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A comparison of the effects of electrical current and penetration enhancers on the properties of human skin using spectroscopic (FTIR) and calorimetric (DSC) methods

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Abstract

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and differential scanning calorimetry (DSC) studies were undertaken to compare the effects of iontophoresis with those of penetration enhancers on the structural properties of the human stratum corneum. These investigations clearly show the combined ability of these two techniques to detect changes in the lipids and proteins of the skin. Our spectroscopic results confirm that treatment with DMSO causes extensive lipid extraction as investigations with a range of other techniques had previously suggested. In addition, the DSC results indicate that DMSO causes stratum corneum protein denaturation. Interestingly, cineole also caused extensive lipid extraction, thus suggesting that lipid extraction may play a role in the transdermal penetration enhancing effect of cineole. Oleic acid/ethanol treatment not only caused an increase in the average lipid acyl chain disorder of treated samples but also led to extensive lipid extraction. In contrast, when ATR-FTIR spectroscopy and DSC were used to evaluate the effect of electrical treatment on the stratum corneum, minimal change was observed on the lipid-cellular matrix; it would appear that electrical treatment exerts a less disruptive influence on the stratum corneum than treatment with penetration enhancers.

Key words: Calorimetry; Spectroscopy; Stratum corneum; Oleic acid; Cineole; DMSO; Electrical field

1. Introduction

The stratum corneum is the major rate-controlling step in the transdermal delivery of drugs. A non-uniform inhomogeneous membrane, it consists of terminally differentiated, keratin rich corneocytes embedded in a multilamellar extracellular matrix (Bommannan et al., 1990; Kurihara-Bergstrom et al., 1990). The relative impermeability of the stratum corneum to the passage of drugs limits the number of potential candidates for transdermal delivery. Hence, a range of different approaches have been investigated in order to increase the permeability of the stratum corneum: these include iontophoresis (LeDuc, 1908; Corish et al., 1989; Srinivasan et al., 1989),

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the use of chemical penetration enhancers (Astley and Levine, 1976; Francoeur et al., 1990; Williams and Barry, 1991) and sonophoresis (Skauen et al., 1984; Tyle and Agrawala, 1989).

DSC and ATR-FTIR spectroscopy provide independent yet complementary information on the structure of the lipid-cellular matrix of the stratum corneum. The IR spectrum provides information on the vibrational modes of its components and hence probes the structure on a molecular level. In contrast, transitions in the DSC thermal profile reflect the 'melting' of extended structural domains which result in changes in heat capacity and so provide information on a macroscopic level (Golden et al., 1986).

The effects of penetration enhancers on the spectroscopic and calorimetric properties of skin are well documented (Goodman and Barry, 1986; Williams and Barry, 1989; Mak et al., 1990; Bommannan et al., 1991). These studies have provided considerable insight into the interactions of penetration enhancers with the lipids and proteins of the lipid-cellular matrix of the stratum corneum and hence on their mechanisms of action. In this study the effects of a range of penetration enhancers on the calorimetric and spectroscopic properties of human stratum corneum are investigated, not only to confirm previous observations but also to evaluate the possible role of lipid removal in their penetration enhancing activities. Against this background the effects of applied electrical fields on the DSC and FTIR profiles of skin were investigated. One of the factors of primary importance in determining the effectiveness of electrically assisted transdermal drug delivery is the iontophoretically induced increase in skin permeability (Srinivasan et al., 1989). This increase in permeability is evident from the marked increases in the passive transdermal fluxes of drugs which have been observed by many workers immediately post iontophoresis in both hairless mouse skin (Bellantone et al., 1986; Srinivasan et al., 1989) and human skin in vitro (Corish et al., 1989). In this study the effects of applied electrical fields on the spectroscopic and calorimetric properties of stratum corneum are investigated and compared with those produced by penetration enhancers in an effort to elucidate the possible effects, if any, induced on the lipid-protein matrix of the skin by iontophoresis.

