Innov. Techn. Biol. Med., Vol 17, n°3, 1996

EVALUATION DE L'EFFET DE PREPARATIONS DE LIPOSOMES SPECIFIQUES PAR LA MESURE DE LA SUSCEPTANCE ELECTRIQUE A BASSE FREQUENCE

MEASUREMENT OF THE EFFECT OF TOPICAL LIPOSOME PREPARATIONS BY LOW FREQUENCY ELECTRICAL SUSCEPTANCE

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RESUME
On présente un nouvel instrument d'évaluation des changements du degré d'humidité de la peau basée sur les mesures de susceptance électrique à basse frequence. Les sites de la peau des onze sujets testés ont éte traités avec des compositions de liposomes et le nouvel instrument a eté comparé avec le Corneometer CM820® généralement utilisé, sous l'angle de leur capacité à détecter les changements induits de l'hydration de la peau. On a trouvé que le nouvel instrument est sensible même a des changements mineurs du niveau d'humidité et donne des résultats plus significatifs que le Corneometer.

MOTS CLES
HYDRATATION DE LA PEAU - HUMIDITE DE LA PEAU - SYSTEME A TROIS ELECTRODES - LIPOSOMES.

ABSTRACT
A new instrument based on low frequency electrical susceptance measurements, is presented for the assessment of changes in skin moisture level. Skin sites on eleven test subjects have been treated with liposome formulations, and the new instrument has been compared with the generally used Corneometer CM820® regarding their ability to detect the induced changes in skin hydration. The new instrument was found to be sensitive even to minor changes in moisture level, and produced more significant results than the Corneometer.

KEY WORDS
SKIN HYDRATION - SKIN MOISTURE - THREE-ELECTRODE SYSTEM - LIPOSOMES.

INTRODUCTION

The monitoring of skin hydration following the application of topical preparations requires the use of a non-invasive technique. Electrical measurements are advantageous because of their general simplicity, and because the electrical properties of skin are influenced by several factors [1]. Electrical measurements are e.g. used to detect skin reactions and irritations [2], and the use of electrical measurements for skin hydration assessment is a natural choice. The instruments and methods presently available makes the results often difficult to interpret. Selective measurement of sweat, water in the different layers of stratum corneum as well as an overall signal of the water content in the skin would be of great interest to the objective evaluation of new topical preparations. Important in this context is a better understanding of an electrical model of the skin barrier.

An increasing number of skin care products use liposomes to facilitate the transport of active substances into the skin. Intact liposomes penetrate

only into the stratum corneum [3], and as they are hygroscopic and able to incorporate water many times their phospholipid weight, they will cause an increase in skin humidity [4].

Variations in the water content of the stratum corneum can be studied by electrical measurements on the skin [5]. In a previous paper, the proper electrical method was found from both an electrical model of the skin, and from comparative measurements with different methods [6]. The conclusion was that such measurements should be carried out using low frequencies to assure that the results are dominated by the stratum corneum. Furthermore, a lock-in amplifier should be used to differentiate between susceptance and conductance (the imaginary and real part of the electrical admittance, respectively). As the conductance is influenced also by sweat gland activity, the susceptance is the proper parameter in the assessment of skin moisture [6].

A small battery operated instrument with lock-in amplifier, three-electrode system and a measurement frequency of 88 Hz has been constructed for the evaluation of the effect of different liposome formulations on the skin. In this study, the effects of two different formulations containing 15 mg/ml and 150 mg/ml liposomes were measured over a period of three hours. The measurements were carried out using both the described instrument, and the Corneometer CM820 ®, a frequently used commercial instrument that measures the capacitive properties of the skin, and displays them in arbitrary units [7]. The Corneometer uses a triangular voltage with a fundamental frequency of about 100 kHz.

Both instruments showed an increase in mean measured value on the treated skin sites throughout the measuring period. For the Corneometer, however, the differences were statistically significant only for the 150 mg/ml formulation, whereas the new instrument measured significant differences for the 15 mg/ml formulation as well. The instrument described in this paper is thus found appropriate for the detection of small changes in stratum corneum hydration.

