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Transdermal iontophoresis of insulin: IV. Influence of chemical enhancers

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Abstract

Transdermal iontophoresis per se may not be able to achieve significant permeation of large peptides like insulin, thereby necessitating the use of combination strategies involving chemical enhancers and iontophoresis. The study investigated effect of pre-treatment with commonly used vehicles such as ethanol (EtOH), propylene glycol (PG), water and their binary combinations, dimethyl acetamide (DMA), 10% dimethyl acetamide in water, ethyl acetate (EtAc) and isopropyl myristate (IPM) on insulin iontophoresis. Solvents, which acted on the lipid bilayer, were able to produce a synergistic enhancement with iontophoresis. The binary solvent systems produced either additive or no effect, when combined with iontophoresis. FT-IR studies showed that EtOH, DMA, EtAc caused lipid extraction and the former two also caused changes in skin proteins, whereas IPM caused increase in lipid fluidity. TGA studies showed that EtOH and PG caused dehydration of skin. Skin barrier property was severely compromised with DMA, followed by EtOH and EtAc, while IPM and PG had relatively minimum skin barrier altering potential. Thus, this study demonstrates the possibility of achieving higher permeation of large peptides like insulin by combining iontophoresis with chemical enhancers that act on the intercellular lipids.

Keywords: Chemical enhancers; Lipid extraction; Intercellular lipids; Pore formation; Iontophoresis; Synergistic enhancement; Additive effect

1. Introduction

Numbers of peptide and protein drugs are increasing exponentially, due to rapid developments in biotechnology. However, their systemic delivery is limited by the large size and charge, resulting in poor biomembrane permeability, apart from their susceptibility to proteolytic degradation at most of the biomembrane interface (Pillai et al., 1999). Transdermal delivery of peptides/proteins has been recognized as an attractive option, due to the fact that skin has less proteolytic enzymes (Pannatier et al., 1978). At the same time to overcome the permeability dogma, several penetration enhancement strategies have evolved to expand the number of drugs delivered by transdermal route (Naik et al., 2000; Barry, 2001). Iontophoresis, which uses a small electric current, offers immense potential for delivery of charged peptide drugs. The advantages include controlled input kinetics with minimum inter-subject variability (Panchagnula et al., 2000). Though, iontophoresis per se may be able to achieve sufficient permeation for small peptides (<1000 Da),

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it is difficult, if not impossible, to achieve meaningful permeation for large peptides like insulin. It is in this context, combination strategies have emerged as a 'new paradigm' in transdermal drug delivery (Mitragotri, 2000). The 'new paradigm' is to utilise 'strengths' of the individual enhancement strategies to overcome their individual 'weaknesses' (Pillai et al., 2001). Srinivasan et al. (1989, 1990) for the first time demonstrated that a dramatic increase in the permeation of polypeptides can be achieved using simple pre-treatment of skin with ethanol before iontophoresis. Apart from achieving synergistic permeation enhancement, the combination of chemical enhancer with iontophoresis, also may help to overcome the skin toxicity issues associated with the use of higher levels of either of them, when used alone (Bhatia et al., 1997). Some of the commercially available iontophoretic systems contain isopropyl alcohol as a cleansing agent before the application of iontophoretic patch; hence, it would not be impractical to pre-treat the skin with chemical enhancers before iontophoresis. In addition to themselves acting as penetration enhancers, the choice of vehicle also has a significant influence on the action of other penetration enhancers (Pillai and Panchagnula, 2003). However, there is no systematic and comprehensive study on influence of commonly used vehicles on the iontophoretic permeation of large peptides. Taking into account all the above considerations, this study was undertaken to systematically investigate the effect of common pharmaceutical solvents in combination with iontophoresis using insulin as a model for large peptides in the molecular weight range of 3–7 kDa. Insulin is a polypeptide (molecular weight 6 k Da) with an isoelectric point of 5.3 and is used in the treatment of type I and majority of type II diabetes mellitus (Pillai and Panchagnula, 2001). There are a number of reports on transdermal iontophoresis of insulin using various animal models and current protocols with claims of varying degrees of enhancement (Siddiqui et al., 1987; Zakewski et al., 1998). However, achieving sufficient permeation to obtain human basal insulin levels is a challenge yet to be accomplished (Panchagnula et al., 2000). Thus, the choice of insulin apart from its immense clinical and commercial importance (Pillai and Panchagnula, 2001) is also based on the need to increase the transdermal permeation by using a combination of chemical enhancers and iontophoresis.

2. Materials and methods

2.1. Materials

Bovine insulin was a *gratis sample* from Knoll Pharmaceuticals (Mumbai, India). ^{125}I-insulin (80–100 μCi/g) was provided by Board of Radiation and Isotope Technology (BRIT, Mumbai, India). Isopropyl myristate (IPM), dimethyl acetamide (DMA), propylene glycol (PG) were purchased from Sigma (St. Louis, MO, USA). Ethanol (EtoH) and ethyl acetate (EtAc) were from Merck (Germany). The binary combinations used in the study were EtoH:water (2:1); PG:water (1:1); PG:EtoH (1:1) and 10% (v/v) DMA. All other chemicals and reagents used were of analytical grade. Ultrapure water prepared by reverse osmosis using Elgastat (ELGA, UK) was used in all experiments and had a resistivity of 18 MΩ or greater.

