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On the correlation between single-frequency impedance measurements and human skin permeability to water

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ABSTRACT

The objective of this study was to quantitatively compare measurements of tritiated water permeability with impedance determined at either 100 or 1000 Hz using an LCR databridge on the same pieces of skin. A previously published expression based on a simple circuit of a parallel resistor and constant phase element (CPE) was used to relate ($R_{PAR}A$) measured at different frequencies to the DC resistance ($R_{skin}A$) and the steady-state skin permeability of tritiated water (k_p). Using this analysis, k_p and ($R_{PAR}A$) data from three laboratories were shown to be consistent with each other, and k_p and ($R_{skin}A$) estimated from ($R_{PAR}A$) were linearly correlated. Compared with urea and mannitol, which are known to permeate skin through a polar pathway, the value of k_p for water was found to be about two times larger than expected for transport through only the polar pathway, suggesting an approximately equal contribution from the lipophilic pathway. Equations relating k_p to ($R_{PAR}A$) and ($R_{skin}A$) were used to compare on a consistent basis proposed tests for identifying and excluding damaged skin from chemical absorption studies. The criterion of 20 kΩ cm^2 for ($R_{skin}A$) corresponds to a tritiated water permeability of 3.2×10^{-3} cm/h, which should exclude damaged skin without screening undamaged but higher permeability skin samples from study.

1. Introduction

In vitro diffusion cell methods have been used extensively to measure the rate and extent of chemical penetration into and through skin from humans or animals. The potential exists for skin samples to be damaged during collection, storage or handling, which may affect the percutaneous absorption measurements (Scott et al., 1991). Therefore, the use of a barrier integrity test for skin penetration studies is advised and often required if data are collected for regulatory submission (Heylings and Esdaile, 2007; International Programme on Chemical Safety (IPCS), 2006; OECD, 2004a). Recommended integrity test protocols include determination of tritiated water permeability, the rate of transepidermal water loss (TEWL), and transcutaneous electrical resistance (International Programme on Chemical Safety (IPCS), 2006; OECD, 2004a). Skin samples are rejected as unreliable for the determination of permeability parameters if the water permeability or TEWL is considered to be too high or if the electrical resistance is deemed to be too low.

With respect to the steady-state permeability coefficient (k_p) of tritiated water, the criteria for rejecting human skin samples have been set variously at 1.5×10^{-3} cm/h (Davies et al., 2004; Fasano et al., 2002; Scott et al., 1987), 2.0×10^{-3} cm/h (van de Sandt et al., 2000), 2.5×10^{-3} cm/h (Bronaugh and Stewart, 1986) and 4.0×10^{-3} cm/h (Buist et al., 2005). Meidan and Roper (2008) suggested that using smaller values of tritiated water permeability as integrity criteria could cause undamaged but higher permeability samples to be rejected, which could bias the permeability measurements toward underestimating absorption. Therefore, they recommended using 4.5×10^{-3} cm/h as the tritiated water k_p criterion based on the observation that 95% of the 2390 skin samples in a study of 112 female volunteers were below this value.

Steady-state determinations of tritiated water permeability require two or more hours, which delays the start of chemical permeation measurements when used to test skin integrity. Alternative tritiated water methods requiring less time have been proposed. Kasting et al. (1994) judged skin to be acceptable for

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diffusion cell measurements if the amount of tritiated water collected in the receptor fluid over 1 h after a 5-min application was less than 1.6 μL/cm^2. By comparing hourly measurements of tritiated water absorption collected for 2 h, Runciman et al. (2009) concluded that an absorption of 2.4 μL/cm^2 (i.e., calculated from 0.6% of the 400 μL/cm^2 application) in the first hour was equivalent to a permeability coefficient of 3.5×10^{-3} cm/h calculated from absorption at 2 h.

Single-frequency electrical impedance measurements are rapid and economical alternatives to tritiated water flux for skin integrity testing. The method involves measuring the potential or current response to a small-amplitude, typically sinusoidal, modulation of an input current or potential. Impedance is the ratio of the change in potential to the change in current, which for skin depends on the modulation frequency. In the limit of low frequency, the impedance measurement approaches the direct current (DC) resistance, designated for skin as R_{skin}.

Proposed criteria for *in vitro* testing of human skin integrity, several of which are listed in Table 1, have been selected by comparing electrical measurements on a series of samples to the percutaneous absorption measurements for tritiated water (Davies et al., 2004; Fasano et al., 2002), polar compounds (Peck et al., 1995), or ionized salts (Kasting and Bowman, 1990). For each of the electrical resistance criteria derived from tritiated water permeability data, the chosen k_p criterion is reported. Electrical resistance test criteria vary widely, from 7 to 45 kΩ cm^2 for the studies listed in Table 1. Even for those derived using the same tritiated water permeability coefficient, the differences in criteria are large (e.g., 11–45 kΩ cm^2 for k_p of 1.5×10^{-3} cm/h).

The variation in the electrical resistance test criteria is due in part to the choice of the percutaneous absorption measurement method. In addition to this, skin resistance has been determined in different ways that affect the magnitude of the measurement. For example, resistances determined using an LCR databridge, like the PRISM or Tinsley instruments (Davies et al., 2004; Fasano et al., 2002; OECD, 2004b), in PAR mode (R_{PAR}) measured at different frequencies are different; in general, these are also different from resistance determined using a DC method (Fasano and Hinderliter, 2004; White et al., 2011).

In assessing skin integrity, the meaningful quantity is electrical resistivity (ρ), which characterizes quantitatively the pathway for transport of ions, which is enhanced for damaged skin. The skin resistivity is proportional to the area-normalized skin resistance measured using a direct current method; i.e., (R_{skin} A) where A is the area. The permeability of polar and ionic chemicals through skin has been shown to be inversely proportional to (R_{skin} A) (Kasting and Bowman, 1990; Peck et al., 1994, 1995; Tang et al., 2001). While impedance measured at low frequencies may provide a good estimate of R_{skin}, and thus be inversely proportional to the permeability coefficient of polar and ionic compounds, direct estimation of R_{skin} from impedance measured at higher frequencies has significant error (Fasano et al., 2002; White et al., 2011).

White et al. (2011) derived a relationship between R_{PAR} measured at a single frequency and electrical properties of the skin by providing a theoretical basis for describing the frequency dependence of R_{PAR}. Based on an analysis of impedance measurements of many skin samples at multiple frequencies, the authors concluded that for skin integrity testing, R_{PAR} measurements at either 100 or 1000 Hz could be used, although measurements at 100 Hz gave more accurate results. In addition, the authors proposed equations for relating (R_{PAR} A) measured at different frequencies to (R_{skin} A). It follows that it should be possible to extend these equations to relate (R_{PAR} A) with k_p for polar compounds, such as small sugars and urea, which are known to permeate skin by the same diffusion pathway as charge carrying ions (Kasting and Bowman, 1990; Peck et al., 1994; Tang et al., 2001). If the transdermal absorption of water is also through this polar pathway, then these equations would apply to water and could be used to relate skin integrity test criteria based on tritiated water k_p with electrical resistance test criteria based on (R_{PAR} A) measured at various frequencies.

Kalia et al. (1996, 1998) observed a correlation between transepidermal water loss and the inverse of impedance (i.e., the admittance) measured at 1.6 Hz (a low enough frequency to represent R_{skin}). This observation is consistent with diffusion through the polar pathway. However, there is other evidence that water permeates through the pathway followed by lipophilic compounds instead. The temperature dependence of water permeability is larger than expected for polar compounds (Mitragotri, 2007; Peck et al., 1995). It is not necessary to invoke a polar pathway to explain skin permeability to water, which is consistent with other lipophilic compounds (Kasting et al., 2003; Potts and Francoeur, 1991; Potts and Guy, 1992), and also, the value of k_p is larger than expected for a polar compound of its size (Mitragotri, 2007 and discussions in Peck et al., 1994, 1995).

Table 1
Recommended values of area-normalized electrical resistance (R A) for testing human skin integrity for *in vitro* determinations of chemical permeation.