2. Materials and methods

2.1. Stratum corneum preparation

Sheets of stratum corneum were prepared from human mid-abdominal skin, obtained within 48 h postmortem, according to the method of Kligman and Christophers (1963). Following isolation from the epidermis, the stratum corneum was dried and stored in a desiccator at room temperature until used. Each experiment required approx. 10 mg dry stratum corneum sheet hydrated to the desired hydration level, H.L., where H.L. = (hydrated sample weight − dry sample weight) × 100%/dry sample weight. Hydration levels of 30% (w/w) were achieved by enclosing the dry stratum corneum in an enclosed chamber of 96% R.H. for several days at room temperature (Potts et al., 1991). Hydration levels of 100% (w/w) were achieved by immersion of the dried stratum corneum samples in phosphate buffer solution, pH 7.4, for approx. 5 h.

2.2. Infrared spectroscopy

The Fourier transform infrared studies of the stratum corneum were performed using a Nicolet 5ZDX FT-IR Spectrometer equipped with an ATR accessory which supported a trapezoidal ZnSe internal reflection element ($7.5 \times 1 \times 0.5$ cm, with a 60° incident angle). All spectra (2 cm^{-1} resolution, representing the average of 64 scans) were obtained in the frequency range 4000–400 cm^{-1}. Unless otherwise stated, the possible role of sample hydration in observed spectral changes was controlled by ensuring that samples were rehydrated before spectra were recorded. The samples were covered during analysis to avoid water loss. Among the effects investigated were the following:

2.2.1. Effect of 1,8-cineole

The spectra of samples were recorded (i) immediately post-immersion in 1,8-cineole for 1 h

(Williams and Barry, 1990) and (ii) after being allowed to rehydrate to 30% hydration post-immersion in 1,8-cineole.

2.2.2. Effects of dimethyl sulphoxide, oleic acid / ethanol and chloroform / methanol

The spectra of 30% hydrated samples were recorded before and after immersion in each of 100% DMSO, 0.15 M oleic acid in ethanol and chloroform/methanol (2:1 v/v) for 24 h (Khan and Kellaway, 1989), 2 h (Potts et al., 1991) and 16 h (Knutson et al., 1985), respectively.

2.2.3. Effect of electrical current

The spectra of 100% hydrated samples were recorded before and after being subjected to current densities of up to 1.25 mA/cm^2 for 60 min using the two-compartmental, isotonic phosphate buffer filled, solution-to-solution iontophoretic procedure shown schematically in Fig. 1a.

2.2.4. Effect of applied voltage

The possible effects of applied voltage on the FTIR spectra of 30% hydrated stratum corneum were examined in situ using two electrode arrangements that are shown in Fig. 1b and c: the second arrangement was adopted to ensure that the current passed directly through the skin. High vacuum grease (Dow Corning®) was applied to the upper surface of the stratum corneum to prevent the developement of shunt pathways between the two electrodes along the surface of the membrane (Burnette, 1989). Voltages in the range 5–30 V were applied for 60 min: the FTIR spectra were simultaneously recorded at regular intervals during that period.

2.3. DSC

The DSC studies of stratum corneum were performed using a Mettler DSC 20 equipped with a Mettler TC 10 A TA processor. All samples were hermetically sealed, as previously advocated (Bouwstra et al., 1991) to avoid evaporation of water. The scan rate was 1 K/min. in the temperature range 303–403 K. Among the processes investigated were: (a) Effect of hydration. (b)