2.2. Skin preparation

All experiments were conducted according to the protocol approved by the Institutional Animal Ethics Committee (IAEC) of NIPER. Female Sprague–Dawley rats (200–250 g) obtained from the central animal facility at NIPER were sacrificed by excessive ether anesthesia. Hair was removed from the dorsal portion using an animal hair clipper (Aesuclap, Germany) and full thickness skin was harvested. Then the fat adhering on the dermis side was removed by using scalpel and isopropyl alcohol. Finally, skin was washed in tap water, packed in aluminum foil followed by storage at −20 °C and was used within a week.

For FT-IR and TGA studies, epidermis was separated from the full thickness skin using 2 M sodium bromide by a procedure described by Scott et al. (1986). The epidermal sheets were peeled off from the full thickness skin after soaking in 2 M sodium bromide solution for 12 h at 37 °C. Epidermal sheets were then dried in a vacuum dessicator for 3–4 days before using for FT-IR and TGA studies.

2.3. Ex vivo permeation studies

Phosphate-buffered saline (0.1 M; pH 7.4) was sonicated for 30 min and was placed in the receptor compartment of unjacketed Franz diffusion cells (area

0.79 cm^2) followed by equilibration for overnight at 37 ± 0.2 °C and 900 rpm in a heating-stirring module (Perme Gear, USA). Skin pieces thawed to 37 °C were mounted with the *Stratum corneum* side facing the donor compartment and were equilibrated for 1 h. Both the donor and receptor solutions consisted of urea (2 mg/ml) as a deaggregating agent and to prevent adsorption of insulin to glass surfaces (Sato et al., 1983) while, sodium azide (0.0025%, w/v) was added to prevent any microbial growth.

To the donor compartment, 500 μl of solvent systems were applied for 2 h (Srinivasan et al., 1989). After 2 h, the solvents were removed and the skin was washed with water followed by blotting with tissue paper. Insulin solution (500 μl of 3 mg/ml insulin; spiked with 80–100 μCi of ^{125}I insulin in pH 3.6 buffer) was placed in the donor compartment and the current was applied through platinum electrodes (2 cm × 0.5 mm diameter) for 6 h using a six-channel power supply unit (Ultrapure Scientifics, Mumbai, India). The anode was placed in the donor compartment and cathode in the receptor compartment. Samples (200 μl) were periodically withdrawn from the receptor compartment followed by replacement with fresh buffer solution upto 48 h and the samples were counted on an automatic gamma scintillation counter (1470, Wallac, Finland). At the end of study, pH of the donor and receptor compartment solutions were measured using a glass microelectrode (Eutech Instruments, USA). Skin area exposed to the drug solution was washed with water, dried, cut and weighed. Tissue solubiliser (NCS-II, Amersham, UK) was used to digest skin samples in a shaker water bath (Julabo, Germany) at 37 °C and 100 rpm for overnight. From the skin homogenates, 200 μl was used for radioactive counting. From the ratio of 'hot' to 'cold' insulin, the amount of insulin in the samples were calculated.

2.4. Tritiated water permeation studies

To study the changes in skin barrier property, tritiated water flux experiments were conducted using full thickness skin after pre-treatment with pure solvents (EtOH, EtAc, DMA, IPM and PG) for 2 h using the same experimental set up as described above. Donor solution consisted of citrate–phosphate buffer (pH 3.6) spiked with ^3H_2O (1 Ci/g) and the receptor solution (200 μl) was collected upto 12 h. The samples were counted in a liquid scintillation counter (1409, Wallac, Finland) after addition of 3 ml of scintillation cocktail (BCS 104, Amersham, UK).

2.5. FT-IR/TGA studies

For FT-IR experiments, the epidermal sheets were cut into pieces of 1 cm^2 and placed in screw-capped vials with 1 ml of solvent system for 2 h. After solvent treatment, the epidermal sheets were blotted and dried at room temperature for 15–30 min. FT-IR spectra were recorded in an IR spectrophotometer (Impact-410, Nicolet Corporation, USA) in the region of 4000–400 cm^{-1}. The spectra were an average of 100 scans with 2 cm^{-1} resolution and Happ-Genzel apodization. Peak area of 2920 cm^{-1} was calculated using OMNIC® FTIR software (Nicolet Corporation). Spectra were recorded before and after treatment with solvents and the percent decrease in area was calculated (Rastogi and Singh, 2001a). As per this procedure, the epidermis sample served as its own control, thereby avoiding inter-sample variation.

The epidermal sheets subjected to EtOH and PG treatment which was used in FT-IR studies were further analysed for water content by thermogravimetry (TGA/SDTA 851, Mettler Toledo, Switzerland) in open aluminium crucible by heating at a rate of 10 °C/min from 25 to 150 °C. Water content in epidermis sample was calculated as percent water loss from the thermograms by using the software attached to TGA (STAR®, Mettler Toledo).

