ELSEVIER

Toxicology in Vitro 18 (2004) 725 729

Toxicology in Vitro

www.elsevier.com/locate/toxinvit

The Tinsley LCR Databridge Model 6401 and electrical impedance measurements to evaluate skin integrity in vitro

W.J. Fasano *, P.M. Hinderliter

The DuPont Company, Haskell Laboratory for Health and Environmental Sciences, P.O. Box 50, Elkton Road, Newark, DE 19714-0050, USA

Accepted 5 January 2004

Abstract

Electrical impedance is used to confirm skin integrity for in vitro dermal regulatory testing and as a tool to evaluate skin condition to determine the irritation and corrosion potential of various chemicals and personal care products. In this experiment, samples of dermatomed human skin were mounted onto static diffusion cells (0.64 cm^2) maintained at 32 °C. Following equilibration with 0.9% saline in the donor and receptor chambers, an impedance measurement was taken with a Tinsley LCR Databridge Model 6401 set in the resistance mode (R) and in (a) the serial-equivalent mode (SER) with an alternating current (AC) frequency of 100 hertz (Hz), (b) SER and 1000 Hz, (c) parallel-equivalent mode (PAR) and 100 Hz, and (d) PAR and 1000 Hz. With the databridge set in the SER-equivalent mode and an AC frequency of 1000 Hz, the minimum (7.2 kΩ), maximum (10.0 kΩ), and median (8.6 kΩ) impedance values exhibited a limited response range (2.8 kΩ). However, when the Tinsley 6401 was set in the PAR-equivalent mode at the lower AC frequency of 100 Hz the minimum (16.7 kΩ), maximum (134.6 kΩ), median (83.2 kΩ), and range (117.9 kΩ) of values were the highest obtained. The results confirm that the operator-selected settings on the Tinsley LCR Databridge Model 6401 affect the impedance measurement and the dynamic range of values observed for dermatomed human skin in vitro.

Keywords: Electrical impedance; Human skin; In vitro static diffusion cell; Stratum corneum; Tinsley LCR Databridge Model 6401

1. Introduction

In a recent publication data was presented that correlated tritiated water permeability with electrical impedance as a rapid method to qualify rat and human epidermal membranes for use on in vitro dermal regulatory experiments (Fasano et al., 2002). In that publication, a number of important factors were presented that affected response when using the Tinsley LCR Databridge Model 6401 (Tinsley 6401). In particular, in vitro cell exposure area, alternating current (AC) frequency, and concentration of the electrolyte bathing solution all had an influence on the impedance measurement. However, the results and discussion in the original paper did not include data and information on the operator-selected settings for the Tinsley 6401 and how these might also affect the impedance measurement.

The Tinsley 6401, a low-voltage, alternating current (AC), automatic LCR databridge, is capable of measuring inductance (L), capacitance (C), and resistance (R) of various electronic circuits. The Tinsley 6401, which is simple to operate, has found its way into the in vitro dermal laboratory and has demonstrated its ability to allow for rapid integrity confirmation of skin mounted on glass static diffusion cells. In addition to its utility to confirm skin integrity, the Tinsley 6401 has seen use in other in vitro dermal tests where a change in skin impedance is used to determine the irritation and corrosivity potential of various chemicals and personal care products (Fentem et al., 1998; Heylings et al., 2003; Oliver et al., 1988). In general, the electrical impedance of skin is a reflection of the condition of the stratum corneum, the principle barrier to diffusion of chemicals, and its long-chain lipids filling the intercellular space between the anucleated corneocytes (Bouwstra, 1997;

Abbreviations: AC, alternating current; Hz, hertz; PAR, parallel-equivalent measurement mode; SER, serial-equivalent measurement mode

* Corresponding author. Tel.: +1-302-366-5216; fax: +1-302-366-5003.

E-mail address: william.j.fasano@dupont.usa.com (W.J. Fasano).

