

# A NEW COSMETIC-CARRIER CHITOSAN-BASED

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## Summary

The polysaccharide chitin/chitosans considered environmentally friendly raw materials, play a pivotal role in many industrial fields cosmetics included. They seem also to be hydrating and immunopotentiating considering their similarities with hyaluronic acid through the common N-acetylglucosamine sugar, and have been shown to be able to scavenge reactive oxygen species.

In the light of above consideration, the aim of this study was to evaluate the property of a PCA-chitosan and a Glycochitosan gel to restore the skin lipid content and to eventually decrease inflammation and skin hydroperoxides alleviating the symptoms of dry skin.

The treatment by the used creams, in a double blind placebo controlled study evaluated by 3C System, induced a significant and progressive improvement in skin hydration (+72%,  $p < 0.05$ ), and skin surface lipids (+38%,  $p < 0.05$ ), and a contemporary decrease in TEWL (-37%,  $p < 0.05$ ) compared to the placebo. The most interesting property obtained is also the intense anti-inflammatory and scavenging activity detected by the SDS challenge test.

The improvement, evident from the first week of treatment, shows how these creams could be considered as useful means to improve skin hydration, and cutaneous elasticity ameliorating also the inflammatory status of subjects affected by skin xerosis.

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## Riassunto

I polisaccaridi, derivati dalla chitina e dal chitosano, considerati prodotti biocompatibili, svolgono un ruolo chiave in molti settori industriali tra cui quello cosmetico. Hanno infatti dimostrato di possedere un'azione reidratante, immunostimolante e antiossidante, grazie soprattutto alle caratteristiche delle loro molecole molto simili alla struttura dell'acido ialuronico.

Scopo di questo lavoro è stato quello di valutare le proprietà reidratanti ed antinfiammatorie di due gel basati sull'uso di due derivati chitosanici, il PCA ed il Glico-chitosano.

Il trattamento effettuato a doppio cieco nei confronti del solo veicolo mediante l'uso del 3C System ha posto in evidenza un incremento dell'idratazione cutanea (+72%,  $p < 0.05$ ) e dei lipidi di superficie (+38%  $p < 0.05$ ) a fronte di un decremento della TEWL (-37%  $p > 0.05$ ). Quel che è interessante rilevare è l'intensa attività antinfiammatoria rivelata da questo tipo di formulazione che sembra essere molto utile nel trattamento delle xerosi anche di natura patologica.

## INTRODUCTION

The polysaccharide chitin/chitosan derived from animal world of crustacean shells obtained from fungi, play a pivotal role in many industrial fields, cosmetic included (1-11).

Moreover, chemically modified chitins, easily recycled by biodegradation operated by microbial genera, are considered environmentally friendly raw materials. They seem also to be hydrating and immuno-potentiating considering their similarities with hyaluronic acid through the common N-acetylglucosamine sugar, and have been shown to be able to scavenge reactive oxygen species. (12-15)

## AIM

In the light of above consideration, the aim of this study was to evaluate the property of a PCA-chitosan and/or a glycochitosan gel (glycolic acid and chitosan) to increase hydration, to restore the skin lipid content and skin hydration and to eventually decrease inflammation and skin hydroperoxides, alleviating the symptoms of dry skin.

## MATERIALS AND METHODS

### MATERIALS

For this study was used a glycochitosan and a PCA-chitosan gel neutralized or not with gelatin-glycine, according with our preliminary studies. (16)

### PATIENTS

The two month trial was a randomized double-blind-placebo-controlled study carried out for 8 weeks on 60 dry skinned female volunteers, aged 29-48 with a moderate xerosis of grade 5 according to Dahl (17). Surface lipids, skin hydration and TEWL were detected by the 3C Sy-

stem (Dermotech Italy) (18,19) meanwhile irritation potential and anti-inflammatory activity were investigated both by SDS challenge test and Pyrexal erythema test (20) using also the Chromameter® C 200.

## TEST PROCEDURE

The subjects were randomly divided into four groups of 15 patients each and received respectively:

- I gel A (active A): glyco-chitosan neutralized with gelatin-glycine at pH 4.8
- II gel B (active B): PCA glyco-chitosan neutralized with gelatin-glycine at pH 4.8
- III gel C (carrier A): glyco-chitosan neutralized with sodium hydroxide at pH 4.8
- IV gel D (carrier B): PCA-chitosan neutralized with sodium hydroxide at pH 4.8.

Each patient, supplied with two identical tubes containing the testing cream (A and C or B and D) was instructed to apply them on their face twice a day for all the study period, and was not allowed to use any other skin care product. Each subject was used as her own control, the testing creams (A and C or B and D) being applied on a randomized basis, on the right or left area of the face.

Moreover they were instructed to apply the same cream always to the designed site after washing, first in the morning and just before retiring in the evening. Subjects were also instructed that only the cleansing cream supplied to each at the beginning of the study should be used to cleanse the test area.

Other instructions included not to apply the testing creams the day of evaluation and to wash their face at least 4 hours before the control.

