. 3(b)). The skin treated by microwave or oleic acid brought about a remarkable rise in permeation propensity of drug dissolved in an aqueous solution, beyond that deliverable by P-SN gel (Fig. 3(c)). Unexpectedly, the transdermal permeation propensity of drug released from gel was negated when the skin was first treated by microwave and/or oleic acid-containing gel was applied onto the skin (Fig. 3(d)). The percent drug permeation of P-SN gel was significantly decreased from $62.97 \pm 1.66\%$ to $21.55 \pm 2.74\%$ (ANOVA: $p = 0.000$) when oleic acid was added into gel. A combination of microwave and oleic acid however did not further reduce the permeation level of drug. Instead, the microwave appeared to be able to antagonize the anti-permeation characteristics of oleic acid on drug in gel.

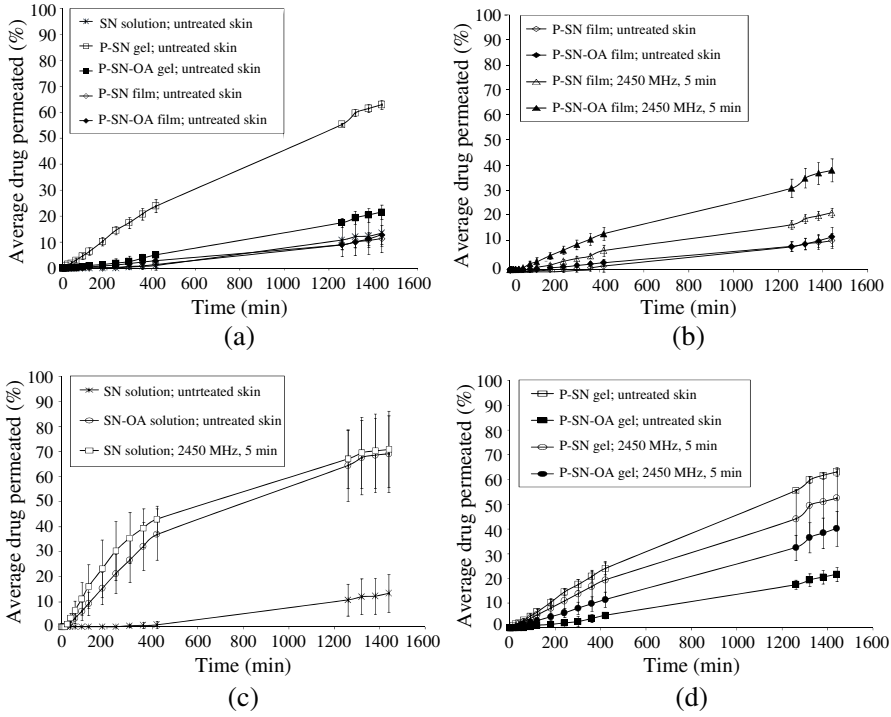


Figure 3. Drug permeation profiles of P-SN and P-SN-OA gels and films, as well as, SN and SN-OA solutions through untreated skin or skin treated by microwave at 2450 MHz for 5 min.

3.3. Mechanisms of Drug Permeation

The skin employed in the drug permeation study constituted of both epidermis and dermis (Fig. 4(a)). Stratum corneum, which is the outer layer of epidermis, has a heterogeneous structure made of dead keratin-rich corneocytes embedded in a ceramide-rich lamellar lipid matrix that is responsible for the impermeable characteristics of skin [42]. It represents the first skin site of contact with microwave and oleic acid. The physicochemical properties of both epidermis and dermis were found to vary with the application of microwave and oleic acid on skin [42]. The drug permeation profiles of drug were a collective response of these changes in skin.

