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THE USE OF A CAPACITANCE DEVICE TO EVALUATE THE HYDRATION OF HUMAN SKIN.

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Synopsis

Directive 93/35/EEC on the testing of cosmetics requires that evidence is provided to support the efficacy claims made for marketed products. In order to fulfil this requirement without resorting to the use of animals, non-invasive skin bioengineering techniques are now being employed with human volunteers. These techniques provide quantitative and objective data, if the measurements are performed under rigorously standardised conditions. In this study, a non-invasive instrument, the Corneometer® CM 820, which measures skin capacitance, has been used to evaluate the short-term effects of three commercially available moisturisers, by monitoring the water content of the stratum corneum at different treated test sites of human skin (inner forearm) in comparison with that at an untreated site. The three products, respective leaders in the perfumery, pharmacy and supermarket distribution, have been confronted with a standard reference material (20% glycerol in distilled water), so that the results can be compared between laboratories and to avoid differences relating to instrumentation and methodologies. Measurements with the Corneometer® CM 820 were taken at the baseline visit, and at 1, 3 and 6 hours post-application, at each test and control site. The results show that all of the test products have a hydrating effect on skin, but that the effect is significantly different between the products at each timepoint, except 3 hours post-treatment, when there was no significant difference between one of the products and the standard reference. If used properly and according to appropriate protocols, the electrical methods for the evaluation of skin hydration, have proved to be useful for the non-invasive evaluation of the efficacy of cosmetic products on human volunteers, and consequently, for the reduction of animal tests.

Riassunto

La Direttiva 93/35/EEC sui test dei prodotti cosmetici dispone che debbano essere fornite delle prove obbiettive per sostenere le rivendicazioni di efficacia fatte per i prodotti venduti sul mercato. Al fine di soddisfare questo requisito senza il ricorso all'utilizzazione di animali, si stanno attualmente impiegando volontari umani e tecniche non-invasive di bioingegneria cutanea. Queste tecniche, se

le misurazioni sono eseguite in condizioni rigorosamente standardizzate, forniscono dati quantitativi e oggettivi. In questo studio e' stato utilizzato uno strumento non-invasivo che misura la capacitanza cutanea, il Corneometer® CM 820, al fine di valutare gli effetti a breve termine di tre idratanti disponibili in commercio, monitorando il contenuto idrico dello strato corneo in differenti sedi cutanee selezionate sull' avambraccio. I tre prodotti, rispettivi leader nella distribuzione nelle profumerie, farmacie e mercato di massa, sono stati confrontati con un riferimento standard (glicerolo 20% in acqua distillata), in modo da poter comparare i risultati con altri ottenuti utilizzando la stessa tecnologia, ma in diversi laboratori. Le misurazioni sono state effettuate alla visita basale, e dopo 1, 3 e 6 ore dall' applicazione. Differenze significative sono state rilevate tra i vari prodotti confermando differenti caratteristiche funzionali dei prodotti testati. I metodi elettrici per la valutazione del contenuto idrico cutaneo, quando opportunamente utilizzati, ed in presenza di protocolli corretti, permettono di essere utili nel valutare l'efficacia dei prodotti cosmetici in modo non invasivo e nel ridurre la necessità di tests sugli animali.

INTRODUCTION

Bioengineering instruments, already widely used in experimental dermatology, have been introduced into cosmetology in order to, *inter alia*, substantiate efficacy claims on cosmetic products, as required by *Directive 93/35/EEC* on the regulation of cosmetic products [1]. These instruments have the advantage of being non-invasive, providing quantifiable and objective information on the mechanical and physiological properties of the skin. However, precautions should be taken in the design and conduct of the study, and in the interpretation of the test results in order to obtain accurate, reproducible and reliable data. Among the various claims made for cosmetics, a very common one is the capacity of the product to restore the water content in the superficial layer of the skin, i.e. the stratum corneum. The Corneometer® CM 820 has been employed in this study to measure the short-term improvement of skin hydration after the application of three commercially available moisturisers at different sites on the skin of the inner forearms of a group of selected human volunteers.

