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Evaluation of the effect of anti-pollution & anti-aging eye cream on the collagen contraction

Gülşah Gedik[1*], Seda Alaca[2]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Trakya University, Edirne, Turkey
[2]Tan-Alize Cosmetics, İstanbul, Turkey

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*Corresponding author:
gulsahgedik@trakya.edu.tr

ABSTRACT

This is a study of the brown algae-derived hydrolyzed algin as used in eye cream formulation. Alginic acid sodium is a gelling and non-toxic anionic polysaccharide, which is used to bone tissue engineering, preparation of alginate hydrogels, encapsulating, hydrating, protective, and de-polluting action. The skin of eye contour is thinner and less dense in support fibres than other parts of the face. Therefore, cutaneous sagging is frequently seen to appear around 40 years of age, very often aggravated by environmental factors such as pollution. The objective of the study was to evaluate the effect of anti-pollution & anti-aging eye cream on the collagen contraction. The outcomes of the characterization analysis indicate the development of successful cream formulation with optimum characteristics. No microbial growth was observed. The collagen lattices were prepared with human dermal fibroblasts, for evaluation of the effect of the cream on the collagen contraction. The lattices were treated or not (control) with the tested cream and then incubated at 37°C for 96 hours. The surface of lattices was measured by image analysis, and the lattices contraction was analyzed at 16, 24, 40, 48, 64, 72, 88, and 96 hours (the measure of lattices area). The treatment with eye cream at 0.5% decreases the surface of the lattice compared to the control (no treatment). According to the results obtained under the conditions of the test, the eye cream tends to increase the collagen contraction and a significative decrease of the lattice surface by 6.68% compared to the control at 16 hours.

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1. INTRODUCTION

This is a study of the brown algae-derived hydrolyzed algin as used in cosmetics. The ingredient in this study is the extract of the whole or a defined part of the seaweed. “Brown algae” is a common name for seaweeds of the class *Phaeophyceae* and classified in about 265 genera with more than 1500 species [1,2].

The actual color varies depending on the proportion of brown pigment (fucoxanthin) to green pigment (chlorophyll). This algal group contains alginic acid and fucoidan in its complex cell walls. Several brown algae constituents, such as phytosterols, phytosteryl ingredients, and alginic acid, were found to be safe [3,4]. The most frequently reported function of brown algae in cosmetics is as a skin-conditioning agent; other reported functions include absorbent, antioxidant, binder, hair conditioning agent, oxidizing agent, and viscosity increasing agent [4].

Alginic acid sodium is a gelling and non-toxic anionic polysaccharide. The carboxylic acid groups on the alginic acid chain render it insoluble in water. However, converting alginic acid to its sodium form enables it to solubilize in water easily [5].

Hydrolyzed algin is used:

- in combination with chitosan, to fabricate a biodegradable porous scaffold for bone tissue engineering [6],
- to study the characteristics of a modified amphiphilic alginate derivative [7],
- to the study the impact of alginate on the rate of lipid digestion by employing an *in vitro* digestion model [8],
- in the preparation of alginate hydrogels [9],
- as an encapsulating agent of β-galactosidase microparticles [10],
- as an occlusive and hydrating agent,
- for protective and de-polluting action [11].

Brown algae have been used for wastewater/effluent treatment and removal of heavy metals (*Sargassum*, *Laminaria*, and *Ecklonia* species) [12].

Alginates, which are membrane polysaccharides taken from the skin of algae, are depolymerized to obtain a high molecular weight oligoalginate. Applied to the skin, their high degree of polymerization enables them to stay on the surface of the epidermis and form a protective mask.

Every day the skin is exposed to all sorts of impurities in the atmosphere, such as heavy metals, cigarette smoke, and

so on. These reduce the levels of hydration and oxygenation and produce free radicals, which lead to cutaneous aging. Alginates minimizes the adhesion of chelates heavy metals such as lead and cadmium to stop particles from encrusting and asphyxiating the skin. Alginates also protects the viability of cells exposed to cigarette smoke [13].

The skin of the eye contour covers 22 muscles, which are continually moving. To make this mobility possible, this area is thinner and less dense in support fibres than other parts of the face. Therefore, cutaneous sagging is frequently seen to appear around 40 years of age, very often aggravated by environmental factors such as pollution [14].

The objective of the study was to evaluate the effect of anti-pollution & anti-aging eye cream on the collagen contraction.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were of reagent and analytical grade. Double-distilled water was used throughout the study. All chemicals CAS No and functions were given in **Table 1**.

