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A new instrument to measure the mechanical properties of human stratum corneum *in vivo*

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Synopsis

The gas-bearing electrodynamometer (GBE) (1) has been used for the last 20 years to obtain sensitive measurements of the stratum corneum. A new instrument for measuring the mechanical properties of the stratum corneum incorporates all of the measurement principles of the GBE but none of its components. A force-controlled miniature d.c. servo, gearing, and leadscrew replace the magnet/solenoid arrangement of the GBE. Error resulting from conversion of an electrical signal to a mechanical force is automatically compensated. Consequently, this control renders the need for a friction-free bearing redundant. The orginal linear variable differential transformer (LVDT) has been replaced with a unit with a sensitivity of 0.01%, and force is now measured by a calibrated 50-g load beam. The function generator, signal conditioner, and storage oscilloscope have been replaced by user-friendly software run by a small portable computer. The new design offers greater inherent accuracy than the GBE and requires minimal servicing. The new instrument (linear skin rheometer, "LSR") has been shown to provide sensitive measurements of stratum corneum mechanics and was used to measure the mechanical responses of the stratum corneum to two topical moisturizing treatments of differing relative hydration performance (as determined by impedance measurements using the Nova™ DPM9003). The relative performance of the two products as measured by the LSR compared favorably with corresponding impedance data, indicating the ability of the LSR to differentiate varying degrees of stratum corneum plasticization in response to hydration.

INTRODUCTION

There is a wide variety of methods available to the dermatological researcher to determine changes in the mechanical properties of human skin *in vivo.* However, to measure sensitive changes in the mechanical properties of the stratum corneum, there is only a small number of instruments and methods that may be used with confidence. This is principally because the majority of available instruments and methods involve relatively large displacements of the stratum corneum, either parallel or perpendicular to the skin surface (1). Consequently, the tissue underneath the stratum corneum will have an unacceptably large effect on the measurement.

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The instrument that appears to have been most widely used over the last 20 years to obtain sensitive measurements of the stratum corneum is the gas-bearing electrodynamometer (GBE) (1–5). It is able to apply a sinusoidal loading stress of less than 5 g parallel to the skin surface, with a resulting displacement of less than 1 mm in each direction. This is achieved by suspending an armature in a gas bearing to create near friction-free movement. Changes in the magnetic field generated by a surrounding coil cause the armature to oscillate at a known frequency and amplitude. The coil is activated by a sinusoidal signal from a low-frequency function generator or from a suitable software trigger. The armature of the instrument is typically attached to the skin surface by a stiff wire probe bent to 90° at its free end. A small plastic stub is usually cemented to the free end of the probe and used to attach the probe to the skin surface with a circular piece of double-sided sticky tape. Displacement of the armature is measured by a sensitive LVDT, mounted coaxially with the coil. Coil and LVDT outputs (force and displacement) are amplified and then supplied for analysis to either a storage oscilloscope or a computer equipped with suitable software. Equipment used in a "classic" GBE workstation is shown in Figure 1.

Results of force and displacement measurements of skin are typically displayed as a hysteresis loop (Figure 2). Analysis of the gradient of the loop (force/displacement or displacement/force) yields derivatives of the dynamic spring rate (DSR), usually expressed as g/mm (a measure of the force required to stretch or compress the skin per unit