## CLINICAL EVALUATION

Clinical and biophysical examinations were performed on the first day (baseline), and at week 2, 4, 6, 8 and 10 (end of the treatment). Clinical evaluations included the efficacy for reduction of symptoms of dry skin as well as the skin tolerance after the application phase. The obtained clinical results are reported on Tab. I

OVERALL SKIN TOLERANCE ON NEW CHITOSAN-BASED GELS

		DAY 15	DAY 30	DAY 45	DAY 30
VERY GOOD	ACTIVE A	32	38	39	39
	ACTIVE B	31	31	35	35
	CARRIER A	30	30	30	30
	CARRIER B	30	30	30	30
GOOD	ACTIVE A	25	21	20	20
	ACTIVE B	27	27	24	24
	CARRIER A	28	28	28	28
	CARRIER B	28	28	28	28
SATISFACTORY	ACTIVE A	2	0	1	1
	ACTIVE B	2	2	1	1
	CARRIER A	1	2	0	0
	CARRIER B	2	2	0	0
POOR	ACTIVE A	0	0	0	0
	ACTIVE B	0	0	0	0
	CARRIER A	1	0	0	0
	CARRIER B	0	0	0	0

Tab. I Patients involved

## BIOPHYSICAL NON-INVASIVE MEASUREMENTS

The determinations were always carried out on four areas of right or left areas (forehead, cheek, chin and nose) and the results are expressed as mean values of the measurements performed. To achieve an higher degree of assurance all evaluations were performed after a 30 minutes acclimatization period in a room at 21°C to 22°C and 45% to 50% humidity, even if the 3C System automatically adjusts environmental conditions to 22°C and 50% relative humidity.

### Skin surface lipids.

Determination is based on photometric measurement of light transmission through a skin surface imprint obtained applying to the designed

skin area a frosted plastic foil. It allows adherence of skin lipids in a 1 cm<sup>2</sup> area.

The obtained readings are automatically converted into (g/cm<sup>2</sup>) and reported in Fig. 1

SUPERFICIAL SKIN LIPIDS AFTER A TWO MONTH TOPICAL TREATMENT WITH GLYCO-CHITOSAN OR PCA-CHITOSAN GEL NEUTRALIZED WITH GELATIN-GLYCINE

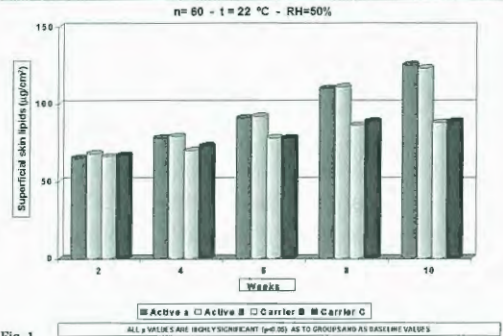


Fig. 1

### Skin Hydration

The hydration of the horny layer was assessed by measuring electrical capacitance of the skin surface by means of the 3C System.

When the probe was applied to the skin (recording time 0.5 s), the capacitance is displayed digitally in arbitrary 3C units. The obtained results are reported in Fig. 2.

SKIN HYDRATION AFTER A TWO MONTH TOPICAL TREATMENT WITH GLYCO-CHITOSAN OR PCA-CHITOSAN GEL NEUTRALIZED WITH GELATIN-GLYCINE

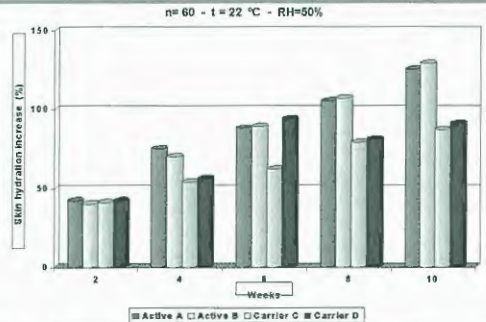


Fig. 2

## Transepidermal Water Loss (TWL)

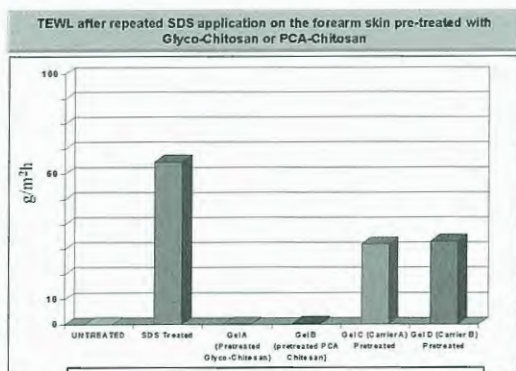
The 3C System probe consists of a cylindrical open chamber measuring system, diameter 14 mm, height 10 mm and skin area 0,95 cm<sup>2</sup>, two sensor units, containing thin capacitive film transducer, are placed at 3 and 7 mm distance from the skin surface. TEWL is calculated digitally in g/m<sup>2</sup> h.

