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Transdermal delivery of naloxone: effect of water, propylene glycol, ethanol and their binary combinations on permeation through rat skin

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Abstract

The effect of the solvent systems water, ethanol (EtOH), propylene glycol (PG) and their binary combinations was studied on the ex vivo permeation profile of the opioid receptor antagonist, naloxone, through rat skin. Fourier transform-infrared (FT-IR) spectroscopic studies were done to investigate the effect of enhancers on the biophysical properties of the stratum corneum (SC), in order to understand the mechanism of permeation enhancement of naloxone by the solvent systems used. The flux of naloxone was found to increase with increasing concentrations of EtOH, upto 66% in water, and PG upto 50% in water. The maximum flux of 32.85 μg cm^{-2} h^{-1} was found with 33% PG in EtOH. The FT-IR spectra of SC treated with EtOH showed peak broadening at 2920 cm^{-1} at all concentrations of EtOH studied indicating that EtOH increases the translational freedom (mobility) of lipid acyl chains. Theoretical blood levels well above the therapeutic concentration of naloxone can be achieved with the solvent system comprising 33% PG in EtOH and hence, provides flexibility in choice of patch size depending on the addiction status of the patient to be treated. © 2001 Published by Elsevier Science B.V.

Keywords: Naloxone; Transdermal permeation; Solvent system; Fourier transform-infrared spectroscopy

1. Introduction

Naloxone, a potent opium antagonist, is used for the treatment of opioid abuse. It is not effective when administered perorally because of its short biological half-life. Presently, it is administered by either subcutaneous or intramuscular injections. The terminal half-life of naloxone after intravenous injection in normal volunteers has been reported to be 64 mim. (Ngai et al., 1976). Therefore, repeated administration is necessary to obtain therapeutic efficacy and infusion may be preferred in some cases of narcosis (Gourlay and

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Coulthard, 1983). This results in patient noncompliance and more so in drug addicts. Development of a transdermal delivery system can circumvent the problems of first-pass metabolism, short half-life and repeated injections and can maintain constant blood levels that will result in better management and treatment of opioid addiction. The use of transdermal drug delivery systems (TDDS) in the treatment of addiction, such as smoking, has been demonstrated earlier with the nicotine TDDS, which has been successful in smoking cessation program. This mode of administration does not require much motivation for a patient thereby contributing for high patient compliance. The feasibility of transdermal delivery of naloxone has been demonstrated earlier (Aungst et al., 1986; Jaiswal et al., 1999)

Fourier transform-infrared (FT-IR) spectroscopy can be used to investigate the biophysical alterations taking place in the lipid bilayer after treatment with solvents or penetration enhancers. The lipid bilayer exists either in gel state (ordered) or sol state (fluidised) and the state of existence of lipid bilayer can be elucidated by studying the vibrational modes of its components using FT-IR. Of particular interest in this context are the IR absorbance regions near 2850 and 2920 cm^{-1} corresponding to symmetric and asymmetric methylene groups (H–C–H) stretching, respectively (Potts and Francoeur, 1993). The increase in lipid bilayer fluidity may be because of increased number of gauche conformers (Potts and Francoeur, 1993) or increase in trnaslational movements (Naik and Guy, 1997). A higher wavenumber shift in FT-IR is an indication of increase in gauche conformers (Potts and Francoeur, 1993). On the other hand broadening of peaks at 2850 and 2920 cm^{-1} is an indication of increased translational movement or mobility of lipid acyl chains (Naik and Guy, 1997).

The selection of a suitable solvent system in development of TDDS is first and most important step as this can affect the transdermal permeability by altering thermodynamic activity of drug and/or barrier nature of skin. Hence, the objective of this investigation was to select an appropriate solvent system for transdermal delivery of naloxone and to investigate the possible mechanism of action of these solvent systems with the help of FT-IR technique. In this study the effect of water, ethanol (EtOH), propylene glycol (PG) and their binary combinations on the permeability of naloxone through rat skin was studied.

2. Materials and methods

2.1. Materials

Naloxone and ^{3}H-naloxone (specific activity, 60 Ci $mmol^{-1}$) were obtained from Du Pont, Willington, DE and Amersham, UK, respectively. PG and EtOH were purchased from Sigma Chemical Co., USA and ACS ISO Merck, Germany, respectively. Tissue solubiliser (NCS II) and scintillation cocktail (PCS 104) were purchased from Amersham International Plc., UK. All other chemicals used were of reagent grade.