2.6. Data treatment

Permeation profiles from insulin through full thickness rat skin was obtained by plotting the cumulative amount permeated versus time. Fluxes were calculated from the regression slopes of the line fitted to the linear portion of each of the permeation profile (Ritschel et al., 1989). The intercept of the straight line on the *x*-axis was taken as the lag time. For calculating the skin affinity values, the method reported by Panchagnula and Patel (1997) was followed. Drug concentration in the skin (μg/mg) was divided by the drug in the receptor compartment (μg/mg, density of receptor solution was considered as 1 g/ml) to get the skin affinity ratio. Different flux enhancement ratios (ER) were calculated as follows.

- ER1: flux with chemical enhancer/passive flux
- ER2: flux with enhancer and iontophoresis/flux with chemical enhancer
- ER3: flux with enhancer and iontophoresis/flux with iontophoresis
- ER4: flux with enhancer and iontophoresis/passive flux.

The ER2 and ER3 values were calculated in order to delineate the role of iontophoresis and chemical enhancer to the overall enhancement obtained with the combination. All experiments were done in triplicate and the values are expressed as mean ± S.E.M., unless specified. Passive permeation experiments were used as control. The skin permeation parameters were subjected to one way ANOVA (SIGMASTAT®, Jandel Scientifics, USA) and was considered as statistically significant at $P < 0.05$.

3. Results

Permeation of insulin through rat skin after pre-treatment with various pure solvent systems alone and in combination with iontophoresis is shown in Fig. 1a and b. Lag time was reduced in presence of all the solvents (except EtOH) compared to passive permeation and iontophoresis further reduced the lag time (Table 1). In absence of iontophoresis, the flux decreased in the following order DMA > EtOH > EtAc > IPM and in presence of iontophoresis flux was DMA > IPM > EtOH > EtAc. Further, all

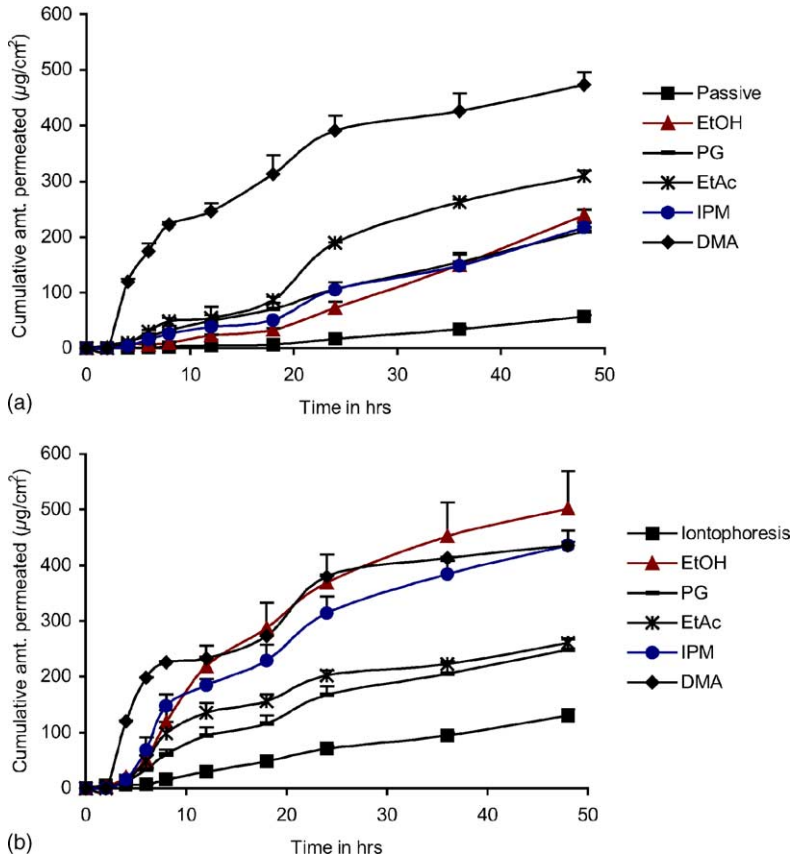

Fig. 1. Transdermal permeation of insulin with pure solvent systems (a) pre-treatment for 2 h followed by passive permeation; (b) pre-treatment followed by iontophoresis (0.5 mA/cm^2 for 6 h). EtOH, ethanol; PG, propylene glycol; EtAc, ethyl acetate; IPM, isopropyl myristate; DMA, dimethyl acetamide. Each data represents mean ± S.E.M. ($n = 3$).

