Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, Nos 10–12, pp. 1089–1094, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00
Pergamon Press plc

The use of FT-IR for the determination of stratum corneum hydration *in vitro* and *in vivo**

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Abstract: Measurement of the water content of stratum corneum plays an important role in physiological and therapeutic inquiries in dermatology. There are many techniques available for non-invasive determination of skin hydration such as measurement of electrical, mechanical, thermal and spectroscopic properties of the skin. Most techniques, however, suffer from the fact that they do not employ a direct measurement of water content rather a property caused by skin hydration. Recently, Potts *et al.*, (*Arch. Derm. Res.* **277**, 489–495, 1985) developed an FT-IR method for the determination of water content of the skin both *in vitro* and *in vivo*. The method employed attenuated total reflectance infrared (ATR-IR) to measure a weak O—H stretch formed by the presence of water at 2100 cm^{-1}. This absorbance is distant from interferences due to skin and most topically applied substances and therefore may be used in the quantitation of skin water content (hydration). This report describes the use of this technique in an investigation into the effect of occlusion on the water content of the skin. Method development and validation employing an *in vitro* system is also discussed.

Keywords: *FT-IR; ATR; stratum corneum; hydration; hydrocolloid dressing.*

Introduction

The water content of the stratum corneum plays an important role in physiological and therapeutic investigations both in terms of its function and cosmetic properties. Recently, many non-invasive techniques have been developed specifically for measurement of stratum corneum hydration. These techniques include measurements of electrical [1, 2], mechanical [3, 4], thermal [5] and spectroscopic [6, 7] properties of the skin. However, most of the aforementioned techniques measure a property caused by hydration rather than direct hydration of the stratum corneum, with the disadvantage that the theoretical relationships between the two changes is not always clearly understood [8].

Infrared spectroscopy has previously been used in the determination of skin hydration in a number of ways with the use of the attenuated total reflectance (ATR) accessory. Early investigations into stratum corneum hydration focused on a peak at ≈3400 cm^{-1} corresponding to strong OH stretching [9, 10]. However, there are interferences which contribute to this peak such as amide bonds and C—H asymmetric stretching of stratum corneum lipids. Therefore, this peak cannot be considered specific for water.

More recently, efforts to determine stratum corneum hydration have focused on measurement of the ratio of Amide I (1645 cm^{-1}) to Amide II (1545 cm^{-1}) [11, 12]. These authors suggested that the Amide I band was due to protein and water, whereas Amide II was due to protein alone. However, this ratio is not a precise measure of water content since both Amide I and II bands of protein (keratin) change on hydration [11]. In addition, topical formulations may interfere in this region, therefore a correction to the intensity of the Amide I band was required [12]. Investigations into the use of this technique in this laboratory and by Gloor and co-workers [12], demonstrated a decrease in hydration through the stratum corneum on removal of surface layers by tape stripping. These results conflict with the known increase of water content with depth in the stratum corneum [13] and demonstrate this method to be of limited use.

In addition to a strong O—H stretch (3400 cm^{-1}), there is also a weak O—H stretch at approximately 2100 cm^{-1} in the stratum corneum spectrum. This peak has been investigated by Potts and co-workers in the quanti-

* Presented at the "Third International Symposium on Pharmaceutical and Biomedical Analysis", April 1991, Boston, MA, USA.

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tative determination of stratum corneum water content [7, 8, 13]. The advantage of employing this technique is that the O—H stretch occurs in part of the mid-IR spectrum in which topically applied substances and stratum corneum exhibit no absorbance.

The present report describes the use of this technique for the analysis of stratum corneum *in vivo* following occlusion by hydrocolloid adhesive patches with varying water uptake properties, and a totally occlusive dressing.

Experimental

Materials and methods

Infrared spectra were obtained on a Nicolet 5ZDX Fourier Transform Infrared (FT-IR) Spectrometer equipped with a Specac ATR sampling device. Due to high signal:noise ratio obtained on this instrument, 10 scans (100 scans min^{-1}) were sufficient to obtain spectra adequate for quantitation. This short analysis time limited the possibility of further stratum corneum hydration due to occlusion of the site during the experiment. The ATR had an internal reflectance element (IRE) crystal composed of zinc selenide set at 45°.