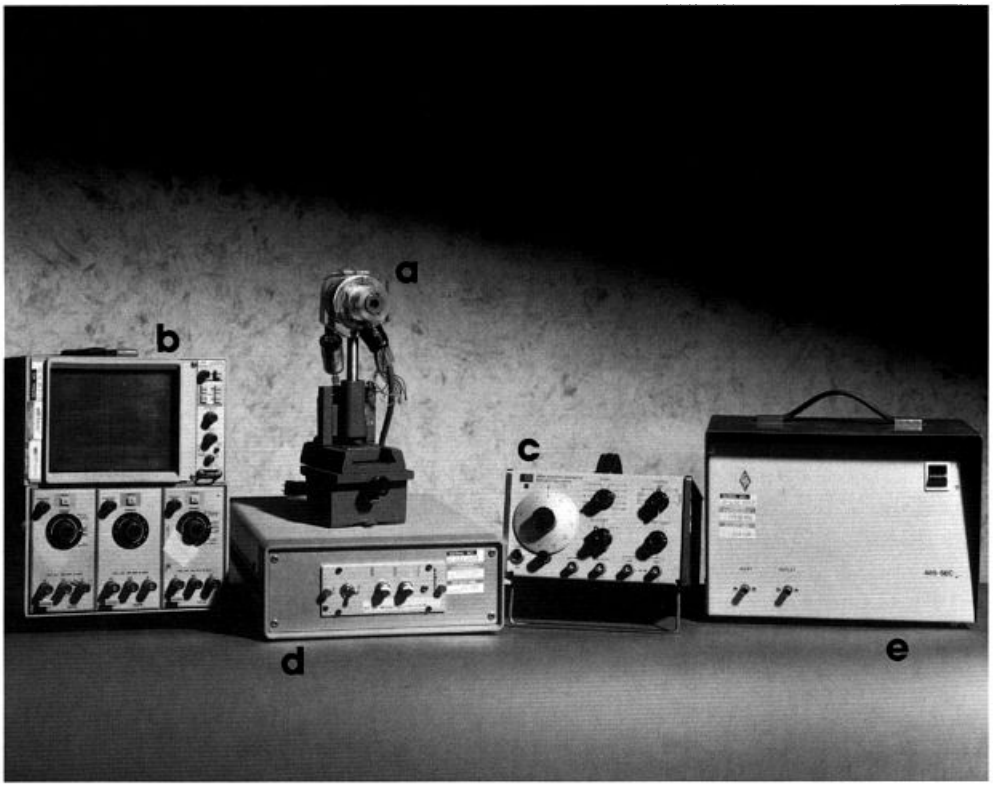

Figure 1. Equipment used in a GBE workstation: a, GBE probe; b, storage oscilloscope; c, function generator; d, signal conditioner; e, air compressor.

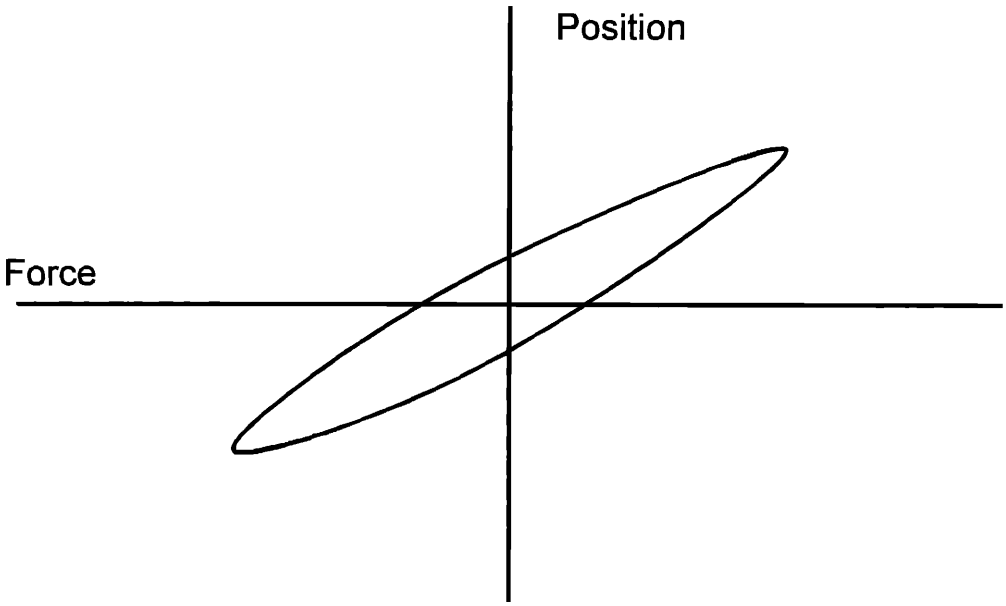

Figure 2. Typical position vs force hysteresis loop produced by a one GBE measurement cycle.

extension), mm/N or μm/g (measures of stretching or compression of the skin in response to a given applied force). Such analysis yields information about the elastic properties of the skin. Analysis of the phase lag between force and displacement responses yields information about the viscous properties of the skin. After 20 years of experience with the GBE within our laboratories, we believe that the principle of the GBE measurement is still the best available for measuring sensitive changes in the mechanical properties of the human stratum corneum *in vivo.* Subtle though important changes in skin elasticity [dubbed "softness" by Maes *et al.* (3)] in response to the application of moisturizing formulae have been measured, as have changes in skin "tightness" due to surfactant damage. Our experience has, however, also highlighted the drawbacks of employing the original Hargens GBE instrument in a modern laboratory. These are as follows:

1. Importantly, the instrument employs an "open-loop" method of control, i.e., during calibration of the instrument, and subsequently in routine operation, one assumes that an applied current equals a given force. As the GBE is calibrated on one point only (3 g), linearity is not guaranteed over the whole measurement range of the GBE. In addition, calibration drift over time is certain.
2. The components needed to run the GBE are bulky and dated (function generator, signal conditioner, storage oscilloscope, compressed gas/air).
3. The probe components are fragile and, in our experience, break easily and require excessive servicing when used routinely (for example, the fine copper wires connecting the armature to the body of the probe).
4. The air-bearing employed in the probe design is inherently susceptible to misalignment, soiling, and malfunction.