2.2. Preparation of whole skin

The protocols for the preparation of whole skin and stratum corneum (SC) were approved by Institutional Animal Ethical Care Committee. Sprague–Dawley rats were sacrificed by excess ether inhalation. The dorsal skin of animal was shaved with the help of animal hair clipper (Aesculap, Germany) and excised from the animal. The subcutaneous tissue was removed surgically and dermis side was wiped with isopropyl alcohol to remove adhered tissue (Panchagnula and Patel, 1997). The skin was washed with phosphate buffer, wrapped in aluminum foil and stored at $-20°C$ till it is used.

2.3. Preparation of epidermis and stratum corneum (SC)

The skin was treated with 1 M sodium bromide solution in water for 4 h (Scott et al., 1986) and the epidermis was separated from the skin using cotton swab moistened with water. SC samples were prepared by floating freshly prepared epidermis membrane, SC side facing upwards, on 0.1% w/v trypsin solution for 12 h. SC sheets were cleaned from digested material by washing with water.

2.4. Ex vivo permeation study

Skin penetration into and permeation across the skin of naloxone was studied using unjacketed Franz diffusion cells with a diffusional surface area of 0.785 cm^2. Phosphate buffer pH 7.4, containing 0.01% w/v sodium azide to retard microbial growth, was used as the receptor medium. The receptor fluid was sonicated to remove dissolved gases. Rat skin was mounted on the diffusion cell with dermal-side in contact with the receptor medium and SC-side facing donor compartment. The skin was equilibrated for 1 h with the receptor medium. A blank sample of 200 μl was withdrawn from the receptor compartment and analysed to ensure that the diffusion cells did not have any residual radioactivity contamination from previous experiments. Naloxone solution (200 μl of 20 mg ml^{-1}) was applied to the SC side in each of the donor compartments. Donor compartment was covered with paraffin film. Samples (200 μl) were withdrawn from receptor compartment for 24 h at regular intervals and analysed for drug content. The sample volume was immediately replaced with fresh receptor medium. It is well known that maximum flux can be achieved by saturated solutions in which thermodynamic activity is maximum, but this imposes practical problem of drug crystallisation in patch or film (Hadgraft, 1999). Therefore, finite dose with solvent combinations was used in this study.

The compositions of drug solutions applied in the donor compartment are given in Table 1.

2.5. Analysis

Radiotracer method of analysis was used in this experiment. Drug solutions were spiked with ^3H-naloxone to obtain 1 μCi ml^{-1} radioactivity. The samples were mixed with 5 ml of scintillation cocktail in a diffuse vial and counted in a liquid scintillation counter (Wallac 1405, Finland). A series of blanks were always run to determine background counts.

2.6. FT-IR analysis of SC sheets

The SC sheets were treated with 5 ml of 100% PG, 100% EtOH, 66% of EtOH in water, 66% of PG in water and 33% of PG in EtOH for 6 h. The treated skin samples were washed with water and blotted dry. The FT-IR spectra of the SC sheet

Table 1
Permeation parameters ($n = 3$, mean ($\pm$ S.D.))

Solvent compositions	Lag time (h)	Flux (μg h^{-1} cm^{-2})	Permeability (cm^2 s^{-1})
Group A (PG:W)			
0:100	–	3.02 (2.24)	1.51×10^{-4} (0.65)
33.5:66.5	1.83 (1.62)	1.87 (1.62)	0.94×10^{-4} (0.38)
50:50	2.40 (1.73)	12.23 (1.89)	6.11×10^{-4} (0.94)
66.5:33.5	4.73 (1.62)	12.27 (3.19)	6.14×10^{-4} (1.59)
100:0	13.77 (11.03)	4.49(0.37)	2.25×10^{-4} (0.18)
Group B (EtOH:W)			
0:100	–	3.02 (2.24)	1.51×10^{-4} (0.65)
33.5:66.5	0.13 (0.23)	5.56 (1.21)	2.78×10^{-4} (0.60)
50:50	1.17 (1.15)	9.08 (2.42)	4.54×10^{-4} (1.21)
66.5:33.5	0.82 (0.47)	23.03 (4.67)	1.15×10^{-3} (2.33)
100:0	3.60 (1.04)	13.18 (5.63)	6.59×10^{-4} (2.81)
Group C (PG:EtOH)			
0:100	3.60 (1.04)	13.18 (5.63)	6.59×10^{-4} (2.81)
33.5:66.5	7.53 (1.85)	32.85 (16.74)	1.64×10^{-3} (8.37)
50:50	5.3 (2.5)	19.20 (8.0)	9.60×10^{-4} (4.00)
66.5:33.5	6.7 (1.06)	10.56 (2.51)	6.70×10^{-4} (1.26)
100:0	13.77 (11.03)	4.49 (0.37)	2.25×10^{-4} (0.18)

was recorded in the range of 3000–1000 cm^{-1} using Nicolet 710 FT-IR spectrophotometer. The FT-IR spectrum of the control SC sheet was also recorded for comparison.

