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Impedance measurements for the non-invasive monitoring of skin hydration: a reassessment

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Summary

A number of reports suggest that skin impedance provides a useful non-invasive method for assessing skin hydration. The technique is reassessed in this report. It has been found that wide inter-subject and intra-subject variations in skin impedance are present. These variations cast some doubts on the validity of some of the earlier reported data. Short-circuiting of the cells may explain some of these data. Experiments involving stripping of the stratum corneum suggest that in measuring skin impedance the area of skin immediately below the electrodes rather than that between the electrodes determine the value obtained. The importance of setting up careful controls is stressed.

Introduction

The assessment of the physicochemical effects of topically applied medication and cosmetics, on the skin, presents a number of difficulties. Unlike other organ systems, except for glandular metabolic rates as evidenced by sweating and greasiness, induced physiological changes are usually subtle and slow. Animal models are

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usually inadequate because of marked inter-species variation. While excised skin adequately models the diffusional properties of in vivo skin, it often fails when other parameters are investigated. Even with diffusional studies, excised skin can be inadequate when trans-follicular transport is important in vivo. The percutaneous absorption of corticosteroids is an example (Flynn, 1977). There is therefore a clear need for non-invasive in vivo methods for such studies. A number of such techniques have been studied and proposed (Rolfe, 1979).

Blanching and hyperaemia induced by pharmacologically active ingredients such as corticosteroids, nicotinate esters and organic nitrates have been successfully studied by visual assessment or more objectively by the use of photometric methods or light-integrating spheres (Feather et al., 1982; Altmeyer and Cremer, 1977). More recently, laser velocimetry has been advocated for such studies (Wahlberg, 1984).

Our interest in non-invasive techniques for assessing the skin arose as a result of the need to devise an objective method for measuring the moisturizing effects of both cosmetic and pharmaceutical formulations. We were also highly conscious of the need to replace animal models whenever alternative methods became available. The importance of the degree of hydration in ensuring the integrity of the skin is beyond dispute since the work of Blank (1952, 1953). There is also little doubt on the cosmetic significance of proper skin hydration. Our initial efforts in monitoring this parameter were directed towards impedance measurements of the skin because of our familiarity with the application of such measurements in other fields (Archer and Armstrong, 1980; Hladky et al., 1980; Mansfeld, 1981; Armstrong et al., 1977), and more importantly, because of the positive results reported by a number of other workers (Clar et al., 1975; Serban et al., 1981a and b; Campbell et al., 1977; Tagami et al., 1980). It was evident to us that in many of the reported studies inadequate controls were carried out to validate the technique and this report deals with a systematic study of factors which may affect the values obtained in the impedance measurement of the skin.

Theory

The composition and metabolic activity of the skin alter its electrical properties. Modelling the skin as an electrical circuit is therefore reasonable. It is now generally accepted that the stratum corneum, the outermost few layers of skin, provides the main barrier to the penetration of exogenous materials and to a large extent imparts the perceptible characteristics such as skin feel and smoothness to the skin. Measurements should therefore focus on the stratum corneum.

The stratum corneum is essentially a dielectric medium being of low conductivity. As it hydrates, its conductivity increases as a result of a combination of factors. Firstly, the hydrated keratin acquires increased flexibility when compared to the dry state. Secondly, the hydrated layer provides a better medium for the conduction of its ionic inclusions, and thirdly, dissociation of the water molecules themselves into the hydronium and hydroxyl ions increases conductivity of the normally oily sebaceous secretions.

To overcome electrode polarization effects, impedance, that is resistance under an alternating current, is measured. The complex impedance Z can be related to the applied voltage and measured current by Eqn. 1.

$$Z = V^*/I \tag{1}$$

with complex V*. The latter can be resolved into a real and imaginary component V′ and V″ and Eqn. 1 rewritten as:

$$Z = V'/I + iV''/I \tag{2}$$

where $i = \sqrt{-1}$ as usual.