Formulations

All hydrocolloid patch formulations employed in *in vivo* hydration studies were obtained from ConvaTec Ltd (Deeside, Clwyd, UK) except Blenderm which was obtained from 3M (Loughborough, UK).

In vitro *spectra*

Human whole skin (epidermis and dermis) was obtained following amputation of the lower leg. The epidermis was then removed by immersion of whole skin in water at 60°C for 45 s. Epidermal cells were subsequently removed from the overlying stratum corneum by digestion with 0.1% trypsin (Sigma, Poole, UK) in phosphate-buffered saline (Sigma) pH 7.4 overnight at 4°C. The resulting stratum corneum was rinsed thoroughly with water and stored on aluminium foil (−20°C) until required.

Hydration of the stratum corneum was achieved by placing the membrane in a covered waterbath (room temperature) overnight which was subsequently removed onto foil for FT-IR and gravimetric analysis.

In vivo *spectra*

The crystal in the flat top ATR system allowed the volar forearm surface to be placed directly onto the sampling device. The volar forearm surface was used for the application of occlusive dressings as this area is relatively hair-free. On removal of occlusive dressings the site was immediately analysed (within 30 s) to prevent skin dehydration. Unoccluded control areas adjacent to experimental sites were also analysed for water content in the *in vivo* studies.

In vitro *water vapour uptake*

Hydrocolloid patches (4 cm × 4 cm) with silicone release paper removed were placed in pre-conditioned polystyrene Petri-dishes and weighed. The samples were subsequently placed in a humidity cabinet (32°C, 97% RH) and removed and weighed after 24, 48, 72 and 96 h. Water vapour uptake was determined as weight gain with time.

Results

Attenuated total reflectance

The ATR phenomenon occurs when radiation propagating through a medium of a certain refractive index (RI) strikes an interface with another medium of lower RI. If the incident beam strikes the interface at an angle greater than the critical angle, the beam penetrates the component of lower RI. The depth of penetration into the medium of lower RI can be calculated as follows:

$$\text{Factor} = \frac{1}{2\pi n_1 [\sin^2\theta - (n_s/n_1)^2]^{1/2}}, \quad (1)$$

where n_1 = RI of the crystal, n_s = RI of sample and θ = angle of crystal.

$$\text{Depth} = \text{Factor} \times \lambda, \quad (2)$$

where λ is equal to the wavelength (μm) of the absorbance band of interest.

In addition, the critical angle can be calculated from the relationship of RI of sample to crystal

$$\theta_c = \sin^{-1}(n_s/n_1). \quad (3)$$

The critical angle must be close to, but less than the beam incident angle in order to obtain adequate penetration into the skin. Also, to achieve good contact of sample to crystal the RI of the crystal should be close to, but greater

than that of the sample. In this report ZnSe was employed with a critical angle of 42°, resulting in a depth of penetration into the stratum corneum of 1.35 μm.

Absorbance ratio (2100 cm^{-1})

The maximum absorbance of the weak O—H stretch occurs at approximately 2100 cm^{-1}. Integration of the peak from baseline points 2280–1900 cm^{-1} produces an absorbance value corresponding to presence of water (A in Fig. 1). To correct for baseline shift, i.e. shift in baseline due to degree of contact of skin with crystal, a background reading from the baseline of the peak to zero baseline (hatched area in Fig. 1) must be taken. This allows for scattered radiation due to the stratum corneum. The ratio of upper to lower areas provides a quantitative measure of water concentration, independent of the stratum corneum–crystal contact. This is defined as the absorbance ratio (2100 cm^{-1}).

In vitro *studies*

Initially, hydration of human stratum corneum was investigated in order to ensure the system was capable of determining water content in a linear fashion. Stratum corneum was floated in a covered water bath overnight, surface uppermost. The hydrated stratum corneum was taken up onto an aluminium foil strip and blotted with tissue paper to remove excess water. The sample was weighed and analysed on the ATR-FT-IR repeatedly until it had reached a constant weight. A plot of absorbance ratio (2100 cm^{-1}) versus weight increase (Fig. 2) demonstrated a linear relationship with a correlation coefficient of r = 0.9813.

