Antiwrinkle and skin-moisturizing effects of a mineral-algal-botanical complex

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Accepted for publication December 15, 1999.

Synopsis

Tests were carried out to compare the skin-smoothing and moisturizing effects of two cosmetic preparations, following two applications a day over a period of four weeks. The skin roughness parameter, Rz, was determined at the beginning and end of the study. The skin hydration was evaluated eight and twelve hours after cream application. At the end of four weeks, the cream enriched with *5%* of Triple D Complex™, composed of Dead Sea Mineral Skin Osmoter™, *Dunaltella salina* algae extract and desert plants, had caused an average teduction of the skin toughness parameter by 43%- This effect was almost twice the improvement that was observed when a control cream was tested. From skin hydration results it can be concluded that the Triple D Complex™ may also be considered as an active moisturizing agent. The role of *Dunaliella salina* biomass enriched with Dead Sea minerals, as an active component of the complex, was examined separately. Several parameters that may affect the mineral biosorption and desorption were evaluated. The relatively low biosorption of calcium and magnesium into the algal biomass and the minimal tendency to release minerals from the biomass at the pH of skin leads to the conclusion that the role of these algae as a vehicle for the tested elements is limited. Similar results in skin smoothing, obtained in two distinct studies, using Dead Sea Mineral Skin Osmoter™ and Triple D Complex™, suggest that Dead Sea minerals play a major role in the proven antiwrinkle effect.

Introduction

Chemists all over the world are eagerly searching for new active agents to benefit the skin health and beauty industries. The global trend toward using natural ingredients is focused on components that are extracted from the botanical world, and from marine algae, commonly known as seaweeds, which have been widely used over the centuries for their nutritional and therapeutic properties. Seaweed is one of the richest natural sources of vitamins and minerals, possessing sea mineral salts in high concentrations and also trace elements. It has been demonstrated that similar minerals from the Dead Sea can act as agents in the reduction of skin roughness, and have a beneficial effect on the natural moisturizing factor, NMF (1—3).

It has been shown in several publications that skin may alter its metabolism and its inner enzymatic activities when exposed to a graded concentration of minerals, especially

calcium, magnesium, and potassium ions. The mode of physicochemical action of the minerals is not yer fully understood (4,5).

Stabilization difficulties, caused by a high electrolyte concentration, are considered as drawbacks in formulating minerals in cosmetic preparations (6). Using mineral carriers as vehicles may enable a better stabilization of the formulations, thus slowing and sustaining the mineral release to the skin surface. Mineral carriers may contribure to the production of mineral-rich long-lasring products-Certain types of microbial biomass can retain relatively high quantities of metal ions by passive sorption and/or complexation. This phenomenon is generally known as biosorp-tion (7). Micro algae from hypersaline habitats have adopted some unique metabolic pathways that enable their survival under relatively high osmotic pressures (8). The green alga *Dunaliella salina* from the order Volvocales/Chlorophyceae, isolated from diluted Dead Sea warer, can accumulate high contents of glycerol and |3-carorene (9,10). This alga also contains vitamins such as thiamine, pyridoxine, riboflavin, vitamin E, and biotin. Its beneficial effects, including immunological enhancement of topical applications of (3-carotene and orher free radical

scavengers, have been reported previously (11). Some plant extracts are recognized as folkloric remedies. Some of these have proven pharmacological activity; othets have pretentious sophisticated claims, but are ineffective (12).

A combination of natural ingredients and blended components from the various sources that were mentioned above may lead to the development of a unique, active composition with potentially synergistically beneficial effects. Meanwhile, the increasing public demand for scientific proof of cosmetic performance claims, as well as new legislation, has encouraged the cosmetics industry to submit studies demonstrating the activity of their preparations. Acceptable testimonies to support a claimed effect for bioacrivity can take the form of a published scientific or clinical study, consumer evaluation, professional observations, and instrumental analysis. Results from *in vitro* tests, mainly cell-culture models, have been used recently to support the bioactivity of some cosmetics (13).