PURPOSE OF THE STUDY

This study has been undertaken in order to investigate if the Corneometer® CM 820 was a valid and reliable instrument to establish a claim on skin hydration and, being used non-invasively and *in vivo* on human skin, if it was contributing to the replacement of animals for cosmetic testing as required by *Directive 93/35/EEC*. Furthermore, it was examined if the instrument was able to discriminate between different moisturisers.

BIOPHYSICAL BASIS

The change in the electrical properties of the stratum corneum induced by hydration involves at least three different types of electroconductive elements [2]:

- conduction by electrons and holes
- conduction exchange of protons all along the H-bonded network of water molecules
- conduction by ions larger than protons ("large ions").

Conduction by electrons and holes is only significant in an abnormally dry skin, and conduction by large ions only if the stimulating frequency is below the MHz range. In consequence, protonic conduction is thought to be the predominant event [3]. Water molecules bound by hydrogen links can mutually exchange protons, which migrate within the network of hydrogen bonds. For that reason, electrical measurements are highly dependent on the water-keratin interaction and hence on the water content of the stratum corneum. The water absorption isotherm describing the quantity of water binding to the stratum corneum at a given temperature has shown that the water-keratin interaction follows the Brunauer-Emmet-Taylor (BET) model [4] and that it is possible to distinguish three types of water according to the type of interaction; "tightly-bound water" for water contents from 0 to around 7%, "bound water" between about 7% and 35%, and "free water" beyond 35% [4,5]. Microcalorimetric measurements have shown the respective adsorption energies which increase with the strength of the binding [4]. Because of the variation in water binding strength, there is no direct proportionality between total water content and electrical conductance. Substances or treatments which interact with the keratin-water network may therefore modify conductance without changing the total water content at the test site [3].

INSTRUMENT

The Corneometer® CM 820 measures the skin capacitance at low frequency (40-75 KHz). It

should be noted that measurements of skin capacitance are frequency-dependent, unlike the electrical capacitance of non-biological materials. The Corneometer consists of a main recording device which displays the hydration values, and a measuring probe. The probe is formed of an interdigital grid of gold-covered electrodes, arranged closely in parallel and functioning as capacitor [6,7,8,9]. The electric scatterfield formed at the edges of the gold lines decreases in proportion to increasing distance between them. The active part of the electrodes has a surface of 7 x 7 mm. The electrodes are 50 µm wide, with an interdigital spacing of 75 µm, covered with a low dielectric vitrified material of 20 µm thickness, to avoid a direct contact between the gold-plated electrodes and the skin surface. No current passes through the skin, but an electric field of variable frequency is formed in the upper part of the skin, i.e. in the stratum corneum and the underlying layer of the epidermis. The depth and the arrangement of the electric field formed in the skin depends on the geometry of the electrodes and the dielectric material covering the electrodes (constant capacitance), and on the capacitance of the biomaterial in contact with the electrodes (variable capacitance). The approximative skin depth measured is 30µm according to the manufacturer [8]. Other authors have reported a depth of about 60-100 µm [10,11]. The total capacitance is only influenced by variations in the dielectric constant of the biomaterial in contact with the electrodes [7]. A dry stratum corneum is a dielectric medium. However, when the stratum corneum is hydrated, a significant change in its dielectrical properties occurs [2]. Increasing the water content of the stratum corneum will increase its relative permittivity. Consequently, the capacitance of the probe in contact with the stratum corneum is increased. A resonating system in the instrument measures the shift in frequency (40 to 75 kHz) of the oscillating probe which results from the changes in the total capacity.

MEASUREMENTS

The capacity of the skin surface is measured by applying the probe (surface area 0.95 cm^2) with a constant pressure of 3.5 N [3] on the skin for about 1.5 s. A graduated spring system is incorporated in the probe to facilitate the measurements and to assure a reproducible pressure. The switch inside the probe turns on when the correct pressure has been applied. A "H" for hydration appears on the screen, to inform the operator that the instrument has been activated. A microprocessor in the device reads the measuring times, compares the value with the stored calibrated data and displays digitally a converted value (arbitrary units of skin hydration) of the variable total capacitance on screen [7,8]. A sound indicates the end of the measurement. The hydration value remains on display for two minutes, after which the instrument automatically switches off after three audible signals.
The instrument is able to measure values from 0-150 arbitrary units (a.u.). In practice, the values of hydration vary from 30-60 for very dry skin, from 60-70 for dry skin, from 70-90 for hydrated skin, and lie above 90 for very moist skin [7].