In vivo *studies*

A series of *in vivo* studies were subsequently initiated to investigate the effect of occlusion on stratum corneum hydration. A series of hydrocolloid adhesive dressings with known water uptake properties were tested against each other and a completely occlusive adhesive tape — Blenderm.

Actiderm versus Blenderm

Actiderm is an adhesive patch containing three hydrocolloids gelatin, pectin and sodium carboxymethylcellulose (NaCMC). It was originally formulated as a dermatological patch

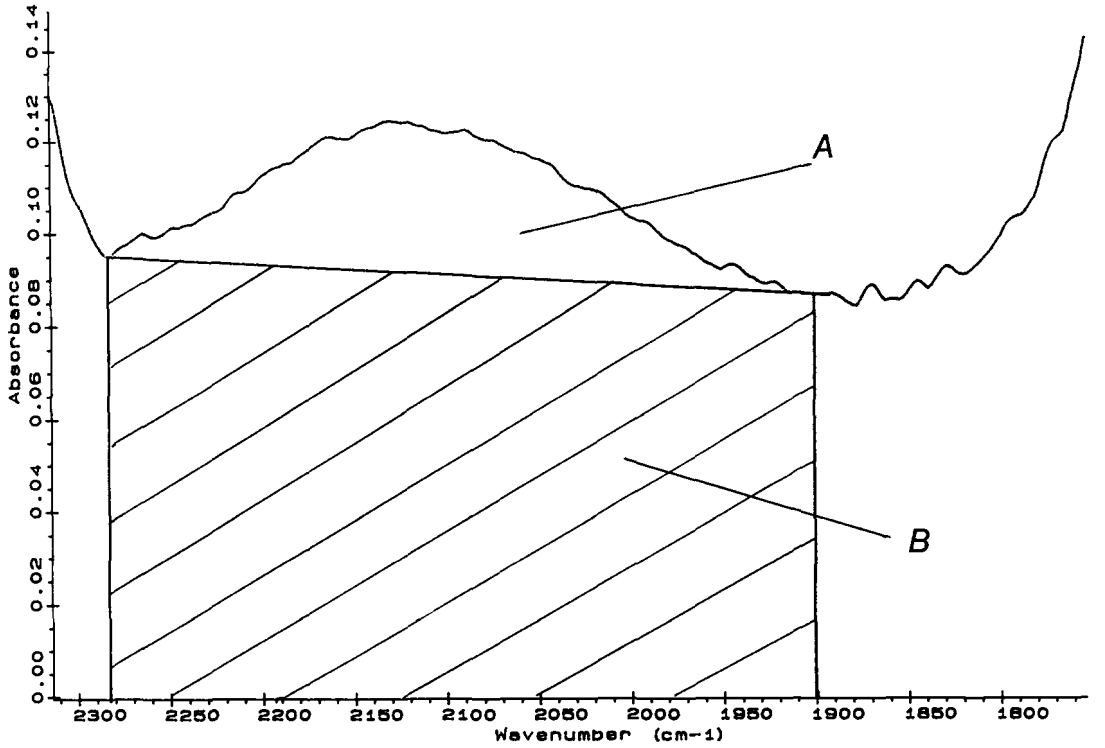

Figure 1
The absorbance ratio calculated from the ratio of the area of the water peak (A) to the area of the background (B). This allows determination of water content of the stratum corneum independent of contact of the sample with the ATR.