In a previous article we have reported that the addition of 1% of Mineral Skin Os-moter™ to a control gel contribured to a significant skin smoothing effect (1). The role of minerals in improving skin roughness and hydration was demonstrated. The aim of the prsent study was to evaluate the effect on the skin of a cosmetic cream with 596 of a mineral-algal-botanical complex (Triple D Complex™). The assumption that *Dunaliella salina* algae, as a part of this unique complex, would serve as an adequate vehicle and a bio-available source for minerals delivered to skin was also examined. The enrichment of *Dunaliella* biomass with Dead Sea minerals, and several parameters that could affect mineral biosorption and desorption, wete studied.

Materials And Methods

Mineral-Algal-Botanical Complex (Triple D Complex™) Preparation

A unique composition, rhe Triple D Complex™ was composed of three elements: Dead Sea Mineral Skin Osmoter™, *Dunaliella salina* algae extract, and desert plant extracts (14). Dead Sea Mineral Skin Osmoter™, is called "sea salt" in the US and INCI-listed as "Maris Sal & Aqua" (supplied by Dead Sea Laboratories Ltd.); *Dunaliella salina* algae extract is INCI-defined as "algae" (supplied by Henkel Cotporation). "Desert plants" are Fenugreek, INCI-defined as "Trigonella Foenum-Graecum extract," and Jujube, INCI-defined as "Ziziyphus jujuba extract" (both botanical extracts supplied by Alban Muller International). Dead Sea Mineral Skin Osmoter™ is a highly concentrated aqueous solution extracted from the Dead Sea via a natural evaporation process developed by the Dead Sea Laboratories Ltd. This ingredient contains a high level of bivalent cations. The composition of this brine is presented in Table 1.

Growth Of *Dunaijella Sauna* And Harvest Of The Biomass

Innoculum of *Dunaliel'la salina* algae, type 19/31, was purchased from the Culture Collection of Algae and Protozoa (CCAP). The algae were cultivated m semi continuous batches based on American Type Culture Collection (ATCC) DA medium No. 1174 (18). The biomass was harvested by continuous centrifugation, lyophilized (Labconco lyophilizer) in the presence of 0.01—0.2% methyl paraben as a preservative, and crushed with a mortar and pestle. The crushed algal powder was utilized for biosorprion tests.

Cosmetic Preparations

Two oil-in-water emulsions, textured as light cosmetic creams, were prepared and comparatively tested: (a) a moisturizing antiwrinkle base cream, serving as a control, and (b) the same moisturizing antiwrinkle base cream, enriched with 5% Triple D Complex™.

Laser Profilometric Test

Each preparation was applied twice a day over a period of four weeks to 20 female volunteers, aged 22 to 63, average age above 36 years, on both right and left forearm. Half of the participants were categorized as having sensitive skin and the rest as having normal skin. At the

beginning and end of the application period, silicone impressions were taken from the same area of skin on the right and left fotearms. Twelve hours before the impressions were taken the participants wete not permitted to apply cream or to use active washing substances. Each subject was acclimatized to room temperature 30 minutes prior to the measurements. Structural changes of the epidermis were quantitatively classified with a computer-aided laser profilometric system according to ISO 4287/1 ("Surface roughness terminology"). Skin surface changes were evaluated by comparing Rz values, a roughness parameter of a surface profile, as measured by the silicone impression, before and following the skin treatments (15).

Table I Typical Chemical Analysis of Mineral Skin Osmoter™ (INCI-Iisted as "Maris Sal & Aqua")

Cation	Mili-equivalent/l	Anion	Mili-equivalent/l
$Na+$	107	Cl^-	9320
$K+$	37	SO_4^{-2}	7
Ca^{2+}	1850	Br^-	150
Mg^{2+}	7430	HCO_3^-	<2

Skin Hydration Test

Skin surface hydration state was assessed by a skin capacitance-based instrument (cor-neometer CM 820, Courage & Khazaka GmbH). The measurements by the interdigital electtode indicate changes Jn skin capacitance due to variations in the moisture content of the stratum corneum (SC). Twenty female volunteers, aged between 22 and 63, applied the tested compositions to three different places on the lower arm. The mean value of the three obtained measurements was calculated. The measurements were taken immediately after application, after 8 hours, and after 12 hours. Untreated skin of the conttalateral lower arm was used as a control. Subjects were acclimatized to an ambient temperatufe of 22°C and relative humidity of 60% for 45 minutes prior to the measurements. The recorded capacitance values were converted into arbitrary hydration units varying from 0—120 rcu (relative corneometer units) (16,17).