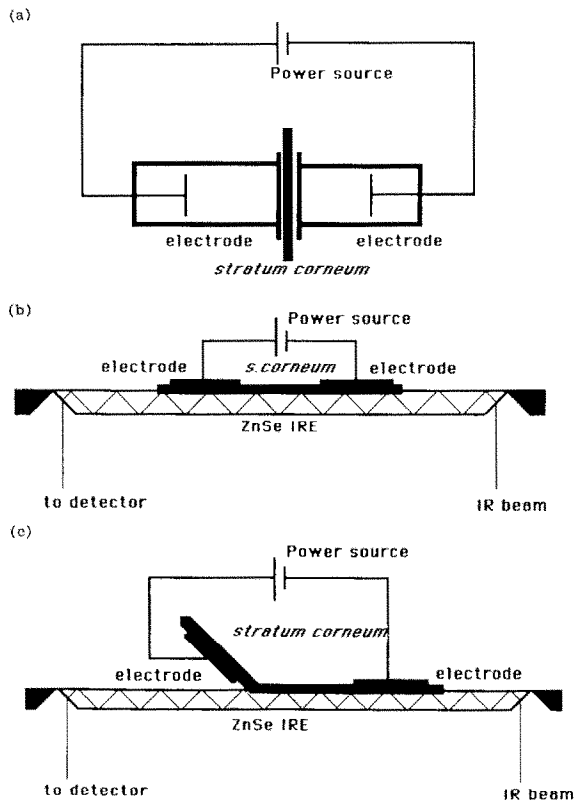

Fig. 1. (a) Schematic representation of the solution-to-solution iontophoretic procedure; (b,c) the two arrangements employed for the in situ spectroscopic study described in the text.

Effect of reheating; 100% (w/w) hydrated samples were heated to 403 K, allowed to cool to 303 K after which the samples were reheated to 403 K. (c) Effect of delipidisation; the DSC thermogram of stratum corneum was recorded following immersion in chloroform/methanol (2:1 v/v) for 16 h and subsequent rehydration to 100% (w/w). (d) Effect of DMSO; a 100% hydrated sample was immersed in DMSO for 2 h following which excess accelerant was removed and the sample sealed and scanned (Goodman and Barry, 1986). (e) Effect of electrical current; the DSC of 100% hydrated stratum corneum through which current densities of up to 1.25 mA/cm^2 had been passed for 60 min was recorded.

3. Results and discussion

The IR absorbance spectrum from 1000 to 4000 cm^{-1} of a sample of human stratum corneum at 296 K and 0.3 g/g water content is shown in Fig. 2. The spectrum is typical of hydrated biological materials with strong amide and water absorbances in the regions of 1500–1700 and 3000–3600 cm^{-1}, respectively. Of particular interest are the peaks near 2850 and 2920 cm^{-1} which are due to symmetric and antisymmetric carbon-hydrogen (C-H) stretching, respectively. The major contribution to the C-H stretching peaks of the stratum corneum is the absorbance of the hydrocarbon chains of the lipids (Golden et al., 1986). Hence, the delipidisation treatment of the stratum corneum with chloroform/methanol (2:1 v/v) dramatically reduced both the antisymmetric and symmetric C-H stretching absorbances as shown in Fig. 3. An increase in the degree of disorder of the lipid acyl chains results in a blue shift of the C-H stretching absorbances, i.e., a shift to higher wavenumber (Casal and Mantsch, 1984). Other FTIR spectral parameters which may be used as indices of relative lipid acyl chain disorder are the bandwidth at 70% height of the C-H stretching absorbance (Knutson et al., 1990) and the ratio of the intensities of the C-H asymmetric and symmetric absorbances (Yau, 1984). DSC thermograms of hydrated stratum corneum show a characteristic irreversible peak attributable to proteins (Goodman and Barry, 1986) at approx. 376 K (Fig. 4a). This peak is thought to relate to the α to β transformation of intracellular keratin (Khan and Kellaway, 1989). Its irreversible nature was easily shown (Golden et al., 1987a) by heating 100% hydrated stratum corneum to 403 K. The protein peak was absent from subsequent thermograms (Fig. 4a). In addition, the protein peak is strongly water dependent and disappears at low water contents in the stratum corneum (Fig. 4a) consistent with the findings of Goodman and Barry (1986). However, as expected (Goodman and Barry, 1986), this peak was not affected by the delipidisation treatment of the stratum corneum (Fig. 4a).