2.7. Thermal gravimetric analysis (TGA)

The epidermal sheets were cut into small pieces and were treated for 6 h with water, 66% PG in water, and 100% PG in individual vials. Then the epidermal samples were blotted dry and percentage water loss was determined by TGA (Mettler, Toledo) between 25 and 250°C at the rate of 10°C min^{-1}.

2.8. Data analysis

Cartesian plots were made, taking time in hours on X-axis and cumulative amount of drug permeated per unit area (μg cm^{-2}), present in receptor fluid, on Y-axis. Flux values (μg cm^{-2} h^{-1}) were calculated from the slopes of the steady states of above plots. Lag times were calculated from the intercepts of extrapolated steady state flux to X-axis. Permeability values are calculated by the following equation;

$$\text{Permeability} = \frac{\text{SSF}}{C_d}$$

where SSF is steady-state flux (μg cm^{-2} h^{-1}) and C_d is the concentration in donor compartment.

The steady-state blood level (C_{ss}) for particular flux value (J_{max}) in a given solvent system was calculated as follows;

$$C_{ss} = \frac{J_{max}A}{Cl_T}$$

where C_{ss} is the steady-state blood level concentration in μg l^{-1}, A is the hypothetical area of the transdermal therapeutic system (cm^2) and Cl_T is the systemic clearance after IV administration (l h^{-1}).

3. Results and discussion

The effect of different solvent systems on ex vivo permeation of naloxone through rat skin is shown in Table 1. The flux of naloxone in group A combinations was found to be increased upto 50% PG in water and there was no further significant increase in flux at 66% of PG as determined by Students Newman Keuls (SNK) test ($P < 0.05$) and with further increase of PG concentration to 100% there was a decrease in flux of naloxone. The flux of naloxone in 33 and 100% were not significantly different from control ($P < 0.053$). In the FT-IR studies, it was found that there was no significant effect of PG at the concentrations studied on either peak shift or broadening at both 2920 and 2850 cm^{-1} (Fig. 1).

PG action as a sorption promoter has been explained in the literature on the basis of its cosolvency effect, where thermodynamic activity is considered as main driving force (Barry, 1983), and also by carrier mechanism, in which PG partitions into the skin and thereby promotes the movement of the drug into and through the skin (Holegaard and Mollgaard, 1985). The parabolic increase in the flux of naloxone with increasing concentration of PG in water is thought to be because of variations in the dehydration of SC at different concentrations of PG, though effect of change in the thermodynamic activity due to change in the cosolvent concentration can not be overlooked. In order to substantiate the above speculation, TGA of epidermis before and after treatment with different concentrations of PG in water was performed. It was observed that with increasing concentration of PG in water, the water loss from the treated epidermis was decreased (Fig. 2).

The first derivative TGA thermogram of epidermis hydrated for 6 h showed a water loss peak in the temperature range of 53.95–94.63°C (Fig. 2a) and total water loss was 57.21%. When epidermis treated with 66% PG in water two peaks were found in first derivative of TGA thermogram (Fig. 2b) in the temperature range of 48–93.3 and 112–155°C and the corresponding water loss was 20.2 and 43.32%, respectively. This implies that only 20% of water is available in epidermis which is liberated first and remaining 37% of water (in comparison to epidermis treated with water) was mixed with PG. In case of 100% PG treated epidermis, no water loss was found below 112°C

