Occlusive Properties of Monolayer Patches: *In Vitro* and *in Vivo* Evaluation

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Purpose. Patches can cause a different grade of skin occlusion, depending on matrix composition and thickness, backing layer material. The aim of this work was to verify if in vitro water vapour permeability (WVP) values are predictive of transepidermal water loss (TEWL) and Fourier-transform infrared (FTIR) spectroscopy values measured *in vivo* after 24 h of methacrylic or acrylic monolayer patches application. The correlation between both *in vivo* methods has been evaluated.

Methods. The WVP, TEWL and FTIR measurements were performed by using four patches made of a methacrylic or an acrylic polymeric system (250 and 500 μm thickness on a polyurethane backing layer). A fifth patch was made of the methacrylic matrix on a polyvinyl chloride backing layer.

Results. A good correlation was found between TEWL values and IR water/lipid absorbance ratios. The *in vitro* WVP values are in a good correlation with the results of both in vivo methods: TEWL = −0.01WVP + 21.31 (R^2 = 0.9312); FTIR water/lipid ratio = −0.01WVP + 27.15 (R^2 = 0.9447).

Conclusions. The *in vitro* method proposed for measuring the WVP is predictive of the degree of occlusion resulting from the in vivo application of monolayer patches.

KEY WORDS: transdermal patches; water vapour permeability; transepidermal water loss; ATR- FTIR spectroscopy.

INTRODUCTION

Monolayer self-adhesive patches are widely used. Clinically both for the protection of injuries and wound healing and also as transdermal drug delivery systems to obtain prolonged and controlled drug release.

However, the application of a patch can reduce the normal evaporative water loss of the skin and risks water accumulation between the skin and the patch. Prolonged application periods of an occlusive material may lead to increases in skin surface P_{CO2} and pH and bacterial infections (1–5), skin irritation and maceration (6–8). With respect to the possibility of a delay in the epidermal barrier repair, different results are reported in the literature. Welzel *et al.* (9) have indicated that permeability barrier repair activities are not significantly delayed by occlusive treatment in human skin, while the use of animal models could be inadequate as there are structural and functional differences from human skin. For example, Proksch and co-workers (10) have shown that occlusive treatment of irritated skin resulted in a reduction of barrier repair activities in hairless mice. Moreover, both the mechanical and electrical properties of stratum corneum are markedly influenced by its water content. Consequently, skin permeability to substances contained in the patch, in addition to xenobiotics, could be markedly altered (8,11,12). For these reasons it is important to determine the degree of occlusion resulting from the prolonged application of adhesive patches.

The total amount of water loss through the skin is generally determined *in vivo* by measuring the transepidermal water loss (TEWL). TEWL is correlated with the state of hydration and it is a well-established method for the evaluation of the epidermal barrier function (13). TEWL is generally considered a passive diffusion phenomenon and is expressed by the steady state flow of water vapor per unit area of surface in unit time, at a specified humidity and temperature. Changes in TEWL may be caused by physical trauma or induced by chemical treatment or by occlusion.

The level of skin hydration *in vivo* can also be studied by using the technique of attenuated total-reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy, since water is an intense absorber of infrared radiation. This non-invasive technique has the advantage that drugs permeating the skin, stratum corneum intercellular lipids and proteins, and changes induced by enhancers or other constituents of a delivery system can be simultaneously investigated. This technique for human skin analysis has been applied since the 1960s and, in particular, the estimation of water content was one of the earliest applications. FTIR has been previously used in a number of ways for determining hydration level including the OH stretching absorbance near 3400 cm^{-1}, the ratio of amide I (1650 cm^{-1}) to amide II (1550 cm^{-1}) bands, and a combination band near 2100 cm^{-1} (14).

The development of a standardized *in vitro* method to evaluate the degree of skin occlusion could be very useful during preformulation and formulation studies of patches. As an *in vitro* indication of occlusion induced by the patch, it is possible to evaluate the patch water vapor permeability, WVP.

The aim of this work was to verify if the WVP values of the patches are predictive of the TEWL and FTIR values measured *in vivo* after 24 h of monolayer patch application. Moreover, the correlation between both *in vivo* methods has been evaluated. In addition, the influence of patch design (matrix composition and thickness, backing layer material) on occlusivity could be ascertained.