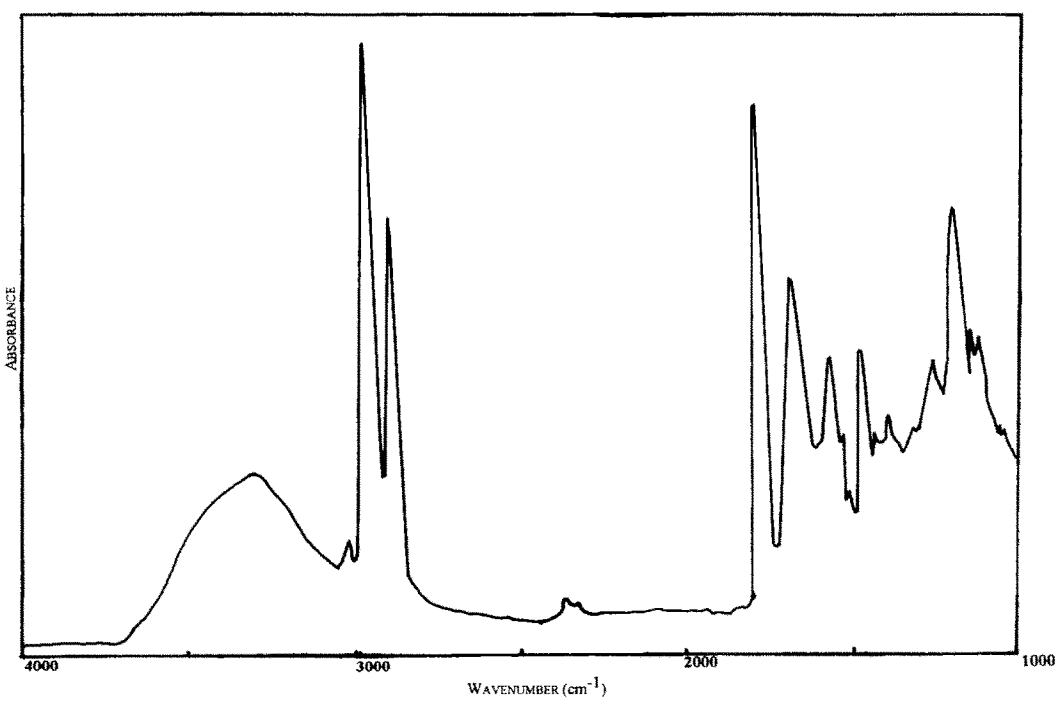

Fig. 2. ATR-FTIR spectrum from 1000 to 4000 cm^{-1} of human stratum corneum at 296 K and 0.3 (g/g) water content.

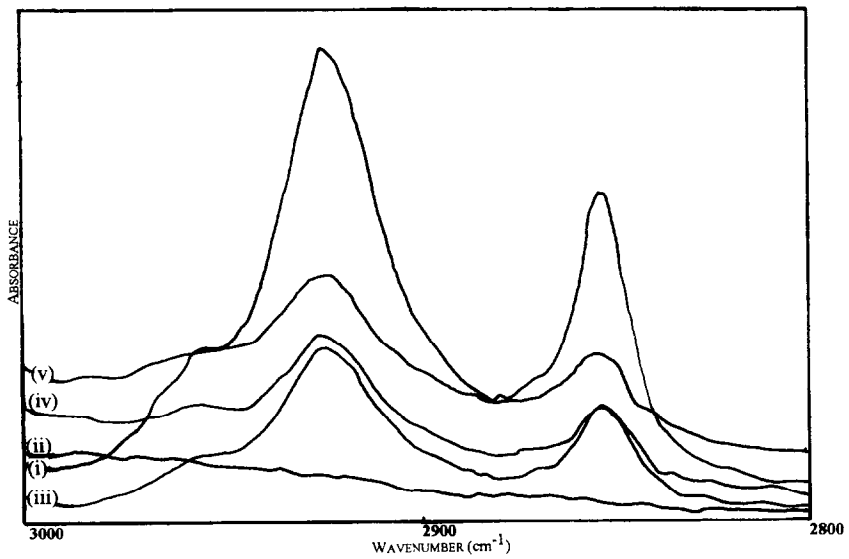

Fig. 3. ATR-FTIR spectra of human stratum corneum in the C-H stretching region between 2800 and 3000 cm^{-1}. (i) Spectrum of an untreated sample. [(ii)–(v)] Spectra obtained following treatment of stratum corneum with each of chloroform/methanol, 1,8-cineole, DMSO and oleic acid/ethanol, respectively. All spectra were obtained at 296 K with samples hydrated to 30% (w/w) water content.