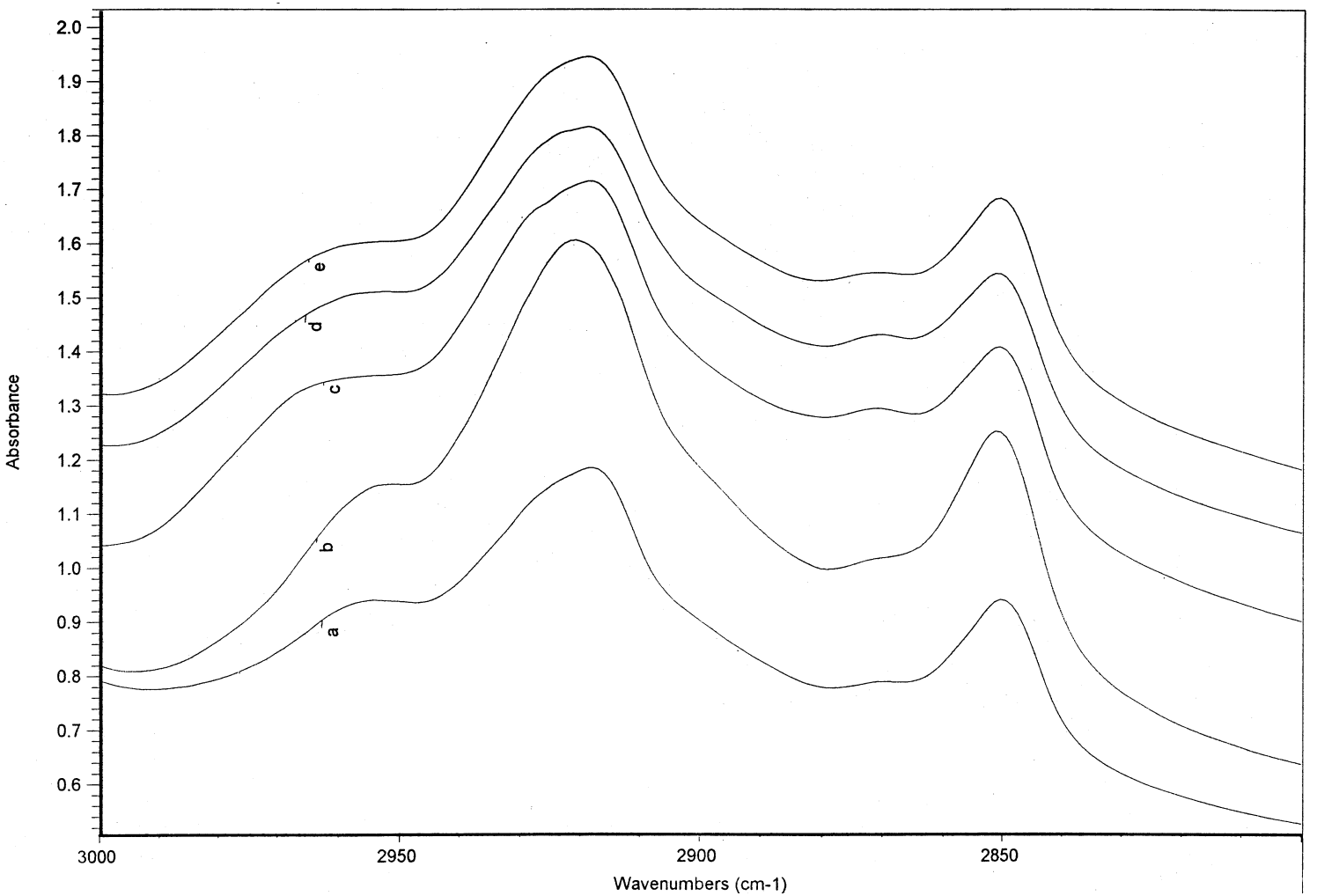

Fig. 1. FT-IR spectra of rat skin after 6 h treatment with PG (a and b), control before and after treatment with water; (c) 50% PG; (d) 66% PG; (e) 100% PG.