0887-2333/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tiv.2004.01.003

Bouwstra et al., 2000; Elias et al., 1981; Tregear, 1962). Removal of or damage to the stratum corneum either by accelerating the desquamation process using a tape stripping technique or upon exposure to various chemicals and organic solvents has been shown to result in a loss of electrical impedance (Sekkat et al., 2002).

The purpose of this brief communication is to present data demonstrating the potential range of impedance measurements, and therefore sensitivities that may be obtained with the Tinsley 6401, by making changes to two operator-controlled parameters: measurement mode, either parallel (PAR) or series (SER), and AC frequency, either 100 or 1000 Hz.

2. Materials and methods

2.1. In vitro static diffusion cell system

The in vitro static diffusion cell system (PermeGear, Inc., Hellertown, PA, USA) was composed of 0.64 cm^2 water-jacketed glass cells with a receptor chamber volume of approximately 5 ml. The individual cells were coupled to a water distribution system, which was connected to a re-circulating water bath adjusted to allow the skin, mounted on the in vitro cells, to be maintained at 32 °C.

2.2. Human skin preparation

Full-thickness human skin (lateral thigh) taken from cadavers within 24 h of death and stored frozen at approximately −20 °C, was thawed at room temperature and dermatomed to approximately 450 μm using a Padgett Electro Dermatome® (Padgett Instruments, Inc., Kansas City, MO, USA). The dermatomed skin was placed onto an aluminum pan and refrigerated at 4 °C until readied for use.

2.3. Electrical impedance measurements

Human skin samples ($n = 14$ replicates from four individuals) were removed from refrigeration storage and hydrated in 0.9% saline for approximately 15 minutes. Following hydration, the dermatomed skin was mounted onto the top of the receptor chamber (stratum corneum uppermost), which was filled with degassed physiologic saline (0.9% sodium chloride injection USP, Baxter Healthcare Corporation, Deerfield, IL, USA). The donor chamber was then clamped in place and filled with 0.9% saline. During equilibration (approximately 30 min), the water-jacketed cells were maintained at approximately 32 °C using a re-circulating water bath system. Following equilibration, an impedance measurement of each skin membrane was taken using a Tinsley LCR Databridge Model 6401 (H. Tinsley, Inc., Croydon, UK). The Tinsley 6401 is a low-voltage AC databridge with a maximum voltage across the circuit under test of 300 mV root-mean-square (rms). The test rigging was standard probe leads with stainless-steel pin tips (Radio Shack, Fort Worth, TX, USA, #278-705A). The input impedance of the Tinsley 6401 is very high as the instrument is capable of measuring up to 100 MΩ (100,000 kΩ). The impedance of each of the 14 dermatomed human skin samples was obtained by immersing the tip of the stainless-steel probe leads, one each into the saline contained in the donor and receptor chambers, with the databridge set in the resistance mode (R) and sequentially in each of the following four user-selectable measurement modes:

(a) Parallel-equivalent (PAR), 100 Hz
(b) Series-equivalent (SER), 100 Hz
(c) PAR, 1000 Hz
(d) SER, 1000 Hz

Impedance values are reported in kilo-ohms (kΩ). Regression analyses of impedance data were performed using Microsoft Excel® 2000, Microsoft Corporation, Redmond, WA, USA.

3. Results

Electrical impedance values for dermatomed human skin mounted on a 0.64 cm^2 glass static diffusion cell, as measured with the Tinsley LCR Databridge Model 6401 (Tinsley 6401) set in the parallel (PAR) and serial (SER) equivalent modes, and each at 100 and 1000 Hz, are presented in Table 1.

With the Tinsley 6401 set in the SER mode and an alternating current (AC) frequency of 1000 Hz, the lowest values were obtained and the range between the minimum (7.2 kΩ) and maximum value (10.0 kΩ) was only 2.8 kΩ. In contrast to this, with the databridge set in the PAR mode operated at the lower AC frequency setting of 100 Hz the range between the minimum (16.7 kΩ) and maximum impedance value (134.6 kΩ) was 117.9 kΩ.