Biosorption Experiments

A series of incubations with 1 g of dry, crushed *Dunaliella* powder and 25 ml of Dead Sea concentrated brine (Mineral Skin Osmoter™) was carried out in various combinations of brine pH, temperature, and exposure times. Algal biomass and brine were mixed and incubated in Erlenmeyer flasks, and mixed with an orbital shaker (EnvironShaker 3328 type, Lab-Line) or with a magnetic lab stirrer. The ratio of mineral biosorption to algal biomass was evaluated in correlation with the pH of the brine, temperature of the solution, and exposute time. After incubation, the brine was separated from the biomass by centrifugation (Sorval RC-58 centrifuge, 10 min, 24,000 g). Non-sorbed salt residues were removed from the algae by two consecutive washing cycles, each of 150 ml distilled water. The algal precipitate was lyophilized, and the dried biomass was digested overnight in 4% nitric acid. The concentrations of acid-soluble calcium, magnesium, and potassium in the biomass were measuted by atomic absorption (Vatian, type Spectra 10 AA).

Desorption Experiments

Dunaliella sahna biomass was saturated with minerals from Dead Sea Mineral Skin Osmoter™ brine under the optimal conditions found previously: pH 5—5.5, 15-minute exposure time, 33°C. The saturated biomass was washed twice with distilled watet, and the slurry was kept at 4°C. Portions of the biomass were introduced into distilled water in a ratio of 0.5% dry weight to water, and exposed to an experimental design matrix of parameters. Design-Ease™ software was used for the experimental design and analysis (Stat-Ease Ltd. 1992, Minneapolis). Residual levels of minerals in the dry algal biomass were evaluated by atomic abosption.

RESULTS

Skin Roughness Evaluation

The surface roughness of the skin was evaluated quantitatively by mean, standard deviation, and minimum and maximum values of the Rz parameter, as observed in the laser profilometric test. The results are shown in Table II.

The improvement in the skin roughness was defined as the difference in the Rz value between two periodic measurements over four weeks, compared to the initial Rz value. In both treatments, the observed improvemenrs were found to be significant ($p < 0.001$). Wilcoxon matched pairs, signed rank, tests were used, comparing Rz values before and after each treatment. It was observed that the mean Rz value was reduced by 43-2% after treatment with the cream enriched with 5% Triple D Complex™, while after treatment with the control cream, the mean Rz value was reduced by only 24.9%. The difference between the two treatments was significant, as analyzed by Kruskal-Wallis one-way non-parametric ANOVA ($p < 0.001$). This significant improvement in the roughness of the skin after four weeks of applications is illustrated in Figure 1.

Skin Hydration Analysis

The SC hydration state was determined by the results of corneometeric analysis of 20 female volunteers treated with cream enriched with 5% Triple D Complex™ and compared to the results of the vehicle cream control. The results are shown in Table III. Small non-significant changes were observed between measurements of untreated skin control areas (data not shown). Wilcoxon matched pairs, signed rank, tests were used to examine changes in values, before and after each treatment. Application of cream with 5% Triple D Complex™ had shown a significant improvement of 10.5% after 8 hours and 6.4% after 12 hours ($p < 0.001$). The control treatment had shown a significant improvement of 7.9% after 8 hours and 5.1% after 12 hours ($p < 0.001$). The difference between the two treatments was analyzed by Kruskal-Wallis one-way non-parametric ANOVA and was found to be significant ($p < 0.001$ for 8-hour measurement, $p < 0.05$ for 12-hour measurement). The resulrs are presented in Figure 2.