3.1. Chemical enhancers

3.1.1. Cineole

As the C-H stretching absorption of cineole caused interference with the corresponding stratum corneum lipid peak, it was not possible to confirm whether or not 1,8-cineole fluidised the highly ordered crystalline structure of the stratum corneum as has been suggested previously (Williams and Barry, 1989). However, 1,8-cineole caused extensive extraction of stratum corneum lipids: both of the lipid C-H stretching absorbances were markedly reduced in the IR spectra of samples rehydrated to 100% (w/w) post-immersion in the terpene, as shown in Fig. 3. This suggests that lipid extraction may play a significant role in the penetration enhancement effects of cineole.

3.1.2. Oleic acid / ethanol

Treatment of human stratum corneum with oleic acid in ethanol produced a blue shift in the asymmetric C-H stretching peak position (Golden et al., 1987b; Potts et al., 1991; Walker and Hadgraft, 1991): the peak position changed from 2921.8($\pm$0.2) cm^{-1} pretreatment to 2923.9($\pm$0.2) cm^{-1} post-treatment. Initial interpretation of these results suggested that incorporation of the *cis* monounsaturated acid into the primarily saturated-like domain of stratum corneum lipids causes increased lipid fluidity due to differences in lipid packing (Guy et al., 1990; Potts et al., 1991). ESR spectroscopy of human stratum corneum treated with oleic acid lends support to this hypothesis (Gay et al., 1991). However, Francoeur et al. (1990) proposed that oleic acid present in stratum corneum is heterogeneously dispersed and not tightly clustered, resulting in an increase in the solid-fluid phase separation of the stratum corneum lipid domains observed at physiological temperatures. It was suggested that the penetration enhancement effect of oleic acid may result from diffusion of drug across these fluid domains or permeable interfacial 'defects'. Contrary to the increase in lipid 'fluidity' previously proposed, this mechanism would account for the significant penetration enhancement observed at physiological temperatures. Further work (Ongpipattanakul et al., 1991) on porcine stratum corneum employing perdeuterated oleic acid con-

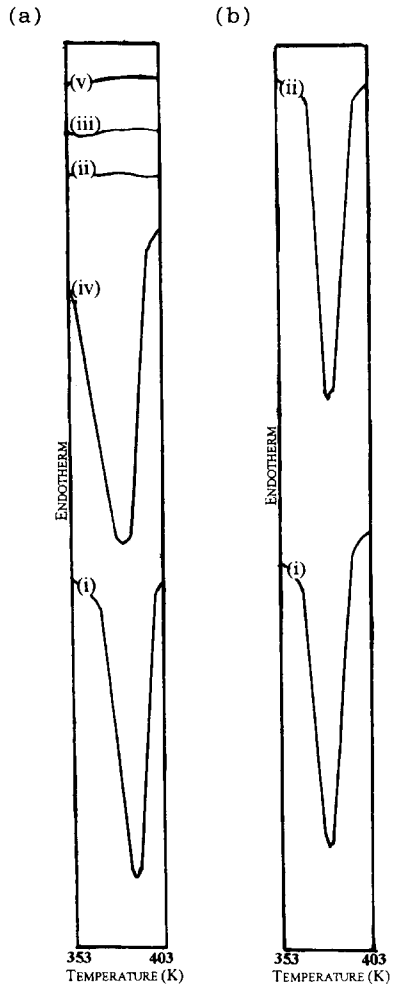

Fig. 4. (a) DSC thermal profiles of human stratum corneum from 353 to 403 K. (i) Thermal profile of an untreated sample hydrated to H.L. 100%. (ii) Thermal profile obtained upon reheating of the same sample. (iii) Thermal behaviour of a sample of dried stratum corneum. [(iv),(v)] DSC thermograms of samples treated with chloroform/methanol and DMSO, respectively. (b) DSC thermal profiles of human stratum corneum from 353 to 403 K. Trace (i) shows the thermal profile of an untreated sample hydrated to H.L. 100% whereas (ii) shows the thermal behaviour of a sample hydrated to H.L. 100% through which constant current of density 1.25 mA/cm^2 was passed for 60 min.