Regression analysis of impedance data used to predict response from each of operator-controlled settings on the Tinsley 6401 showed a good correlation ($R^2 > 0.93$) for the default mode of PAR-1000 Hz to SER-100 Hz (Fig. 1A), and also for PAR-1000 Hz to PAR-100 Hz (Fig. 1B). The data presented in Fig. 1C also confirm a good fit between PAR-100 Hz and SER-100 Hz. Regression analysis of SER-1000 Hz with all other databridge settings was unsuccessful.

Table 1
Individual impedance values (kΩ) using a Tinsley LCR Databridge Model 6401 operated in the parallel (PAR) and serial (SER) mode with an alternating frequency (AC) of 100 and 1000 Hz for dermatomed human skin mounted in a 0.64 cm^2 static diffusion cell

Skin sample	SER-1000 Hz	PAR-1000 Hz	SER-100 Hz	PAR-100 Hz
A	7.2	25.0	42.4	75.6
B	10.0	13.9	18.2	19.9
C	8.9	26.9	45.5	84.2
D	9.8	30.4	54.1	94.2
E	8.4	35.4	52.6	134.6
F	7.5	29.2	47.1	98.5
G	7.7	20.4	37.4	63.4
H	8.2	26.9	45.7	82.1
I	9.3	11.8	16.0	16.7
J	8.7	31.7	50.5	105.7
K	9.9	33.4	54.4	89.3
L	7.4	31.1	46.6	124.7
M	9.1	22.5	38.3	48.0
N	7.9	14.9	27.4	36.7
Minimum	7.2	11.8	16.0	16.7
Maximum	10.0	35.4	54.4	134.6
Median	8.6	26.9	45.6	83.2
Range	2.8	23.6	38.4	117.9