Table 1. *The complete formula of eye cream formulation*

Chemical Analysis Properties			
Components	**% w/w**	**CAS No**	**Function**
Water	q.s.	7732-18-5	solvent
Glycerin	5.000	56-81-5	humectant
Cetearyl Alcohol	4.500	67762-27-0	emulsifying
Glyceryl Stearate Ceteareth-20 Ceteareth-12 Cetyl Palmitate	4.000	31566-31-1 68439-49-6 68439-49-6 540-10-3	emollient emulsifying
Caprylic/Capric Triglyceride	2.500	73398-61-5	skin conditioning
Dicaprylyl Carbonate	2.000	1680-31-5	emollient
Seawater, Hydrolyzed Algin, Phenethyl Alcohol, Sucrose	1.500	- - 60-12-8 57-50-1	 humectant skin conditioning
Phenoxyethanol Ethylhexylglycerin	0.765 0.085	122-99-6 70445-33-9	preservative skin conditioning
Cera Alba	0.600	8012-89-3	emollient
Mica	0.500	12001-26-2	opacifying
Butyrospermum Parkii (Shea Butter)	0.500	194043-92-0 91080-23-8	skin conditioning
Sodium Polyacrylate	0.300	9003-04-7 25549-84-2	emollient emulsion stabilizing film forming
Xanthan Gum	0.050	11138-66-2	viscosity controlling
Tocopheryl Acetate	0.001	7695-91-2 58-95-7	antioxidant

2.2. Preparation of Eye Cream Formulation

The complete formula is reflected in **Table 1**. All the aqueous phase materials and the oil phase ingredients were placed in two separate containers and heated to above 75°C. The water phase was then added to the oil phase using continuous agitation. Preservative, antioxidant, humectant agents and hydrolyzed algin mixture were added after cooling. The formulation was kept at 25±1°C for 48 hours to see a possible phase separation.

2.3. Characterization of Formulation

The formulation was evaluated for its sensorial parameters and physicochemical parameters like pH, density, and viscosity. The sensorial parameters of the formulation, such as appearance, odor, color, were determined. The pH of the formulation was detected by a digital pH-meter (Mettler Toledo S 220, Switzerland), the density was detected by a pycnometer (Mettler Toledo 30330857, Switzerland), and the viscosity measurements were performed with a vibro viscometer (AND, SV-10, Japan). The experiments were repeated five times at 25°C.

2.4. Microbial Contamination Tests

The microbiological contamination of formulations was evaluated by validated test kits from AFNOR using cultures of *Staphylococcus aureus, Escherichia coli, Pseudomonas Aeruginosa*, total bacteria, yeast, and mold.

2.5. Evaluation of The Effect of an Eye Cream on The Collagen Contraction

For technical reasons, the tested formulation was previously extracted. It was first diluted to 10% in the medium; the insoluble mixture was stirred for 1 hour to extract the active ingredients. After centrifugation, the supernatant containing the active principles (aqueous phase) was collected and diluted in culture medium at specified concentrations. This study was done with the support of ARERKO.

2.5.1. Biological model

The collagen lattice model was used, which corresponds to a three dimensional (3D) reconstituted dermis, with a population of fibroblasts synthesizing new fibers and interacting with collagen fibers network (confidential composition).

2.5.2. Preparation of the lattices

The cells were Normal human dermal fibroblasts from a donor of 30 years old, passage 12.

The collagen lattices were prepared with these fibroblasts in 5% of serum. The lattices contraction was analyzed after 16, 24, 40, 48, 64, 72, 88, and 96 hours of culture (photographs and measure of lattices area).

2.5.3. Treatment

The lattices were treated or not (control) with the tested cream and then incubated at 37°C for 96 hours. The lattices contraction was analyzed at 16, 24, 40, 48, 64, 72, 88, and 96 hours (measure of lattices area). The surfaces of lattices were measured by image analysis at a different time of incubation (16, 24, 40, 48, 64, 72, 88, and 96 hours).

2.5.4. Data expression

Results were expressed as the percentage of the surface after the contraction in the function of the initial surface (measured at t_0 before incubation). The more the percentage is low, the more the contraction is good.

2.6. Statistical Analysis

The raw data were transferred and processed using MS Excel Software. The different conditions were compared using the Student's t-test. A difference between the two groups was considered statistically significant if the p-value was less than 0.05, which was noted *p<0.05. If the p-value is lower than 0.01 and 0.001, it was noted **p<0.01 and ***p<0.001, respectively.