Source	f (Hz)[a]	(R A) test criteria (kΩ cm^2)	Tritiated water k_p test criteria ($\times 10^3$ cm/h)[b]	Estimated (R_{skin} A) (kΩ cm^2)[c]	Estimated tritiated water k_p ($\times 10^3$ cm/h)[d]
Lawrence (1997)	12.5[e]	45[f]	1.5[f]	45	1.4
Fasano et al. (2002)	1000	11	1.5	26	2.4
Davies et al. (2004)	100[g]	25	1.5	31	2.0
Kasting and Bowman (1990)	0	35	NA[h]	35	1.8
Horne et al. (2010)	100	13	3.5	15	4.1
Horne et al. (2010)	1000	7	3.5	12	5.1
Peck et al. (1995)	0	20	NA	20	3.2

[a] Frequency of the impedance measurements upon which the recommended electrical resistance test criteria is based. A frequency of zero indicates DC measurements. All measurements at non-zero frequencies were determined in PAR mode using an LCR databridge.
[b] The tritiated water permeability coefficient criterion that was used to derive the indicated electrical resistance test criterion.
[c] (R_{skin} A) value calculated using Eq. (4) and the corresponding electrical resistance (R A) test criterion.
[d] Estimate of the tritiated water k_p corresponding to the electrical resistance test criteria calculated using (R_{skin} A) and the optimized value of b (63 kΩ cm^2) in Eq. (8).
[e] Measurement frequency was not given in the paper; this value was determined from the operation manual for the instrument used in the study (World Precision Instruments, 2008).
[f] The authors did not specify a test criteria; however, they report observing that all but 4 of 111 skin samples with k_p less than 1.5×10^{-3} cm/h exhibited a resistance of at least 45.4 kΩ cm^2.
[g] Davies et al. (2004) incorrectly listed the measuring frequency as 100 kHz rather than 100 Hz (J. Heylings, personal communication, 2009).
[h] Not applicable. The electrical resistance test criterion was not derived by comparing to tritiated water k_p.

The objective of this study was to compare (R_{PAR} A) determined at 100 and 1000 Hz to the cumulative tritiated water absorption during 1 h of skin exposure measured on the same pieces of skin. These new measurements were then compared to previously reported correlations of steady-state tritiated water permeation and R_{PAR} determined at 100 and 1000 Hz by Davies et al. (2004) and Fasano et al. (2002), respectively. The relationship between tritiated water absorption and the estimate of skin resistivity was examined using published equations relating the DC resistance to R_{PAR} measurements determined at different frequencies using an LCR databridge (White et al., 2011). The observed linear correlation between the DC resistance estimates and tritiated water permeability coefficients was consistent among the three laboratories. Compared with the correlation in human skin of the permeability coefficient for two model polar compounds and skin resistance, the permeability of water is larger than would be expected by assuming permeation through only the polar pathway.

2. Theory

Impedance spectroscopy probes in a different way, the barrier skin presents to the absorption of hydrophilic compounds. An overview of the theory behind the two measurement techniques and the relationship between them is presented in this section.

2.1. Skin impedance

Skin impedance is measured by applying a small-amplitude alternating current or potential signal across the skin and measuring the responding potential or current. The impedance is the ratio of the change in potential to the change in current. The dielectric material in the skin causes a phase shift in the measured signal relative to the applied signal, and, as a result, the measured impedance varies with the frequency of the applied signal. Mathematically, this means that the impedance is a complex number containing both real and imaginary parts. The frequency response of skin is represented reasonably by a simple equivalent circuit model consisting of a frequency-independent (Ohmic) resistance (R_e) in series with a parallel skin resistance (R_{skin}) and constant phase element (CPE) as shown in Fig. 1 (Hirschorn et al., 2010; Kontturi and Murtomaki, 1994; Yamamoto and Yamamoto, 1976a,b, 1981). For *in vitro* determinations of skin impedance, R_e includes contributions from the electrolyte solution on both sides of the skin, the wires, and possibly the dermis and viable epidermis.

In the skin integrity studies described below, an LCR databridge (i.e., the Tinsley 6401 or AIM 6401) was used to measure impedance at a frequency of 100 or 1000 Hz. These instruments report a resistance in parallel mode (R_{PAR}), which is equal to the inverse of the real part of the skin admittance (White et al., 2011). The admittance is the alternating current analog of the DC conductance and it is equal to the inverse of the impedance. Therefore, the frequency dependence of R_{PAR} is described by the following expression derived from the R-CPE equivalent circuit model (White et al., 2011):

$$R_{PAR} = \frac{R_e + R_{skin} + \frac{(R_{skin}\, Q\, R_e (2\pi f)^{\alpha})^2}{R_{skin}+R_e} + 2\, Q\, R_{skin} R_e (2\pi f)^{\alpha} \cos(\frac{\alpha\pi}{2})}{1 + \frac{R_e (Q\, R_{skin} (2\pi f)^{\alpha})^2}{(R_{skin}+R_e)} + Q\, R_{skin} (2\pi f)^{\alpha} \cos(\frac{\alpha\pi}{2})} \quad (1)$$

where f is the frequency (in units of Hz, cycles per second) of the applied alternating current or potential, and Q and α are parameters quantifying the behavior of the CPE (Orazem and Tribollet, 2008). For human skin, α is approximately constant at 0.8 (Hirschorn et al., 2010; Poon and Choy, 1981) and Q can be related to the resistance (R_{skin}) and the effective capacitance of the skin ($C_{skin,eff}$) by (Hirschorn et al., 2010):

$$Q = C_{skin,eff}^{\alpha} R_{skin}^{(\alpha-1)} \quad (2)$$

If R_e is small compared to R_{skin}, then Eq. (1) can be simplified to give

$$R_{PAR} = \frac{R_{skin}}{1 + Q R_{skin} (2\pi f)^{\alpha} \cos(\frac{\alpha\pi}{2})} \quad (3)$$

which can be written, after substituting for Q using Eq. (2), as

$$R_{PAR} = \frac{R_{skin}}{1 + (2\pi f\, R_{skin}\, C_{skin,eff})^{\alpha} \cos(\frac{\alpha\pi}{2})} \quad (4)$$

According to Eq. (4), R_{PAR} is only equal to R_{skin} when it is measured at low frequency. At higher measurement frequencies, R_{PAR} is less than R_{skin} and it approaches zero in the limit of large frequency. If R_e is not small relative to R_{skin}, then R_{PAR} will approach R_e at high frequency and the sum of R_{skin} and R_e at low frequency.

2.2. Skin permeability

Skin permeability to a chemical is often characterized by the permeability coefficient (k_p). It is calculated from the ratio of the steady-state flux (J_{ss}) to the driving force for diffusion, which is the chemical concentration in the vehicle (C) when the concentration in the receptor fluid is kept close to zero, i.e.,

$$k_p = \frac{J_{ss}}{C} \quad (5)$$

In many *in vitro* diffusion cell experiments, the cumulative mass of chemical delivered into the receptor solution (M) per area (A) is determined as a function of time (t). If the concentration in the vehicle C is held constant, M/A increases linearly with t after a delay that is approximately 2.4 times the lag time (t_{lag}) as described by (Bunge and Cleek 1995):

$$M/A = J_{ss}(t - t_{lag}) \quad \text{for } t > 2.4 t_{lag} \quad (6)$$

in which J_{ss} is the slope and t_{lag} is the time intercept. Experimental estimates for J_{ss} that include M/A measurements at times less than about 2.4 t_{lag} in a linear regression based on Eq. (6) are systematically lower than the actual value (Bunge et al., 1995).