Both microwave and oleic acid increased transdermal permeation of drug released from solid film through inducing lipid/keratin fluidization at hydrophobic C-H and hydrophilic O-H, N-H, C-O, C=O, C-N regimes of skin [42]. In the case of pectin gel, its drug permeation

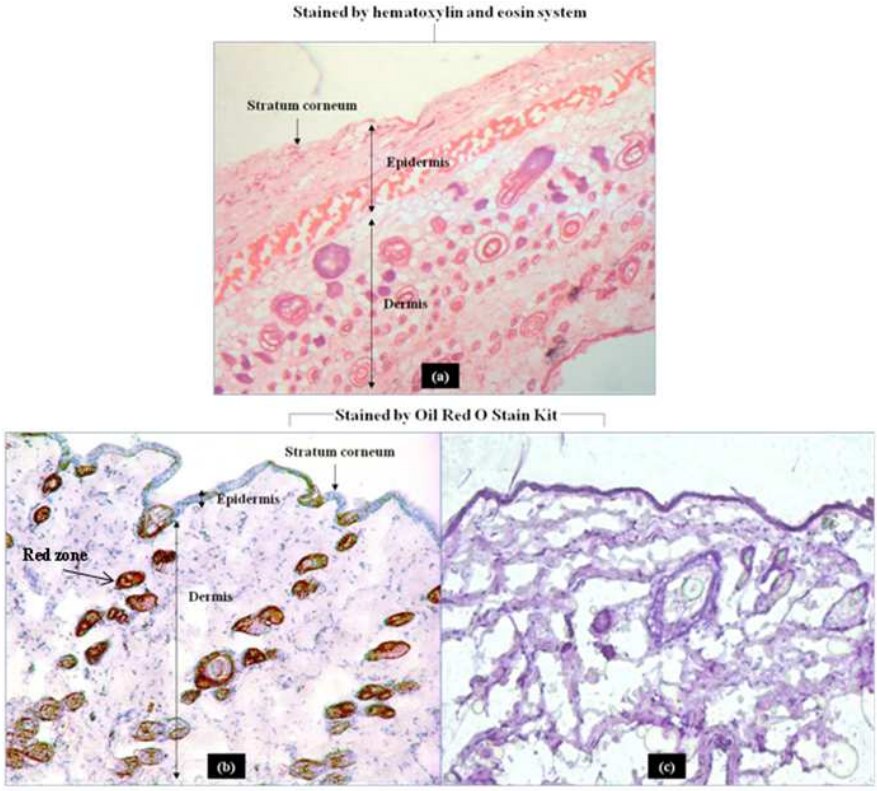
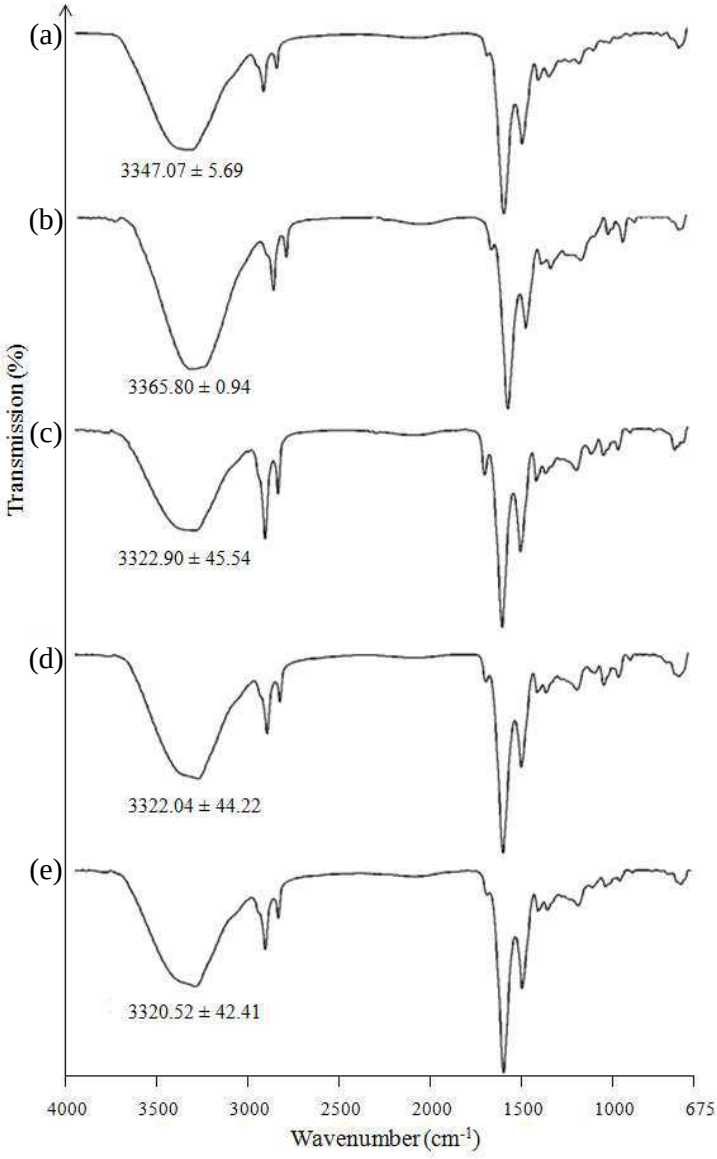