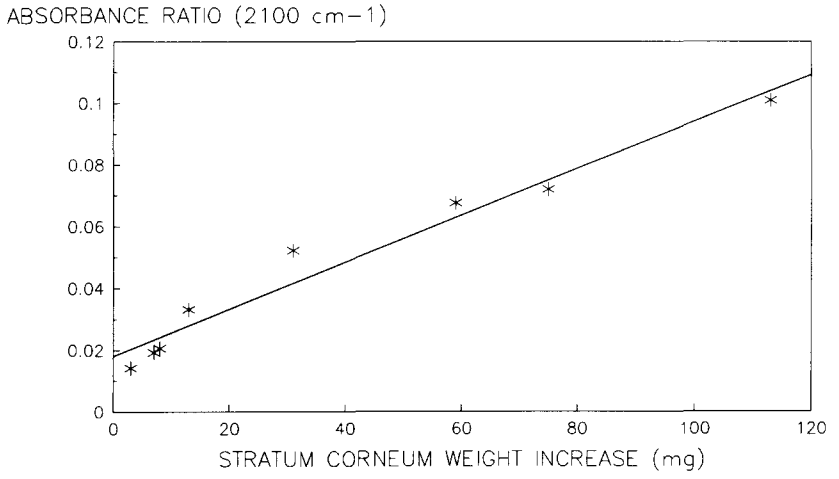

Figure 2
Linearity of absorbance ratio versus weight of water in the stratum corneum. Good linearity with a correlation coefficient of $r = 0.9813$ was achieved employing this technique to assay water in stratum corneum *in vitro*.

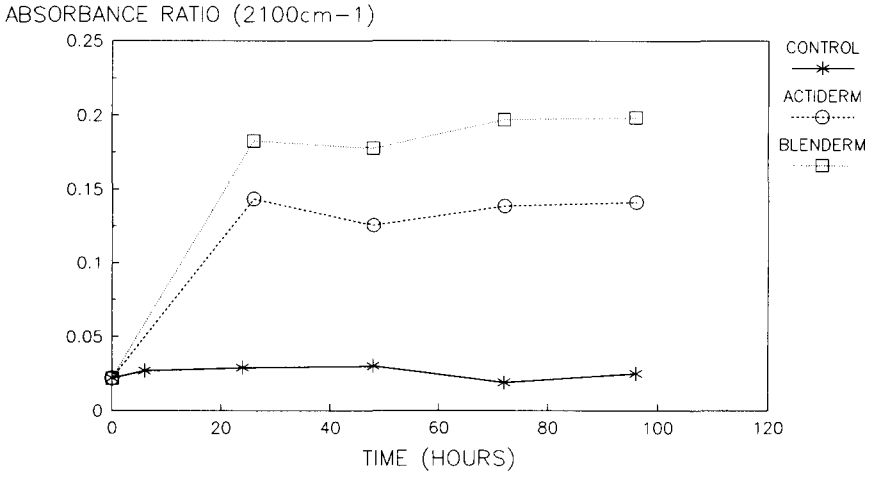

Figure 3
Stratum corneum hydration *in vivo* following occlusion for 0, 24, 48, 72 and 96 h with the Actiderm hydrocolloid patch and Blenderm occlusive tape.

and was designed to be worn for 2–4 days. Therefore, skin hydration, which can cause deleterious effects such as skin maceration or microbial proliferation was controlled by the moisture absorbing hydrocolloids present in the adhesive matrix. In order to demonstrate the water absorbing properties of the patch, Actiderm was compared to Blenderm over a period of 96 h. Figure 3 shows the resulting plot of absorbance ratio ($2100\ cm^{-1}$) versus time in two healthy volunteers following removal of the occlusive materials from their forearm volar surface at 24, 48, 72 and 96 h. The sites treated with Blenderm clearly resulted in increased hydration from 24 h onwards, as demonstrated by the higher absorbance ratio ($2100\ cm^{-1}$) when compared with the water absorbing hydrocolloid patch. Both treatments resulted in a marked increase in stratum corneum hydration when compared to the control unoccluded sites.

It was observed from this study that the initial rate of stratum corneum hydration following occlusion with both Blenderm and Actiderm appeared similar. Therefore, a further study was conducted with the same samples over the initial period of occlusion, i.e. 0–10 h. The results of this study (Fig. 4) demonstrated the initial rate of stratum corneum hydration to be completely independent of the nature of the occluding material.