2.3. Relationship between skin impedance and permeability

The electrical resistivity (ρ), which quantitatively characterizes the resistance to ionic transport through skin, is related to the area-normalized DC skin resistance R_{skin} as

$$R_{skin} A = \rho \ell \quad (7)$$

where ℓ is the thickness of the skin layer primarily responsible for electrical resistance, which is usually the stratum corneum. The rate of ionic transport through the skin is proportional to the electrical conductivity, which is the inverse of ρ. Since k_p for an ionic compound is also proportional to ionic transport through skin, the ratio

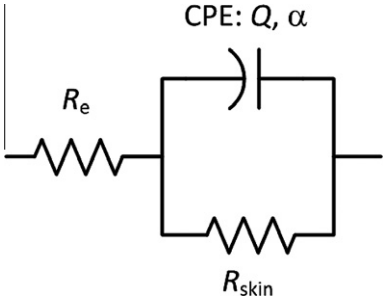

Fig. 1. A simple R-CPE circuit model of skin.

of k_p and $(\rho\ell)^{-1}$ is expected to be a compound-specific constant (b), which is defined as

$$b = \frac{k_p}{1/(\rho\,\ell)} = k_p(R_{skin}A) \tag{8}$$

The relationship between k_p and (R_{skin} A), as described by Eq. (8), should hold for any compound that penetrates through skin primarily *via* the pathway followed by ions. For these compounds, it follows that, for $R_e/R \ll 1$, Eqs. (2), (4), and (8) can be combined to give

$$R_{PAR}\,A = \frac{b/k_p}{1 + (2\pi f\,\frac{C_{eff}}{A}\,\frac{b}{k_p})^{\alpha}\cos(\frac{\alpha\pi}{2})} \tag{9}$$

which specifies the relationship between k_p and the frequency-dependent value of (R_{PAR} A).

3. Materials and methods

The data used in this study were taken from new experiments as well as previously published studies comparing: (a) tritiated water permeation to (R_{PAR} A) measured at 100 or 1000 Hz (Davies et al., 2004; Fasano et al., 2002), (b) (R_{skin} A) to (R_{PAR} A) measured at frequencies between 1 and 10,000 Hz (White et al., 2011), and (c) (R_{skin} A) to permeation of two model hydrophilic compounds, urea (Peck et al., 1995) and mannitol (Tang et al., 2001). In this section, the materials and methods are described for the new experiments and are summarized for the previously published experiments.

3.1. New experiments

Skin impedance and tritiated water absorption studies were conducted at Charles River (Edinburgh, Scotland, UK) using Scott-Dick diffusion cells (0.64 cm^2 diffusion area) and an automated flow-through system (Newcastle University, Newcastle, UK) described previously (Meidan and Roper, 2008). The receptor fluid flow rate was set to 1.5 mL/h. Full thickness human skin obtained from 20 different donors (8 male abdomen, 7 female abdomen, and 5 female breast, ages 19–66 years old) was acquired with full informed consent from the Plastic Surgery Unit of St. John's Hospital (Livingston, Scotland, UK) and stored at about −20 °C as described elsewhere (Meidan and Roper, 2008). After removal from frozen storage, samples were dermatomed (Zimmer, Swindon, UK) to a depth of 200–400 μm. Each sample was placed onto aluminum foil and its thickness confirmed using a micrometer (Pocket Thickness Gauge, Mitutoyo Corporation, Japan) and subtracting off the thickness of the foil. The samples were used immediately. Five skin samples from each subject were mounted with the stratum corneum facing the donor chamber. The receptor solution was minimum essential medium eagle (Sigma, Product No. M4655) with added glucose (1%, weight per volume), polyoxyethylene 20 oleyl ether (6%, weight per volume), penicillin G (100 units/mL) and streptomycin (0.1 mg/mL), which is a standardized receptor fluid used at Charles River in studies using both fresh and frozen skin. The skin surface temperature was maintained at 32 ± 1 °C (Meidan and Roper, 2008).

One milliliter of phosphate buffered saline (PBS) from Sigma (Product No. P4417) was applied to the skin surface in the donor chamber and allowed to equilibrate for 0.5 h, after which R_{PAR} was measured at 1000 Hz and then 100 Hz with the Tinsley 6401 LCR databridge (Surrey, England) using two 2-mm diameter stainless-steel wire electrodes (Low Voltage Meter Probe, RS Components Limited, Corby, Northamptonshire, UK) placed in the receptor and donor chambers, respectively. The donor solution was then removed by pipette; the skin surface was dried with tissue paper and left exposed to ambient air for 0.5 h while a blank sample of the receptor solution was collected. Next, 250 μL (390 μL/cm^2) of tritiated water (Amersham Pharmacia Biotech UK Limited, Little Chalfont, Buckinghamshire, UK) at 400,000 dpm/mL was applied to the stratum corneum surface of the skin within the donor chamber for 1 h and the receptor fluid collected. This sample was combined with 10 mL of liquid scintillation cocktail (Aquasafe 500 plus, Zinsser Analytic, Frankfurt, Germany) and analyzed by liquid scintillation counting using a Packard 2100-TR liquid scintillation analyzer (Perkin–Elmer, Beaconsfield, UK).

The fraction of the applied tritiated water collected in the receptor solution (f_{abs}) was calculated for each skin sample. The cumulative volume of water that penetrated through the skin during the 1-h exposure (equal to M divided by the density of water) was calculated from the product of f_{abs} and the volume of the tritiated donor solution applied to the skin sample (V), which was 250 μL.

For comparison to studies by Davies et al. (2004) and Fasano et al. (2002), k_p was estimated as

$$k_p = \frac{f_{abs}\,V}{A\,(t - t_{lag})} \tag{10}$$

where t was 1 h and t_{lag} was estimated to be 0.5 h from the results of a separate 2-h absorption study, as described below. In this study (Runciman et al., 2009), which followed procedures similar to those described above, the amount of tritiated water in the receptor solution was determined at 1 and 2 h after 400 μL/cm^2 of tritiated water was applied to 1082 split-thickness (*ca* 400 μm thick) skin samples acquired from 96 different donors (34 abdomen, 60 breast and 2 upper arm, aged 19–87 years old) with 2–47 samples from a given donor.

3.2. Literature experiments

Davies et al. (2004) reported paired *in vitro* measurements of tritiated water k_p and R_{PAR} at 100 Hz for whole and heat-separated human skin (59 and 53 samples, respectively) determined at 32 °C in diffusion cells with an area of 2.54 cm^2. In these experiments, the receptor fluid (0.9% sodium chloride) was sampled at 3, 4, 5 and 6 h after application of tritiated water (also in 0.9% sodium chloride solution) and k_p was calculated from the steady-state slope of M/A versus time. Electrical impedance was measured at least 0.5 h after application of the tritiated water solution using the PRISM Electronics AIM 6401 LCR databridge connected to stainless-steel wire electrodes, one each placed in the donor and receptor solutions. Skin samples that exhibited "very high" tritiated water absorption at 3 h were rejected for comparison with the electrical resistance measurements. Data from this study were derived by digitizing Figs. 1A and D in Davies et al. (2004) using Grapher (Version 8.5, Golden Software, Inc., Golden, CO, USA).

In the study from Fasano et al. (2002), *in vitro* measurements of tritiated water k_p and R_{PAR} determined at 1000 Hz using the Tinsley model 6401 LCR Databridge (Surrey, UK) were collected on split-thickness human skin from 11 subjects in vertically oriented diffusion cells at 32 °C with an area of 0.64 cm^2. The experimental procedures were similar to those followed by Davies et al. (2004), except for the timing of the receptor solution sampling, which occurred at 0.5, 1 and 2 h (Fasano et al., 2002). The data for 63 samples, which are plotted in Fig. 4 of Fasano et al. (2002), were provided by W. Fasano (private communication, August 2010).

The new tritiated water permeability and R_{PAR} data presented in this paper as well as the studies from Davies et al. (2004) and Fasano et al. (2002) were compared to multi-frequency impedance scans of 145 samples of split-thickness (approximately 300 μm thick) human cadaver skin collected from six different subjects as described previously (White et al., 2011). In this study, impedance of skin equilibrated for several hours with phosphate buffered saline (Sigma, Product No. P-3813) at 32 °C was determined in

horizontal diffusion cells as a function of frequency using four Ag/AgCl electrodes (In Vivo Metric, Healdsburg, CA, USA) by modulating the potential 10 mV rms with a Gamry potentiostat (model PCI4/300, Warminster, PA) for a range of frequencies (typically, between 1 Hz and 10 kHz) with 10 measurement frequencies per logarithmic decade. The real part of the skin impedance determined at the lowest measured frequency was taken to be the DC skin resistance; this value was estimated to be within 4% of the actual DC skin resistance for skin samples in the study.