STUDY MATERIAL

The three test materials used in the clinical investigation were current commercial moisturisers. Product A is an oil-free serum enriched with ceramides, hydrating agents and vitamins. Products B and C are both oil-in-water (O/W) emulsions, enriched, respectively, with Jojoba oil and Lecithin (product B) and by a moisture collector (product C).

STUDY DESIGN

The clinical trial was conducted on twenty healthy Caucasian human volunteers, including

females and males. They were chosen according to precise inclusion and exclusion criteria. One week before starting the study, the subjects were asked to sign an informed consent form and to provide details of their medical histories. At the same time, the skin test sites were visually examined by the dermatologist in order to confirm subject eligibility.

The subjects were instructed to discontinue the use of all topical products on their forearms, including moisturisers and medicated cleansers three days before the study, but were asked to continue their normal cleansing routine.

Each of the subjects' forearms was divided into four sites of 4x5 cm each, plus a fifth site on both upper forearms, by using a template. They were all treated with a single application of each product, with one site serving as the control. The site on the upper forearm served as the standard reference and was treated with an aqueous solution containing 20% glycerol. The standard reference is used to compare results within the same laboratory and between different laboratories, avoiding differences related to instrumentation, environmental conditions and methodologies. Test products were applied symmetrically on each forearm, according to a randomisation table, at a rate of $2\mu l/cm^2$, as small, evenly spaced blobs within the delineated areas. Different fingers were used for applying different products.

After the baseline measurement, readings were taken at 1, 3 and 6 hours post-application. Instrumental assessment was performed under standardised environmental conditions (at a room temperature of 19-21° C, with 40-50% relative bumidity), following a period of relaxation and acclimatisation for the human volunteers of at least 15 min. At each timepoint, four readings were taken at different areas of each test site to provide a meaningful value. Any cream remaining on the probe was removed at every change of site.

Each untreated site value (the control) was subtracted from the treated site values for the respective forearms at each timepoint (A1-control1=A1norm, B1-control1=B1norm, etc...). This has been carried out to normalise the data. The mean value of the four measures at each of the treated test sites was calculated. The three products plus the control were assigned to the different test sites on each human volunteer at random, so that any differences among the sites were minimal.

Left and right forearms of each human volunteer have been treated the same way, to allow left-right comparisons.

The normally distributed data were analysed by using analysis of variance (ANOVA) to compare the hydration values of the different products at the same time interval. Multiple subgroup comparisons were calculated by using the Neumann-Keuls test to detect where the differences between the products lay. The software Statistica 3.0 for Macintosh was used to analyse the data.

RESULTS

The results are presented in Table I and Fig.1.

Table I

Products[1]	Mean ± SD
A1	16 ± 9.4
B1	7.7 ± 10.9
C1	21.6 ± 9.5
Gly1	19.1 ± 10.4
A3	13.6 ± 9.6
B3	4.7 ± 11.3
C3	16.2 ± 10.9
Gly3	16.3 ± 10.1
A6	10.4 ± 10.1
B6	3.1 ± 11
C6	12.4 ± 10.6
Gly6	14.5 ± 8.6

Table I: [1]normalised hydration values of the products at 1, 3 and 6 hours post-application (mean and standard deviation)