In recent years, the cost of precision has improved greatly. We can now achieve with conventional technology what was achieved previously through Hargens' (1) considerable ingenuity. We have designed and built a new instrument that retains and builds on

all the principles of the original GBE, but contains *none* of the components. This instrument, designated the linear skin rheometer (LSR) is described in the following sections.

MATERIALS AND METHODS

INSTRUMENT HARDWARE AND DESIGN

A schematic diagram of the new instrument is shown in Figure 3. A force-controlled miniature d.c. servo (Maxon 23-12, 0.5 W rating, supplied in U.K. by Trident Engineering), gearing, and leadscrew now replace the GBE solenoid arrangement, and drive the LSR probe. The original Schaevitz 050 HR LVDT in the GBE has been replaced with a unit of linearity 0.3% (15 μm) and sensitivity 0.01% (0.5 μm) (Solartron type DF2.5, Schlumberger Industries). The force exerted on the probe is now measured directly by a calibrated load beam (Minigram Beam Load Cell, type MBH50, rated 50 g, supplied in U.K. by RDP Electronics) with an overall accuracy of <20 mg. The load beam is mounted vertically within the instrument casing.

All components fit into one casing measuring 20.0 × 14.8 × 6.9 cm, and the whole unit weighs 1.7 kg. The probe housing itself is a light-weight machined perspex chuck mounted on a low-friction swivel assembly allowing 360° movement (analogous to the GBE). This is protected from damage during routine usage by a metal collar. The chuck contains wire grips to allow the wire probes to be inserted or withdrawn by a simple, firm push or pull. A single lead connects the unit to a PC via a 25-pin D-type connector. Power for the LSR unit is taken from the PC via the connector. The unit can be seen in Figure 4.

INSTRUMENT CONTROL

An IBM (or compatible) PC is used to control the movement of the probe and to log

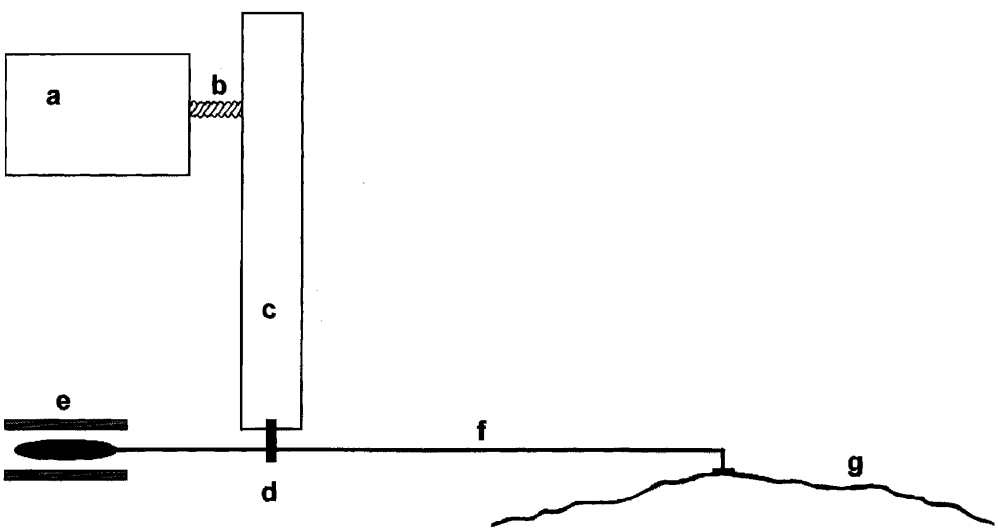

Figure 3. Schematic diagram of the LSR sensing head: a, miniature motor; b, load screw; c, lead cell; d, load cell sensing head; e, LVDT; f, probe; g, skin surface.

Figure 4. The linear skin rheometer.

force and displacement data. Both force and displacement are monitored continuously at a rate of 1 KHz using a 12-bit ADC plug-in card (National Instruments MIO16). The motor is controlled with an analogue output signal also generated by the PC. The desired force/time cycle, which is normally a single sinusoid, is calculated initially and then stored in memory as a table of values. The actual force applied to the probe is compared with the desired value in the table 1000 times a second. A feedback loop is used to control the motor that moves the load cell in such a way as to minimize any discrepancy. The force applied thus follows the desired force/time cycle extremely closely. The control loop uses an algorithm with proportional and integral terms, whose relative weighting can be varied.