V′/I and V″/I are elements of the set of all real numbers although they are normally referred to as the resolved real and imaginary components of Z. Eqn. 2 can therefore be rewritten as:

$$Z = R_s + i\,X_s \tag{3}$$

or $Z = Z' - i\,Z''$ in some literature.

With a change in frequency the point Z in the complex impedance plane changes to produce a part-circular locus for most biological materials. The points on the complex plane can, of course, be represented in polar form in terms of modulus |Z| and the argument θ as defined by Eqns. 4 and 5.

$$|Z| = \left[X_s^2 + R_s^2\right]^{1/2} \tag{4}$$

$$\theta = \arctan(X_s/R_s) \tag{5}$$

|Z| is known as the impedance and θ as the phase angle. R_s is the resistance and X_s

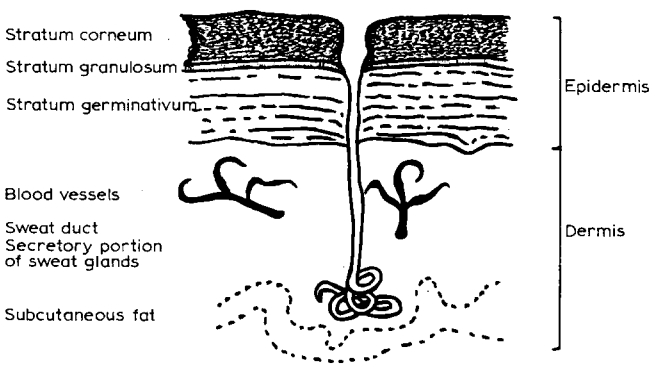

Fig. 1. Schematic structure of the skin.

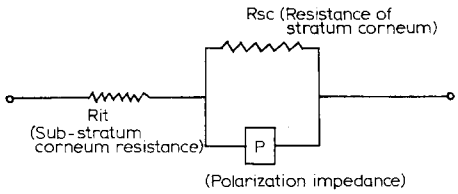

Fig. 2. Equivalent electrical circuit of the skin.

the reactance. Figs. 1 and 2 are schematic diagrams of the skin and its most widely accepted equivalent circuit (Salter, 1979; Clar et al., 1975; Tagami et al., 1980; Barnett, 1938; Tregear, 1966; Yamamoto and Yamamoto, 1976; Allenby et al., 1969). This equivalent circuit assumes one electrode is placed on the skin surface, whereas the other is within the deeper body tissues. Fig. 3 is a typical impedance plot obtained from a pair of electrodes applied on the skin surface. In this electrode configuration, as used by us, the hypothetical equivalent circuit should be as shown in Fig. 2 with an additional Rsc and P parallel element.

In assessing the effects of topical applications on the stratum corneum, the Rsc term is therefore of prime importance, if the model is satisfactory. P is referred to as a polarization impedance in the literature and takes account of the frequency dependence of the capacitive elements of the skin: a more detailed explanation can be found elsewhere (Salter, 1979; Tregear, 1966; Barnett, 1938).

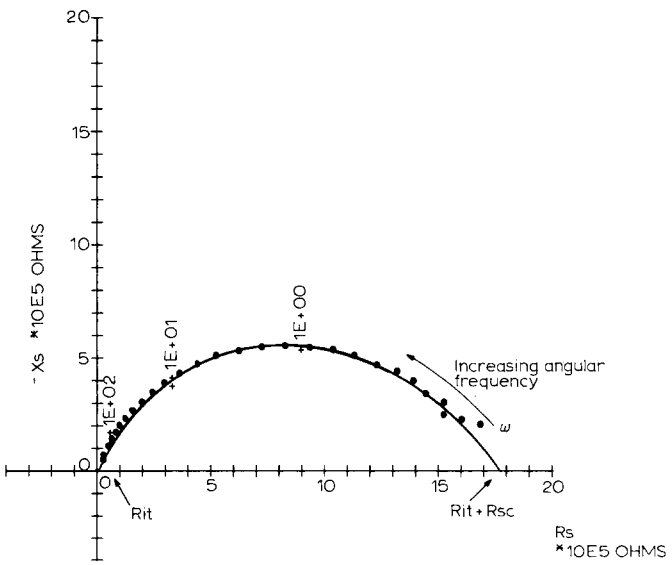

Fig. 3. Resultant impedance plot for in vivo skin measurements.