The Influence Of Various Parameters On The Passive Mineral Biosorption To The *Dunaliella Sauna* Biomass

The ratio of mineral biosorption to algal biomass was evaluated according to changes in the pH of the Mineral Skin Osmoter™, temperature of the solution, and exposure time. When exposed to Dead Sea concentrated brine (Mineral Skin Osmoter™), *Dunaliella salina* dry biomass became enriched with calcium and magnesium, with a preference for

Table II

Rz Values of Skin Treatments With Basic Cream (control) and the Same Cream Enriched With 5% Triple D Complex™, as Observed by Computer-Aided Laser Profilometric Analysis, Lasting 4 Weeks

	Variable	Mean	SD	Minimum	Maximum	N
Control	Rz before	235.22	57.10	166.20	393.10	20
	Rz after	175.81	40.65	122.70	278.00	20
Cream + 5% Triple D Complex	Rz before	231.53	43.12	137.60	339.20	20
	Rz after	131.18	25.14	75.90	174.10	20

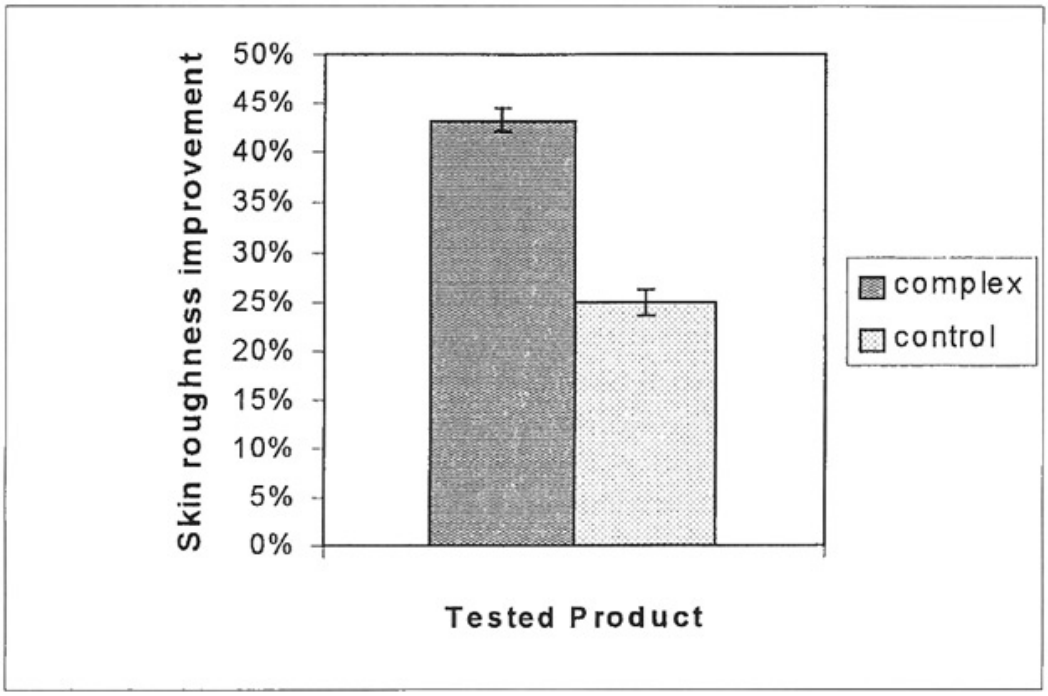


Figure I. Skin-smoorhing effects of [wo cream preparations; percentage of relative improvement after four weeks of skin treatment. Results determined by a computer-aided laser profilometric analysis of the skin surface. Mean of 20 subjects + SEM.

Table III

tion State After Application of Control (vehicle cream) and the Same Cream Enriched With 5% Triple D Complex™, as Determined by Corneometer Analysis

	Time	Mean	SD	Variance	N
Control (vehicle cream)	Initial values	81.8	5.47	28.09	20
	After 8 hr	88.2	5.42	28.53	20
	After 12 hr	85.9	5.52	31.44	20
Cream +5% Triple D Complex	Initial values	83.4	5.3	26.4	20
	After 8 hr	92.1	5.2	25.59	20
	After 12 hr	88.6	4.8	21.84	20

calcium. The passive uptake of these elements occurred simultaneously with the release of potassium from the algal biomass. Typical biosorption values are shown in Table IV.