The PC logs all the force and displacement values over a complete measurement cycle, which is usually set at 0.33 Hz, thus generating 3000 pairs of points over a three-second cycle. Two waveform plots are then obtained (Figure 5). Three parameters may be obtained from these curves:

F_{max}: the peak force that is applied to the skin surface

P_{max}: the peak displacement occuring as a result of that force

T: the phase shift between the two signals

The dynamic spring rate (DSR) of the stratum corneum is given simply by the formula F_{max}/P_{max}. Derivatives are calculated and expressed as g/mm, mm/N, and μm/g.

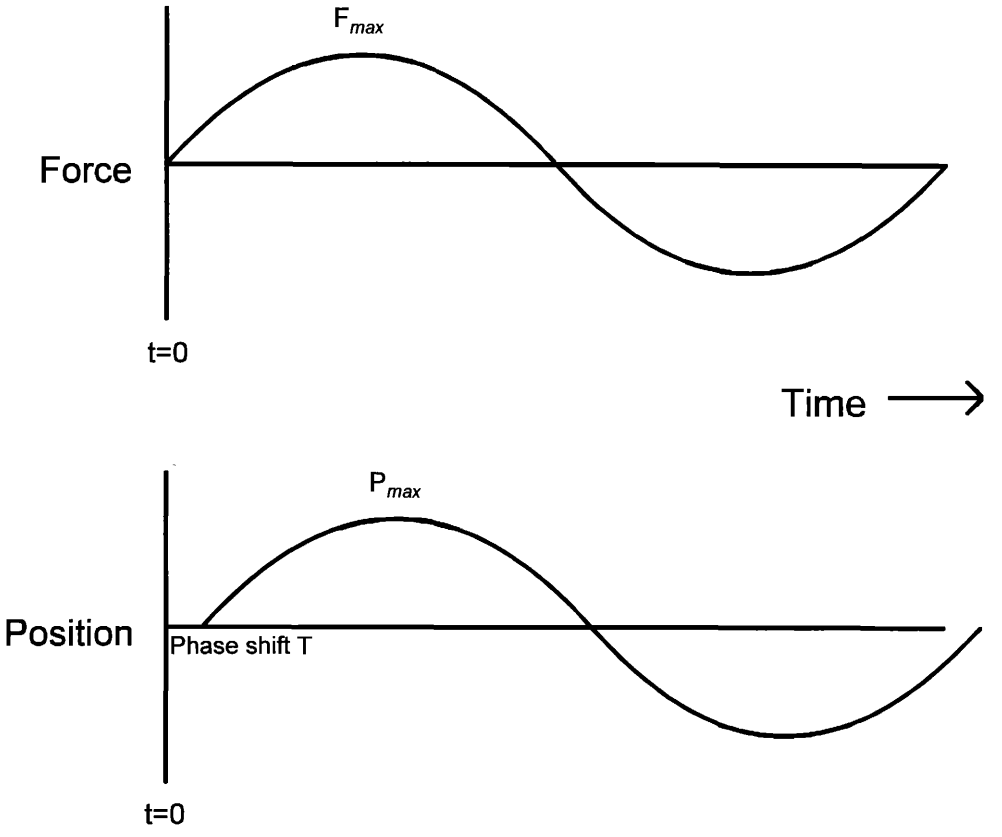

Figure 5. Waveform plots demonstrating a complete LSR measurement cycle.

The viscous component of the stratum corneum is often inferred by calculating the area of the ellipse shown in Figure 2. A more rigorous approach is to perform a regression on the original sinusoidal data in order to solve the equations:

$$F = F_{max}\,\mathrm{Sin}(t) \tag{1}$$

$$P = P_{max}\,\mathrm{Sin}(t + T) \tag{2}$$

where F = instantaneous force, F_{max} = peak force, t = time for one complete cycle in seconds, P = instantaneous displacement, P_{max} = peak displacement, and T = phase shift in radians.

Having solved for these equations, it is then a straightforward problem to solve the integral over one cycle that represents the area of the ellipse:

$$\int_0^{2\pi} F_{max}\mathrm{Sin}(t)P_{max}\mathrm{Cos}(t + T) \tag{3}$$

The LSR software solves the above equations for both elastic and viscous components of the data. These are subsequently displayed, directly after measurement. Note: units of μm/g (1/DSR, a measure of stretching or compression of the stratum corneum in response to a given applied force) will be used as a convenient expression of skin elasticity/softness in the rest of this paper.

