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Quantitative FT-IR determination of skin hydration following occlusion with hydrocolloid containing adhesive dressings

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Summary

A non-invasive FT-IR method was employed for the quantitative determination of stratum corneum (s.c.) water concentration both in vitro and in vivo. The method, modified from Potts et al. (*Arch. Dermatol. Res.*, 277 (1985) 489–495), relied on the determination of the area ratio of a peak corresponding to weak O–H stretching at approximately 2100 cm^{-1} (4–8 μm). This absorbance is distant from interferences due to other skin components and most topically applied substances and may therefore be used in the quantitation of s.c. water content. This report describes the use of this technique in an investigation into the effect of occlusion of the skin with hydrocolloid adhesive dressings having a range of water uptake properties. In addition to s.c. hydration in vitro and in vivo gravimetric analysis was employed to determine hydrocolloid dressing water vapour uptake. These results were subsequently related to s.c. hydration and demonstrated that dressings with a high in vitro water vapour uptake exhibited a significantly higher in vivo water uptake and significantly lower s.c. hydration than those with low in vitro water vapour uptake as determined by parametric paired *t*-test.

Introduction

The water content of the stratum corneum (s.c.) plays an important role in physiological and therapeutic investigations. This is markedly affected by environmental, pharmacological and physiological factors. These influence the subjective assessment of skin feel and appearance which is involved in dermatological disorders. (Tagami et al., 1982).

There are numerous non-invasive techniques for the determination of skin hydration both in vitro and in vivo. These include measurement of electrical (Clar et al., 1975; Tagami et al., 1980), mechanical (Potts et al., 1983), thermal (Grice et al., 1972), and spectroscopic (Potts, 1986; Salter, 1987) properties of the skin. However, most of the aforementioned methods do not measure s.c. hydration directly, rather a property caused by hydration. This has the added disadvantage that the theoretical relationships between the two

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changes are not always clearly understood (Potts et al., 1985).

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 focused on a peak in the s.c. spectrum at approx. 3300 cm^{-1} corresponding to strong O–H stretching (Comaish, 1968; Puttnam, 1972). However, there are interferences which contribute to this peak such as amide bands and C–H asymmetric stretching of s.c. lipids. Therefore, this peak cannot be considered specific for s.c. water.

To overcome this substantial increase in the broad peak at approx. 3300 cm^{-1} Mak et al. (1991) used deuterated water to investigate the influence hydration has on the intercellular lipids domains. They suggest that there is no overall increase in intercellular disorder, a fact confirmed by studies performed by Bouwstra et al. (1990) recently which demonstrated, using small-angle X-ray scattering, that up to 40% hydration (w/w) resulted in no swelling of the lipid bilayer. They also observed that, at 60% hydration w/w (fully hydrated) there is reduced ordering of a part of the lipids at these high water contents (Bouwstra et al., 1991). Other recent FTIR studies have suggested that there is a correlation between the frequency of the C–H stretching vibrations and water permeability over a wide temperature range (22–90°C) (Potts and Francoeur, 1990).

Skin hydration has also been estimated by FT-IR using the ratio of amide I (1645 cm^{-1}) to amide II (1545 cm^{-1}) (Bendit, 1956; Gloor et al., 1981). These workers suggested that the amide I band was due to protein + water, whereas amide II was due to protein alone. However, this method has inherent problems since both amide I and II bands of protein (keratin) change on hydration (Bendit, 1956). In addition, topical formulations may interfere in this region, therefore a correction to the intensity of the amide I band would be required (Gloor et al., 1981). Investigations into the use of this technique demonstrated a decrease in hydration through the s.c. on removal of surface layers by tape stripping (Gloor et al., 1981). These results conflict with the known increase of water content with depth in the s.c. (Potts, 1985) and demonstrate this method to be of limited use.

In addition to a strong O–H stretch (approx. 3300 cm^{-1}), there is also a weak O–H stretch at approx. 2100 cm^{-1} in the s.c. spectrum. This peak has been investigated by Potts and coworkers in the quantitative determination of s.c. water content (Potts, 1985, 1986; Potts et al., 1985; Potts and Francoeur, 1990; Mak et al., 1991).