The overall concentrations of the adsorbed elements were relatively low. The pH value, exposure time, and brine temperature were evaluated for their effects on the uptake of the elements. The pH value was found to be the most influential parameter. Biosorption of calcium and magnesium went up as the pH value increased. At pH values above 5.5, the obtained results may have been distorted by precipitation of mineral hydroxides. Thus, the optimal pH range for biosorption was defined as 5-0-5.5. Increased biosorption was obtained between 10°C and 33°C. Minor changes were measured between 33°C and 40°C. The maximum mineral uptake was observed after 10—15 minutes exposure to the brine. A longer exposure period contributed only minor changes. The observed results, as shown in Figure 3, are in agreement with published data (6). Blank values relate to non-treated algae.

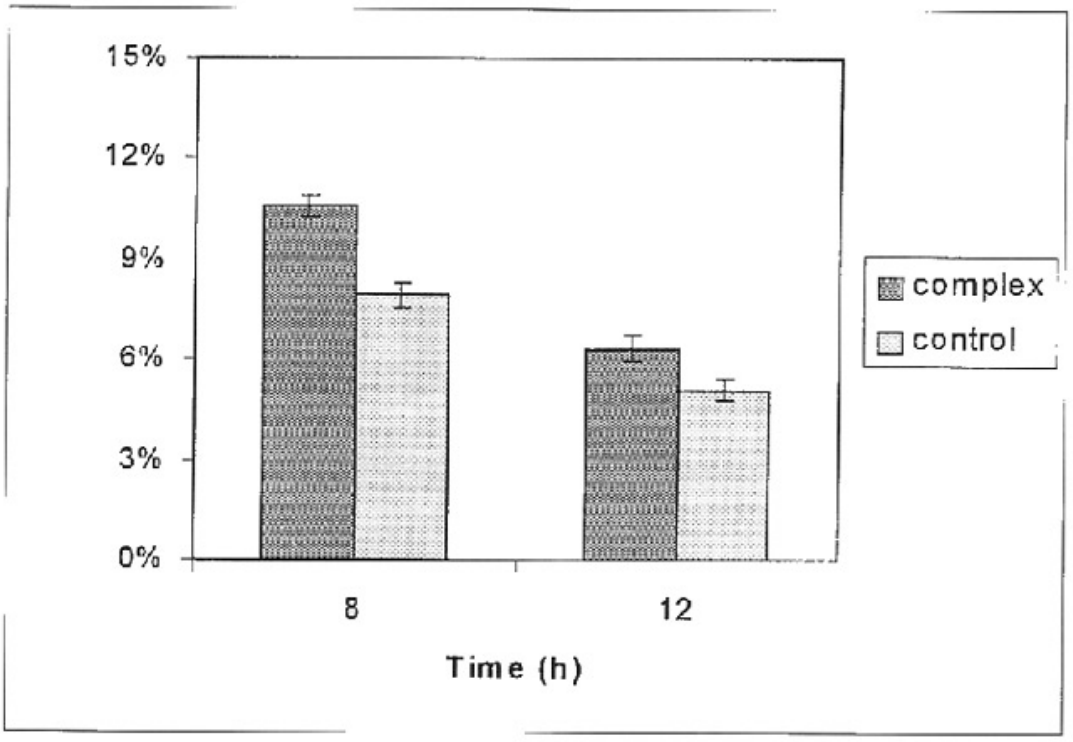
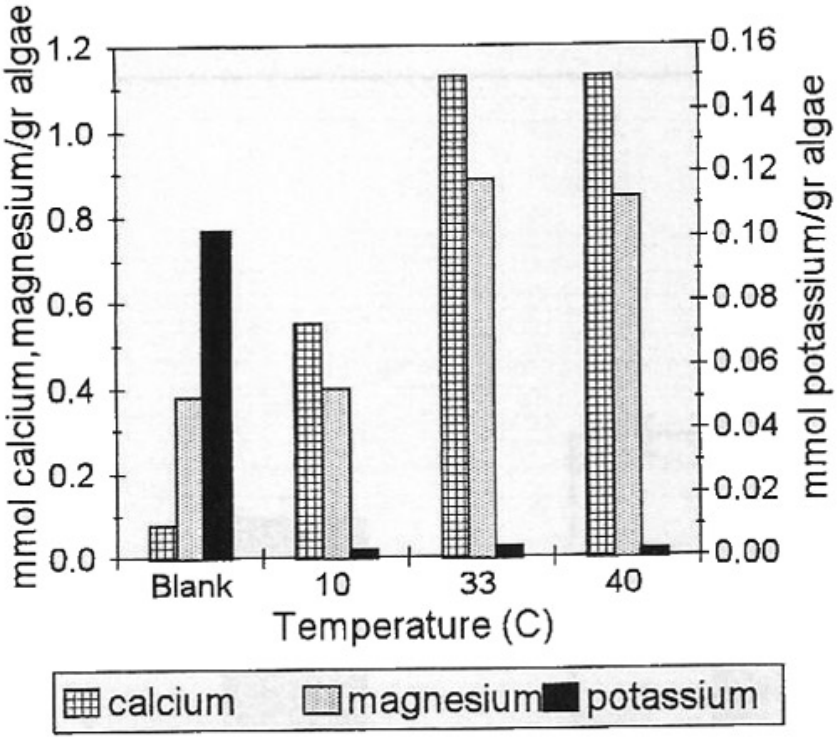