MATERIAL AND METHODS

Materials

Eudragit® NE40D (EUNE) and Plastoid® E35L (PLE) were supplied by Röhm, Darmstadt, Germany. Duro-Tak® 387-2287 (DT) were supplied by National Starch and Chemical B.V., Zupthen, The Netherlands. Faitex® 01 polyurethane (PU) film: thickness 20 μm; weight 22.8 g/m^2; elongation at break 500%; tensile strength 44 MPa (FAIT, Brescia, Italy).

Polyvinyl chloride (PVC) film: thickness 90 ± 1 μm; weight 90 ± 9 g/m^2; elongation at break: longitudinal 300 ± 15% and transversal 400 ± 15%; tensile strength: longitudinal

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2.3 ± 0.23 Kg/cm and transversal 2.0 ± 0.20 Kg/cm (Bouty, Milan, Italy).

Preparation of Mixtures

PLE and EUNE in the ratio 70/30 w/w were mixed using an under vacuum paddle stirrer at 50 rpm for 1 h. The mixture was used after 12 h of rest. DT was used as received.

Preparation of Patches

Patches were prepared by using a laboratory coating unit Mathis LTE-S(M) (Mathis, Zurich, Switzerland). Mixtures were spread on the backing layer at the constant rate of 1 m/min and with a constant thickness of 250 or 500 μm (Table I). Systems were dried at 60°C for 15 min, covered with the protecting layer and stored in an airtight container until used.

Water-Vapor Permeability (WVP) Evaluation

The WVP measurements were performed using a modified British Pharmacopoeia method (15).

Approximately 20 ml of water was poured into the chamber of the apparatus (16) and the sample was mounted at the center of the top surface of the cell (circular opening of 40 mm diameter). The chamber was placed into a natural air circulating oven containing silica gel and maintained at 37 ± 1°C for 24 h. The chamber was weighed before the test and 1 h after removal from the oven.

The WVP is given by the following equation:

$$WVP = W/A$$

where WVP is expressed in $g/m^2 \times 24$ h, W is the amount of vapor permeated through the patch expressed in g/24 h and A is the effective area of the exposed samples expressed in m^2.

Each WVP value represents the average of five sample readings.

Transepidermal Water Loss (TEWL) Measurement

Twelve healthy volunteers females (age 23–53 years, non-atopics) from whom informed consent was obtained participated in this study. The five different types of patches (Table I), cut in 2.54 cm^2 segments, were applied on the left forearm (17), sufficiently far from both wrist and elbow. The site of application was randomized between volunteers and the application was carefully checked. Patches remained on the skin for 24 h and measurements were recorded on a control site nearby (on the same arm), on the patch just before being removed and on the skin 2 min after removing the patch. Measurements on the application site were repeated after 3 days.

TEWL was measured using an evaporimeter with a probe consisting of an open chamber (12 mm in diameter) and mounted with sensors for determination of temperature and relative humidity (Tewameter TM 210®, Courage-Khazaka electronic, Köln, Germany). Measurements were performed in two consecutive days of the month of April (RT: 19–22°C; RH: 60 ± 10%). All TEWL readings were conducted 30 s after application of the probe onto the skin, when the level was stabilized, for a duration of 90 s. The mean value from the 90 s was indicated by the instrument and used for the discussion.

All measurements were conducted according to the guidelines of the Standardization group of the European Society of Contact Dermatitis (13). TEWL was expressed in $g/m^2/h$.

Table I. Characteristics of the Patches Used

Patch no.	Matrix components	Coating thickness (μm)	Backing layer
1	PLE:EUNE	250	PVC
2	DT	500	PU
3	DT	250	PU
4	PLE:EUNE	500	PU
5	PLE:EUNE	250	PU

Attenuated Total-Reflectance Fourier-Transform Infrared (ATR-FTIR) Spectroscopy

A Nicolet 730 FTIR spectrometer (Nicolet, Madison, WI, USA) was used to record the *in vivo* measurements. The spectrometer was equipped with an ATR accessory that supported a ZnSe internal reflectance element. All spectra reported were the average of 64 scans, measured at a resolution of 4 cm^{-1}. Omnic® FTIR software was used to calculate the peak areas associated with the OH and CH_2 stretching, and amide I and II bands. For each stratum corneum site, peaks area was measured before and after the 24 h patch application. This experimental strategy allowed each sample to serve as its own control.

Data Analysis

Tests for significant differences between means were performed by one-way ANOVA and Student-Newman-Keuls test (SigmaStat 1.0 Jandel Corporation, USA). Differences were considered significant at the $p < 0.05$ level.