MATERIALS AND METHODS

The liposome formulations

Liposomes were produced using egg L-α-phosphatidylcholine (PC, approx. 99%) and L-α-phosphatidyl-DL-glycerol (PG, approx. 99%) in the ratio of 10 to 1. The phospholipids were supplied by Sigma Chemical Co.

Preparation of liposomes were done by the film method, and the suspension of handshaken liposomes were extruded 10 times through 100 nm polycarbonate membrane filters (Nuclepore) with a Lipex extruder. Photon correlation spectroscopic measurements (Coulter N4MD) showed a mean diameter of 85 nm. The lipid film was suspended in a 10 mM acetic acid / sodium acetate buffer with a pH of 5.0 ± 0.1. This pH is close to that of the skin, and the liposomes will have a stable, negative surface charge due to the PG content. Liposomes were stored under N_2 atmosphere in a refrigerator until the day of use.

The instrument

Figure1 shows a block-scheme of the instrument. The three-electrode system described by Grimnes [8], is based on the fact that the low frequency electrical admittance of the stratum corneum is considerably lower than the admittance of the underlying, living tissue [9]. The large volume of the living tissue compared to the stratum corneum increases this effect, and the living tissue below the stratum corneum can thus at low frequencies be regarded equipotential. The instrument is implemented with a 88 Hz quadrature-oscillator, supplying the three-electrode system with a sine-voltage and the lock-in amplifier with a cosine reference signal. The current through the measuring electrode is converted to a voltage with a transresistance amplifier, and errors caused by the use of shunt resistor are therefore avoided. Any 50/60 Hz noise, caused by stray capacitance between the test subject and power lines, is also eliminated by the three-electrode system [10]. The instrument measures the electrical susceptance of the part of the stratum corneum located under the M-electrode. The measurements are not influenced

by susceptance changes under the other two electrodes, and are thus true monopolar.

All measurements were performed with a concentric electrode, similar in construction to the one described by Yamamoto et al. [11], but with Platinum electrodes. Inner electrode area was 20 mm^2 and outer electrode area was 236 mm^2. There was furthermore separate cables to the two electrodes. The cable to the inner electrode was a coaxial type with grounded shield. The inner electrode was used as the M-electrode, the outer as the R-electrode, and a 3M Ag/AgCl solid gel electrode attached to the dorsal side of the forearm as the C-electrode. The polarisation impedance between the platinum electrode and the skin has previously been found to be negligible [6, 8]. DC potentials due to different electrode materials and skin potentials are possible sources of error in skin admittance measurements. However, this electrode arrangement with adjacent M- and R-electrodes of the same type of metal, eliminates these difficulties [6].

When using this electrode, a short breath was always applied in advance to the measured skin site, in order to achieve a more stable contact the first seconds after application [8]. Readings were done 5 sec. after electrode application, and the electrode was then immediately removed. The measurement voltage was 200 mV rms.

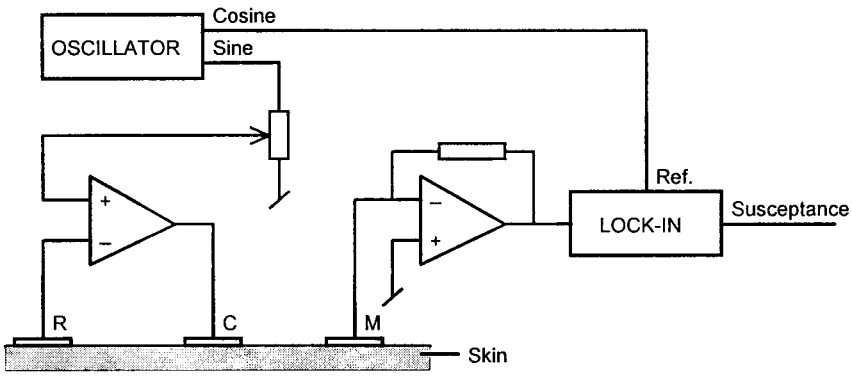

Figure 1.
Block-scheme of the instrument.

Experimental procedure

Eleven healthy Caucasian volunteers, five women and six men, were studied. Age range was 30 ± 6 years. They had not used any skin moisturisers the last 48 hours before the experiment. Relative humidity was 27 - 29 % and room temperature was 21 - 23°C. The experiment was carried out in October, starting each measuring series at 08.30 a.m.