Figure 2. Skin-moisturizing effects of two cream preparations. Percentage of relative improvement after eight and twelve hours of skin treatment, observed by corneometeric analysis of skin moisture. Mean of 20 subjects ± SEM.

Table IV
Mineral Biosorption to *Dimaliella ialina* CCAP 19/31 Biomass: Typical Values (measured in dry, acid-soluble biomass)

	Calcium (mg/g D.W. algae [mmol/g D.W. algae])	Magnesium (mg/g D.W. algae [mmol/g D.W. algae])	Potassium (mg/g D.W. algae [mmol/g D.W. algae])
Before biosorption	2.5 (0.06)	6.1 (0.25)	6.7 (0.01)
After biosorption	30 (0.75)	12 (0.5)	0.28 ($4.3 * 10^{-3}$)
% Relative biosorption	1100%	100%	-95%

The Influence Of Various Parameters On Element Desorption From *Dunaliella Sauna*
The conditions for mineral desotption from saturated *Dunaliella salina* biomass were evaluated in order to determine and to predict the release ratio of minerals from the biomass to human skin. The influence of the pH of the solution (1.5—5), brine temperature (10°-40°C), and exposure time (5—30 minutes) were simultaneously assessed. The relative desorption of calcium and magnesium were subjected to a linear analysis, and a model, predicting the desorption of minerals from the *Dunahella* biomass, was suggested. Since the potassium concentratoin in the saturated biomass was very low, its measured values were excluded. The pH value was found to be the most influential parameter for both calcium and magnesium: as the pH decreased, desorption from the biomass correspondingly increased ($p < 0.01$).