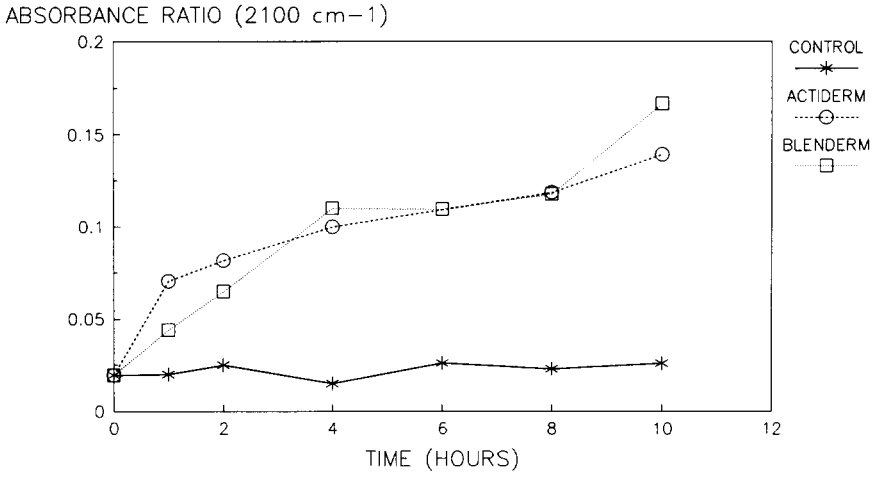

Figure 4
Initial rate of hydration (0–10 h) *in vivo* following occlusion with Actiderm and Blenderm tape.

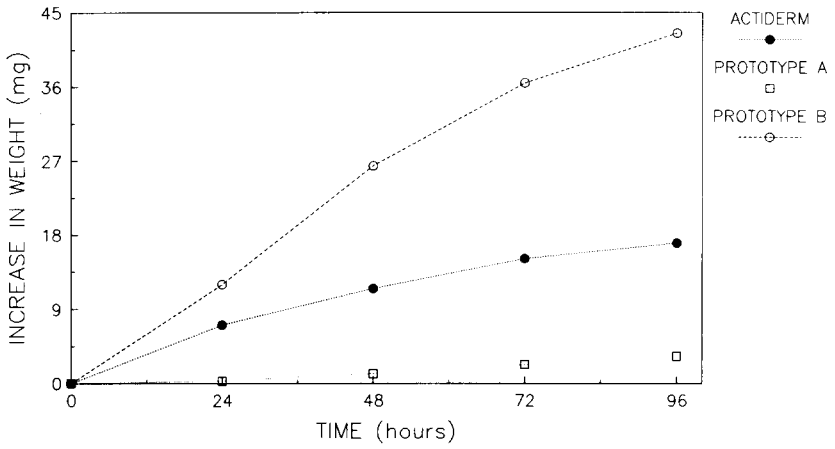

Figure 5
Water vapour uptake of three different hydrocolloid patch formulations having low (Prototype A), medium (Actiderm) and high (Prototype B) water uptake values.

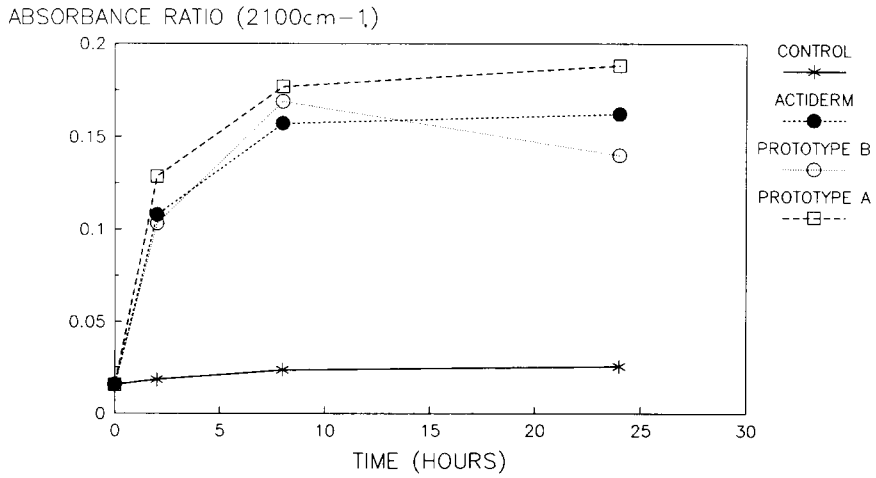

Figure 6
Stratum corneum hydration following occlusion for 0, 2, 8 and 24 h with hydrocolloid patches having low (Prototype A), medium (Actiderm) and high (Prototype B) water vapour uptake values.