Figure 4. Cross-sectional histology of (a) untreated skin stained by hematoxylin and eosin system, skin treated by microwave at 2450 MHz for 5 min and applied with (b) OA and (c) P-OA gel, stained by Oil Red O Stain Kit.

enhancement effect was brought about primarily through reducing the strength of hydrogen bonding between keratin and/or polar moieties of ceramide and other lipid/protein materials in epidermis and dermis. This was indicated by ATR-FTIR findings of untreated skin where its wavenumber corresponding to O-H and/or N-H at $3347.07 \pm 5.69\,\mathrm{cm}^{-1}$ in epidermis and $3318.68 \pm 2.64\,\mathrm{cm}^{-1}$ in dermis increased to $3365.80 \pm 0.94\,\mathrm{cm}^{-1}$ and $3367.42 \pm 0.52\,\mathrm{cm}^{-1}$ respectively when P gel was applied onto the skin (Figs. 5(a)–(b), (f)–(g)). Interestingly, pre-treatment of skin by microwave followed by P gel application onto skin or use of P-OA gel defluidized the skin structure. This negated the permeation of drug released from P-SN gel (Fig. 3(d)). The hydrophilic O-H and/or N-H regimes in epidermis and dermis exhibited a greater strength of

interaction when P-OA gel was applied onto skin or P gel was applied following the treatment of skin by microwave (Figs. 5(b)–(d) and (g)–(i)). This was inferred from reduction in wavenumbers of ATR-FTIR peaks at $3365.80 \pm 0.94\,\mathrm{cm}^{-1}$ and $3367.42 \pm 0.52\,\mathrm{cm}^{-1}$, corresponding to OH and/or N-H moiety of epidermis and dermis respectively.



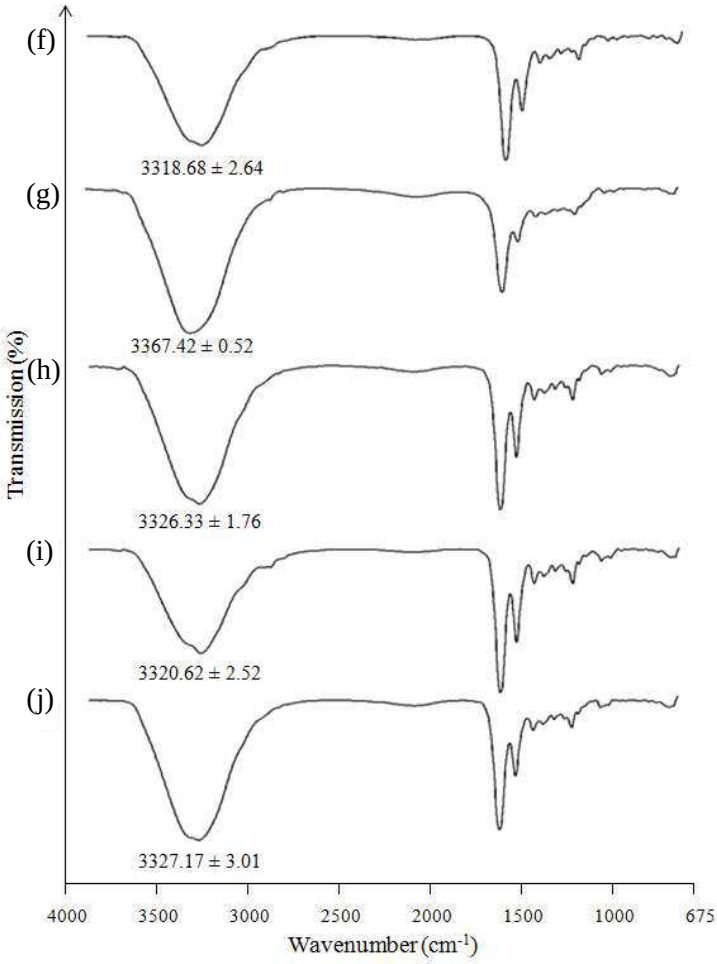
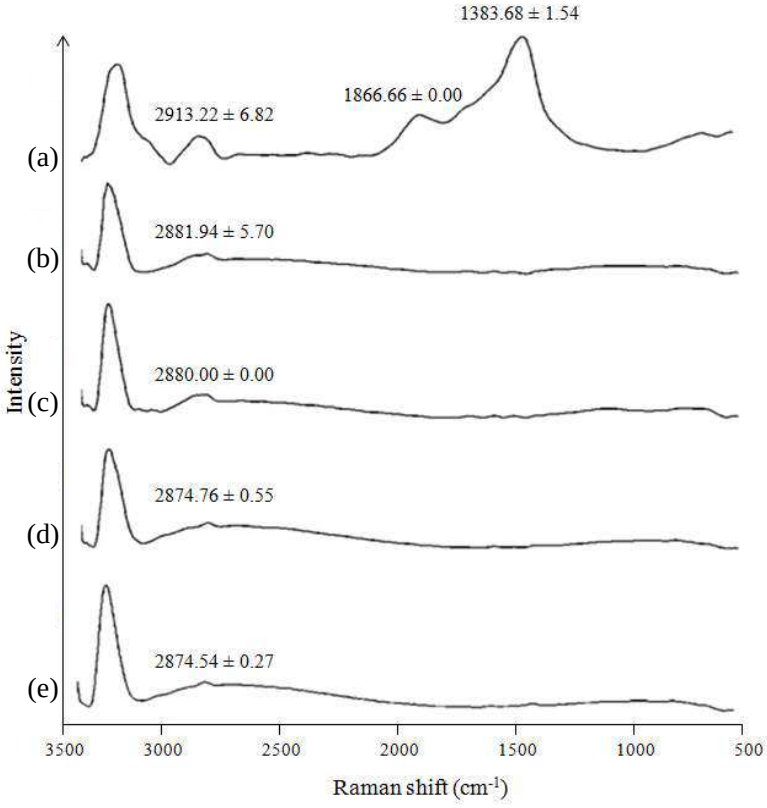