Table 1
Skin permeation parameters after pre-treatment[a] with chemical enhancers followed by passive and/or iontophoretic[b] permeation

Chemical enhancer	Lag time (h)		Flux (μg/cm^2/h)		Cumulative amount permeated (μg)	
	Without TI	With TI	Without TI	With TI	Without TI	With TI
Control	12.91 (0.92)	5.24 (1.57)	1.57 (0.11)	3.74 (0.13)	40.53 (2.34)	94.50 (3.06)
EtOH	13.85 (2.19)	3.63 (0.43)	6.93 (0.03)	22.51 (0.02)	150.23 (6.86)	265.93 (17.81)
EtOH:water[c]	9.97 (1.25)	7.90 (2.42)	2.06 (0.40)	7.50 (0.12)	59.89 (4.64)	203.01 (5.76)
EtAc	3.00 (1.01)	2.19 (1.23)	5.00 (0.73)	13.67 (0.34)	195.13 (10.54)	206.53 (4.23)
IPM	3.22 (0.92)	3.10 (0.87)	4.57 (0.16)	24.53 (0.50)	148.12 (0.63)	300.12 (39.79)
DMA	0.08 (0.02)	0.07 (0.01)	10.84 (0.73)	32.98 (0.82)	298.27 (13.98)	274.46 (10.45)
DMA[d] (10%, v/v)	9.89 (0.74)	0.73 (0.73)	2.00 (0.13)	4.26 (0.12)	57.72 (4.01)	176.48 (9.74)
PG	1.05 (0.55)	0.80 (0.50)	3.71 (0.21)	4.06 (0.40)	166.00 (5.87)	167.90 (2.77)
PG:water[e]	11.23 (2.42)	2.44 (1.80)	3.16 (0.11)	6.11 (0.39)	71.71 (3.00)	192.35 (4.21)
PG:EtOH[e]	0.52 (0.26)	3.82 (0.54)	2.86 (0.22)	10.01 (0.48)	136.79 (16.68)	205.94 (6.34)

EtOH, ethanol; EtAc, ethyl acetate; IPM, isopropyl myristate; DMA, dimethyl acetamide; PG, propylene glycol; TI, transdermal iontophoresis. All the values are mean ($n = 3$) with S.E.M. in parentheses.

[a] Pre-treatment was done for 2 h.

[b] Current strength was 0.5 mA/cm^2 for 6 h.

[c] The composition was 2:1.

[d] DMA was 10% (v/v) in water.

[e] The composition was 1:1.

these solvent systems per se produced a flux which was higher than iontophoresis. There was no significant difference ($P > 0.05$) in the flux between PG and PG with iontophoresis, while the flux seen with EtOH alone was comparable to the flux in presence of iontophoresis for PG:water and EtOH:water systems. Similarly, there was no significant difference ($P > 0.05$) in the flux produced by DMA alone and PG:EtOH with iontophoresis whereas, minimal flux was seen in case of 10% DMA.

In presence of iontophoresis, there was no significant difference ($P > 0.05$) in cumulative amount of insulin permeated with DMA, EtOH and IPM, but was higher than all other solvent systems. Furthermore, there was no significant ($P > 0.05$) influence of iontophoresis on total amount of insulin permeated with DMA, EtOH, EtAc and PG, while in case of IPM, iontophoresis resulted in a significant ($P < 0.05$) increase in the total amount of insulin permeated. On the other hand, among the binary solvent systems, there was no significant difference ($P > 0.05$) in amount of insulin permeated with PG:EtOH; PG:water; EtOH:water and 10% DMA in presence of iontophoresis. However, all solvent systems except PG:water, resulted in significantly ($P < 0.05$) higher amounts of insulin permeation compared to iontophoresis alone. DMA pre-treatment resulted in highest ($P < 0.05$) cumulative amount permeated among the solvent systems tested. The solvents reduced the skin affinity of insulin compared to passive permeation except PG, PG:water, EtOH:water and 10% DMA. Iontophoresis further reduced the skin

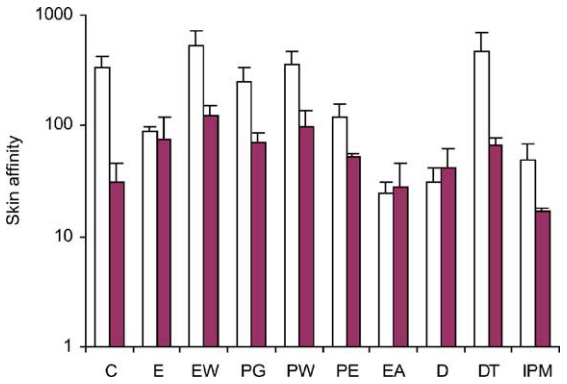

Fig. 2. Skin affinity values (expressed as ratio of amount of drug in skin to that in the receptor compartment) of insulin after pre-treatment with various solvent systems. White bars represent values in absence of iontophoresis and dark bars represent values in presence of iontophoresis. Values are mean ± S.E.M. ($n = 3$). The *y*-axis is represented in logarithmic scale for better clarity as the skin affinity values differed by more than one order of magnitude. C, control; E, ethanol; EW, ethanol:water; PG, propylene glycol; PW, propylene glycol:water; EA, ethyl acetate; D, DMA; DT, 10% DMA; IPM, isopropyl myristate.