INSTRUMENT SOFTWARE

The LSR software runs on a standard IBM (or compatible) PC of at least 386 33 MHz speed, and is sourced in C programming language. The closed loop control employed by the LSR is achieved as follows: As the LSR control loop is a sampled data system, it is essential that a fast real-time clock is generated that triggers the measurement of data samples and updates the control signal output. All IBM PCs have as standard a user interrupt (on interrupt vector 0 × 1c) called the "timer tick," which is available for programmers to use as a regular timing source. This timing signal is generated from the 4.192 MHz system clock via an Intel 8253 programmable interval timer. The BIOS presets this timer to its full scale of 65536 and, therefore, the "timer tick" interrupt is normally 18.188 Hz. This is far too slow for a sampled data system.

This problem is overcome by reprogramming the 8253 divider to give the desired frequency during the measurement cycle, in this case 1 KHz. In order, however, not to disrupt important internal functions such as monitoring disk drive heads, the timing of interrupt 0 × 8 (the interrupt number actually triggered by the 8253 output) needs to be restored. This may be achieved by not using interrupt 0 × 1c, but replacing the BIOS interrupt function at 0 × 8 with the control program itself. The original timing is derived within the new interrupt 0 × 8 by installing a simple counter and calling the BIOS interrupt at the correct interval. In this way, a fast timer is generated that allows data sampling at 1 KHz but that does not harm other internal PC operations.

CALIBRATION OF THE LSR

A simple calibration jig has been designed and built that allows rapid, absolute calibration of *actual* force and displacement (Figure 6). Displacement is measured by a 10-μm–resolution digimatic indicator [Mitutoyo (UK) Ltd, Warwick, U.K.], traceable to NAMAS calibration standards. Force is measured by a 10-g load beam (Maywood load beam, type 49034, Maywood Instruments Ltd, Basingstoke, U.K.) with <10 mg accuracy, also traceable to NAMAS calibration standards.

The load cell calibration factor is expressed in terms of mV signal per volt per gram measured. The output signal is then amplified through a proprietary amplifier (Maywood amplifier, type D2000, Maywood Instruments Ltd) set to a nominal gain of 325. The amplified signal is then converted via the MIO16 interface card (ADC) such that a full-scale reading of 2048 is equal to an input of 10 V. The calibration factor is expressed in terms of an ADC input reading that equals 1 g. The following values are required:

The load cell calibration value taken from its certificate, L

The bridge supply voltage measured with a NAMAS calibrated digital volt meter, B

The gain of the amplifier measured with a calibrated digital volt meter, G

The correction for the digital volt meter taken from its certificate, C

Thus, L = mV per V per g at a nominal voltage of 10 V; output from the sensor for a 1-g load = (L * B * C)/10; output from the amplifier for a 1-g load = (L * B * C * G)/10; and the ADC input reading for a 1-g load = (L * B * C * G * 2048)/(10 * 10).