The effects of the two liposome formulations were studied using the instrument described above and the Corneometer CM820®. The test subjects rested in the laboratory for at least 20 min. before the experiment. During this time, four circular test areas (d= 2.5 cm) were marked on randomly chosen sites on the ventral side of the forearm, and initial values were measured on these sites immediately before treatment with moisturiser. On three of the test areas, 35µl of 15 mg/ml liposome formulation, 150 mg/ml liposome formulation and buffer respectively, was the applied, and the treated areas were intermittently massaged during the next 5 min. The massage was very gentle to avoid any frictional trauma. Any redundant solution was left on the skin. The fourth area was left untreated as a reference. Recordings were done with both instruments at 30, 60, 90, 120, 150 and 180 min. after treatment, and the electrodes were cleaned after each measurement to avoid transfer of lipids between test areas.

Test subject comparison

A proper comparison of the results from different test subjects is difficult because of the natural inter- and intrapersonal variations in skin susceptance. The measured susceptance B is given by:

$$B = \omega C = \omega \varepsilon_0 \varepsilon_r \frac{A}{d}$$

where ε_0, ε_r, A and d are empty space permittivity, relative permittivity, surface area and thickness of the measured skin, respectively. Furthermore, ω equals $2 \cdot \pi \cdot f$ where f is the frequency of the applied signal, and C is the capacitance of the measured tissue. This capacitance is by nature frequency dependent in biological materials. Differences in B between persons or skin sites can be due to ε_r or d. When comparing the results from the eleven test subjects, there is a significant tendency that the relative increase in susceptance value is smaller on test subjects with high initial susceptance. In other words, a small initial value before treatment yields a larger relative increase after treatment. It is therefore assumed that the differences in initial values are mainly due to differences in ε_r, because a high susceptance caused by thinner stratum corneum (small d) would according to the equation above, result in a larger relative susceptance increase when exposed to a certain amount of moisturiser.

It is furthermore assumed that the relation between the water concentration of the stratum corneum, C_W , and ε_r is linear within the small region ΔC_W measured in this study. Hence, the relation between C_W and ε_r is given by:

$$\varepsilon_r = k_1 C_W + k_2$$

where k_1 and k_2 are constants. It is then obvious that the addition of a certain amount of water to the skin will result in a relative susceptance increase that is dependent of the initial C_W , where as the absolute susceptance increase is independent of this value. When comparing the results from different skin sites or test subjects, the initial values measured before treatment should thus be subtracted from the subsequent values.

RESULTS

The mean and standard deviation (SD) of the susceptance readings from the new instrument are listed in tab.1 as functions of time, and correspondingly for the arbitrary units of the Corneometer in tab.2. The unit $\mu S/cm^2$ represents susceptance per electrode area. The SD represent the standard deviation on the measurements on the different test subjects, and not the repeated measurements on each subject. Repeated measurements were avoided in order to minimise occlusion. The results are also presented graphically in figs.2 and 3 as mean absolute increase in measured values after treatment, i.e. mean of recorded values minus initial values for each test subject.

Both methods show a mean increase in recorded values on the skin sites treated with liposome formulations. No effect from the buffer was detected. It should be noted that the ordinate in figure2 and figure3 are totally different, and that the main difference between the two figures is not the measured behaviour of the reference and buffer areas, but rather that the increased hydration of the treated areas have a considerably higher influence on the low frequency susceptance than on the Corneometer units. This is easily seen from tables 1 and 2. The mean increase in recorded values after application of 15 mg/ml liposome formulation, was significant for the 88 Hz instrument ($4 \cdot 10^{-5} < p < 1 \cdot 10^{-3}$), but not significant for the Corneometer ($0.1 < p < 0.8$). The increase after application of the 150 mg/ml liposome formulation was significant for both the 88 Hz instrument ($5 \cdot 10^{-6} < p < 8 \cdot 10^{-6}$) and the Corneometer ($3 \cdot 10^{-4} < p < 1 \cdot 10^{-2}$).