Figure 5. ATR-FTIR spectra of Epidermis: (a) untreated skin, (b) untreated skin-P gel, (c) microwave treated skin-P gel, (d) untreated skin-P-OA gel and (e) microwave treated skin-P-OA gel. Dermis: (f) untreated skin, (g) untreated skin-P gel, (h) microwave treated skin-P gel, (i) untreated skin-P-OA gel and (j) microwave treated skin-P-OA gel.

The influence of microwave and oleic acid in association with the use of pectin gel on skin was greater with epidermis than dermis, when compared to tests using solid film where both epidermis and dermis were similarly affected by microwave and oleic acid [42]. Using

pectin gel, the extent of changes in hydrophilic regime of dermis was lesser than that of epidermis. The ATR-FTIR wavenumbers of dermis, applied with P-OA gel or P gel following skin treatment by microwave, were comparable to that of untreated and gel-free skin characterized by a wavenumber of $3318.68 \pm 2.64\,\mathrm{cm}^{-1}$ (Figs. 5(f), (h) and (i)). On the other hand, the ATR-FTIR wavenumbers of epidermis, applied with P-OA gel or P gel following skin treatment by microwave, were markedly lower than that of untreated and gel-free skin characterized by a wavenumber of $3347.07 \pm 5.69\,\mathrm{cm}^{-1}$ (Figs. 5(a), (c) and (d)).

The use of pectin gel as a transdermal drug delivery system was envisaged to allow freely soluble pectin molecules to act as a permeation enhancer [42]. Nevertheless, skin fluidization by microwave or oleic acid could have promoted a greater extent of interaction between pectin and skin through increasing its binding to skin tissue particularly at the epidermis level. The pectin is a binder of plant cell walls [44]. Its extensive interaction with the skin tissue would mean