The LVDT calibration factor is expressed in terms of mV output per volt per mm

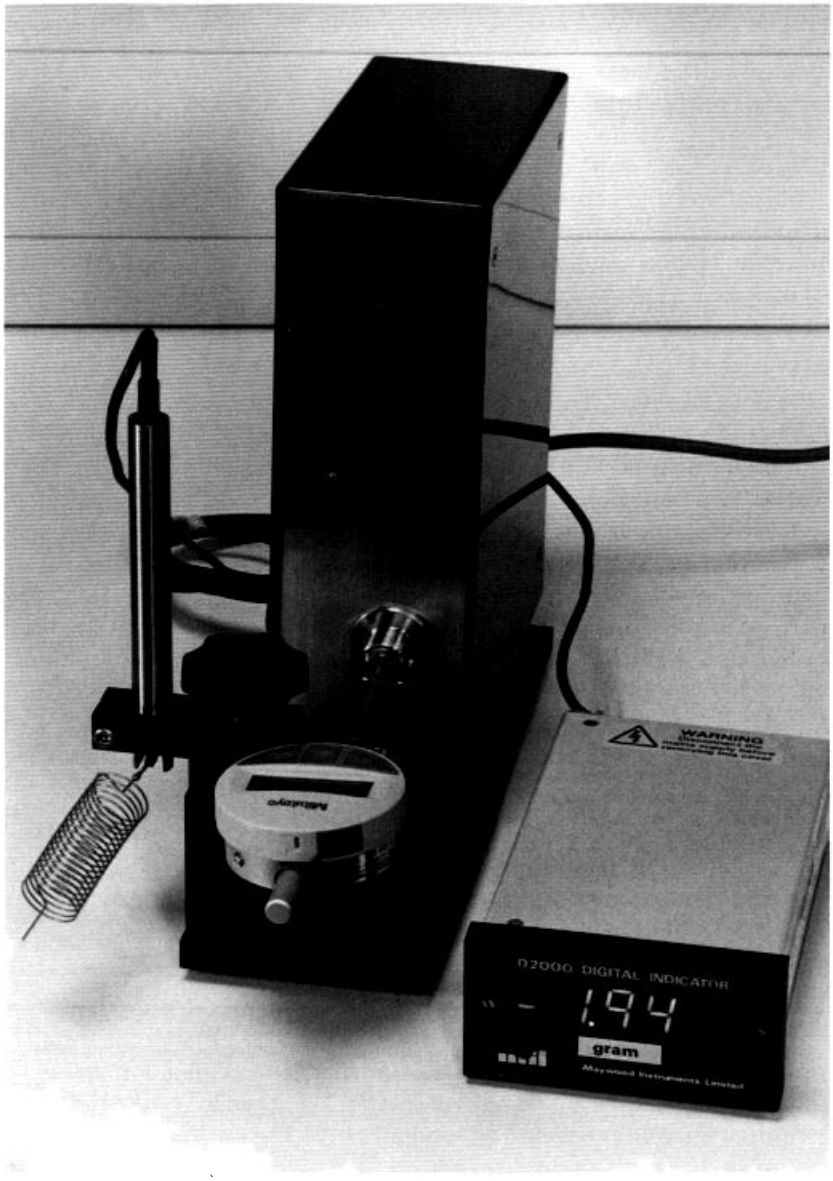

Figure 6. LSR calibration jig.

displacement. The final slope is expressed in terms of ADC input per mm displacement. The following values are required:

The LVDT calibration value from its certificate, L

The excitation voltage measured with a NAMAS calibrated digital volt meter, V

The correction factor for the digital volt meter taken from its certificate, C

The slope value is derived as follows: output voltage for 1-mm displacement = (L * V * C), and the ADC input reading for 1-mm displacement = (L * V * C * 2048)/10.

In practice, these calculations are performed automatically by a simple software program, allowing rapid and simple calibration of absolute force and displacement.

SKIN MEASUREMENT USING THEIR LSR

For direct comparison with the GBE reproducibility data obtained by Maes *et al.* (3), the reproducibility of the LSR was estimated by the same method. Forty consecutive identical measurements were performed on the back of the hand of a female volunteer. Results were analyzed to determine the coefficient of variation of the measurement.

To determine the ability of the LSR to measure sensitive changes in stratum corneum mechanics in response to simple hydration, the following study was performed: Two moisturizing formulae of differing hydration performance (products A and B; hydration performance was determined by impedance measurements using a Nova™ Dermal Phase Meter 9003, see below) were applied to the back of the hands of 13 female subjects (aged 18–35). The dorsal surface of the hand was chosen for mechanical measurements (a) to conform to previous measurement sites using the GBE (3) and (b) because it is relatively simple to immobilize the hand effectively. The study was performed in a controlled-environment chamber (temperature 20 ± 1°C; relative humidity 45 ± 5%). The plastic stub on the end of the LSR wire probe was attached to skin on the back of the hand via a circular piece of double-sided tape (5 mm diameter). LSR measurements were then performed in triplicate. Baseline measurements were performed before product application. Test products were then applied at a rate of 2 $\mu l/cm^2$ to the entire back of the hand according to a predetermined randomization schedule. LSR measurements were performed at one, three, and six hours after product application. As the whole dorsal surface of each hand was used for product treatment, inclusion of an untreated control was not possible. Results were, therefore, expressed as mean difference to initial pretreatment baseline.

Hydration performance of products A and B was assessed by randomized application at the same rate as above (2 $\mu l/cm^2$) to 5 × 5-cm sites on the volar forearms of 12 female subjects (aged 18–35; the volar forearm was chosen as the site for hydration measurements because of its smooth, hairless morphology and its utility as a standard in this type of testing (6). Each forearm also contained an untreated 5 × 5-cm control site. The study was performed within a controlled-environment chamber (temperature 20 ± 1°C; relative humidity 45 ± 5%). Impedance measurements were performed using a Nova™ Dermal Phase Meter 9003 with the standard measuring probe DPM 9103 (Nova Instruments, USA) at one, two, four, and six hours after application, and results were expressed as mean difference to untreated control.