Experimental

Measuring assembly

Impedance measurements were made using a Solartron 1174 frequency response analyzer (Solartron Electronics Group, Farnborough, Hampshire, U.K.). The frequency response analyzer consists of a programmable generator which provides the perturbing sinusoidal signal, a digital correlator to analyze the response of the system, and a display to present the results. The use of such equipment has been fully covered elsewhere (Armstrong et al., 1977).

Fig. 4 shows a typical experimental arrangement using the Solartron 1174 linked with an HP9845B (Hewlett Packard, CO, U.S.A.) desk top computer and associated peripherals. A real time complex plane impedance diagram is displayed on the CRT of the computer as the measurements progress, and the data is stored on flexible discs for subsequent manipulation.

Measurements were carried out over the frequency range 1–500 Hz with a perturbing signal of 100 mV RMS. A logarithmic frequency sweep was employed with 10 points per frequency decade being measured.

Electrodes

Ag–AgCl electrodes (E223, In Vivo Metric Systems, Healdsburg, CA, U.S.A.) of 8 mm sensor diameter were used. The electrodes are mounted in a solid epoxy housing with a cavity 2 mm deep. Fig. 5 shows the type of electrodes used and the complete electrode assembly. Two electrodes were held, 39.5 mm apart from their centres, in a adjustable elastic bracelet. This ensured a comfortable and secure contact with the skin. The electrical contact between the body surfaces and electrodes was further promoted using a salt free electrode contact gel (Spectra 360, In Vivo Metric Systems).

Procedure

The subjects chosen were Caucasians in the age range 21–25 years. No distinction was made between the sexes. Subjects were placed in a room on an adjustable

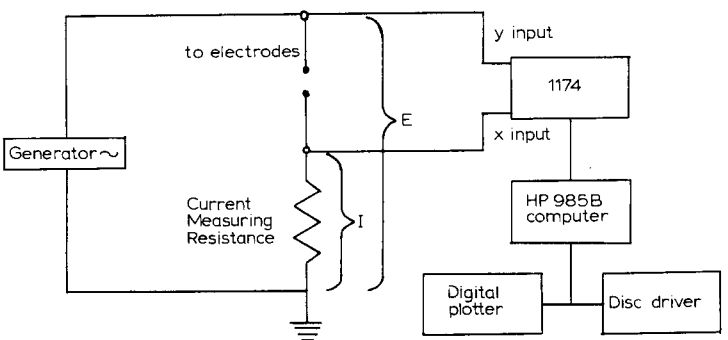

Fig. 4. Schematic diagram of the experimental arrangement for automated impedance measurements.

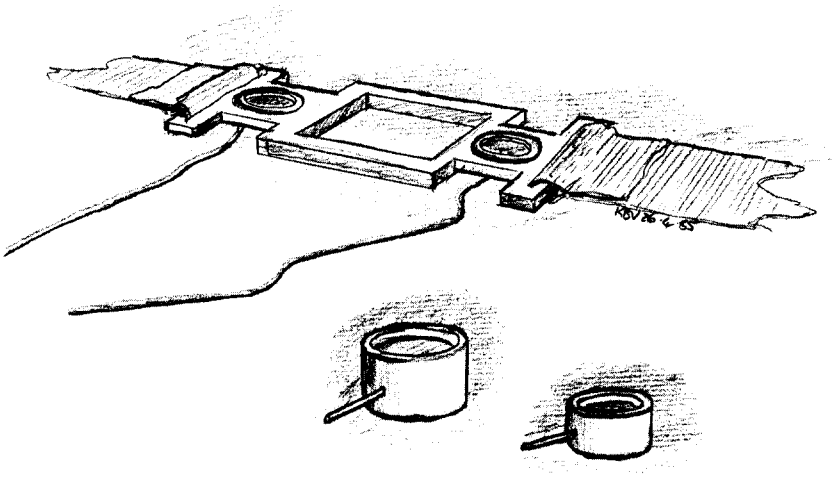

Fig. 5. Electrodes and electrode assembly for impedance measurements.

reclining chair. The electrodes were applied on the volar area of the forearm. The subjects were then left to 'equilibrate' for a period of 30 min prior to commencement of measurements.