Table 2
Enhancement ratios of insulin with solvents and/or iontophoresis

Chemical enhancer	ER1[a]	ER2[b]	ER3[c]	ER4[d]
Iontophoresis	2.38[e] (0.08)	–	–	–
EtOH	4.41 (0.83)	3.25 (0.10)	6.02 (0.18)	14.34 (0.43)
EtOH:water	1.31 (0.60)	3.64 (0.08)	2.00 (0.31)	4.78 (0.07)
EtAc	3.16 (0.27)	2.73 (0.07)	3.65 (0.09)	8.71 (0.21)
IPM	2.91 (0.69)	5.37 (0.11)	6.56 (0.13)	15.63 (0.39)
DMA	6.91 (1.72)	3.04 (0.08)	8.82 (0.22)	21.01 (0.52)
DMA (10%, v/v)	1.28 (0.13)	2.13 (0.06)	1.14 (0.03	2.71 (0.08)
PG	2.36 (0.53)	1.09 (0.11)	1.08 (0.11)	2.58 (0.26)
PG:water	2.00 (0.31)	1.93 (0.12)	1.63 (0.10)	3.89 (0.25)
PG:EtOH	1.82 (0.54)	3.50 (0.08)	2.67 (0.13)	6.37 (0.31)

EtAc, ethyl acetate; IPM, isopropyl myristate; DMA, dimethyl acetamide; PG, propylene glycol. All the values are mean ($n = 3$) with S.E.M. in parentheses.

[a] ER1: flux with enhancer/passive flux (enhancement with chemical enhancer over that of passive control).

[b] ER2: flux with CE + TI/flux with CE (contribution of iontophoresis over that of chemical enhancer in the combination).

[c] ER3: flux with CE + TI/flux with TI (contribution of chemical enhancer over that of iontophoresis in the combination).

[d] ER4: flux with CE + TI/passive flux (enhancement with combination over that of passive control).

[e] Iontophoretic flux/passive flux; CE, chemical enhancer; TI, transdermal iontophoresis; EtOH, ethanol.

affinity of insulin, though not significant ($P > 0.05$) in case of EtAc, EtOH and DMA (Fig. 2).

Table 2 shows the various enhancement ratios calculated to delineate the contribution of chemical enhancers and iontophoresis to the overall enhancement achieved by the combination. ER2 is an indicator of the enhancement achieved by iontophoresis over that of chemical enhancer in the combination, while ER3 indicates the enhancement contributed by the chemical enhancer over iontophoresis in the combination. DMA produced the highest enhancement per se as well as when combined with iontophoresis. Further, DMA, EtOH, IPM and EtAc produced an enhancement that was higher than iontophoresis. On the other hand, PG, PG:water and PG:EtOH produced comparable enhancement ($P > 0.05$) to iontophoresis alone, whereas, EtOH:water and 10% DMA produced the least enhancement (ER1) among the solvent systems.

The higher ER3 values seen with DMA, EtOH, IPM and EtAc signify that these solvent systems contribute to a major extent to the overall enhancement seen with the combination. In case of binary solvent systems EtOH:water, PG:EtOH and 10% DMA (where ER2 > ER3), the total enhancement seen is mainly due to the contribution of iontophoresis rather than the solvent system. While with PG and PG:water systems, there is an equal contribution of iontophoresis and solvent system. From the ER4 values, it is evident that DMA, IPM, EtOH and EtAc showed a synergistic enhancement of insulin when combined with iontophoresis,

Table 3
Changes in asymmetric stretching (2920 cm^{-1}) peak area in FT-IR spectra and percent water loss in TGA after pre-treatment[a] with solvents

Chemical enhancer	Before treatment	After treatment	Decrease in peak area[b] (%)	Water loss[c] (%)
EtOH	4.73 (0.37)	0.88 (0.05)	81.39	3.46 (0.53)
EtAc	6.86 (0.025)	4.1 (0.100)	40.23	ND
DMA	5.96 (0.06)	3.31 (0.38)	44.46	ND
PG[d]	–	–	–	1.57 (0.32)

ND, not done. EtOH, ethanol; EtAc, ethyl acetate; DMA, dimethyl acetamide; PG, propylene glycol. All the values are mean ($n = 3$) with S.E.M. in parentheses.

[a] Pre-treatment was done for 2 h.

[b] Decrease (%) = 100 − [(peak area after treatment)/(peak area before treatment) × 100].

[c] Percent water loss determined by theromogravimetric analysis (TGA).

[d] No changes were observed in FT-IR in lipid stretching region.