The effective capacitance of each sample ($C_{skin,eff}$) was calculated using

$$C_{skin,eff} = \frac{1}{2\pi f_c R_{skin}} \quad (11)$$

where the characteristic frequency of each skin sample (f_c) is the frequency at which the negative of the imaginary component of the impedance is maximized (Orazem and Tribollet, 2008). The log-mean average of $C_{skin,eff}$ was 39.8 nF/cm^2.

The observed relationship between tritiated water absorption and the estimate of skin resistivity was compared to experimental measurements of urea and mannitol through heat-separated human skin, as examples of compounds known to permeate skin through the polar pathway. These data were chosen because pairs of k_p and (R_{skin} A) measurements through human skin were provided. It is anticipated that an analysis of measurements for other polar compounds would have produced similar results.

Peck et al. (1995) measured the DC resistance and k_p at 27 and 39 °C for three polar compounds: urea, mannitol, and tetraethylammonium bromide. Permeability coefficients were determined from steady-state flux measured in side-by-side diffusion cells with PBS (0.1 M ionic strength, buffered at pH 7.4) starting at either 27 or 39 °C, and then alternating between the two temperatures every 12 h for a total of five permeability measurements on each piece of skin. The DC resistance was calculated as the ratio of the applied voltage (100 mV) and the current response determined after the flux measurements at each temperature using Ag/AgCl electrodes in a four-electrode configuration. The authors report the average of the two or three measurements of k_p and (R_{skin} A) on each piece of skin at each temperature for urea and corticosterone, as example hydrophilic and lipophilic compounds, respectively. Data for urea were digitally derived from Fig. 7 in Peck et al. (1995) using Grapher.

Tang et al. (2001) measured the steady-state k_p of mannitol at 23 °C in vertical Franz-type diffusion cells filled with PBS. Impedance of skin was calculated from the current response to 100 mV applied across two Ag/AgCl electrodes (adjusting for the resistance from the PBS solution) at 10 Hz, which is a low enough frequency for the impedance measurement to represent R_{skin}. Human skin data were digitized from Fig. 1 of Tang et al. (2001) using Grapher.

4. Results and discussion

Experimental verification of the proposed relationship between electrical and permeability properties of skin are developed here for fluxes of tritiated water and the results compared to prior studies of two polar compounds. This work is then extended to quantify the criteria employed to assess skin integrity.

4.1. Application to tritiated water

The lag time for water permeation was estimated from measurements, originally reported by Runciman et al. (2009), of the area-normalized volume of tritiated water collected in the receptor fluid (f_{abs} V/A) in the first hour after tritiated water was applied compared to (f_{abs} V/A) collected over 2 h (Fig. 2). Under the assumption that a steady-state flux was reached within 1 h, (f_{abs} V/A) at 1 h and at 2 h should be related according to the following expression derived from Eq. (10):

$$k_p = \frac{f_{abs} V/A}{t - t_{lag}} = \frac{[f_{abs} V/A]_{at\ 1\ h}}{1\ h - t_{lag}} = \frac{[f_{abs} V/A]_{at\ 2\ h}}{2\ h - t_{lag}} \quad (12)$$

It follows then that a plot of (f_{abs} V/A) at 1 h compared to (f_{abs} V/A) at 2 h should be represented by

$$\left[\frac{f_{abs} V}{A}\right]_{at\ 1\ h} = \frac{1\ h - t_{lag}}{2\ h - t_{lag}} \left[\frac{f_{abs} V}{A}\right]_{at\ 2\ h} \quad (13)$$

which represents a line with a slope of (1 h − t_{lag})/(2 h − t_{lag}). The slope of the best-fit line through the data in Fig. 2 is 0.34. From this t_{lag} was calculated to be about 0.5 h, which indicates that a steady-state flux should be achieved in about 1.2 h (Bunge et al., 1995). In general, inclusion of data that were not at steady state in the analysis will cause t_{lag} to be underestimated. However, the difference between 1 and 1.2 h is small enough that the 0.5 h estimate for t_{lag} is reasonable within the accuracy of the measurements.

Measurements of (R_{PAR} A) determined at 100 and 1000 Hz from the Charles River study are plotted in Fig. 3 as functions of the inverse of k_p estimated from tritiated water absorption over 1 h under the assumption that t_{lag} was equal to 0.5 h for all skin samples. The assumption that t_{lag} was the same for all samples increases the variability of the estimated k_p. The data reported by Davies et al. (2004) and Fasano et al. (2002) for (R_{PAR} A) measured at 100 and 1000 Hz, respectively, are represented in Fig. 3 as triangles. In the Davies et al. (2004) study, there was no apparent difference in measurements from whole skin (filled triangles; (R_{PAR} A) = 37 ± 13 kΩ cm^2 and k_p = 0.97 × 10^{-3} ± 0.41 × 10^{-3} cm/h, reported as mean ± one standard deviation) compared with human epidermal membranes (HEM) prepared by heat separation (open triangles; (R_{PAR} A) = 53 ± 18 kΩ cm^2 and k_p = 0.81 × 10^{-3} ± 0.56 × 10^{-3} cm/h).

The curves in Fig. 3 represent the theoretical relationship for (R_{PAR} A) versus 1/k_p calculated according to Eq. (9) for b equal to 63 Ω cm^3/h, which is the value of b that minimized the sum of the square residuals for all data from the three laboratories weighted equally. In the calculation, $C_{skin,eff}/A$ was assigned a value of 39.8 nF/cm^2, which is the log-mean average from White et al. (2011), and α was assumed to be 0.8, which is a typical number for skin (Hirschorn et al., 2010; Poon and Choy, 1981).

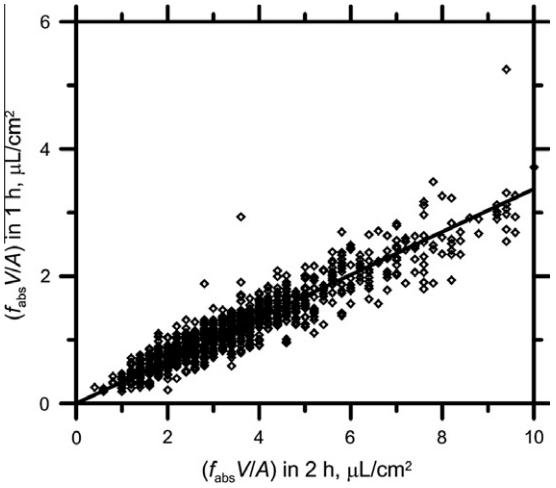

Fig. 2. Tritiated water absorption at 1 h compared with absorption at 2 h for all samples with less than 10 µL/cm^2 of water absorbed in 2 h. The line representing the best-fit linear regression of the data forced through the origin has a slope of 0.34 (r^2 = 0.86).