Effect of patch water uptake on stratum corneum hydration

The water uptake properties of the Actiderm patch can be markedly varied by formulation changes. Theoretically, if the water vapour uptake of the adhesive dressing is high, the level of hydration of the stratum corneum should be low, as surface water will be absorbed effectively. The opposite effect should occur with patch exhibiting low water vapour uptake. Therefore, by adjusting the adhesive patch water uptake capability by formulation modification, three hydrocolloid dressings were manufactured with very low (Prototype A), medium (Actiderm) and high (Prototype B) water vapour uptake. Figure 5 demonstrates water vapour uptake properties of the three formulations by increase in weight with time on hydration. The three formulations were employed in an *in vivo* skin hydration study. Patches were applied to the volar forearm surface of healthy volunteers and removed after 2, 8 and 24 h. The level of hydration was determined by absorbance ratio (2100 cm^{-1}) and plotted against time (Fig. 6). These results were in good agreement with those predicted, i.e. patches with low water vapour uptake resulted in high levels of skin hydration and *vice versa* demonstrating the method to be of value not only for assessing skin hydration but for product differentiation.

Conclusion

In summary, the present report demonstrates the use of FT-IR in the determination of levels of hydration in the stratum corneum, both *in vitro* and *in vivo*. This method has been successfully employed in a number of hydration studies to investigate the effect of occlusion with water absorptive hydrocolloid dressings and completely occlusive tape. In addition, differentiation between formulations was possible both *in vitro*, employing water vapour uptake, and *in vivo* using FT-IR, the *in vitro/in vivo* results exhibiting good correlation.

It is clear that further *in vivo* studies employing a statistically valid number of volunteers and *in vitro* studies to quantitate water concentration are required and are currently being carried out in our laboratory. However, the use of this technique may be a valuable tool in the initial screening of topically applied occlusive formulations, in terms of their effects on skin hydration.

References

[1] E.J. Clar, C.P. Her and C.G. Sturelle, *J. Soc. Cosmet. Chem.* **26**, 337–361 (1975).
[2] H. Tagami, M. Ohi, K. Iwatsaki, Y. Kanamam, M. Yamada and B. Ichijo, *J. Invest. Dermatol.* **75**, 500–507 (1980).
[3] M.S. Christensen, C.W. Hargens, S. Nacht and E.H. Gans, *J. Invest. Dermatol.* **69**, 282–286 (1977).
[4] R.O. Potts, D.A. Chrisman and E.A.A. Buras, *J. Biomech.* **16**, 365–372 (1983).
[5] A. Dittmar, M.C. Martini, L. Planchette and J. Lotte, in *5th International Symposium on Bioengineering and the Skin* (1983).
[6] D.C. Salter, *Cosmetics and Toiletries* **37**, 9–33 (1986).
[7] R.O. Potts, *J. Soc. Cosmet. Chem.* **37**, 9–33 (1986).
[8] R.O. Potts, D.B. Guzek, R.R. Harris and J.E. McKie, *Arch. Dermatol. Res.* **277**, 489–495 (1985).
[9] S. Cornaish, *Br. J. Dermatol.* **80**, 522–528 (1968).
[10] N.A. Puttnam, *J. Soc. Cosmet. Chem.* **23**, 209–226 (1972).
[11] E.G. Bendit, *Biopolymers* **4**, 539–559 (1965).
[12] M. Gloar, G. Hirsch and U. Willebraudt, *Arch. Dermatol. Res.* **271**, 305–313 (1981).
[13] R.O. Potts, *Cosmetics and Toiletries* **100**, 27–31 (1985).

[Received for review 29 April 1991]