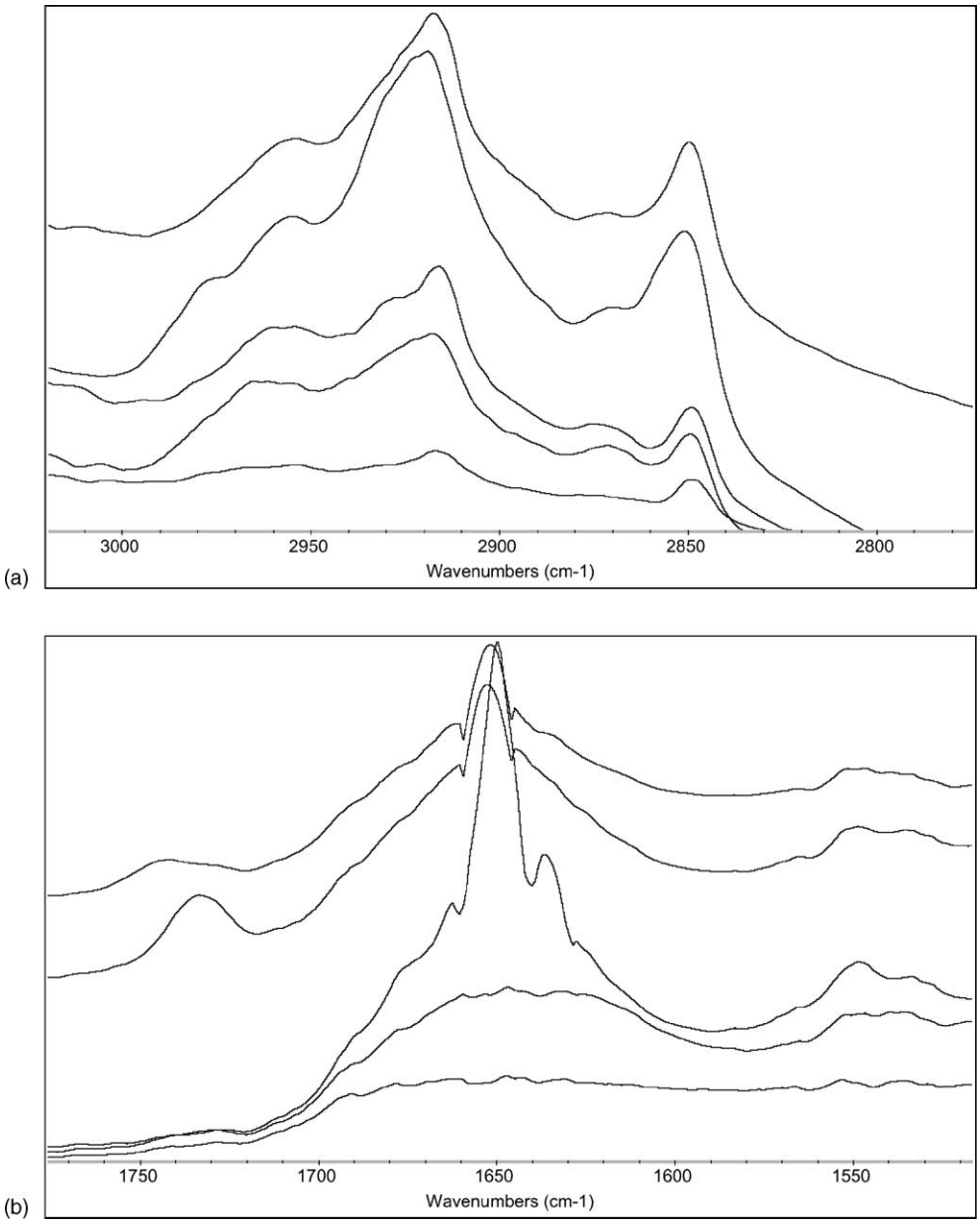

Fig. 3. FT-IR spectra after pre-treatment with solvent systems. (a) Changes in lipid C–H stretching (2920 cm^{-1}) vibrations; (b) changes in amide I (1640 cm^{-1}) and amide II (1550 cm^{-1}) vibrations. The spectra are arranged from top to bottom; epidermis pretreated with control (pH 7.4 buffer), isopropyl myristate, ethyl acetate, dimethyl acetamide and ethanol.

while PG:EtOH and PG:water showed an additive effect. In comparison to iontophoresis alone, no additional advantage was gained by combining either PG or 10% DMA with iontophoresis.

FT-IR spectroscopic studies showed that EtOH reduced the C–H stretching (2920 cm^{-1}) peak area to the maximum extent, followed by DMA, EtAc (Fig. 3a and Table 3), whereas IPM caused a shift

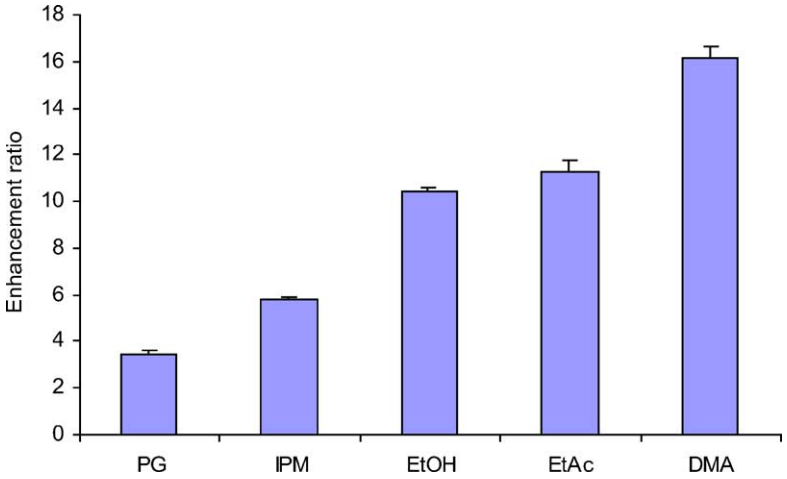

Fig. 4. Enhancement of $^{3}H_2O$ flux after pre-treatment with various solvent systems for 2 h. PG, propylene glycol, IPM, isopropyl myristate; EtOH, ethanol; EtAc, ethyl acetate; DMA, dimethyl acetamide. Values are mean ± S.E.M. ($n = 3$).