In the present study, a suitable template for excised s.c. attachment to the FT-IR for routine reproducible in vitro analysis was identified, and data generated was subsequently used to compare two methodologies for quantitation of s.c. hydration. The reproducibility of the most appropriate in vitro method was investigated, and the standard curve generated was employed in the estimation of s.c. water concentration in vivo. Finally, an in vivo study was conducted to investigate the effect of occlusion on s.c. hydration. A series of hydrocolloid adhesive dressings with known water vapour uptake properties were compared with each other and a completely occlusive adhesive tape – Blenderm™.

Materials and Methods

In vitro stratum corneum preparation and hydration

Stratum corneum (s.c.) was prepared from human full thickness epidermis by digestion with 0.1% trypsin (Sigma Chemical Co. Ltd) in phosphate-buffered saline (Sigma) at 4°C overnight. The resulting s.c. was hydrated by attaching a strip of s.c. (8 cm × 2 cm) to an acetate film (0.1 mm thickness (Nobo)) template with glue (cyanoacrylate based) and floating the sample s.c. face down in a covered water bath at room temperature overnight. Prior to analysis, the template and s.c. were removed from the water bath and blotted dry with tissue paper. The sample was subsequently weighed and analysed on the FT-IR and this procedure was repeated until no weight loss occurred.

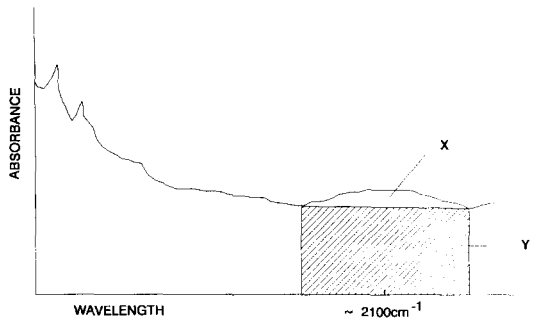

Fig. 1. FT-IR spectrum to demonstrate the use of absorbance ratio at approx. 210 cm^{-1} in the determination of stratum corneum hydration. The ratio of x/y is proportional to stratum corneum water content independent of contact with the ATR crystal.

In vitro water vapour uptake measurements

Water vapour uptake of hydrocolloid containing adhesive dressings and Blenderm^{TM} tape (16 cm^2) was performed using a temperature controlled oven at 32 ± 1°C, 97% relative humidity (± 0.5%). Three adhesive dressings were chosen with different water uptake properties. One had low water uptake capacity (prototype B), one medium (Actiderm^{TM}) and one high water uptake (prototype A). Five gravimetric determinations were made for each material at 24 h intervals over a 4 day period.

In vivo hydration study

Pre-weighed samples were applied to the volar forearm surface of 11 healthy non-patient human volunteers, of either sex for a period of 48 h. All four dressings were randomly applied to each individual and subsequently removed at 6, 24 and 48 h. On removal of each dressing, the s.c. water content was determined by placement of the forearm onto the accesory (for details see below) FT-IR attenuated total reflectance (ATR). The