3. RESULTS AND DISCUSSION

The physicochemical and sensorial characterization parameters of the formulation are reported in **Table 2**. The density, pH, and viscosity of all formulations were found to be satisfactory. The pH of the developed cream ranged between 5.5-6.0. The density of the developed cream found 0.98 ± 0.02 g/mL. The viscosity found 14.10 ± 0.22 P. The density, pH, and viscosity of the cream was appropriate for the dermal application.

Table 2. *The physicochemical and sensorial characterization parameters of the eye cream formulation*

Physicochemical Parameters	
Density (g/mL)	0.98 ± 0.02 (25°C)
pH	5.75 ± 0.10 (25°C)
Viscosity (P)	14.10 ± 0.22 (25°C)
Sensorial Parameters	
Appearance	Cream
Odor	Characteristic
Color	White

3.1. Microbial Contamination Tests

All results about microbiological contamination studies were given in **Table 3**. After the incubation period, the tests were checked for microbial growth. No microbial growth was observed. The obtained results had confirmed the microbial study of our formulation.

Table 3. *The microbiological study results*

Test Microorganisms	Microbiological Parameters
Total Bacteria	< 100 CFU/mL
Yeast and Mould	-
Escherichia coli	-
Staphylococcus aureus	-
Pseudomonas aeruginosa	-

3.2. Evaluation of The Effect of an Eye Cream on The Collagen Contraction

The treatment with eye cream (118-08) at 0.5% decrease the lattice surface compared to the control (no treatment). The cream visually decreased the lattice surface over time, which means an increase in the collagen contraction (**Figure 1-3**).

3.3. Discussion

This study evaluated an eye cream on the collagen contraction *in vitro* in human dermal fibroblasts cultured in a lattice of collagen that appeared to be a model close to the living dermis. Today, the use of extracts as anti-pollutant and anti-aging from various plants and marine organisms has become popular. The objective of the study was to evaluate the effect of anti-pollution & anti-aging eye cream on the collagen contraction.

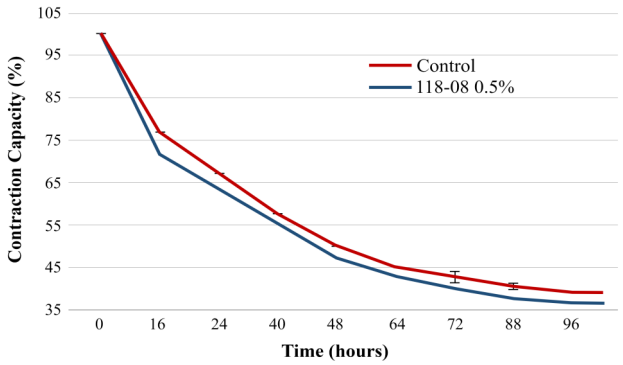

Figure 1. *Effect of the eye cream (118-08) at 0.5% on the lattices surface over time*

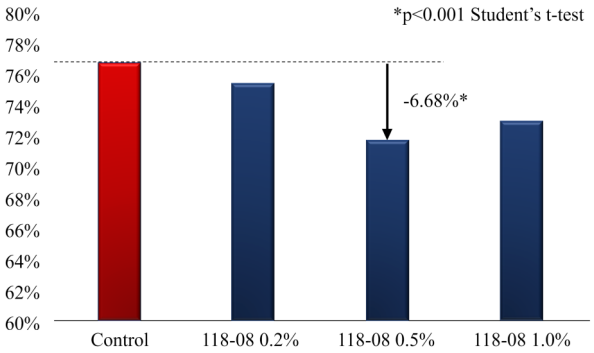

Figure 2. *Effect of the eye cream (118-08) at 0.5% on the lattice surface at 16 hours*