DISCUSSION

Both methods indicate that the hydration of the untreated reference sites increases somewhat during the three hours of measurement. As room temperature and relative humidity was controlled, this is presumably due to daily, natural variations in sweat gland activity, corresponding to the natural variations in other physiological parameters, e.g. body temperature and blood pressure. As mentioned in the introduction, the new technique is insensible to sweat duct filling, but increased sweat activity will lead to increased stratum corneum hydration, which will be detected by the instrument.

Time [min]	Reference [µS/cm2]		Buffer [µS/cm2]		15 mg/ml liposome [µS/cm2]		150 mg/ml liposome [µS/cm2]	
	mean	SD	mean	SD	mean	SD	mean	SD
0	1,25	1,18	1,28	1,21	0,95	0,65	0,95	0,60
30	1,28	1,15	1,17	1,18	3,16	1,42	4,71	1,53
60	1,42	1,21	1,52	1,10	3,57	2,06	5,13	1,92
90	1,53	1,10	1,70	1,02	3,70	1,39	4,93	1,41
120	1,81	1,54	1,89	1,39	3,86	1,61	4,88	1,05
150	1,90	1,47	1,90	1,12	3,78	1,26	4,95	1,09
180	1,94	1,42	1,88	0,85	3,65	1,14	4,73	0,87

Table 1.
Results from the 88 Hz instrument

Time [min]	Reference [a.u.]		Buffer [a.u.]		15 mg/ml liposome [a.u.]		150 mg/ml liposome [a.u.]	
	mean	SD	mean	SD	mean	SD	mean	SD
0	73,91	12,29	78,18	16,30	82,18	14,74	78,64	9,05
30	74,18	14,91	79,36	13,03	86,27	11,37	99,82	6,01
60	82,36	14,75	87,36	12,31	98,73	12,69	103,18	7,57
90	87,00	13,50	90,82	12,40	96,09	10,76	103,64	6,77
120	84,09	14,81	88,73	10,13	98,36	7,00	101,18	8,41
150	84,55	13,22	89,45	11,47	92,00	7,67	101,91	9,55
180	85,82	13,46	91,27	14,29	96,73	7,80	102,27	9,27

a.u. = arbitrary units

Table 2.
Results from the Corneometer CM820

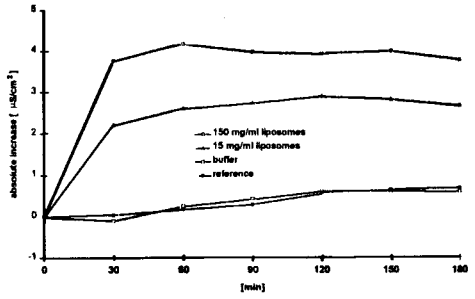

Figure 2.
Increase in 88 Hz electrical susceptance after treatment with moisturisers. Mean of eleven test subjects

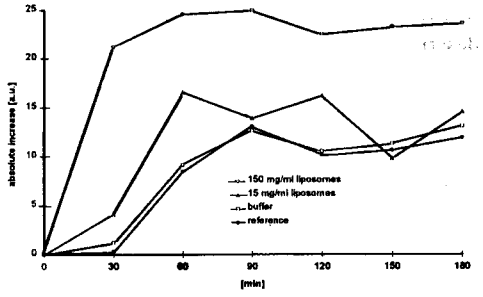

Figure 3.
Increase in Corneometer units after treatment with moisturisers. Mean of eleven test subjects

The results from the reference sites and the sites treated with buffer are equal, indicating that the skin has re-established its balance with the environment after 30 min. The effect of adding 15 mg/ml liposomes to the buffer is significant, however, caused either by the hygroscopic liposomes binding water inside the stratum corneum, or by lipids left as an occlusive film on top of the stratum corneum after the massage. The three-electrode system assures that the electric current must pass the stratum corneum. Excess lipid or moisture on the skin surface will hence not be able to produce increased total admittance values. This proves that increased susceptance reflect the changes in the stratum corneum itself. The proper mechanism is not revealed through this study, however, and this will be a topic for further research.

CONCLUSION

The results of this study show that the described instrument is well suited for the detection of variations in stratum corneum hydration, as it is found to be sensitive even to minor changes in the moisture level. Assessment of basal (absolute) water content of the stratum corneum, however, requires data on the relationship between low frequency susceptance and water content. This is the topic for an ongoing study at our laboratory.

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