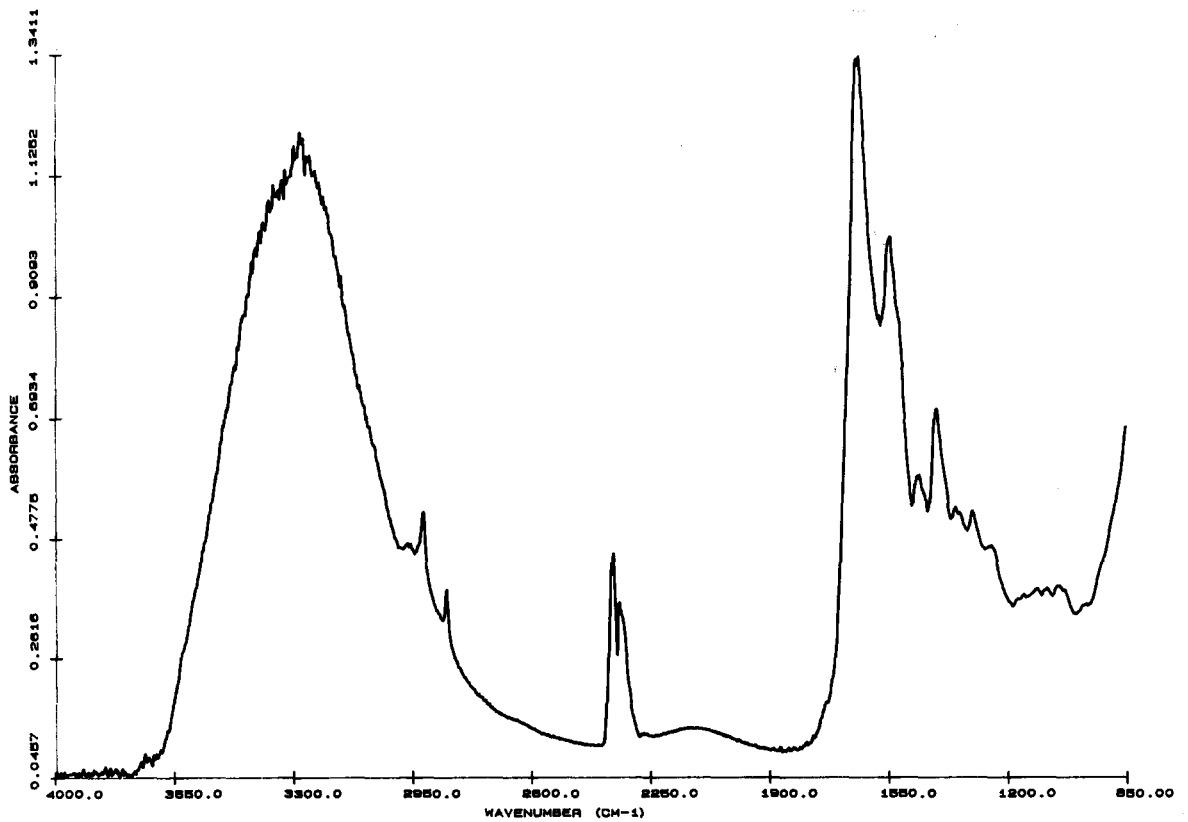

Fig. 2. A typical infrared spectrum of hydrated stratum corneum.

dressings were re-weighed to evaluate in vivo patch water uptake.

Analytical determinations

All analysis was conducted on a Nicolet 5ZDX FTIR (Nicolet Instruments) with a flat top ATR accessory (Specac Ltd) with a zinc selenide (ZnSe) crystal having a 45° incidence angle. Data handling was performed on Advantage software and scanning parameters were as follows: scan rate, 100 min^{-1}; number of scans, 10; wavelength range, 4000–650 cm^{-1}; resolution, 4 cm^{-1}. Peak area measurements were made at approx. 2100 cm^{-1} (weak O–H stretch) and approx. 3300 cm^{-1} (strong O–H stretch). The ratio of the area to the background absorbance results in a measure of s.c. hydration independent of s.c. contact with the ZnSe crystal in the ATR accessory (Fig. 1).

Statistical analysis

Statistical analysis of data generated from the in vivo, study was performed using Instat software using parametric paired *t*-test with the assumption that results conformed to a normal distribution.

Results and Discussion

In vitro stratum corneum hydration

A typical infrared spectrum of hydrated s.c. is shown in Fig. 2. It is clear from this spectrum that, although the peak corresponding to strong O–H stretching at approx. 3300 cm^{-1} is much larger than that corresponding to weak O–H stretching at approx. 2100 cm^{-1}, there is interference of the 3300 cm^{-1} peak by C–H stretching attributed to s.c. lipids, in addition to amide bands which are present in this region of the s.c. spectrum. The weak peak at approx. 2100 cm^{-1}, however, exhibits no interference from s.c. or other topically applied substances (Potts, 1985, 1986).

To investigate further the utility of the two methods hydrated excised s.c. attached to an acetate template was assayed for water content (as measured by area ratio at 3300 or 2100 cm^{-1}). Plots of area ratio vs weight of s.c. demonstrated the 2100 cm^{-1} peak area-ratio to have a more linear relationship with weight of s.c. than the 3300 cm^{-1} area ratio and this weak O–H stretch was subsequently employed in all in vitro and in vivo hydration determinations.