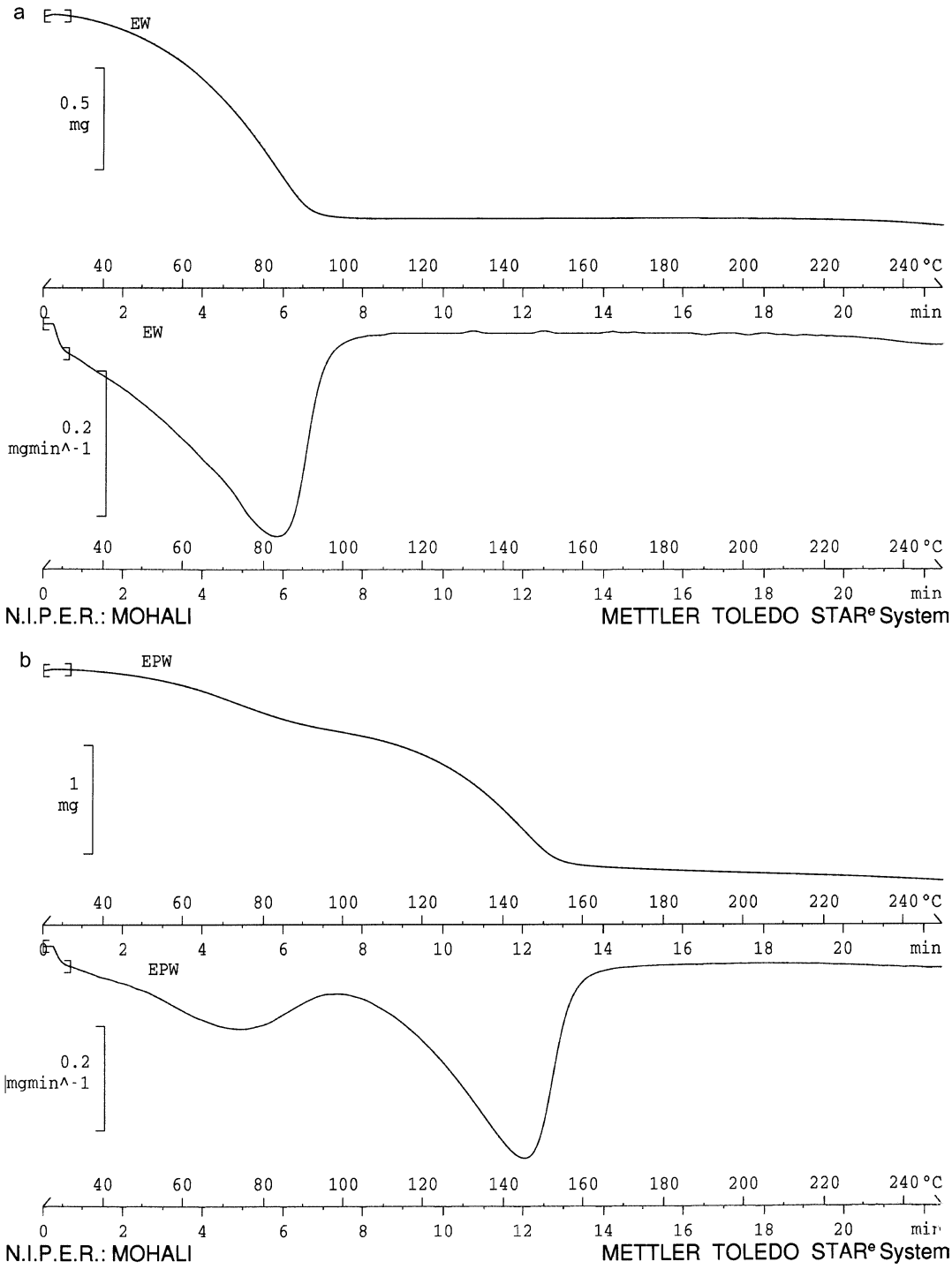

Fig. 2. TGA of epidermis treated with (a) water; (b) 66% PG in water; (c) with PG; (d) with PG and solvent PG.