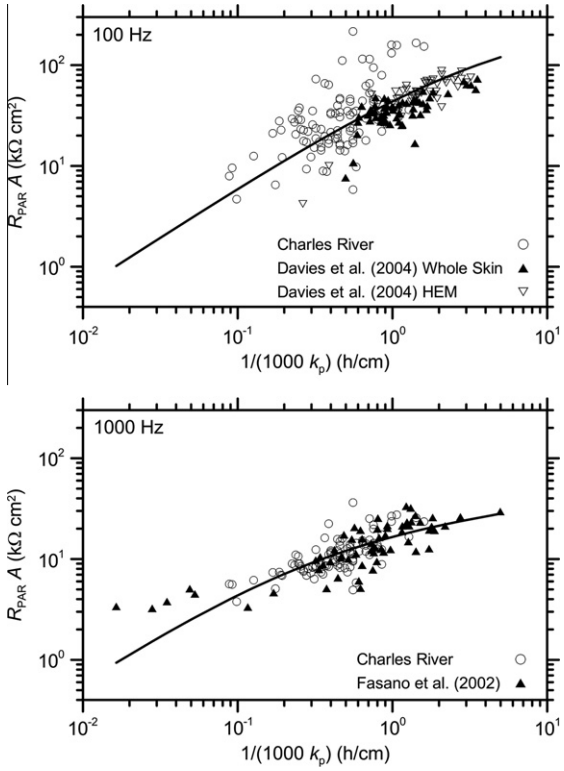

Fig. 3. (R_{PAR} A) measured at 100 and 1000 Hz plotted as a function of the inverse k_p for tritiated water determined in this study (circles) compared to data reported by Davies et al. (2004) and Fasano et al. (2002) for (R_{PAR} A) measured at 100 and 1000 Hz, respectively (triangles). For Davies et al. measurements collected on whole skin and heat-separated human epidermal membranes are distinguished by filled and open triangles, respectively. The curves represent the theoretical relationship for (R_{PAR} A) versus $1/k_p$ described by Eq. (9) for the value of b (63 Ω cm³/h) that minimized the sum of the square residuals for all the data assuming α is 0.8 and $C_{skin,eff}$ is 39.8 nF/cm², which is the log-mean average determined in the multi-frequency impedance study of White et al. (2011).

Consistent with the impedance theory, the experimental values of (R_{PAR} A) for a given skin sample were smaller at 1000 Hz than at 100 Hz. As a result, for the same range of k_p values, the range of observed (R_{PAR} A) values is compressed at 1000 Hz compared with 100 Hz. Therefore, changes in the skin barrier are detected with greater sensitivity using (R_{PAR} A) determined at lower measurement frequency (Fasano and Hinderliter, 2004).

Mean values and 90% confidence intervals, ignoring the influence of different subjects, are presented in Table 2 for the (R_{PAR} A) and k_p data shown in Fig. 3 as well as the values of (R_{PAR} A) reported by White et al. (2011) for frequencies of 1000, 100 and approximately 0 Hz (which represents R_{skin} A). In several cases, the mean value of the same measurement collected in a different laboratory differed by a statistically significant amount. This indicates that there were differences in the skin samples between laboratories with respect to skin resistance and tritiated water permeability. One source of these differences may be the exclusion in Davies et al. (2004) of skin samples with very large tritiated water concentrations in the receptor at 3 h.

Despite the differences between laboratories, as shown in Fig. 3, the relationship between k_p and (R_{PAR} A) determined at both 100 and 1000 Hz are consistent between laboratories and are also consistent with the theoretical model, Eq. (9), which includes two parameters (α and $C_{skin,eff}$ /A) derived from an impedance study of a different set of skin samples (White et al., 2011).

Five data points from the Fasano et al. (2004) study, all with (R_{PAR} A) determined at 1000 Hz that were less than 5 kΩ cm², appear to deviate systematically from the model prediction. This different behavior of low resistance skin samples compared to higher resistance samples has been observed previously. From the ratio of urea permeability measured at 39 and 27 °C, Peck et al. (1995) discovered that heat-separated human skin (epidermis) with (R_{skin} A) less than 20 kΩ cm² at 27 °C behaved more like a porous Nuclepore membranes than like higher resistance skin. Thus, Peck et al. (1995) selected 20 kΩ cm² as the minimum value of (R_{skin} A) for skin with acceptable integrity, which is one of the test criteria listed in Table 1. Notably, the value of b determined by best-fit regression of the data in Fig. 3 to Eq. (9) was unaffected by the presence of the five deviant points in the Fasano et al. data set.

In the derivation of Eq. (9), which relates (R_{PAR} A) and k_p, the product of the area-normalized DC skin resistance (R_{skin} A) and k_p for a given chemical was assumed to be a constant, b. This hypothesis is tested directly in Fig. 4, in which R_{PAR} measurements, determined at either 100 or 1000 Hz from each of the three laboratories, are plotted as a function of b/k_p for b at the optimum value of 63 kΩ cm³/h. The figures also include (R_{PAR} A) data at 100 and 1000 Hz from White et al. (2011) plotted as a function of (R_{skin} A). Within the variability of the measurements, the four different sets of (R_{PAR} A) and k_p data pairs from three different laboratories were in remarkable agreement with the (R_{PAR} A) and (R_{skin} A) data pairs from White et al. (2011). This shows that k_p for water is correlated linearly with the inverse of (R_{skin} A), as would be expected for compounds that permeate the stratum corneum primarily through the polar pathway.

The solid curves in Fig. 4 are plots of the frequency-dependent relationship between (R_{PAR} A) and (R_{skin} A) predicted by Eq. (4), which represents the R-CPE circuit model for α = 0.8 and $C_{skin,eff}$/A = 39.8 nF/cm² with the reasonable assumption that (R_e A) (typically, of order 0.1 kΩ cm² for PBS solutions in these diffusion cells)

Table 2
(R_{PAR} A) and k_p data from four different laboratories.[a]

Study[b]	ID	f (Hz)[c]	n[d]	(R_{PAR} A) (kΩ cm²)	(R_{PAR} A) differs from study ID[e]	k_p (cm/h)	k_p differs from study ID[e]
White et al.		0	145				
Charles River	A	100	97	39.4 ± 17.0%	C	2.78 ± 11.6%	B,E
Davies et al.	B	100	112	45.2 ± 6.0%	C	0.90 ± 8.5%	A,E
White et al.	C	100	145	54.4 ± 9.7%	A,B		
Charles River	D	1000	97	11.7 ± 8.1%	E,F		
Fasano et al.	E	1000	63	14.9 ± 11.2%	D,F	1.56 ± 18.6%	A,B
White et al.	F	1000	145	23.1 ± 8.8%	D,E		

[a] Results are reported as mean ± 90% confidence interval (reported as percent of mean), which was calculated using the Student's t-statistic.
[b] Full citation for the listed studies are: White et al. (2011), Davies et al. (2004), and Fasano et al. (2002).
[c] Frequency of the impedance measurement; for White et al. (2011), determinations at the lowest frequency measured (all less than or equal to 1 Hz) are designated as a frequency of zero.
[d] Number of skin samples in study.
[e] Means are considered different if the difference is statistically significant at the 5% level using the Student's t-test with different sample variances (Welch's test).