firmed the coexistence of fluid oleic acid and ordered stratum corneum lipids at physiological temperatures. Interestingly, it has been observed that oleic acid does not distribute homogeneously within dipalmitoylphospatidylcholine (DPPC) liposomes, thus further supporting the theory that oleic acid forms pools within structured lipids and hence the solid-fluid phase separation theory of penetration enhancement (Watkinson et al., 1991). However, to date, neither theory has been unequivocably accepted. Penetration enhancement could result from a combination of both mechanisms (Walker et al., 1991). In addition, it was noted that pretreatment with oleic acid/ethanol also resulted in stratum corneum lipid extraction as shown in Fig. 3. This is consistent with the observation that treatment of human skin with oleic acid/ethanol dramatically reduces the skin's resistance (Nolan et al., 1993). Thus, it is not inconceivable that lipid extraction plays a role in the penetration enhancing effects of oleic acid/ethanol.

3.1.3. DMSO

Characteristically (Goodman and Barry, 1986; Khan and Kellaway, 1989), immersion in pure DMSO caused the removal of the protein peak from the DSC thermogram of stratum corneum (see Fig. 4a). In addition, lipid extraction, consistent with earlier observations employing different experimental techniques (Allenby et al., 1969; Khan and Kellaway, 1989), was observed in our spectroscopic study as is shown in Fig. 3 although no significant change was effected in the C-H stretching absorbance peak positions.

As a transdermal penetration enhancer, DMSO exhibits an unusual concentration dependence: usually only high concentrations (above 60%) promote steady-state drug permeation through the skin. A similar concentration dependence has been observed in interactions of DMSO with stratum corneum lipids, thus implying that it is this interaction that is important in its enhancing action (Barry, 1987). However, as the results shown here suggest, delipidisation and protein denaturation may also play significant roles in its accelerant action (Khan and Kellaway, 1989). The dramatic and irreversible decrease in the absolute magnitude of the impedance of skin treated with DMSO/water mixtures of greater than 70% DMSO also supports the role of delipidisation (Foley et al., 1992).

3.2. Electrical treatment

The above studies clearly show the combined ability of these two techniques to detect changes in the lipids and proteins of the stratum corneum. Solution to solution iontophoresis did not effect any significant changes in any of the spectroscopic parameters investigated as indices of stratum corneum lipid disorder in spectra of samples examined post-iontophoretically, even though relatively high current densities (Sanderson et al., 1989) produced by voltages of up to 8 V were employed. Because of the possible reversible nature of any induced changes (Corish et al., 1989; Foley et al., 1992), it was necessary to design an in situ spectroscopic analysis technique. The ZnSe crystal employed in the ATR-FTIR studies allows the IR beam to penetrate to a depth of about 0.8 μm into the stratum corneum (Mak et al., 1990). Hence, in view of the lack of knowledge relating to the distribution of applied electrical field lines within the skin (Leveque and De Rigal, 1983), two different in situ systems were employed to ensure that the applied field lines passed through the region of the stratum corneum being analysed (Fig. 1b and c). However, no significant changes were observed in the resulting spectra even though voltages of up to 30 V were employed (Fig. 5). Combining these results, it is possible to conclude that even relatively high current densities and relatively strong applied fields did not cause detectable fluidisation in the highly ordered lipid structure of the stratum corneum.

Passage of constant current through the skin did not appear to affect the endotherm attributed to proteins in DSC thermograms, i.e., even relatively large current densities did not cause detectable denaturation of the stratum corneum proteins (see Fig. 4b). In situ investigations were obviously not possible in the DSC studies.