in wavenumber to higher frequencies. This indicated that all these solvents increased insulin permeation by acting on intercellular lipids in skin. On the other hand, DMA and EtOH also had an effect on skin protein as seen by the absence of prominent amide I and II stretching peaks compared to control (Fig. 3b). At the same time, TGA studies showed that EtOH and PG had a dehydrating effect on skin and the later was more dehydrating than the former (Table 3). Tritiated water permeation studies showed that the skin barrier was severely compromised in case of DMA followed by EtAc and EtOH (Fig. 4), while IPM and PG had relatively minimal effects on skin barrier.

4. Discussion

The unique structure of SC compromise of keratin-rich cells embedded in multiple lipid bilayers, which pose an excellent barrier to the transport of large and charged molecules. Therefore, to administer peptides at an appropriate rate, the lipid domain must be breached. Transport of hydrophilic solutes has been found to mainly take place via the transfollicular and polar routes through the highly tortuous pathways of the intercellular lipids (Rastogi and Singh, 2001b). These pores are supposedly located in the intercellular lipids, which comprise of aqueous region surrounded by lipids with the hydrophilic groups forming the walls of microchannels, through which amino acids and zwitter ions are transported (Sznitowska et al., 1998). On the other hand, there is ample evidence to suggest that iontophoretic transport also takes place through intercellular lipids involving perturbation of lipid bilayer as a function of current strength and duration (Van-Hinsberg et al., 1997). Thus, it was hypothesized that a combination of chemical agents that alter the intercellular lipids will lead to decreased diffusive resistance for the iontophoretic transport of large peptides like insulin. Further, to achieve synergistic permeation enhancement, it is necessary to couple strategies which act by different rather than same mechanisms (Mitragotri, 2000). In this study, solvents were selected which act at different sites in the skin according to the classical lipid–protein partition theory (Barry, 1991).

EtOH is one of the most commonly used solvents in transdermal drug delivery systems and is used in some of the commercial transdermal patches to improve solubility and enhance the permeation of drugs (Bommannan et al., 1991). There are several mechanisms that have been proposed for EtOH action on skin depending on its concentration and the physicochemical properties of the permeant (Ghanem et al., 1987; Bommannan et al., 1991; Suhonen et al., 1999). However, it has been generally observed from literature, that at lower concentrations of EtOH (20–70%, v/v), permeation of lipophilic drugs are enhanced (Williams

and Barry, 1992), while at higher concentrations, permeability of both hydrophilic and hydrophobic drugs are enhanced (Manabe et al., 1996). At higher concentrations of EtOH (>70%), lipids are extracted from the skin leading to formation of pores (Inamouri et al., 1994; Manabe et al., 1996). In addition, EtOH extracts water from the intercellular spaces resulting in interdigitation of lipid bilayer, which is attributed to the long lag time observed with 100% EtOH compared to 66% EtOH in water (Table 1). FT-IR and TGA results support the lipid extraction and dehydrating properties of absolute EtOH (Fig. 3a and Table 3).

At the same time, EtOH also cause swelling of the keratin fibrils and extract proteins from SC at higher concentrations (Bergstrom et al., 1990; Suhonen et al., 1999). This can be seen from the absence of amide I and II peaks in EtOH pre-treated epidermis in Fig. 3b. The decrease may also be attributed to the extraction of ceramides (Pershing et al., 1990), as they also show amide stretching vibrations in the same wavenumber region. Nevertheless, with 100% EtOH, the porosity is increased although, the tortuousity remains high (due to extraction of lipids) and the 'new' pores are small or comparable size to existing pores in the skin (Peck et al., 1994). This increased porosity reduces the diffusive resistance and increases the free volume for transport of large molecules like insulin, which may be appreciated from the fact that skin resistance has been reported to be reduced drastically on EtOH pre-treatment (Lankjaer et al., 1998). On the other hand, comparable enhancement observed with EtOH and iontophoresis (Table 2) signifies that iontophoresis per se may not be able to achieve sufficient permeation (Srinivasan et al., 1990). Hence, the ideal situation is to increase the porosity as well as the size of the pores to achieve synergistic increase in enhancement (Peck et al., 1994), which may be attributable to EtOH and iontophoresis, respectively, when combined together. Similarly, other investigators have also reported synergistic enhancement with this combination on insulin permeation using human and hairless mouse skin (Srinivasan et al., 1989; Lankjaer et al., 1998).