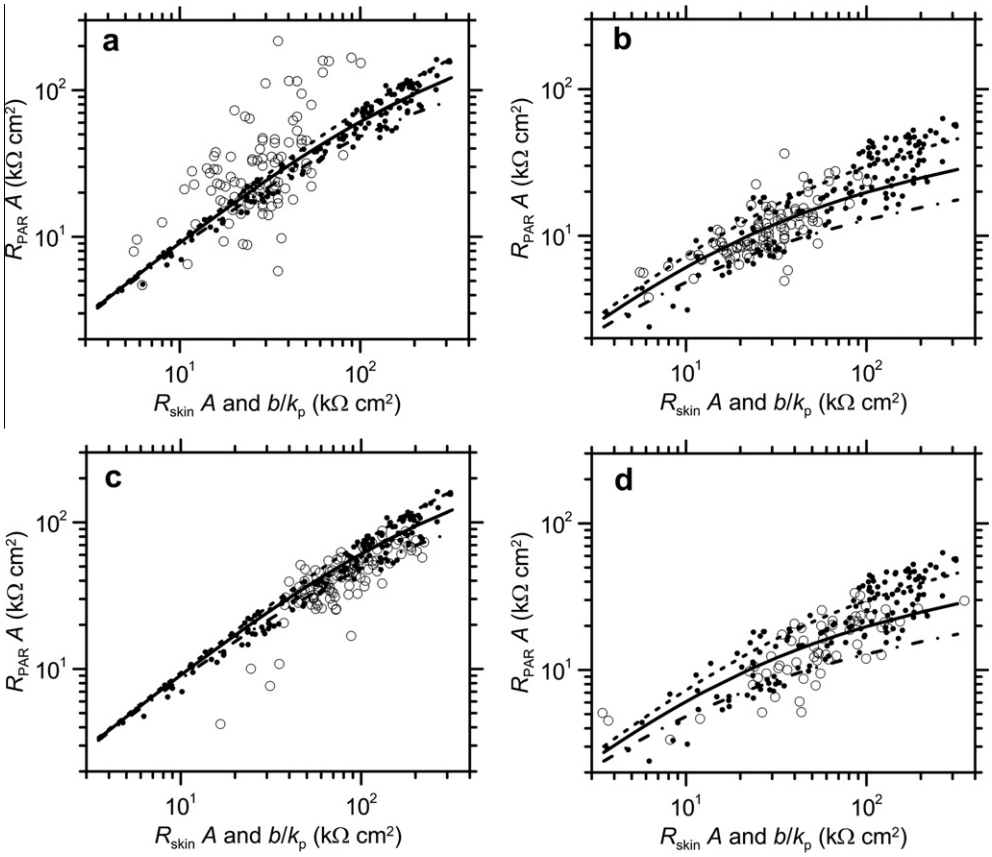

Fig. 4. A comparison of (R_{PAR} A) versus (R_{skin} A) data (small filled circles) from the multi-frequency impedance study of White et al. (2011) with (R_{PAR} A) measured at 100 and 1000 Hz versus b/k_p data from three different laboratories (open circles) for b equal to 63 Ω cm³/h: (a) Charles River data measured at 100 Hz, (b) Charles River data measured at 1000 Hz, (c) Davies et al. (2004) data measured at 100 Hz, and (d) Fasano et al. (2002) data measured at 1000 Hz. The theoretical relationship for (R_{PAR} A) plotted as a function of (R_{skin} A) was calculated using Eq. (4) for α equal to 0.8 and $C_{skin,eff}$ /A equal to 39.8 nF/cm² (solid curves), 75.8 nF/cm² (dot-dashed curves), and 20.2 nF/cm² (dashed curves), which represent the mean and plus and minus one standard deviation of log($C_{skin,eff}$/A) determined in the multi-frequency impedance study of White et al. (2011).

is small relative to (R_{skin} A) (typically, larger than 10 kΩ cm²). (Identical curves would be generated using Eq. (9) to calculate (R_{PAR} A) as a function of b/k_p for b at the optimum value of 63 kΩ cm³/h.) Although the curve closely represents the (R_{PAR} A) versus (R_{skin} A) data measured at 100 Hz, there is a systematic deviation between the theory and data measured at 1000 Hz for (R_{skin} A) larger than approximately 100 kΩ cm².

A likely cause of this deviation is that the R-CPE circuit model does not represent perfectly the skin impedance at all frequencies (Grimnes and Martinsen, 2005; Martinsen et al., 1997; Yamamoto and Yamamoto, 1976b). As a result, different values of $C_{skin,eff}/A$ and also α are derived when the R-CPE model equation is regressed to data from different frequency ranges. The 39.8 nF/cm² value for $C_{skin,eff}/A$ is the log-mean average of $C_{skin,eff}/A$ determined from the characteristic frequency for the 145 skin samples in White et al. (2011). Upper and lower bounds calculated by adding or subtracting one standard deviation, 0.3, to the logarithm of 39.8 nF/cm² corresponded to 75.8 and 20.2 nF/cm², respectively (White et al., 2011). The curve calculated using $C_{skin,eff}/A$ equal to 20.2 nF/cm² rather than 39.8 nF/cm² in Eq. (4) more closely represented the large impedance skin samples at 1000 Hz (see dashed curve in Fig. 4). Since there were only a few k_p data points with R_{PAR} determined at 1000 Hz that are larger than 100 kΩ cm², the systematic deviation of Eq. (4) calculated using 39.8 nF/cm² to the 1000 Hz data at large (R_{skin} A) had little effect on the estimate of b. Moreover, because (R_{skin} A) values of interest for skin integrity testing are much less than 100 kΩ cm², Eq. (4) with $C_{skin,eff}/A$ equal to 39.8 nF/cm² can be used reliably to relate integrity test criteria given as (R_{PAR} A) to (R_{skin} A).

4.2. Comparison to polar compounds

The values of the proportionality constants b for different chemicals that permeate skin through the same pathway as ions should be related to their permeability coefficients. The specific DC conductance of ions, which is equal to the inverse of (R_{skin} A) for a given skin sample, should equal the ratio of the values of k_p and b. Therefore, for two polar chemicals, it follows that

$$\frac{1}{R_{skin}A} = \left(\frac{k_p}{b}\right)_{\text{polar chemical 1}} = \left(\frac{k_p}{b}\right)_{\text{polar chemical 2}} \quad (14)$$

If water permeates the skin through only the polar pathway, then b for tritiated water compared to b for a known polar compound should equal the ratio of the permeability coefficients of tritiated water to the chemical:

$$\frac{b_{water}}{b_{\text{polar chemical}}} = \frac{k_{p,water}}{k_{p,\text{polar chemical}}} \quad (15)$$

However, if water also permeates the stratum corneum through the lipophilic pathway, then k_p/b for water should be smaller than k_p/b for a known polar chemical. In performing this analysis, the temperature dependence of (R_{skin} A) and $1/k_p$ should be the same, and therefore, b should be independent of temperature. However, k_p values do vary with temperature, and k_p measurements used in Eq. (15) should be at the same temperature.

Permeability coefficient and DC resistance data for urea (MW = 60.0 and $\log K_{o/w} = -2.11$; Hansch et al., 1995) from Peck et al. (1995) are shown in Fig. 5 along with the best-fit linear

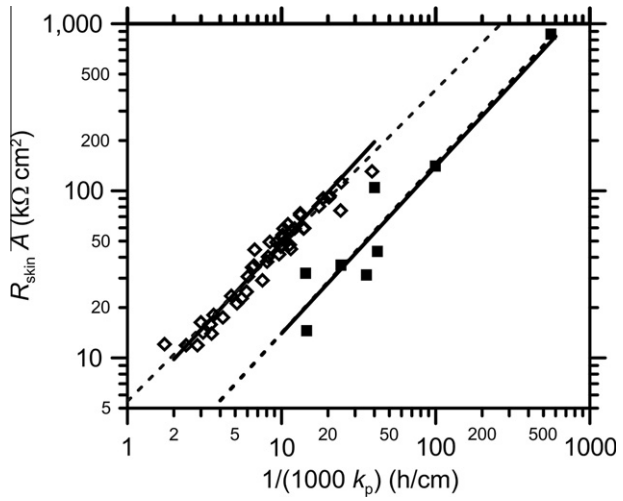

Fig. 5. (R_{skin} A) plotted as a function of $1/k_p$ for urea (diamonds) measured at 27 °C and 39 °C (Peck et al., 1995) and for mannitol (squares) measured at 23 °C (Tang et al., 2001). The solid lines have a slope of 1 and intercepts, log(b), equal to (log(R_{skin} A) – log($1/k_p$)) averaged over all data for each chemical: b is 4.9 Ω cm^2 and 1.4 Ω cm^2 for urea and mannitol, respectively. For comparison, the best-fit linear regressions to the data (dashed lines) are included: for urea, log[(R_{skin} A), kΩ cm^2] = 0.93 log[1/(1000k_p), h/cm] + log(5.5 Ω cm^2), r^2 = 0.95; for mannitol, log[(R_{skin} A), kΩ cm^2] = 1.01 log[1/(1000k_p), h/cm] + log(1.4 Ω cm^2), r^2 = 0.91.

regression of log(R_{skin} A) with log($1/k_p$), which, consistent with Eq. (8), has a slope close to 1. The expectation that temperature should not affect the relationship between (R_{skin} A) with ($1/k_p$) is supported by these results, which include pairs of (R_{skin} A) and k_p data determined at both 27 and 39 °C (Peck et al., 1995). Under the assumption that the slope has a value exactly equal to one, log(b) was determined from the log(R_{skin} A) intercept for each data point as

$$\log(b) = \log(R_{skin}\ A) - \log(1/k_p) \quad (16)$$

and then averaged over all data points. The resulting estimate for b was 4.9 ± 0.7 Ω cm^3/h (mean ± 1 standard deviation).