RESULTS

Forty consecutive measurements on the same subject and same site indicated that the coefficient of variation of the measurement was only 2.9% (Figure 7). This demonstrates very good reproducibility of the measurement technique and compares very favorably with the value of 3% obtained by Maes *et al.* (3) for the GBE. The variation measured is almost certainly due to movement of the subject during the probe cycle. This has always been the main source of error in these types of sensitive measurements, and

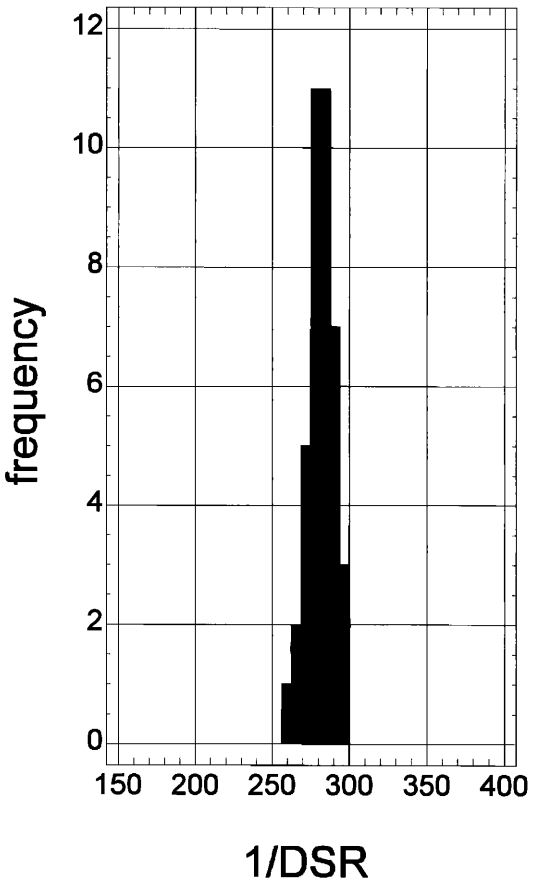

Figure 7. Reproducibility of LSR measurement (n = 40; coefficient of variation = 2.9%; mean = 281.8 μm/g; SEM = 1.3 μm/g).

various means have been employed to minimize subject movement during readings [e.g., use of a precast plaster mold by Maes *et al.* (3)]. However, like Cooper *et al.* (4), we have found that the use of no restraint is preferable and we employ a simple sloping table on which subjects rest their hands.

The results of the study using the Nova DPM 9003 to measure the hydration efficacy of products A and B can be seen in Figure 8. Both products induced significant increases ($p < 0.05$; paired t-test vs untreated control) in apparent stratum corneum hydration (as measured by impedance changes) up to, and including, six hours after application. Moreover, product A increased stratum corneum hydration significantly more ($p < 0.05$; paired t-test) than product B at all time points up to and including six hours after application. Water exerts considerable influence on the mechanical properties of the human stratum corneum due to its complex interactions with keratin (7,8). This plasticization of the stratum corneum, an essentially viscoelastic material, has been described as skin "softening" (2,3). In the case of topical application of a moisturizing formula, the extent of this softening effect is directly related to the ability of the product to deliver and maintain increased water concentrations within the stratum corneum. This is usually achieved by the delivery of humectant compounds such as glycerol and/or use of

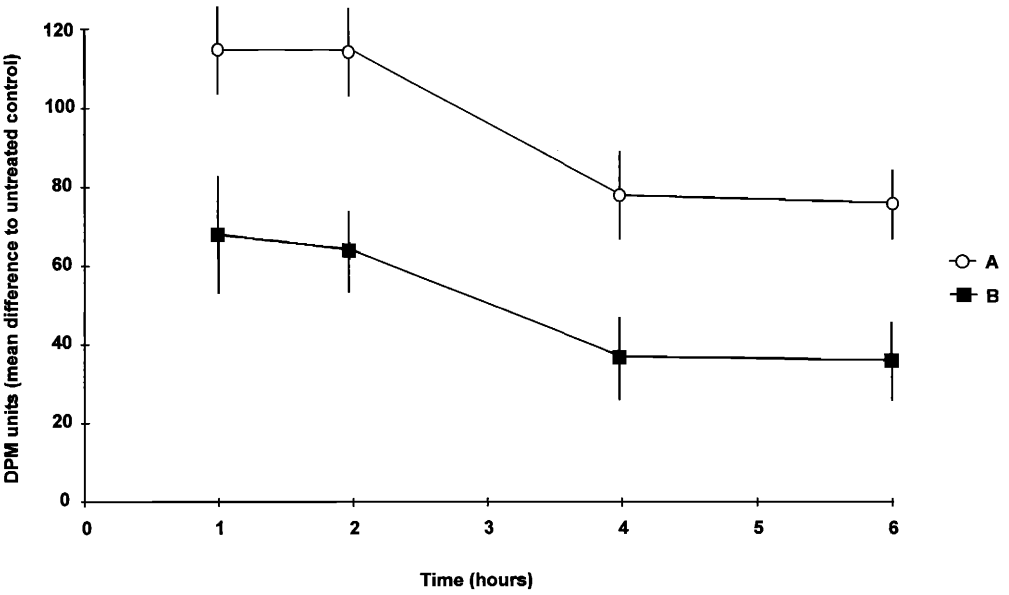

Figure 8. Relative hydration performance of products A and B (as measured by impedance).