EtAc is a GRAS solvent, which is used in transdermal drug delivery to improve solubility and enhance the permeation of many drugs (Friend and Heller, 1993). It is reported to act as permeation enhancer by extracting the skin lipids and thereby reduces the diffusive resistance for transport of hydrophilic molecules (Rastogi and Singh, 2001a). The lower lag time with EtAc compared with EtOH is attributed to the fact that rodent skin is more permeable to EtAc than EtOH (Friend and Heller, 1993). Moreover, EtAc is more volatile than EtOH, accounting for the relatively lower ER than the later. However, skin barrier property was altered to the same extent with both the solvent systems as found from tritiated water permeation studies (Fig. 4). Rastogi and Singh (2001a) have shown in case of LHRH, that pre-treatment with EtAc resulted in 31 times enhancement, while with insulin we observed 8 times enhancement and these differences is attributed to physicochemical properties of the permeant in addition to the type and composition of skin (porcine versus rat skin).

In comparison to EtAc and EtOH, IPM had lesser effects on skin barrier property (Fig. 4). The FT-IR studies showed that IPM caused increased fluidity of lipid bilayers, as there was a frequency shift with no effect on peak area. But, IPM still showed a comparable ER to EtOH in presence of iontophoresis. This is probably because, IPM gets interdispersed in the lipid bilayer (Poulsen et al., 1968) and stays for longer time (as it is also viscous compared to ethanol), modulating the iontophoretic transport of insulin. Unlike EtOH, the ER2 and ER3 values were comparable, implying that IPM and iontophoresis contributed to an equal extent in causing synergistic enhancement of insulin permeation.

DMA, which is a powerful aprotic solvent, is the first compound from the class of amides to be used as skin penetration enhancers (Santus and Baker, 1993). It is reported to show a concentration-dependent effect with lower concentration acting on skin proteins and loosening the structure, while at higher concentrations, disrupt the lipid packing as a result of shell formation around the polar head group of lipids (Williams and Barry, 1992; Walker and Smith, 1996). This was evident from the FT-IR results, where changes were observed in both lipid C–H stretching region and protein amide stretching absorbance regions (Fig. 3a and b). Further, delipidzation by DMA has also been reported to result in dramatic decrease in skin impedance (Allenby et al., 1969). On the other hand, Singh and Singh (1995) have shown that iontophoresis in combination with dimethyl group create pores on the skin and cause increased transport of permeants. All these

events are attributed to the dramatic increase in permeation of insulin with DMA, although its practical use is limited by its high skin irritation potential (Santus and Baker, 1993).

PG, a commonly used vehicle in transdermal formulations has been shown both to enhance as well as inhibit drug permeation depending on the drug and the delivery environment (Walker and Smith, 1996). This discrepancy is mainly attributed to the differences in experimental conditions and in several instances, it has been observed that the effect of PG is more evident, when skin is not fully hydrated (Barry, 1987). Not unexpectedly, lesser enhancement was observed with PG in combination with iontophoresis, as PG is known to dehydrate the skin (Ward and Osborne, 1993). The removal of water pools in the skin by PG, consequently creates an environment that is less conducive for iontophoretic transport. This dehydration effect was evident from TGA studies (Table 3). Similar negative effects of PG on permeation have also been reported with mannitol, hydrocortisone, progesterone and amino acids (Ruland and Kreuter, 1992). Moreover, it has been reported in literature, that even after 24 h pre-treatment, PG has no influence on the rate of transport of desglycinamide vasopressin (Hoogstraate et al., 1991), which implies that peptides are very unlikely to penetrate across corneocytes; suggesting the need to use only those solvents which act on intercellular lipids.

On the other hand, in case of PG:water systems, the presence of water satisfies hygroscopicity of PG, which in turn spares the water from skin. The lesser dehydrating property of PG:water systems has been earlier proved by TGA studies in our laboratory (Panchagnula et al., 2001). With PG:EtOH system, the dehydrating effects of PG and EtOH is compensated by the lipid perturbation property of EtOH, resulting in relatively higher amounts of insulin permeation compared to other binary solvent systems. Similar results with PG:EtOH have been obtained earlier with naloxone and FT-IR studies showed that PG:EtOH combination increased the lipid fluidity in skin (Panchagnula et al., 2001).

Among all the solvents investigated, EtOH is a useful solvent to combine with iontophoresis, due to the fact that it is compatible with most of the penetration enhancers and further it showed a synergistic enhancement with iontophoresis for insulin. On the other hand, IPM although, produced comparable enhancement is viscous and is relatively less compatible with other penetration enhancers.

5. Conclusions

This study demonstrates the potential of various commonly used solvents to enhance the permeation of large peptides like insulin. Synergistic enhancement with iontophoresis is achieved by combining solvents that act on intercellular lipids. Use of binary solvent systems was not much useful, as they either produced no effect or at the maximum showed an additive effect, when combined with iontophoresis. Hence, by use of chemical enhancers, it is possible to modulate the iontophoretic regimen to achieve the desired permeation. Alternatively, the current profile may also modulate the delivery over and above the effect of chemical enhancer to achieve meaningful permeation for large peptides. However, the combined influence of chemical enhancers and iontophoresis on skin morphology need to be further investigated, with the use of lower levels of chemical enhancer and iontophoretic current to understand the skin toxicity implications of such combination strategies.

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