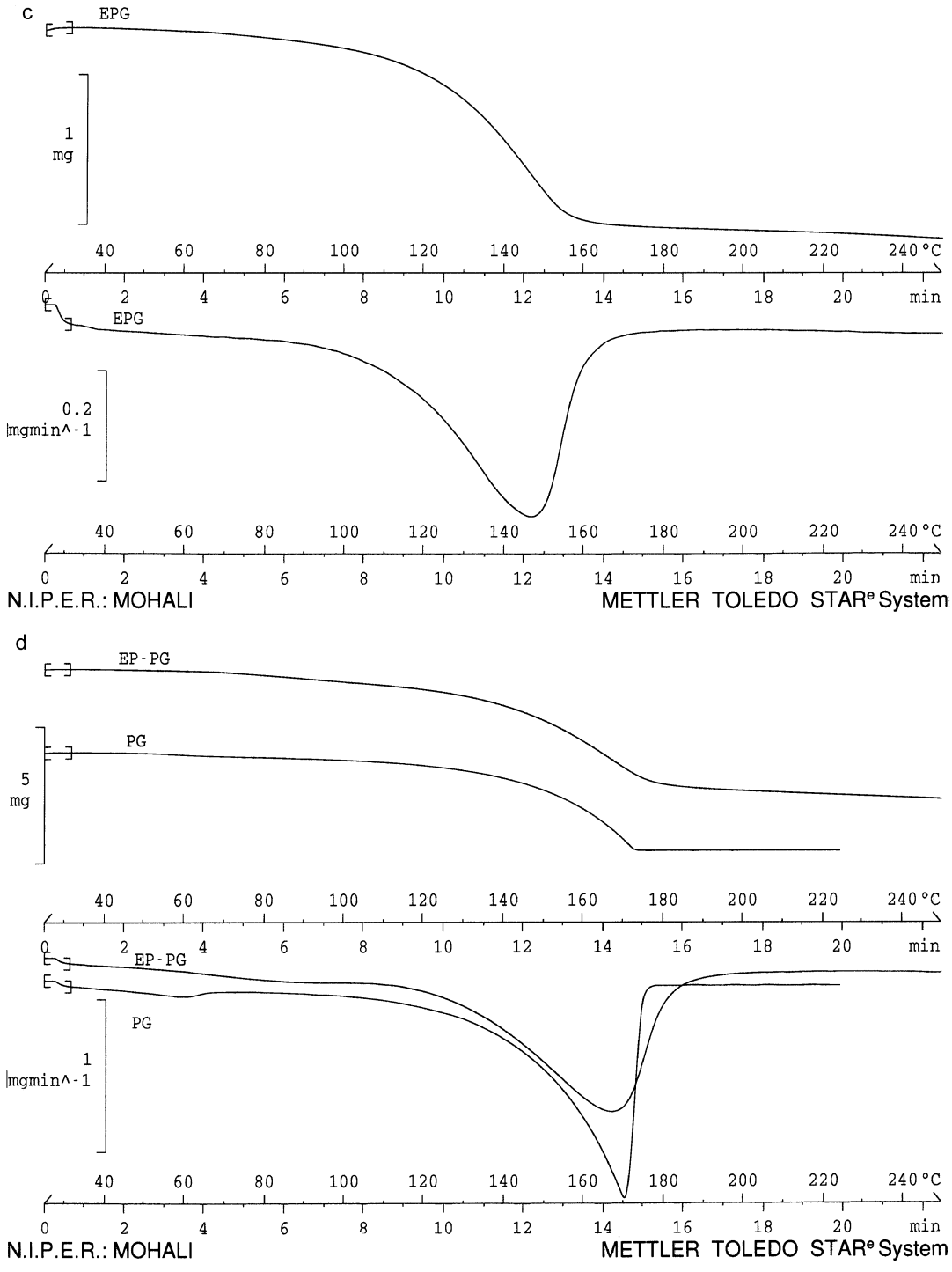

Fig. 2. (*Continued*)

(Fig. 2c) and its first derivative TGA was similar to that of PG (Fig. 2d), indicating that the epidermis treated with 100% PG is completely dehydrated. Which indicates that PG is responsible for complete dehydration of epidermis and results in higher barrier property for drug permeation. At 66% of PG the hygroscopic requirements of PG are compensated by the water present in the solvent system and therefore, in this case, PG takes up water from lipid bilayer and corneocytes to a minimum extent and hence the natural barrier property of skin is not altered. Moreover, at 66% of PG in water, PG acts as a penetration enhancer by either of the aforementioned mechanisms. Whereas, at 100% of PG, there is no free water in solvent system thus PG extracts water from the lipid bilayer and corneocytes and thereby leading to an increase in the barrier property of SC.

The flux of naloxone in group B was greatest with 66% of EtOH in water ($P < 0.05$). At 100% of EtOH, flux declined and there was no significant difference between flux of naloxone at 100, 50 and 33% of EtOH as determined by SNK test ($P < 0.05$). In the FT-IR investigations it was found that there was broadening of peaks at 2920 cm^{-1} with increasing concentration of EtOH (Fig. 3) which indicates that EtOH increase the rotational freedom of lipid acyl chains leading to increase in fluidity of lipid bilayer.

Penetration enhancing activity if EtOH can be attributed to two effects; (a) increase in thermodynamic activity due to evaporation of EtOH, known as 'push effect' (Kadir et al., 1987) and (b) 'pull effect', in which permeation of drug molecule is increased due to reduction in barrier property of SC by EtOH. Berner et al. (1989) reported the concentration dependent effect of EtOH. Many reports have supported this concentration dependent effect of EtOH and it was hypothesised that at low concentration (< 33% or 0.3 v/v) of EtOH, only lipoidal pathway is affected while at higher concentration polar pathway is also affected. Though naloxone is hydrophilic compound, its flux with 100% EtOH was found lesser than that of 66% EtOH ($P < 0.05$). This may be due to stabilisation of gel phase of lipid bilayer at 100% of EtOH and leading to rigidisation of lipid bilayer (Rowe, 1983).

Table 2
Patch size and theoretical blood levels that can be achieved[a]

Patch size (cm^2)	Theoretical blood levels (ng ml^{-1})
5	2.1
10	4.2
20	6.3
25	10.5
40	16.8
50	21.0

[a] With the input rate of 33 μg cm^{-2} h^{-1}, found in 33% PG in EtOH.

In group C, maximum flux of naloxone was found with 33% PG in EtOH ($P < 0.05$). As the concentration of PG increased beyond 50% in EtOH, significant reduction in flux of naloxone was found ($P < 0.05$) and minimum flux was found with 100% of PG. Among all the solvent compositions studied, maximum flux was obtained with 33% PG in EtOH. This result was supported by FT-IR data, and it was found that broadening of peak was greatest with 33% PG in EtOH (Fig. 4). Increased thermodynamic activity of naloxone by both EtOH and PG, and reduction in the barrier property of SC by EtOH are thought to be the reasons for the higher flux of naloxone in 33% PG in EtOH.