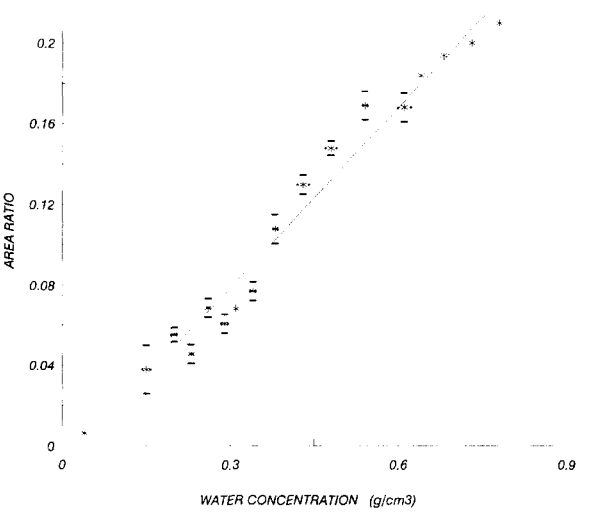

Fig. 3. A plot of area ratio (2100 cm^{-1}) vs stratum corneum water concentration (in vitro data). Each point represents the average of value obtained using six templates of excised stratum corneum. Density of stratum corneum was assumed as 1.33 g/cm^3.

Reproducibility of the technique

The reproducibility of this in vitro model was performed using six different strips of s.c. attached to six acetate templates. Hydration was performed as described above and assayed using peak area ratio at 2100 cm^{-1}. As the weight of s.c. was known, and assuming s.c. density of 1.33 g/cm^3 (Wu, 1983), the mass of water could be calculated and related to the volume of s.c. to give a water concentration in g/cm^3 (Potts, 1985, 1986).

A plot of area ratio (2100 cm^{-1}) vs s.c. water concentration (Fig. 3) demonstrated good reproducibility and linearity (correlation coefficient $r = 0.9868$) of the technique. These in vitro data were subsequently used in the calculation of s.c. water concentrations in vivo by fitting area ratios into the equation of the line.

In vivo stratum corneum hydration

To investigate the use of this technique for the evaluation of s.c. hydration in vivo following oc-

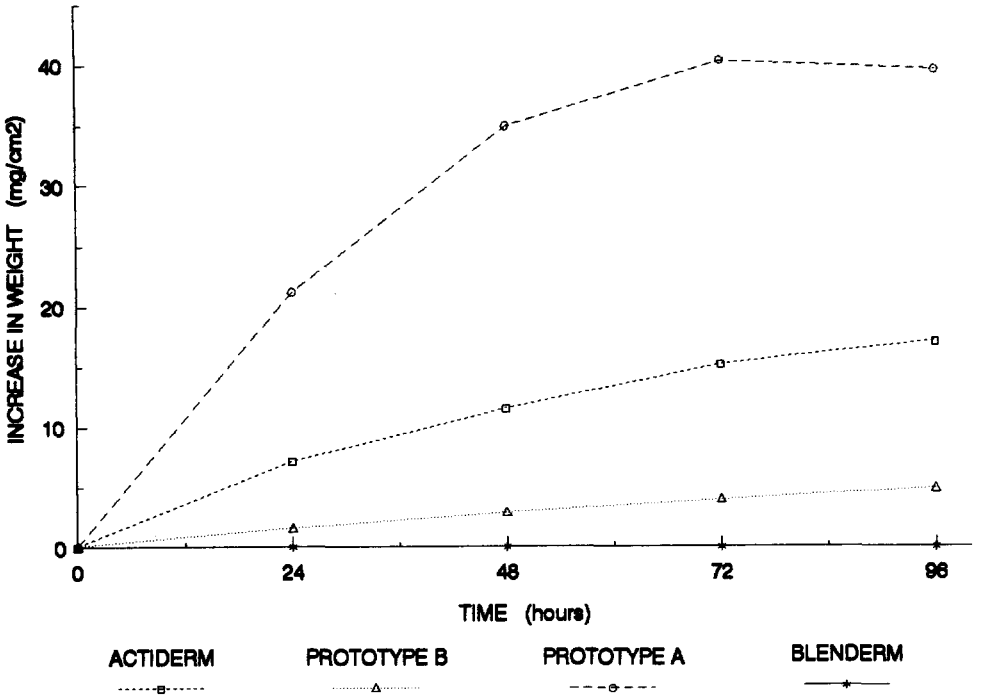

Fig. 4. Comparison of in vitro water vapour uptake properties of adhesive dressings measured as weight increase time.