The measurement room was air-conditioned and the relative humidity closely monitored. The temperature of the room was maintained at $22 \pm 2°C$ while the humidity was measured to be 40–60% during the period of experimentation. Daily humidity changes were found to be minimal.

Results and Discussion

Since impedance methods for studying moisturization of the skin are fairly widely used, one would have expected a high degree of concordance in the reported results. This is in fact not so and many of the factors contributing to the observed variability have been discussed by Leveque and de Rigal (1983). Single frequency measurements are clearly inadequate as discussed by Salter (1979) and in the present studies resistance–reactance profiles were constructed over the frequency range of 1–500 Hz. Fig. 3 gives a typical complex impedance plot. To obtain the impedance the measurements were subjected to a least squares fit. The value for Rsc was then calculated from the intercepts of the semi-circle on the ordinate.

Fig. 6 gives an example of the time-dependence of the computed parameter. Measurements were made approximately every 2 min, after the application of the electrodes, for nearly an hour. Despite the fact that subjects were rested for 30 min in an air-conditioned room, prior to measurement, it can be seen that reasonably steady values were not observed until after about ten minutes of electrode application. Wide inter- and intra-subject variations were seen in both the rate of decrease

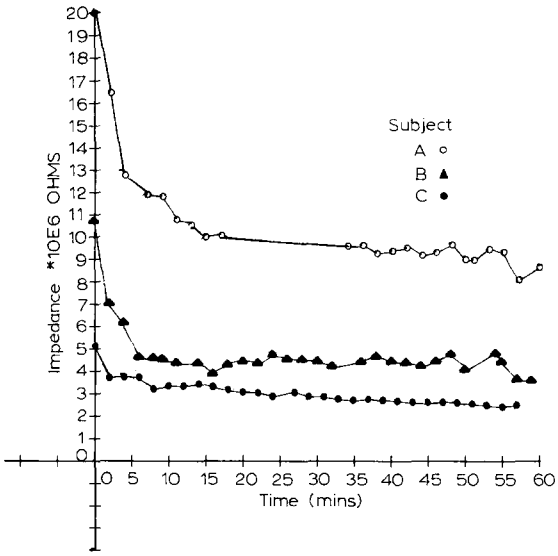

Fig. 6. Variation of impedance with time.

of impedance with time and the steady state average impedance. Fig. 7 gives specimen results to illustrate the extent of the observed variability of the impedance. The values were averaged from about 10 to 40 min of measurement.

The initial unsteady-state has been referred to by a number of authors and indeed numerous electrode modifications have been reported in attempts to overcome this problem (Campbell et al., 1977). Isherwood (1965), for example, described the use of electrodes made of pins which would therefore not interfere with transepidermal water loss. This approach assumes that the observed rapid initial drop in impedance is the result of increased hydration of the skin as a result of accumulation of transepidermal water in the area of stratum corneum immediately beneath the

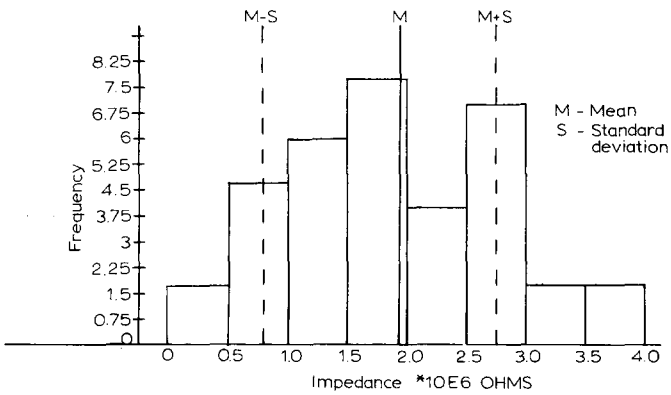

Fig. 7. Variability of mean skin impedance in human volunteers.