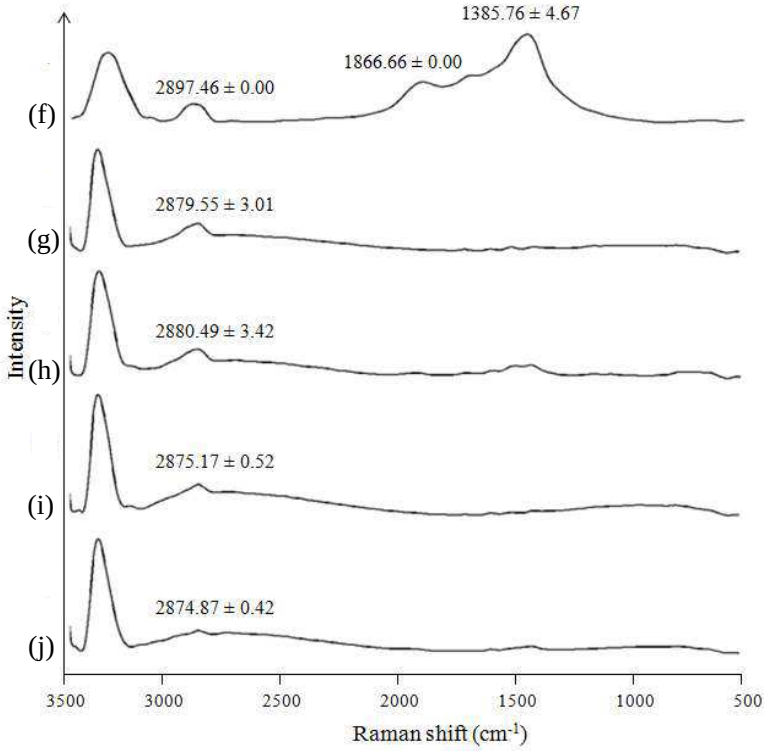


Figure 6. Raman spectra of Epidermis: (a) untreated skin, (b) untreated skin-P gel, (c) microwave treated skin-P gel, (d) untreated skin-P-OA gel and (e) microwave treated skin-P-OA gel. Dermis: (f) untreated skin, (g) untreated skin-P gel, (h) microwave treated skin-P gel, (i) untreated skin-P-OA gel and (j) microwave treated skin-P-OA gel.

structuring and strengthening of skin domains, thereby defluidizing skin and inhibiting drug permeation. Its widespread interaction with epidermis instead of dermis could aptly explain that the dermis was less responsive in its ATR-FTIR characterization against that of untreated and gel-free skin.

The interaction of pectin with dermis at the lower parts of skin was deemed possible, though it could be less than that at the epidermis level. In comparison to ATR-FTIR findings of skin applied with solid film [42], the dermis of skin applied with pectin gel demonstrated no marked conversion of *trans* lipid to *gauche* conformers though the skin was first treated by microwave. This was characterized by the absence

of peaks between 2800 and 2900 cm^{-1} in ATR-FTIR spectra (Figs. 5(f) and (h)). In addition, the raman spectra of skin applied with pectin gel were characterized by diminishing peaks related to C-H, C=O and C-O moieties of lipid and/or protein at wavenumbers between 1300 and 1900 cm^{-1} as well as 2800 and 2900 cm^{-1} (Fig. 6) with the latter wavenumbers ascribing the C-H moiety decreased when compared to the case of untreated skin. This observation was different from that of skin treated by microwave and applied with solid film where the chemical environment of C-H, C=O and C-O moieties of skin was re-organized at a smaller scale [42]. The pectin gel could immobilize the matrix of dermis via pectin-lipid and pectin-protein interaction at C-H, C=O and C-O moieties. Inferring from ATR-FTIR and raman studies, it could negate the drug permeation enhancement property of microwave and oleic acid through rigidifying the epidermis and to a smaller extent, the dermis at their hydrophilic as well as hydrophobic regimes.