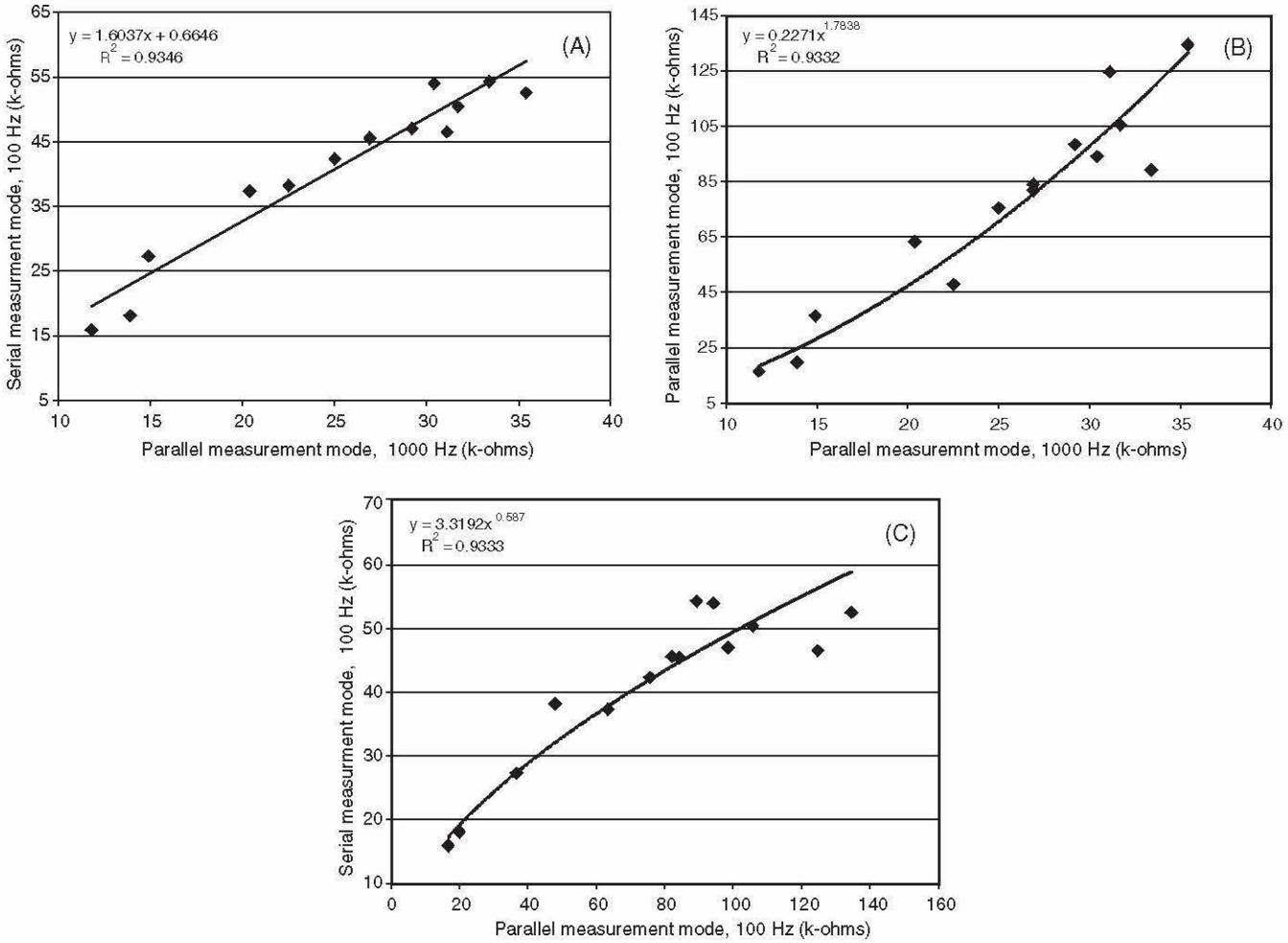

Fig. 1. Regression analysis of impedance data taken with a Tinsley LCR Databridge Model 6401 for dermatomed human skin mounted in a 0.64 cm^2 static diffusion cell: (A) parallel measurement mode (PAR) 1000 Hz vs. serial measurement mode (SER) 100 Hz; (B) parallel measurement mode (PAR) 1000 Hz vs. parallel measurement mode (PAR) 100 Hz; (C) parallel measurement mode (PAR) 100 Hz vs. serial measurement mode (SER) 100 Hz.

4. Discussion

When we conducted our original laboratory work to correlate tritiated water permeability with electrical resistance (impedance) as a means to rapidly qualify rat and human epidermal membranes for in vitro dermal regulatory testing, our data were generated with the Tinsley 6401 set in the default mode of PAR with an AC frequency of 1000 Hz; the data presented in Table 1 for PAR at 1000 Hz is in line with our original impedance data (Fasano et al., 2002). The essential conclusions from that work showed that electrical impedance of human skin was greater than rat skin, and that the range of impedance responses was found to fit a distribution, log-normal for rat skin and Weibull for human skin. Upon evaluation of that original data with similar data found in the open literature it was noted that impedance was also dependent upon skin exposure area (increase the area and decrease the impedance), and the concentration of sodium chloride used in the donor and receptor chambers (increase the ionic strength of the bathing medium and decrease the impedance). Although we were aware of the two mode settings of PAR and SER, and the two frequency settings of 100 and 1000 Hz on the Tinsley 6401, and the possible influence these settings might have on skin impedance, this was not explored in our original research. However, on one subsequent occasion, the frequency was inadvertently set to 100 Hz yielding higher values then had previously been observed and reported. Subsequently, this resulted in an examination of how the databridge functions and the potential impact of the various databridge settings on the impedance measurements that can be obtained. However, a discussion of how the Tinsley 6401 determines skin impedance in vitro is not complete without a brief review of a few basics principles regarding electrical circuits. The reader is encouraged to seek a primer on the subject if a more in-depth review is required.