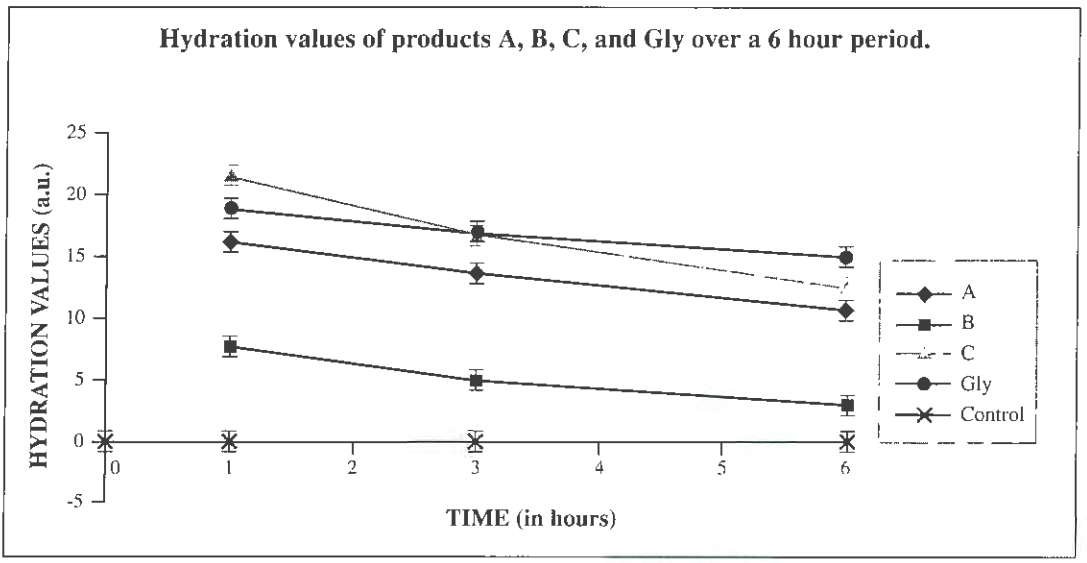

Fig. 1: The normalised hydration values are all greater than zero, i.e. each treated test site gives higher hydration values (in arbitrary units) than the control site at the respective timepoint. All the products show a maximum hydration effect one hour post-application. Product C and glycerol in water give the best results and have a very similar effect at three hours post-application.

It has been found that:

- All 4 products have a hydrating effect at each timepoint (Fig. 1). This has been checked by calculating the mean ± 2x (standard error). All such confidence intervals are strictly greater than zero
- Some treatments are better than others. This is confirmed by time-by-time analysis of variance (ANOVA).

The significance level of the test was set at 0.05. By comparing products A, B, C and glycerol at 1, 3 and 6 hours post-treatment, significant differences between the products were found ($p<0.01$). Follow-up comparisons with the Neumann-Keuls test showed where such differences lay. The effects of all the products were significantly different ($p < 0.05$), except at 3 hours post-treatment, when there was no significant difference between product C and glycerol ($p=0.88$).

DISCUSSION

In general, product C and glycerol had the highest hydrating effects. Product B had the least effect. The standardised conditions in the laboratory avoided a rapid evaporation of the glycerol. Furthermore, the cosmetic properties (smell, feeling, aspect) of glycerol are unpleasant and would not be appreciated by the consumer.

The usual hydration profile of a cosmetic moisturiser depends on whether it is an oil-in-water (O/W) emulsion or a water-in-oil (W/O) emulsion. Capacitance measurements for an O/W emulsion show an immediate and significant increase in hydration (capacitance values) after application of the product [13]. This increase in capacitance is thought to be due to the water content of the product, which itself is dependent on the nature and the quantity of humectant which keeps the water in the formulation. After 10 or 15 minutes, a rapid decrease in hydration follows, due to the evaporation of excess water from the skin surface [3, 7, 14]. After a certain

time, the capacitance values are maintained at an increased level in relation to the control, even for several hours, depending on the efficacy of the cosmetic product [7].

The control has been subtracted from each value to increase the sensitivity of the study by avoiding individual-related differences and allowing a more precise comparison of the products (smaller standard deviation).

A short-term study was chosen rather than a long-term study, to avoid the influence of changes in the environment (e.g. changes of climate or season during the experiment) and to permit better discrimination between the products tested [3]. However, because the products were applied in a single application on the inner forearm, a useful subjective evaluation of their effects by the human volunteers themselves was not possible.

CONCLUSION

In this study, the Corneometer® CM 820 has been shown to be a sensitive and useful tool, able to quantify skin hydration in a rapid and inexpensive way. The study has been designed in such a manner as to avoid as much as possible the limitations of the instrument. However, even then the results have to be interpreted with caution, bearing in mind that the instrument only gives relative information on the water content of the stratum corneum and not absolute values [3].

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