mmol calcium,magnesium/gr algae
1.2
1.0
0.8
0.6
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0.2
0.0
0.16
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0.10
0.08
0.06
0.04
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mmol potassium/gr algae
Blank
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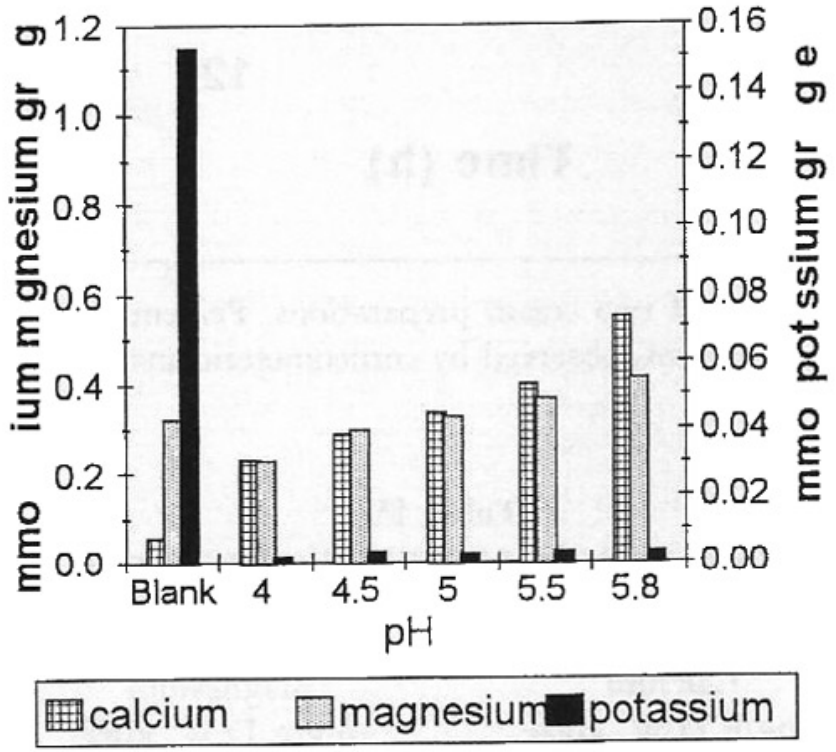
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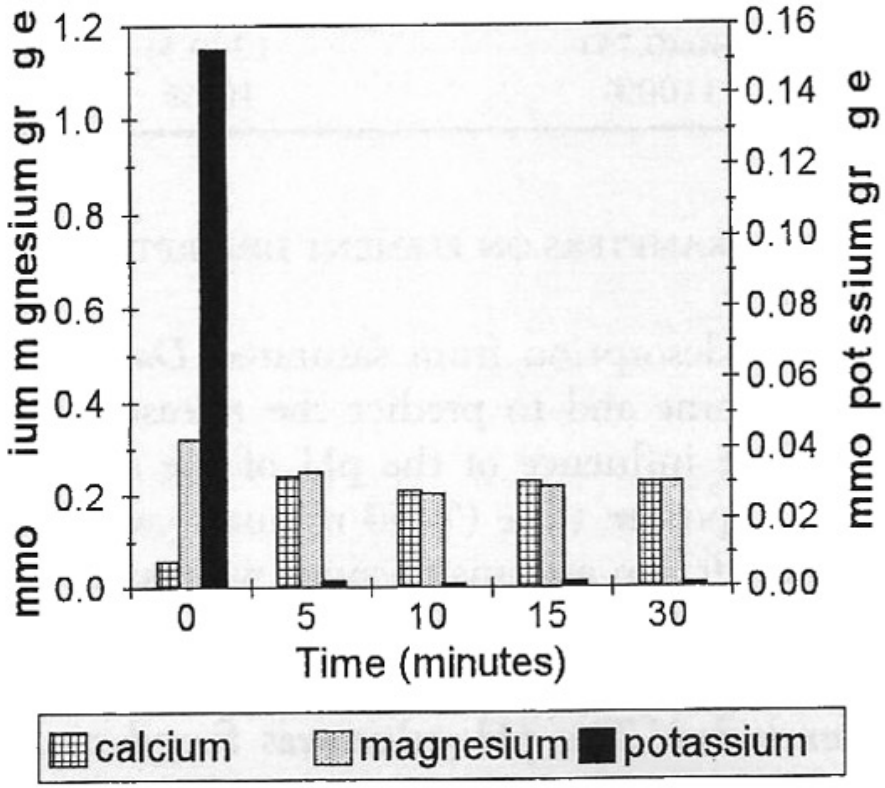

Figure 3- The effect of pH value, temperature, and exposure time to Dead Sea concentrated brine (Mineral Skin Osmoter™) on the biosorption of elements to *Dunaliella salma* algal biomass. Blank values relate to non-treated algae.