Peck et al. (1995) reported k_p values for urea of 0.201 × 10^{-3} cm/h and 0.272 × 10^{-3} cm/h at 27 and 39 °C, respectively, from which k_p at 32 °C was estimated by interpolation to be approximately 0.23 × 10^{-3} cm/h at 32 °C. This is slightly larger than 0.15 × 10^{-3} cm/h, which was measured by Barber et al. (1992) at 32 °C. Thus, k_p/b for urea is between 0.031 and 0.047 kΩ^{-1} cm^{-2}. Using a k_p for tritiated water between 1.5 × 10^{-3} and 2.0 × 10^{-3} cm/h (see Table 2 and Meidan and Roper, 2008), k_p/b for water is between 0.024 and 0.031 kΩ^{-1} cm^{-2}, which is either equal to or smaller than k_p/b for urea.

From a similar analysis of mannitol data from Tang et al. (2001), also shown in Fig. 5, b was determined to be 1.4 ± 0.6 Ω cm^3/h. For mannitol (MW = 182.2 and log$K_{o/w}$ = −3.10; Hansch et al., 1995), k_p at 32 °C was estimated to be 0.078 × 10^{-3} cm/h from measurements of 0.067 × 10^{-3} cm/h and 0.093 × 10^{-3} cm/h at 27 °C and 39 °C, respectively (Peck et al., 1995). For comparison, the average k_p from Tang et al. (2001) was 0.034 × 10^{-3} cm/h measured at 23 °C and Scott et al. (1991) reported a value of 0.061 × 10^{-3} cm/h at 30 °C. Therefore, k_p/b for mannitol is approximately 0.056 kΩ^{-1} cm^{-2}, which is similar to k_p/b for urea, as it should be for two polar compounds. Significantly, k_p/b for mannitol is about two times larger than k_p/b for water, which suggests that k_p for water is approximately twofold larger than expected for skin permeation by only the polar pathway used by mannitol. A twofold larger than expected water permeability also could be consistent with the urea results, although this is less clear since the range of k_p values is larger for urea.

The observation that k_p for water is linearly correlated with (R_{skin} A) is consistent with water permeation through the polar pathway. However, on average, the permeability of water through skin is about two times larger than expected for the polar pathway alone, suggesting an approximately equal contribution from the lipophilic pathway. Nearly equal contributions of the polar and lipophilic pathways may explain the apparent ability of water to function as both a lipophilic and polar skin permeant. Given this dual behavior, water permeation might be a better predictor of skin's permeability to lipophilic compounds than electrical conductance, which should represent only the polar pathway. However, ionic transport across skin that has enough damage to potentially affect permeation measurements of lipophilic compounds would exceed greatly the ionic transport through undamaged skin. As a result, tritiated water flux and appropriate measurements of skin resistance should be equally capable of identifying damaged skin.

4.3. Application to tests of skin integrity

A rearrangement of Eq. (10) along with Eqs. (8) and (4) provide a means of estimating, for a given tritiated water k_p, the corresponding values that are expected at a given frequency for tritiated water absorption after 1 h, (R_{skin} A), and (R_{PAR} A). The results are presented in Table 3 for the various tritiated water k_p values that have been used as criteria for rejecting skin that was potentially damaged. Also listed in Table 3 are values for (R_{PAR} A) calculated using two equations derived by linear regression of the logarithms of (R_{PAR} A) measurements at 100 and 1000 Hz, respectively, to the logarithm of (R_{skin} A) measured on the same piece of skin (White et al., 2011). Generally, these values of (R_{PAR} A) are smaller than those estimated from Eq. (4), although the difference was usually less than 10%. The chief advantage of Eq. (4) over the linear regression equations from White et al. (2011) is that Eq. (4) can be used to estimate (R_{PAR} A) at any frequency. Based on the results shown in Fig. 4, it is appropriate to restrict the use of Eq. (4) to frequencies less than about 1000 Hz.

Table 3
Calculated values of the 1-h absorption, (R_{skin} A), and (R_{PAR} A) determined at 100 and 1000 Hz corresponding to different values of tritiated water permeability.

Tritiated water		(R_{skin} A) = b/k_p (kΩ cm^2)[b]	(R_{PAR} A) from Eq. (4)		(R_{PAR} A) from White et al.[c]	
k_p × 10^3 (cm/h)	1-h absorption (μL/cm^2)[a]		100 Hz (kΩ cm^2)	1000 Hz (kΩ cm^2)	100 Hz (kΩ cm^2)	1000 Hz (kΩ cm^2)
1.5	0.8	42	32	14	28	14
2.0	1.0	32	25	12	21	11
2.5	1.3	25	21	11	18	10
3.0	1.5	21	18	9.7	15	8.6
3.5	1.8	18	15	8.9	13	7.8
4.0	2.0	16	14	8.2	12	7.1
4.5	2.3	14	12	7.6	11	6.6

[a] Equal to f_{abs} V/A at 1 h calculated using Eq. (10) and assuming t_{lag} = 0.5 h.
[b] For b equal to 63, which is the optimum value determined by minimizing the sum of the squared residuals to the prediction from Eq. (9) equally weighting all measurements from all studies.
[c] log[($R_{PAR}A$) at 100 Hz] = 0.837 log($R_{skin}A$) + 0.092; log[($R_{PAR}A$) at 1000 Hz] = 0.668 log($R_{skin}A$) + 0.053 (see White et al., 2011).

Using Eq. (4), the (R_{skin} A) values that corresponded with the various electrical resistance integrity test criteria listed in Table 1 have been calculated. These are listed in Table 1 along with tritiated water k_p values estimated using Eq. (8), which can be compared to the k_p values that were used to derive the test criteria. Of the criteria in Table 1, the recommendation of 20 kΩ cm^2 for (R_{skin} A) from Peck et al. (1995) was based on membrane behavior that is consistent with damage rather than correlation to a selected permeability coefficient value. Significantly, 20 kΩ cm^2 as a test criterion for (R_{skin} A) corresponds to 17 kΩ cm^2 for (R_{PAR} A) at 100 Hz and a tritiated water k_p of 3.2×10^{-3} cm/h. This k_p may be large enough so that undamaged but higher permeability skin samples are not screened from study.

5. Conclusions

Impedance measurements collected at a single frequency not larger than 1000 Hz on human skin *in vitro* are related in a predictable way to DC skin resistance (R_{skin} A), based on a simple model circuit of a parallel resistor and constant phase element (CPE) in parallel, and therefore, after normalizing for area, to the steady-state permeability coefficient (k_p) for ionic or polar chemicals. The nonlinear relationship between k_p values for tritiated water and resistances reported in parallel (PAR) mode obtained in three different laboratories at either 100 or 1000 Hz were predicted using a single equation, even though the mean values of the skin samples for permeability and resistance were significantly different for the different laboratories. The success of this equation supports the hypothesis that k_p values for tritiated water are linearly correlated with (R_{skin} A), which are a measure of the barrier function to ions. However, the magnitude of k_p relative to (R_{skin} A) for water compared with two polar chemicals, urea and mannitol, is consistent with water permeation at comparable rates through both the polar and lipophilic pathways. This may explain the apparent ability of water to behave as both a lipophilic and polar skin permeant.

Using the equation relating water k_p and (R_{PAR} A), previously published impedance criteria for identifying skin as acceptable for chemical absorption studies were compared on the basis of water k_p. Overall, (R_{PAR} A) determined at 100 Hz can be used reliably to test for skin integrity with better sensitivity than (R_{PAR} A) determined at 1000 Hz. The test criterion of 20 kΩ cm^2 for (R_{skin} A) has been shown to correspond with a tritiated water permeability of about 3.2×10^{-3} cm/h. This criterion could be able to exclude damaged skin without screening undamaged but higher permeability skin samples from study.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

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References

Barber, E.D., Teetsel, N.M., Kolberg, K.F., Guest, D., 1992. A comparative study of the rates of in vitro percutaneous absorption of eight chemicals using rat and human skin. Fundam. Appl. Toxicol. 19, 493–497.