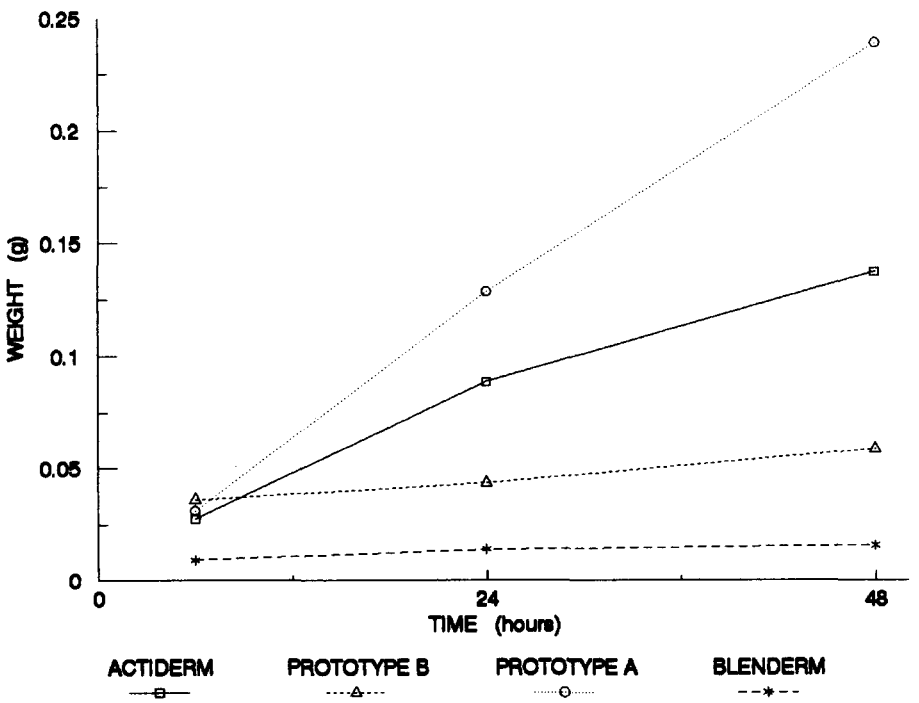

Fig. 5. Comparison of in vivo water uptake properties of adhesive dressings after they had been removed from the skin of volunteers at 6, 24 and 48 h.

clusion, a volunteer study was performed. The effect of occlusion with three hydrocolloid containing adhesive patches with low, medium and high water vapour uptake, prototype B, Actiderm™ and prototype A, respectively, were compared to Blenderm™ tape (totally occlusive). Performance of these dressings was assessed based on (a) in vitro water vapour uptake (b) in vivo patch hydration and (c) in vivo s.c. hydration. Initially, samples of the dressings were hydrated in vitro and their water vapour uptake assessed gravimetrically. Fig. 4 clearly demonstrates the differences in water vapour uptake between the four dressings.

Pre-weighed dressings (samples from the same batches as those used in in vitro hydration study) were applied to the volar forearm surface of 11 volunteers and removed after 6, 24 and 48 h. On removal, the dressings were re-weighed and the site of occlusion was immediately assayed for water using FTIR-ATR. A plot of dressing weight increase (a measure of their hydration) vs time (Fig. 5) demonstrated good agreement with the water vapour uptake in vitro data, i.e., prototype A exhibited greatest hydration followed by Actiderm™, prototype B and Blenderm™ (which exhibited minimal weight increases).

The effect of occlusion on s.c. hydration in vivo as measured by FTIR-ATR is shown in Fig. 6. The results following 48 h occlusion were not representative, as poor adhesion and creasing of the patches probably led to surface moisture loss, thus decreasing skin hydration levels. Therefore, statistical analysis was only performed following 6 and 24 h occlusion (good adhesion).