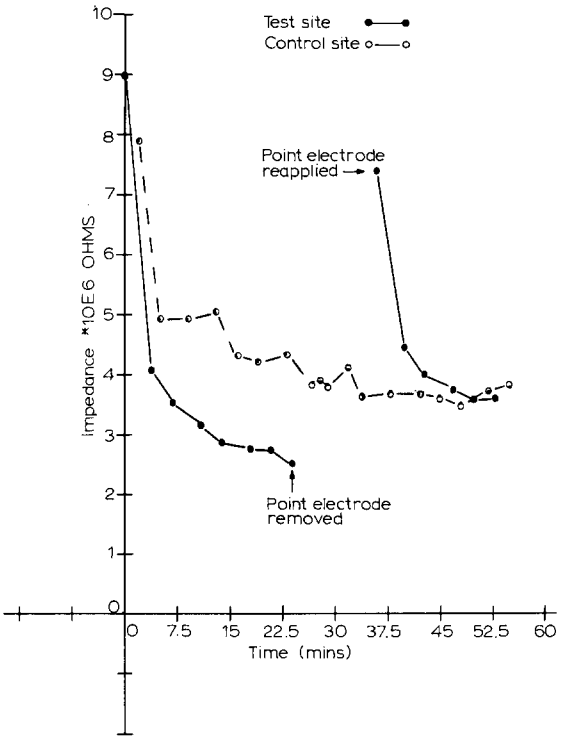

Fig. 8. Typical impedance values following electrode removal and re-application.

electrodes. Serban et al. (1981a and b), on the other hand, used electrodes equipped with grids which enabled partial evacuation. Clearly then, using our electrode system, steady-state must be reached before meaningful data can be obtained.

Fig. 8 shows a typical result obtained when the electrodes are removed after equilibration and re-applied in exactly the same position after approximately 10 min. As can be seen from Fig. 8, re-application of the electrodes causes the measured impedance to rise and a decay of the type seen upon initial application of the electrodes is observed once more. This suggests that the time taken to achieve a steady state is a function of electrode–skin equilibration rather than moisture equilibration within the stratum corneum on its own. If the latter was the cause, one would anticipate a much more rapid equilibration upon re-application of the electrodes. However, this was not the case.

Site-to-site variability was also observed. As the thickness of the stratum corneum varies with its position on the body, positional differences were to be expected. Preliminary data indicated that as one moved away from the wrist, the impedance of the skin on the volar area of the forearm increased. An increase was also observed between the region from the thumb to the elbow compared with the region from the little finger to the elbow. However, no data have been found in the literature to confirm that these impedance variations are due to the variation of the stratum corneum thickness over the volar area of the forearm.

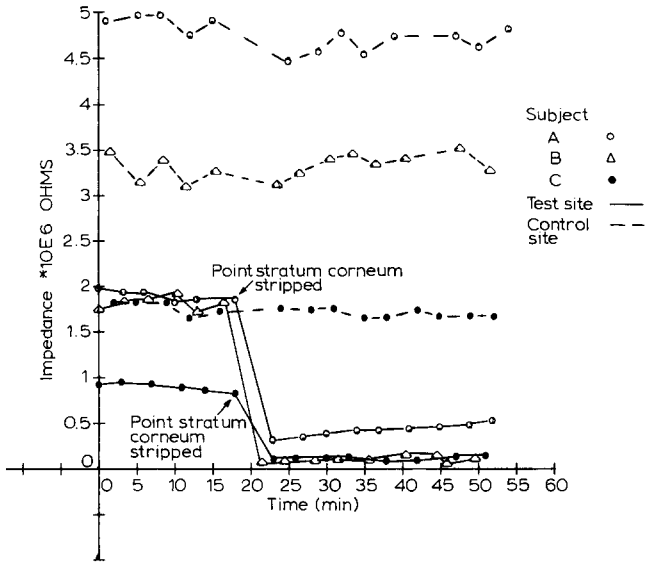

Fig. 9. Effect of stripping the stratum corneum below the electrodes, on skin impedance.