Bronaugh, R.L., Stewart, R.F., 1986. Methods for in vitro percutaneous absorption studies VI: preparation of the barrier layer. J. Pharm. Sci. 75, 487–491.

Buist, H.E., van de Sandt, J.J.M., van Burgsteden, J.A., de Heer, C., 2005. Effects of single and repeated exposure to biocidal active substances on the barrier function of the skin in vitro. Regul. Toxicol. Pharmacol. 43, 76–84.

Bunge, A.L., Cleek, R.L., 1995. A new method for estimating dermal absorption from chemical exposure. 2. Effect of molecular weight and octanol-water partitioning. Pharm. Res. 12, 87–94.

Bunge, A.L., Cleek, R.L., Vecchia, B.E., 1995. A new method for estimating dermal absorption from chemical exposure. 3. Compared with steady-state methods for prediction and data analysis. Pharm. Res. 12, 972–982.

Davies, D.J., Ward, R.J., Heylings, J.R., 2004. Multi-species assessment of electrical resistance as a skin integrity marker for in vitro percutaneous absorption studies. Toxicol. In Vitro 18, 351–358.

Fasano, W.J., Hinderliter, P.M., 2004. The Tinsley LCR Databridge Model 6401 and electrical impedance measurements to evaluate skin integrity in vitro. Toxicol. In Vitro 18, 725–729.

Fasano, W.J., Manning, L.A., Green, J.W., 2002. Rapid integrity assessment of rat and human epidermal membranes for in vitro dermal regulatory testing: correlation of electrical resistance with tritiated water permeability. Toxicol. In Vitro 16, 731–740.

Grimnes, S., Martinsen, O.G., 2005. Cole electrical impedance model – a critique and an alternative. IEEE Trans. Biomed. Eng. 52, 132–135.

Hansch, C., Leo, A., Hoekman, D., 1995. Exploring QSAR: Hydrophobic, Electronic, and Steric Constants. American Chemical Society, Washington, DC.

Heylings, J.R., Esdaile, D.J., 2007. Percutaneous absorption of pesticides. In: Roberts, M.S., Walters, K.A. (Eds.), Dermal Absorption and Toxicity Assessment, second ed. Informa Healthcare, New York, pp. 575–591.

Hirschorn, B., Orazem, M.E., Tribollet, B., Vivier, V., Frateur, I., Musiani, M., 2010. Determination of effective capacitance and film thickness from constant-phase-element parameters. Electrochim. Acta 55, 6218–6227.

Horne, A., Blackstock, C., Roper, C.S., 2010. Evaluation of electrical resistance for use as a rapid method for human skin barrier integrity assessment in the flow through diffusion cell. In: Proceedings of the Skin Forum, 11th Annual Meeting, Edinburgh, Scotland.

International Programme on Chemical Safety (IPCS), 2006. Dermal Absorption, Environmental Health Criteria 235. World Health Organization, Geneva.

Kalia, Y.N., Pirot, F., Guy, R.H., 1996. Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo. Biophys. J. 71, 2692–2700.

Kalia, Y.N., Nonato, L.B., Lund, C.H., Guy, R.H., 1998. Development of skin barrier function in premature infants. J. Invest. Dermatol. 111, 320–326.

Kasting, G.B., Bowman, L.A., 1990. DC electrical properties of frozen, excised human skin. Pharm. Res. 7, 134–143.

Kasting, G.B., Filloon, T.G., Francis, W.R., Meredith, M.P., 1994. Improving the sensitivity of in vitro skin penetration experiments. Pharm. Res. 11, 1747–1754.

Kasting, G.B., Barai, N.D., Wang, T.-F., Nitsche, J.M., 2003. Mobility of water in human stratum corneum. J. Pharm. Sci. 92, 2326–2340.

Kontturi, K., Murtomaki, L., 1994. Impedance spectroscopy in human skin. A refined model. Pharm. Res. 11, 1355–1357.

Lawrence, J.N., 1997. Electrical resistance and tritiated water permeability as indicators of barrier integrity of in vitro human skin. Toxicol. In Vitro 11, 241.

Martinsen, O.G., Grimnes, S., Sveen, O., 1997. Dielectric properties of some keratinised tissues. 1. Stratum corneum and nail in situ. Med. Biol. Eng. Comput. 35, 172–176.

Meidan, V.M., Roper, C.S., 2008. Inter- and intra-individual variability in human skin barrier function: a large scale retrospective study. Toxicol. In Vitro 22, 1062–1069.

Mitragotri, S., 2007. Temperature dependence of skin permeability to hydrophilic and hydrophobic solutes. J. Pharm. Sci. 96, 1832–1839.

OECD, 2004a. OECD Guideline for testing of chemicals. Guideline 428: skin absorption: in vitro method (Original Guideline, adopted 13th April 2004).

OECD, 2004b. OECD Guideline for the testing of chemicals. Guidline 430: in vitro skin corrosion: Transcutaneous Electrical Resistance test (TER) (Original Guideline, adopted 13th April 2004).

Orazem, M.E., Tribollet, B., 2008. Electrochemical Impedance Spectroscopy. Wiley-Interscience, Hoboken, NJ.

Peck, K.D., Ghanem, A.H., Higuchi, W.I., 1994. Hindered diffusion of polar molecules through and effective pore radii estimates of intact and ethanol treated human epidermal membrane. Pharm. Res. 11, 1306–1314.

Peck, K.D., Ghanem, A.H., Higuchi, W.I., 1995. The effect of temperature upon the permeation of polar and ionic solutes through human epidermal membrane. J. Pharm. Sci. 84, 975–982.

Poon, C.S., Choy, T.T.C., 1981. Frequency dispersions of human-skin dielectrics. Biophys. J. 34, 135–147.

Potts, R.O., Francoeur, M.L., 1991. The influence of stratum corneum morphology on water permeability. J. Invest. Dermatol. 96, 495–499.

Potts, R.O., Guy, R.H., 1992. Predicting skin permeability. Pharm. Res. 9, 663–669.

Runciman, J., Roper, C.S., Madden, S., 2009. Evaluation of a rapid tritiated water skin barrier integrity method for use in regulatory toxicology testing in vitro, Occupational and Environmental Exposure of the Skin to Chemicals (OEESC), Edinburgh, Scotland, June 14–17.

Scott, R.C., Dugard, P.H., Ramsey, J.D., Rhodes, C., 1987. In vitro absorption of some ortho-phthalate diesters through human and rat skin. Environ. Health Perspect. 74, 223–227.

Scott, R.C., Corrigan, M.A., Smith, F., Mason, H., 1991. The influence of skin structure on permeability: an intersite and interspecies comparison with hydrophilic penetrants. J. Invest. Dermatol. 96, 921–925.

Tang, H., Mitragotri, S., Blankschtein, D., Langer, R., 2001. Theoretical description of transdermal transport of hydrophilic permeants: application to low-frequency sonophoresis. J. Pharm. Sci. 90, 545–568.

van de Sandt, J.J., Meuling, W.J., Elliott, G.R., Cnubben, N.H., Hakkert, B.C., 2000. Comparative in vitro-in vivo percutaneous absorption of the pesticide propoxur. Toxicol. Sci. 58, 15–22.

White, E.A., Orazem, M.E., Bunge, A.L., 2011. A critical analysis of single-frequency LCR databridge impedance measurements of human skin. Toxicol. In Vitro 25, 774–784.

World Precision Instruments, 2008. EVOM & EVOMX Epithelial Voltohmmeters, Instruction Manual, Sarasota, FL.

Yamamoto, T., Yamamoto, Y., 1976a. Dielectric constant and resistivity of epidermal stratum corneum. Med. Biol. Eng. Comput. 14, 494–500.

Yamamoto, T., Yamamoto, Y., 1976b. Electrical properties of the epidermal stratum corneum. Med. Biol. Eng. 14, 151–158.

Yamamoto, T., Yamamoto, Y., 1981. Non-linear electrical-properties of skin in the low-frequency range. Med. Biol. Eng. Comput. 19, 302–310.