Current, which is expressed in amperes (A), is the flow of electrons in a circuit. The electromotive force or pressure that causes electrons to flow is known as voltage (V), and resistance (R) is the friction that limits flow. In direct current (DC) circuits the current flows in a single direction and the ratio of voltage to current is called resistance ($R = V/I$). If the ratio V/I remains linear over a range of voltages or current densities when subjected to a DC potential, the material under test is said to obey Ohm's Law and is therefore "ohmic". However, when the relationship becomes non-linear, that is an increase in V does not yield a proportional increase in I, the material under test is considered "non-ohmic" and its resistance is no longer constant. Commercially available resistors, like those we often use to check the response of the databridge, are typically ohmic. Skin on-the-other-hand exhibits non-ohmic behavior, for which R decreases as current or voltage increases (Kasting and Bowman, 1990).

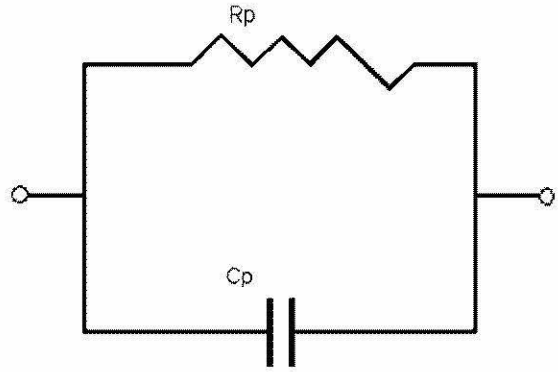

Fig. 2. An equivalent circuit model of skin where R_p is the resistor and C_p is the capacitor in parallel.

A simple equivalent model of skin is presented in Fig. 2 (Lai and Roberts, 1998; Yamamoto and Yomamoto, 1976) and is composed of a resistor (R_p) and capacitor (C_p) in parallel. Under the influence of AC, where the voltage and current constantly reverse direction as an oscillating sine wave, a frequency-dependent form of resistance called capacitance reactance (X_c) is introduced into the circuit. For circuits under the influence of AC, X_c is given by the following equation:

$$X_c = \frac{1}{2\pi f C} \tag{1}$$

where f is the frequency of the AC sine wave and C is the capacitance; the capacitor will store and release energy as the current and voltage fluctuate with each AC cycle. The parallel combination of resistance and reactive capacitance yields electrical impedance (Z), which is given by the following equation:

$$|Z| = \frac{1}{\sqrt{R^2 + X_c^2}} = \frac{R_p}{\sqrt{1 + 4\pi^2 f^2 C_p^2 R_p^2}} \tag{2}$$

For a pure resistor, $C_p = 0$ and Eq. (2) reduces to $Z = R$. However, for skin which is a non-ohmic material with a reactive component, $Z \neq R$.

When the Tinsley 6401 is set in the resistance measurement mode (R), exclusive of frequency, the displayed value for skin is actually Z not R. As given by Eq. (2), increasing the frequency from 100 to 1000 Hz will decrease the influence of the capacitor in the parallel equivalent model of the skin, which in turn results in a lower electrical impedance. In essence, the sensitivity of the skin impedance measurement is diminished when higher frequencies are employed making it difficult to resolve small perturbations to the stratum corneum. Further, if the databridge were measuring R instead of Z, changing the frequency would not change the displayed value.

The Tinsley 6401 also has a parallel equivalent measurement mode (PAR) and a serial equivalent measurement mode (SER) that roughly corresponds to a parallel and series equivalent circuit, respectively. Parallel and series resistance can be related by the following equation:

$$\frac{R_p}{R_s} = 1 + Q^2 \quad (3)$$

where R_p is the resistance in the PAR mode, R_s is the resistance in the SER mode, and Q is defined as a measure of quality when capacitance reactance (X_c) is introduced into the circuit under test ($Q = R/X_c$). For an ohmic material, $Q = 0$, and R_s will equal R_p. However, for non-ohmic materials like skin, Q increases as the AC frequency increases from 100 to 1000 Hz, also increasing R_p/R_s. The impedance value displayed by the Tinsley 6401 in the PAR measurement mode will always be greater than or equal to the impedance value when set in the SER measurement mode. Only if the component under test is a pure resistor will the value displayed by the Tinsley 6401 be identical in either the PAR or SER equivalent measurement modes.