The lag time with 100% of PG was found to be maximum and was significant ($P < 0.05$). The lag time of 66% EtOH in PG was significantly higher than that of 66% EtOH in water ($P < 0.05$). However, there was no significant difference between flux of these solvent combinations ($P < 0.05$). Maximum lag time was found with PG because it increases the tortuosity of skin by dehydration of SC, which will results in increased diffusional path length (Ostrenga et al., 1971).

Ex vivo permeation data of 33% PG in EtOH (33 μg cm^{-2} h^{-1}) can be translated into meaningful blood levels of naloxone, (0.42 ng ml^{-1} × surface area). The systemic pharmacokinetic parameters used in calculations of blood levels were taken from the literature (Hardman and Limbard, 1996). Since the theoretical blood levels that can be achieved were well above the therapeutic requirements, there is flexibility in choice of patch size depending on the addiction status of the patient to be treated (Table 2).

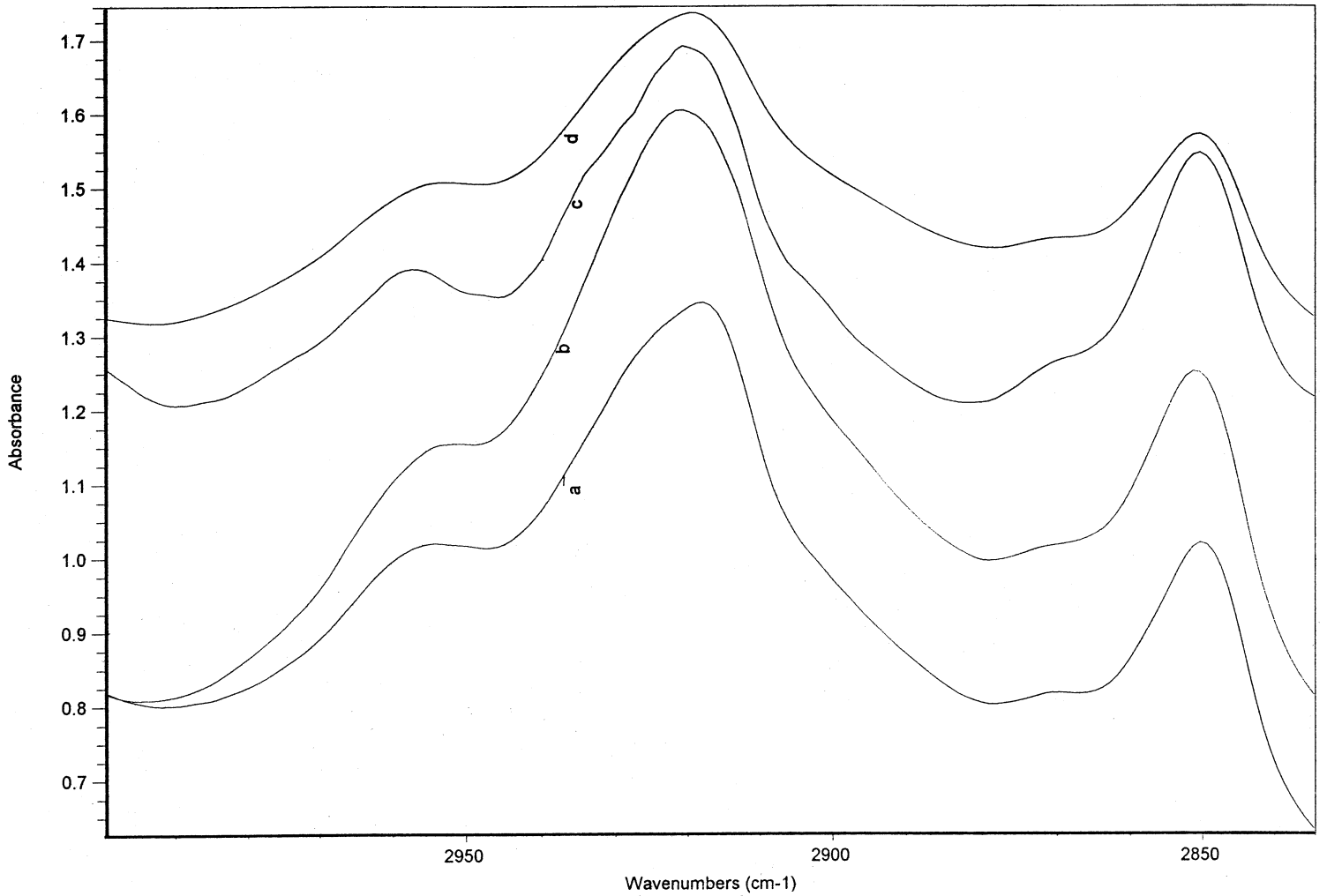

Fig. 3. FT-IR spectra of rat skin after 6 h treatment with EtOH (a and b) control before and after treatment with water; (c) 50% EtOH; (d) 66% EtOH.