The microwave could exert spacing of lipid architecture of skin in

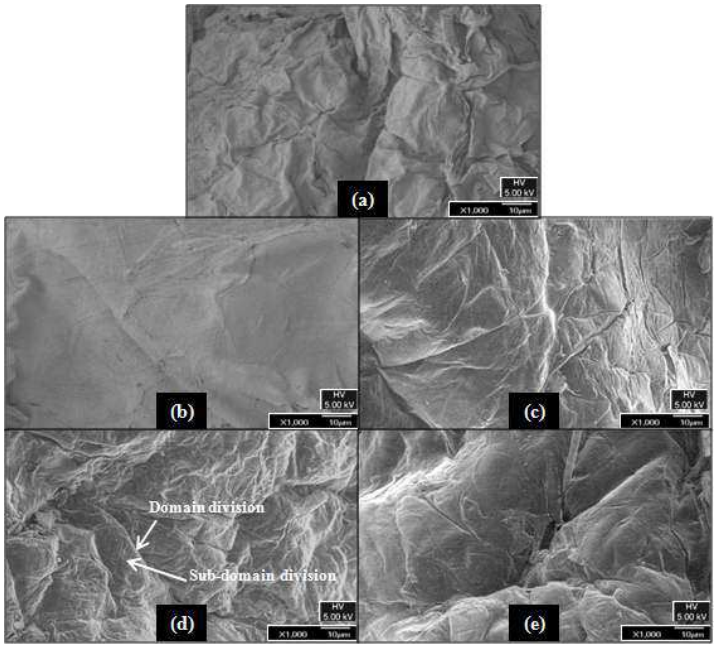


Figure 7. SEM surface morphology of epidermis: (a) untreated skin, (b) microwave treated skin, (c) untreated skin-P-OA gel, (d) untreated skin-OA and (e) microwave treated skin-P-OA gel.

a random manner with no build-up of specific structured domains [42]. The barrier to drug transport which formed between the structured domains can be destroyed by microwave. This converted the skin surfaces into a smooth texture (Figs. 7(a)–(b)). The definition of

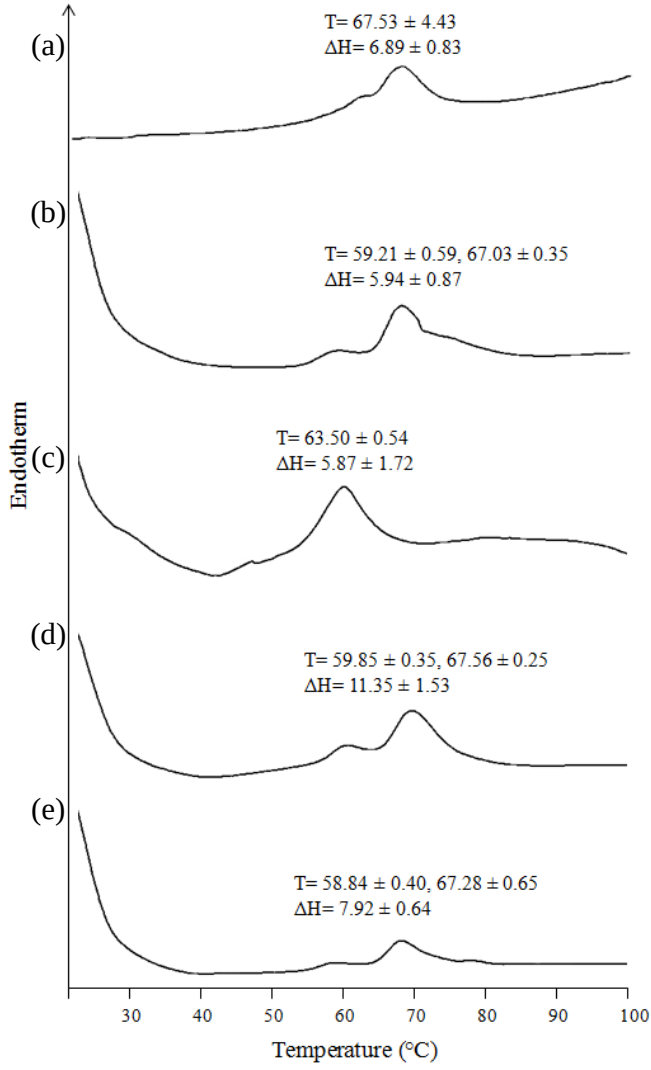


Figure 8. DSC thermograms of (a) untreated skin, (b) untreated skin-P gel, (c) microwave treated skin-P gel, (d) untreated skin-P-OA gel and (e) microwave treated skin-P-OA gel.

structure domains nonetheless was prominent and accompanied by a large number of sub-domain divisions when pectin gel loaded with oleic acid or oleic acid was applied on the skin (Figs. 7(a), (c), (d)). Oleic acid is an unsaturated fatty acid with a *cis* double bond at C9 induces a kink in its hydrocarbon chain [45–47]. Owing to the

Table 1. Derivative data from drug permeation study of P-SN and P-SN-OA gels and solutions using untreated skin or skin treated by microwave as the permeation barrier.