occlusive lipidic films. Indeed, in the case of products A and B, product A might be expected to leverage a greater increase in stratum corneum hydration due to its higher glycerol content (4% [w/w] glycerol in A, in contrast to 3% [w/w] in B) and formulation (gel network, in contrast to a simple oil-in-water emulsion in B). For products A and B, therefore, one would expect to be able to measure (a) absolute significant increases in softness for both treatments and (b) differing relative changes in skin softness for both treatments in accordance with their apparent hydration performance.

Results of the study using the LSR to measure the effects of products A and B on stratum corneum mechanics are presented in Figure 9. Both products induced significant increases ($p < 0.05$; paired t-test vs pretreatment baseline) in skin softness at all time points up to and including six hours after application. Moreover, product A induced greater increases in skin softness than product B throughout the time course, significantly so ($p < 0.05$; paired t-test) at six hours after application. These results compare favorably with the relative hydration profiles of the two products (Figure 8). The LSR is, thus, able to measure subtle changes in stratum corneum mechanics in response to hydration and to distinguish between the effect of topical application of moisturizing products and differing relative hydration performance.

CONCLUSIONS

The control system used for the LSR provides the instrument with an inherently more accurate and reliable measurement capability because it employs closed-loop (feedback) control. The GBE, in contrast, uses an open-loop method of control whereby a predetermined current is applied to the solenoid and assumed to be transformed into the desired force. As no determination of the actual force generated is made at the time of measurement, it is difficult to know with certainty the true force applied to the skin.

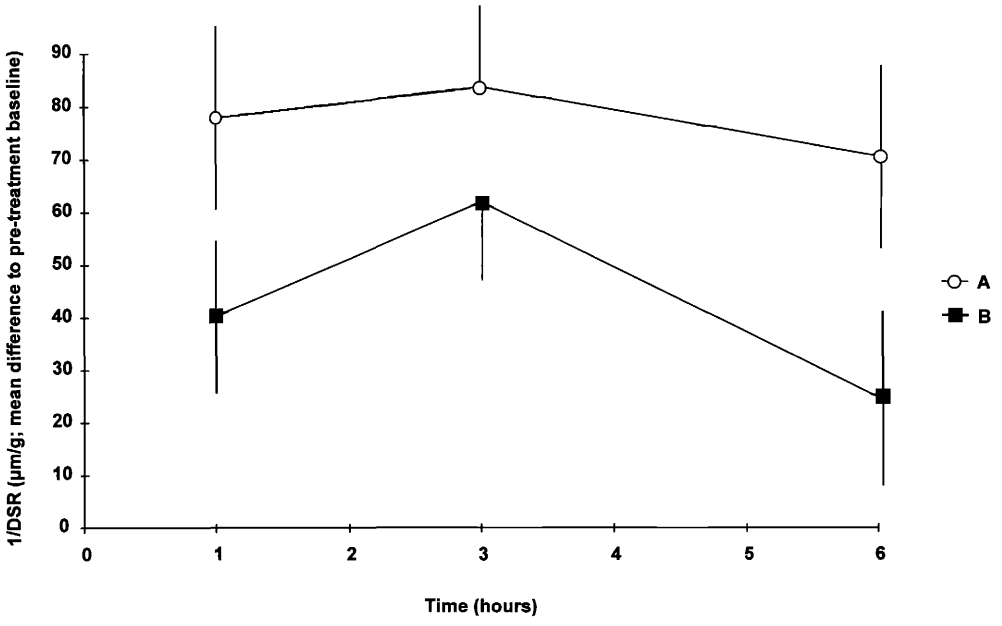

Figure 9. Relative performance of products A and B as measured by LSR.

While open-loop techniques can be used successfully in perfectly stable environments, no account can be taken of instantaneous fluctuations in such a system (notably, in this case, subject movement). With the LSR closed-loop system, the true force applied to the skin is measured at a rate of 1 KHz and corrective action taken within 1 ms to restore that measured force to the required value. This system helps ensure that the test sequence is reliable, repeatable, and can dynamically adjust for the inevitable variations that occur during *in vivo* testing. Put another way, because this system allows instanteous compensation of error resulting from the conversion of an electrical signal to a mechanical force, the need for the friction-free gas-bearing arrangement of the GBE is eliminated. This allows the deployment of a compact, efficient, and flexible new instrument.

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