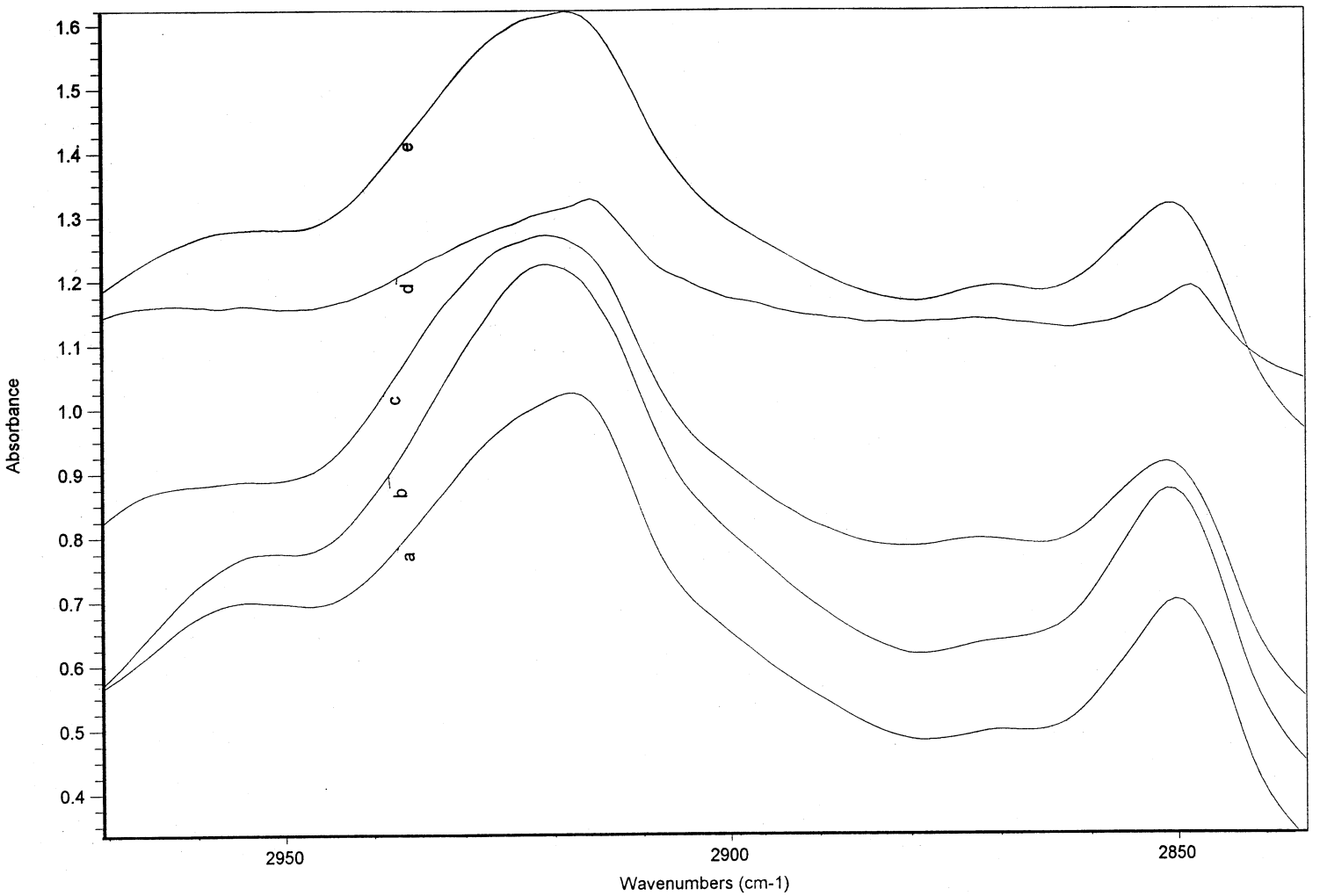

Fig. 4. FT-IR spectra of rat skin after 6 h treatment with PG in EtOH (a and b) control before and after treatment with water; (c) 50% PG; (d) 66% PG; (e) 100% PG.

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