On the basis of these results, it appears that the effect of the passage of current on the lipid-cellular matrix of the stratum corneum is minimal. This suggests that the structural basis for iontophoretically induced increases in skin permeability is more probably associated with the pores of the skin. Analysis of the relevant literature strongly supports the importance of pores in

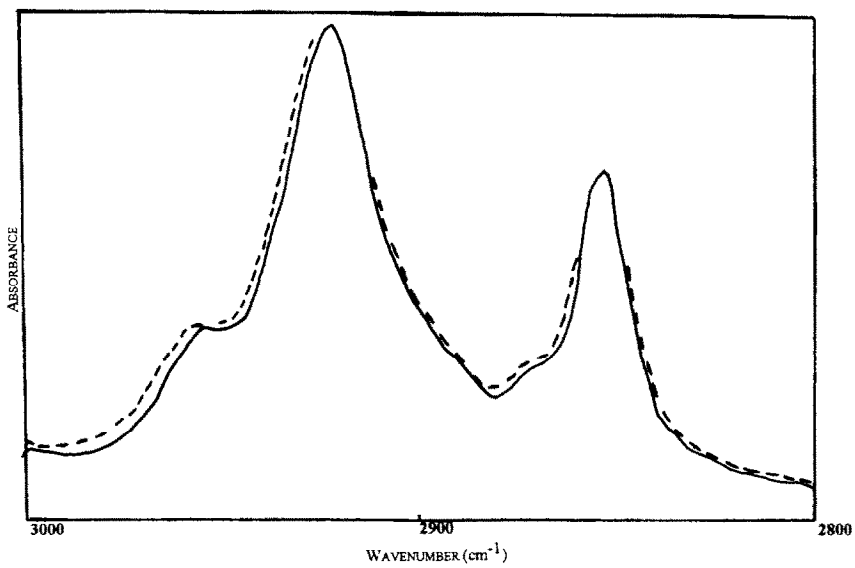

Fig. 5. ATR-FTIR spectra of human stratum corneum (hydrated 30% (w/w)) from 2800 to 3000 cm^{-1}. The spectra were obtained from the same sample before (solid line) and during (broken line) the application of a constant voltage of 30 V using the procedure illustrated schematically in Fig. 1b.

the current assisted transdermal flux of charged solutes (Abramson and Gorin, 1940; Grimnes, 1984; Burnette and Bagniefski, 1988; Foley et al., 1992). Initially, these pores were reported to be the hair follicles and sweat glands, i.e., of appendageal origin (Abramson and Gorin, 1940; Abramson and Engel, 1941; Grimnes, 1984; Cullander and Guy, 1992). However, the true identity of the pores has not been definitively elucidated through complementary morphological studies. One cannot ignore the possibility that molecular-sized pores may be present or may be formed in the lipid matrix of the stratum corneum during iontophoresis treatment (Wearley et al., 1989). Electroporation – the creation by an electrical field of transient pores in lipid bilayers and cell membranes – has been suggested as a phenomenon which occurs in lipid bilayers (Prausnitz et al., 1992).

It would be premature, however, to suggest that all iontophoretic transport occurs though appendages in the skin: there has been increasing evidence of the role of the intercellular route in the percutaneous transport, current assisted or otherwise, of charged solutes (Sharata and Burnette, 1988; Bodde et al., 1991; Potts et al., 1992). It should be remembered that iontophoresis, by increasing transdermal flux, possibly increases skin drug loading which may consequently lead to the higher post-iontophoretic fluxes observed (Corish et al., 1989).

4. Conclusion

These biophysical investigations clearly illustrate the disruptive influences of the chemical penetration enhancers studied on the lipid-protein matrix of the stratum corneum. Although previous work suggested that cineole fluidises the highly ordered crystalline structure of the stratum corneum, our results indicate that cineole treatment also leads to extensive stratum corneum lipid extraction. Our FTIR results confirmed previous suggestions that DMSO causes lipid extraction. In addition, the results confirm that DMSO causes stratum corneum protein denaturation. Oleic acid/ethanol treatment not only results in lipid extraction but also causes an increase in the average acyl lipid chain disorder of treated samples. In contrast, our studies on the effect of electrical treatment on the stratum corneum indicate minimal influences on the lipid-cellular matrix. This suggests that the structural basis for iontophoretically induced increases in skin permeability is more probably associated with the pore pathways of the skin. Hence, one may conclude that electrical treatment exerts a less disruptive effect on the lipids and proteins of the stratum corneum than the penetration enhancers investigated.

5. Acknowledgement

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