## SDS CHALLENGE TEST

This test is generally assessed to evaluate protective and anti-irritation potential efficacy of the studied products. Thereafter, one occlusive patch with 20% sodium dodecyl sulfate (SDS) solution were applied in a randomized way to left or right forearm of each subject for 3 days and different hours before and after 8 weeks of twice daily treatment with the different kind of gels used.

Two SDS treated and untreated sites were left as control.

There was no further application of the studied gels after removal of the last patch. Patches were applied to the pre-marked forearm areas, removed after 4 hours and reapplied to the same sites for two consecutive days for 4 hours each. After 1 hour and 24 hours of removal of the last set of patches, the skin reaction was graded and evaluated by TEWL and Chromameter® 200 measurements.



The intensity of the irritation was assessed by visual scoring/chromametry and TEWL measurements 5 hours and 24 hours after application of the patches.

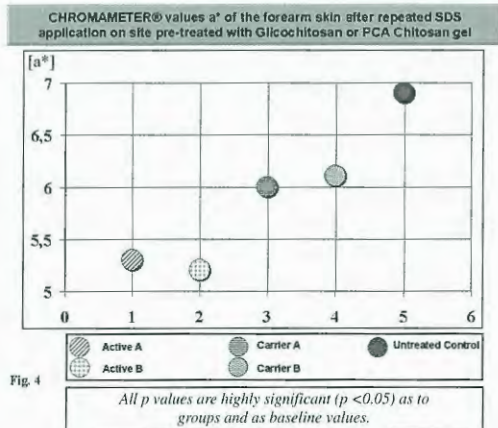
(0 = no-erythema; 0.5 = equivocal reaction; 1 = slight erythema; 2 = moderate, uniform erythema; 3 = intense; 4 = fiery redness with edema.) The obtained results are reported on fig. 3 and 4.

## ERYTHEMA TEST

This inflammation is obtained injecting intracutaneously 0.1 ml. Pyrexal (lipo-polysaccharide from salmonella abortus equii) into dorsal skin of the volunteer subjects, according to Heilmeyer and Hiemejer (20)

These bacterial pyrogens induce an inflammation. The morphologic signs take the form of a sharply defined erythema whose surface area is measured over time. Simultaneous application of an anti-inflammatory cream inhibits its spread. By this injection 5 vials were induced in each subject. These were then topically treated in randomized succession with 0.2 ml. of the 4 different preparations and covered with transparent film.

Two of these areas were treated with the carriers to serve as a control and another was treated by 0.2 ml. of betamethasone valerate 0.1% and co-



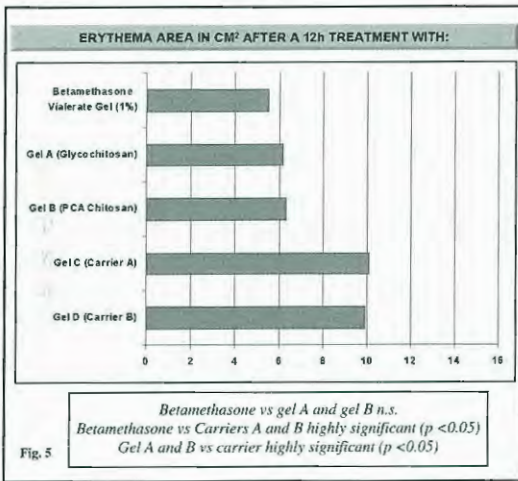
vered also with transparent film.

In this way the erythema is visible at all times and can be measured through the film. The extent of the erythema was determined 6,8,10 and 12 hours after application, the maximum (a) and minimum (b) diameter being measured and the surface area calculated by the elliptic formula:

$$F = \frac{a \times b \times \pi}{4}$$

The efficacy was classified by determining the sum of the erythema surface areas from the 4th to the 12th hour. A small area means that the preparation is highly effective. The comparative evaluation was performed with the aid of bifactorial analysis of variance

The obtained results are reported on Fig. 5



## STATISTICAL ANALYSIS

Results are the mean  $\pm$  S.E.M. of at least 5 measurements. Data were analyzed by one way *Anova*, followed by inspection of all difference by Duncan's new multiple-range test.

Differences were considered significant at ( $p < 0.05$ ).

## RESULTS AND COMMENTS

The treatment by used creams induced a significant and progressive improvement in skin hydration (+72%,  $p < 0.05$ ), and skin surface lipids (+38%,  $p < 0.05$ ), and a contemporary decrease in TEWL (-37%,  $p < 0.05$ ), and on skin redness compared to the placebo. The first and the most interesting property obtained is undoubtedly the intense anti-inflammatory activity comparable and close to beta-methasone valerate, used as parameter. As clearly see on Fig. 4 and 5.

The improvement, evident from the first week of treatment, shows how these creams could be considered as useful means to improve skin hydration, to rebalance skin lipids tone and elasticity ameliorating also the inflammatory status of subjects affected by skin xerosis caused by environmental or pathological reasons.

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