A number of authors have shown that stripping of the stratum corneum reduces the barrier properties of the skin against diffusion of applied substances (Tregear, 1966). This in fact led to the identification of the stratum corneum as the main diffusion barrier of the skin (Monash, 1957). It can therefore be expected that stripping of the skin would reduce its impedance.

Using Sellotape the stratum corneum was stripped 15 times below each electrode (Fig. 9) and in a separate experiment it was stripped 16 times between each electrode (Fig. 10). Sharp impedance drops were obtained when the stratum corneum was stripped below the electrodes, whereas no significant changes were observed when the region between the electrodes was stripped. This suggests that, in the case of untreated skin, the deeper, more conductive, epidermal layers are being measured in the area between the electrodes and that the stratum corneum is contributing to the impedance only in the region immediately below the electrodes.

The data discussed so far shows the wide variability of the resting hydration levels of the stratum corneum in vivo. The next question is whether the method available to us was sufficiently sensitive to detect changes induced by topical applications. Here it is noteworthy that successes in the use of impedance measurements for quantifying skin hydration have only been well documented in situations modelling skin pathology (Tagami et al., 1980) or when moisturizing products are used (Clar et al., 1975; Serban, 1981a and b). In these cases it may be argued that the baseline variability is negligible compared with changes due to topical applications.

Fig. 11 shows sample data obtained when solvents were applied in the area between the electrodes. The effect of ethanol and a 60% aqueous solution of dimethyl sulphoxide are shown. We were unable to correlate the observed impedance

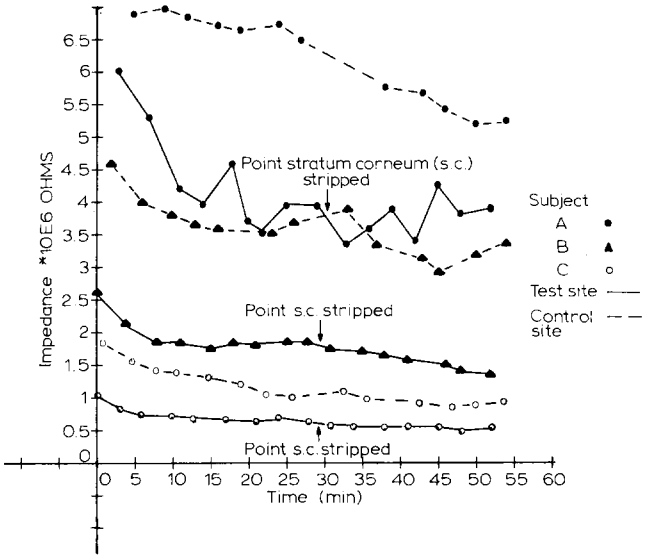

Fig. 10. Effect of stripping the stratum corneum between the electrodes, on skin impedance.

drops with any physicochemical property of the applied substance as one would expect if hydration was an end effect.

The solvent effects observed were probably due to short circuiting between the electrodes. To test for this possibility a thin film of white soft paraffin was applied to the area immediately next to the electrodes, as shown in Fig. 12. The results shown