Dosage form type	Microwave condition	Lag time (min)	Flux ($\mu g\ cm^{-2}\ h^{-1}$)	Permeability coefficient ($\times 10^{-5}$ cm h^{-1})
Gel				
P-SN	Untreated	10.1 ± 6.6	18.55 ± 0.57	14.17 ± 0.44
P-SN	2450 MHz 5 min	8.2 ± 3.5	15.35 ± 3.4	11.72 ± 2.6
P-SN-OA	Untreated	31.9 ± 8.8	6.41 ± 0.70	4.89 ± 0.53
P-SN-OA	2450 MHz 5 min	21.2 ± 3.7	11.71 ± 1.85	8.94 ± 1.41
Solution				
SN	Untreated	723.6 ± 80.3	4.04 ± 2.32	3.08 ± 1.77
SN	2450 MHz 5 min	12.8 ± 2.8	23.58 ± 1.57	18.44 ± 1.20
SN-OA	Untreated	31.9 ± 10.0	20.55 ± 4.23	15.69 ± 3.23

Dosage form type	Enhancement ratio	Extent of drug permeation at 24 h (%)
Gel		
P-SN	-	62.97 ± 1.66
P-SN	0.83	52.41 ± 11.45
P-SN-OA	0.35	$21.55 + 2.74$
P-SN-OA	0.63	40.14 ± 7.06
Solution		
SN	-	13.45 ± 7.55
SN	5.99	70.94 ± 15.28
SN-OA	5.09	69.30 ± 15.30

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.

bent *cis* configuration, oleic acid has been reported to be able to disturb the highly ordered intercellular lipid packing of predominantly hydrophobic saturated chains [45–48]. It could extract a fraction of endogenous lipid and form separate phases within the intercellular lipid lamellae [45, 48–50]. This accounted for the formation of an extensive number of sub-domain divisions which were readily rigidified by freely soluble pectin from gel to form a large drug-impermeable area that led to low drug permeation. The pre-treatment of skin by microwave prior to applying P-OA gel reduced the formation propensity of sub-domain divisions (Figs. 7(c) and (e)). The treatment of skin by microwave reduced the rigidifying opportunity of pectin gel which was brought about by oleic acid. This aptly explained that oleic acid failed to act synergistically with microwave to further reduce the drug permeation degree of gel. The destruction of structured domains by microwave was characterized by coalescence of DSC dual-peak endotherms into a peak with reduced enthalpy value (Figs. 8(a)–(c)). The treatment of skin by microwave followed by P-OA gel was translated to dual endothermic peak formation (Figs. 8(c) and (e)), with a lower enthalpy gain than untreated skin with applied P-OA gel due to reduced development of sub-domain divisions (Fig. 8(d)).

The synergistic transdermal permeation enhancement action of microwave and oleic acid was not noted when pectin was formulated as gel instead of solid film [42]. The freely soluble pectin in gel could act as a binding agent for epidermis and dermis, thereby establishing an impermeable skin barrier for drug diffusion. The skin binding effect of pectin from gel was remarkable to the extent that there was no substantial fraction of oleic acid diffused through epidermis to dermis and accumulated at sebaceous gland (red zone) (Figs. 4(b)–(c)). The negative influence of gel on drug permeation through skin treated by microwave or applied with pectin gel loaded with oleic acid was supported by the corresponding increase in lag time, and decrease in flux, permeability coefficient, enhancement ratio and permeation extent of drug at 24 h (Table 1).

4. CONCLUSION

Unlike solid film, the application of pectin gel on skin was accompanied by reduced drug permeation when the skin was treated by microwave or administered with gel loaded with oleic acid. Using gel with freely soluble pectin molecules instead of solid film with entangled chains, the pectin molecules could interact readily with epidermis and dermis. An extensive skin intercellular space-pectin binding could take place thereby remarkably decreasing drug permeation. The

outcome of study implies that microwave-enhanced transdermal drug delivery may not be achieved using gel dosage form and gel formulation enriched with oleic acid. The associated knowledge can be extended to telecommunication, environment, cosmetics, agriculture and food technology sectors in the context of toxic exogenous substances invading skin or related tissue structures due to the influences of microwave or penetration enhancer may be preventable by topical pectin gel application.

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