Albeit serendipitous, it is now clear that the default setting of PAR-1000 Hz does not afford the greatest response range, and therefore sensitivity, to evaluate the condition of human skin in vitro. Nonetheless, the setting of PAR-1000 Hz offers acceptable response as evidenced by good correlation with the most sensitive databridge setting of PAR-100 Hz. Also, it can further be concluded that the response values for the setting of SER-1000 Hz must be considered useless since they lack a meaningful dynamic range and therefore offers little value as an endpoint measurement.

Upon review of the results from this brief experiment, we have shown that when using the Tinsley 6401 to determine the electrical impedance of human skin in vitro the operator-selected modes have an influence on the response and therefore the sensitivity of the evaluation method used where impedance (resistance) is an endpoint. The inter-setting results are well correlated and each skin replicate, without exception, followed the trend of PAR-100 Hz > SER-100 Hz > PAR-1000 Hz > SER-1000 Hz. Although only data for human skin is presented here, it is reasonable to expect that a similar response profile will be observed for skin from others species. Nonetheless, evaluation of rat skin in vitro, which is typically more permeable (lower electrical impedance) compared to human skin, may require the most sensitive databridge setting (PAR-100 Hz) in order to reveal minor yet possibly important changes to the stratum corneum, particularly where rat skin is used to predict the irritation and corrosive potential of chemicals and personal care products.

The Tinsley 6401 and its utility to perform rapid integrity checks of skin in vitro make it a valuable asset in the in vitro dermal laboratory. Information has been presented on the range of possible responses obtained for human skin based on the operator-selected settings, and therefore the potential sensitivity of the measurement, which are important considerations for evaluating the electrical impedance of skin in vitro.

Acknowledgements

We wish to thank Ms. LaRue A. Manning for her dedication to our in vitro dermal studies, and for pushing all the databridge buttons, especially those that allowed us to learn.

References

Bouwstra, J.A., 1997. The skin barrier, a well-organized membrane. Colloids and Surfaces, A: Physicochemical and Engineering Aspects 123 124, 403 413.

Bouwstra, J.A., Dubbelaar, F.E., Gooris, G.S., Ponec, M., 2000. The lipid organization in the skin barrier. Acta Dermato-Venereologica 208, 23 30.

Elias, P.M., Cooper, E.R., Korc, A., Brown, E.E., 1981. Percutaneous transport in relation to stratum corneum structure and lipid composition. Journal of Investigative Dermatology 76, 297 301.

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. Toxicology in Vitro 16, 731 740.

Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Esdaile, D.J., Holzhutter, H.G., Liebsch, M., 1998. The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicology in Vitro 12, 483 524.

Heylings, J.R., Diot, S., Esdaile, D.J., Fasano, W.J., Manning, L.A., Owen, H.M., 2003. A prevalidation study on the in vitro skin irritation function test (SIFT) for prediction of acute skin irritation in vivo: results and evaluation of ECVAM Phase III. Toxicology in Vitro 17, 123 138.

Kasting, G.B., Bowman, L.A., 1990. DC Electrical properties of frozen, excised human skin. Pharmaceutical Research 7, 134 143.

Lai, P.M., Roberts, M.S., 1998. Iontophoresis. In: Roberts, M.S., Walters, K.A. (Eds.), Dermal Absorption and Toxicity Assessment, vol. 91. Marcel Dekker, New York.

Oliver, G.J., Pemberton, M.A., Rhodes, C., 1988. An in vitro model for identifying skin-corrosive chemicals. I. Initial validation. Toxicology in Vitro 2, 7 17.

Sekkat, N., Kalia, Y.N., Guy, R.H., 2002. Biophysical study of porcine ear skin in vitro and its comparison to human skin in vivo. Journal of Pharmaceutical Sciences 91, 2376 2381.

Tregear, R.T., 1962. The structures which limit the permeability of the skin. Journal of the Society of Cosmetic Chemists 13, 145 151.

Yamamoto, T., Yomamoto, Y., 1976. Electrical properties of the epidermal stratum corneum. Medical and Biological Engineering 14, 151 158.