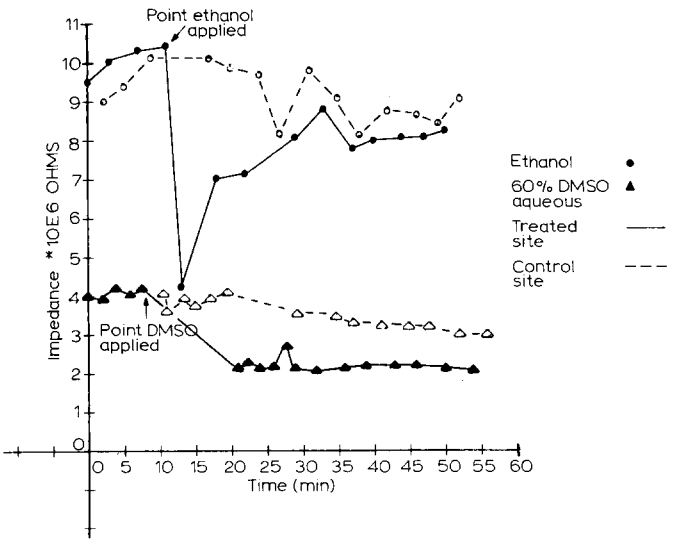

Fig. 11. Effect of solvents on skin impedance.

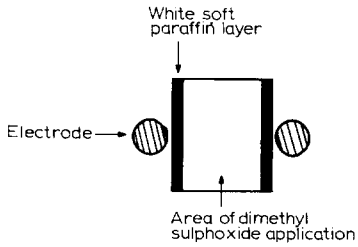

Fig. 12. Diagram showing electrode shielding with white soft paraffin.

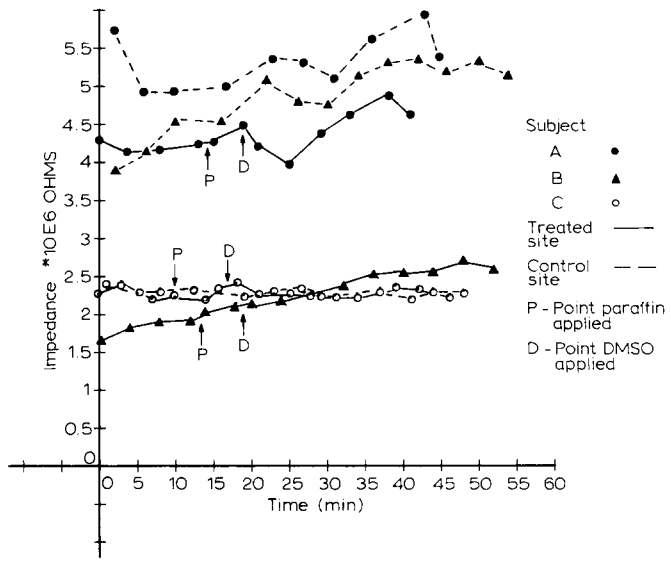

Fig. 13. Effect on impedance of electrode shielding using white soft paraffin prior to application of 60% aqueous dimethyl sulphoxide.

in Fig. 13 lend support to this observation. Similar results have been noted in the case of oil in water-based skin moisturizing lotions applied between the electrodes. Significant drops in the impedance were observed, but when a white soft paraffin "barrier" was applied near the electrodes no change occurred. It therefore seems likely that the topical product on the skin surface induces a short circuit, leading to the impedance drops perceived.

Conclusions

This investigation into the application of impedance measurements for monitoring skin hydration has shown that the method is affected by two main parameters: (a) baseline variability; and (b) short-circuiting of the electrodes.

Baseline variability is high both inter- and intra-subject, such that the current method will require considerable modification to successfully quantify in vivo measurements. Even the possibility of using a subject as his own control is complicated by inter-variability between measurement sites. Such variability is, however, not unusual in biological systems.

Monitoring of skin impedance immediately after the application of a topical product results in an erroneous impedance value. This value arises from a short-circuiting of the electrodes by the product, giving a measurement of the product's electrical properties rather than its moisturizing attributes. This problem of short-curcuiting puts serious restrictions on the use of this technique for the rapid assessment of topical products.

If the problem of baseline variability could be overcome, it may be possible to use this technique to measure the long-term effects, say 1–3 weeks, of applying a topical product. The effects of such a long-term application may then be far larger in comparison with an untreated control.

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