A small increase in the desorption of calcium was found with either temperature elevation or a longer exposure rime ($p < 0.1$). A minor connection between the exposure time and the temperature $ip < 0.5$) was also detected for calcium. No significant effect was observed for magnesium desorption with these parameters.
Magnesium was released more readily from the biomass than calcium. In order to predict the magnesium and calcium release from saturated *Dunaliella* biomass, the following equations, based on linear models, are suggested:

$$\% \text{ Desorption of calcium} = 105.1 - 15.5 \times \text{pH} - 0.15 \times \text{Temperature (°C)} - 0.283 \times \text{Time(min)} + 0.021 \times \text{Temperature} \times \text{Time} \quad (1)$$

$$\% \text{ Desorption of magnesium} = 98.1 - 7.1 \times \text{pH} + 0.1 \times \text{Temperarure (°C)} + 0.043 \times \text{Time(min)} \quad (2)$$

Discussion
Our study has conclusively shown that using 5% of Triple D Complex™, when added to a cosmetic cream, had a beneficial effect on skin roughness. A 43.2% improvement of the skin roughness, as indicated by a reduction in the Rz parameter, was achieved over four weeks of application time. This effect was almost twice the improvement of skin roughness observed when a control cream was topically applied. All results were calculated from a double-blind test, performed on contact-free computer-aided laser pro-filometry, which can be considered as a non-invasive and highly accurate technique.

Based on skin hydration results after eight and twelve hours from application time, it can be concluded that the Triple D Complex™ can be also used as an active moisturizing ingredient. Skin hydration and skin smoothing are two essential parameters of the anti-aging effects of cosmetic products. Biosorprion and desorprion of minerals from Dead Sea concentrated brine (Dead Sea Mineral Skin Osmoter™) to the *Dunaliella saltna* algae biomass were investigated. Results indicated that overall concentrations of the adsorbed elements were lower than the reported results for other species of algae: about 45 vs. 100 mg mineral/g dry weight biosorbent (7). This difference is mainly attributed to the absence of a rigid cell wall in *Dunaliella* alga. Biosorption of magnesium and calcium occutred at the same time as desorption of potassium from the biomass. The pH value was found to be the most influential parameter for the biosorption and desorption of elements: higher pH values enhanced the biosorption, while desorption from the saturated biomass was enhanced at lower pH values. Mineral biosorption may contribute to the watet capacity within the skin tissue due to its patticipation in the natutal moisturizing factor, NMF.

It was assumed that the biomass of *Dunaliella sahna,* originating ftom a hypersaline habitat, would be a suitable vehicle for delivery of minerals to skin. However, the relatively low biosorption of calcium and magnesium obtained for the algal biomass, and the tendency to a low release of minerals from the biomass at the normal pH of human skin (5-5), led to the conclusion that the advantage of these algae as a mineral vehicle for the tested elements is limited.

In a previous publication, we have reported thar adding 1% of Mineral Skin Osmoter™ to a control gel contributed to an increase in the smoothing effect of the skin, to 40.3%.

This phenomenon was reported as the "Dead Sea Anti-Wrinkle Effect" (1). The present' study differed from the previous one by the use of a cream application, and by the addition of 5% Triple D Complex™. This Triple D Complex™ contains Mineral Skin Osmoter™. The similar improvement achieved in skin smoothing in both studies suggests that the Dead Sea minerals play a significant role in the mechanism of the proven effect of antiwrinkling. Thus the minerals may be assumed to be a potent antiwrinkle agent. The mechanism of the beneficial activity of the minerals on the skin, and the role of other components of the Triple D Complex, namely the algae and plant extracts, will be further investigated.

Acknowledgments
The authors would like to thank Dr. W. Voss of Dermatest for skillful profilometric and corneometric clinical measurements; Dead Sea Works Ltd. for financial support; the Pharmacist, Dr. I. Iaccony from AMI, for her essential support in formulation of the Complex; Dr. E. Kvallen of IMI for his statistical analyses; Dr. E. Cohen of Ben-Gurion University, Beer-Sheba, for supplying the algae; and Ms. A. Alzaradel, Ms. M. Weis-buch, and Ms. M. Friedman of IMI for their technical assistance.

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