RESULTS AND DISCUSSION

WVP Evaluation

The WVP values of the backing layers were: 162 ± 4 $g/m^2/24h$ for PVC and 1535 ± 72 $g/m^2/24h$ for PU. As expected, the WVP values of the patches (Table II) were always lower than the values of the backing layer.

The patch prepared on a PVC film showed a low WVP quite similar to that of the backing layer alone and the patches prepared using the PU backing layer showed a wide

Table II. WVP Values and TEWL Values Measured on the Patch Just before Being Removed and on the Skin 2 min after Removing the Patch (Mean ± Standard Deviation)

Patch no.	WVP[a] (g/m^2/24 h)	TEWL (on the patch)[b] ($g/m^2/h$)	TEWL (on the skin)[b] ($g/m^2/h$)
1	141 ± 4	5.1 ± 0.6	22.0 ± 6.3
2	289 ± 23	6.1 ± 1.3	17.6 ± 6.8
3	451 ± 11	6.5 ± 1.1	16.7 ± 7.4
4	1182 ± 53	7.4 ± 1.6	12.2 ± 5.6
5	1432 ± 38	6.4 ± 2.0	9.7 ± 2.6

[a] n = 5.
[b] n = 12.

range of water vapor permeability due to difference in matrix polymeric composition and thickness. DT matrices, obtained by using an organic polymeric system, were less permeable to water vapor than the PLE-EUNE matrices, obtained by using an aqueous polymeric system. Additionally, the highest spreading thickness reduced the WVP significantly in the case of both polymeric systems (Table II). All the WVP values were statistically different among them.

TEWL Measurements

The mean value of TEWL recorded on the control sites ($n = 60$) was 9.0 ± 2.1 g/m^2/h. This value is in agreement with the baseline value for an adult at rest, which is reported in the 5–10 g/m^2/h range (18–20).

TEWL measurements conducted on the patches before removing them indicated that none of patches were completely occlusive; in every case the TEWL values were lower and statistically different from the control value (Table II).

The TEWL values measured on the skin 2 min after removing the patches was significantly different from the control value, except for patch no. 5 (Table II). These data allow us to conclude that the skin hydration was not modified by the application of patch no. 5, while it was significantly modified following application of all the other patches. No statistical difference was found between patches no. 2 and no. 3, while for all other patches the values were significantly different.

TEWL can be considered as a marker of the functional state of the cutaneous barrier and it may be used to monitor the level of skin irritancy (17). A red area appeared only for one subject after the removal of patches no. 2 and no. 3. After 3 days TEWL values returned to the baseline values in each subject involved in this study with the exception of the subject, which had red area. In this case the values remained higher with respect to the control values indicating barrier perturbation.

FTIR Spectroscopy

Gloor *et al.* (21) proposed the ratio amide I/amide II as a relative measure for the level of stratum corneum (SC) hydration, because the amide I and II peaks are considered to be due to keratin and water, and protein alone, respectively. However, the SC amide I/amide II ratios (Table III, ratio amide I/amide II) determined in this study were not significantly different following the application of various patches, in contrast to the measured TEWL values. It has been suggested that both the amide I and II vibrational bands are affected by water (22), which limits this method.

In addition to a strong OH vibration at 3400 cm^{-1}, a weaker absorbance near 2100 cm^{-1} is also present in SC. The latter OH stretching vibration is of interest (23), as it occurs in a spectral region where skin or commonly applied substances show no IR absorbance. For the patches studied, this peak increased significantly only for those patches, which gave TEWL values above baseline values, i.e. only the more occlusive patches (patch no. 1 and 2, Table I). Moreover, these peak areas were not statistically different among the patches studied (Table III, ratio water above/below baseline).

The intense OH stretching absorbance at 3400 cm^{-1} has also been used in the evaluation of SC hydration. Although this absorbance is considered to be a nonspecific indicator of SC water content, it is clearly influenced by the presence of water. As skin hydration increases, this band becomes more intense and broad, with the CH_2 stretching absorbance (predominantly from the SC lipids) near 2940–2850 cm^{-1} appearing on the shoulder of the broad OH peak (Fig. 1). In this study, the area under the OH band was normalized by the integrated intensity of the CH_2 peaks, to correct for inter- and intra-individual variation in skin crystal contact (which is highly dependent on skin hydration). The OH peak area was determined by integrating the intensity of the entire region encompassed by the OH band (including the CH_2 peaks on the shoulder), followed by subtraction of the area corresponding to the CH_2 peaks alone.