The level of s.c. hydration after 6 h occlusion was not significantly different ($p < 0.05$) with any treatment. However, after 24 h occlusion with prototype A (high water vapour uptake) s.c. hydration was significantly lower ($p < 0.05$) than all other treatments. Occlusion with Actiderm™ and Blenderm™ produced similar results with no significant difference. However, occlusion with prototype B (low water vapour uptake) produced s.c.

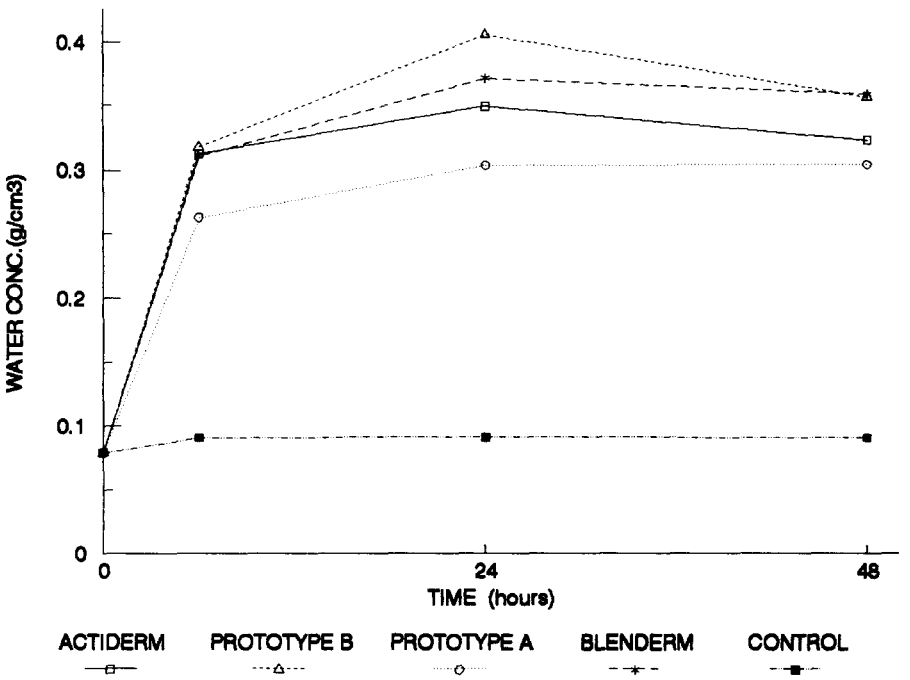

Fig. 6. A plot of stratum corneum hydration (measured as water concentration using FT-IR-ATR) against time following occlusion with adhesive dressings.

hydration which was significantly higher ($p < 0.1$) than the Actiderm™, Blenderm™ and prototype A ($p < 0.001$) treatments.

The results of this study agree well with those predicted from in vitro dressing hydration data in that s.c. hydration is greater following occlusion with low water vapour uptake dressings than the level observed with highly water vapour absorbent dressings.

Previously, Potts (1986) demonstrated an in vitro model for determination of s.c. water content by FTIR-ATR up to a concentration of approx. 0.2 g/cm^3. In the present study, a similar FTIR-ATR method was employed and linearity of the assay to a s.c. water concentration of approx. 0.5–0.6 g/cm^3 was achieved. These differences may be explained by the method of s.c. hydration and the use of a more powerful FT-IR spectrometer. Potts and co-workers used humidity chambers for s.c. hydration, whereas the present study employed a covered water bath. Hydration of excised s.c. is difficult to achieve in a high humidity cabinet, hydration being complete in days rather than hours. The use of a water bath allowed complete saturation of s.c. in approx. 16 h with good reproducibility. The FT-IR spectrometer used in this study has a high signal/noise ratio enabling data collection to be performed in seconds rather than minutes. This is also contributed to the sensitivity of the assay.

Results obtained using FT-IR and gravimetric analysis demonstrated that a quantitative and reproducible in vitro assay of s.c. water content could be used to calculate in vivo s.c. hydration following occlusion. The levels of dressing hydration in vivo were in good agreement with those predicted from in vitro data and resulting s.c. hydration in vivo agreed well with that predicted.

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