This ratio provided useful information about the hydration level reached after the application of patches (Table III, ratio water/lipid). Based on these measurements, patches no. 1, 2, 3 gave results statistically different from patches no. 4 and 5.

Table III. FTIR Values as Ratio of Spectrum Peaks (Mean ± Standard Deviation)

Patch no.	Ratio amide I/amide II	Ratio water above/below baseline	Ratio water/lipid
1	2.28 ± 0.11	0.51 ± 0.20	24.8 ± 3.5
2	2.11 ± 0.39	0.49 ± 0.21	26.3 ± 4.8
3	1.92 ± 0.26	0.38 ± 0.25	22.3 ± 4.6
4	2.49 ± 0.39	0.27 ± 0.18	14.8 ± 2.3
5	1.96 ± 0.14	0.28 ± 0.019	14.4 ± 2.2

Data Correlation

A good correlation was found between the results of both *in vivo* methods, TEWL measured on the skin (Table II) and IR water/lipid absorbance ratio (Table III):

$$\text{TEWL} = 1.04\ \text{FTIR water/lipid ratio} + 4.21 \quad (1)$$
$$R^2 = 0.8041 \quad F = 12.31 \quad p = 0.0392$$

The in vitro WVP values are in a good correlation with the results of both in vivo methods:

$$\text{TEWL} = -0.01\ \text{WVP} + 21.31 \quad (2)$$
$$R^2 = 0.9312 \quad F = 40.63 \quad p = 0.0078$$
$$\text{FTIR water/lipid ratio} = -0.01\ \text{WVP} + 27.15 \quad (3)$$
$$R^2 = 0.9447 \quad F = 51.23 \quad p = 0.0056$$

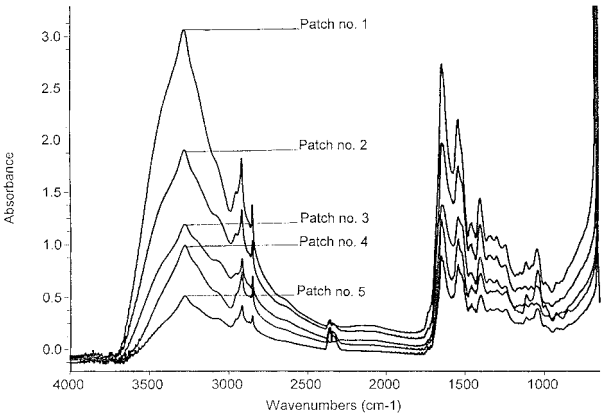

Fig. 1. ATR-FTIR spectra after the application of patches.

The TEWL values measured on the patch and the WVP values had no statistically sound correlation ($R^2 = 0.6935$, $F = 6.79$, $p = 0.08$).

The discrimination between the 250 and 500 μm patches was possible by using the *in vitro* test, while differences were less evident by using *in vivo* methods. In fact, changing the coating thickness, TEWL values were statistically different only in the case of methacrylic patches (no.4 and 5, Table I) and by using the FTIR method, it was never possible to distinguish between acrylic patches (no.2 and 3, Table I) and methacrylic patches (no.4 and 5, Table I).

CONCLUSIONS

Despite the high interindividual variability, TEWL and FTIR can be considered suitable techniques to discriminate the occlusive properties of acrylic and methacrylic patches.

The method of choice could be considered TEWL, as it is easier to perform and able to discriminate the occlusive properties of methacrylic patches.

Among the many features of the FTIR spectra, the occlusive properties of patches could be evaluated by using the ratio between OH stretching area, at approximately 3400 cm^{-1}, and the area under the CH_2 stretching vibrations, at approximately 2940-2850 cm^{-1} and located on the shoulder of the broad OH peak. The OH stretching area was considered without the lipids area.

A good correlation was found between TEWL values on the skin and IR water/lipid absorbance ratios and a sound correlation was found between these values and those obtained by using the WVP.

Therefore, the *in vitro* method proposed in this work for measuring the water vapor permeability is predictive of the degree of occlusion resulting from the *in vivo* application of monolayer patches made of methacrylic or acrylic copolymers. Furthermore, the *in vitro* methodology described is able to provide valuable information regarding patch occlusivity being simpler and less expensive to perform than *in vivo* techniques.

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