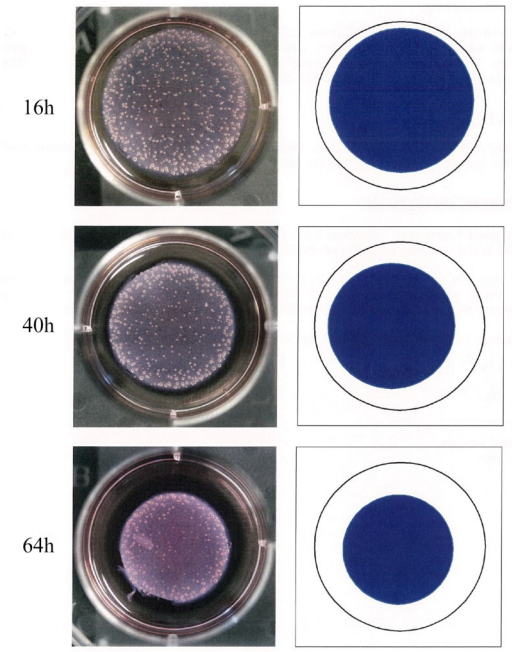

Figure 3. *Observation of one lattice treated with the eye cream (118-08) at 0.5% (16, 40, and 64 hours)*

Environmental pollutants have a negative effect on human health and human skin. Exposure to pollutants can cause aging, pigmentation, acne, atopic dermatitis, psoriasis, and even skin cancer, or acne [15].

Pollution has a negative impact that can be observed at the stratum corneum, which is generally colonized with residual microorganisms. In the presence of pollutants, the skin microbiome changes for the benefit of pathogenic bacteria [16]. Moreover, contamination enhances the production of reactive oxygen species and causes disturbance in the redox balance. Some pollutants also tend to permeate through the stratum corneum into deeper skin layers. There, they act as a ligand for the aryl hydrocarbon receptor (AhR), which takes part in mediating the toxic effects of pollutants. All of them cause the induction of an inflammatory cascade in the skin. The increased production of pro-inflammatory cytokines, such as interleukin1β or interleukin 8, greatly impacts the biological function of the cells, resulting in skin lesions and deterioration of skin appearance [17,18].

Vierkötter et al. showed a direct link between chronic exposure to traffic-related particulate matter and the occurrence of prominent skin aging signs, especially pigment spots, but also wrinkles in Caucasian women [19].

Li et al. then reported epidemiological evidence that indoor air pollution from cooking with solids fuels was associated with wrinkles in Chinese women [20]. A recent study has found that exposure to NO_2 was associated with the formation of lentigines in Caucasian and East Asians [21].

Particles can serve as carriers for organic chemicals and metals that are capable of localizing in mitochondria and generating ROS directly in mitochondria leading to collagen degradation in human skin and thereby cause wrinkle formation [22,23].

In recent years, there has been an increasing interest in products that protect us from the negative impact of pollutants, and that helps to restore the skin barrier function.

The cream was made for this purpose, and depolymerized oligoalginate of high molecular weight was used as an active agent. Applied on human skin, it protects against heavy metals, particles, air pollution, and cigarette smokes. Alginate chelate metal ions, reduce inflammatory mediators, and cytokines. Shanura et al. showed alginic acid also reduced the levels of COX-2, interleukin 6, TNF-α, and inhibited specific key molecular mediators of the NF-κB and MAPK pathways in keratinocytes. Alginic acid substantially reduced the levels of metal ions like Pb2+ and Ca2+ in keratinocytes attributable to its metal ion chelating properties. These cells presented with increased levels of NO, iNOS, COX-2, PGE2, and pro-inflammatory cytokines [24].

The cream formulation characterized based on its pH, density, and viscosity. Physicochemical characterization of the formulation is an important subject to be considered in the formulation part, especially those intended for dermal application. The cream has good appearance, color, and odor on sensorial inspection. The pH of the developed cream ranged between 5.5 and 6.0. Ideally, dermal formulations should possess pH in the range of 5.0-6.0. The basic formulation does not include the hydrolyzed algin, which served as the control for density analysis, presented a density value of 1.01 g/mL, which is similar to the values registered for the remaining formulation that 0.98 g/mL. The hydrolyzed algin incorporated into the eye cream formulation did not affect the density of the basic formulation.

Microbial contamination study is crucial to evaluate the microbial stability of formulation to ensure its safety. No microbial growth was observed for the eye cream formulation.

Cellular stiffness is significantly increased in dermal fibroblasts during aging *in vivo*. This increase in stiffness has a direct impact on cellular contraction capacity.

Human dermal fibroblasts cultured in a lattice of collagen appeared to be a model close to the living dermis. The collagen polymerizes into anchoring fibrils known to bind to cells. Then, the fibroblasts pull on these fibrils thanks to their migratory movement and thus reorganize the matrix.

The lattice decreases its diameter, which results in a phenomenon called contraction or retraction, indicative of the contractile activity of the fibroblasts [25].

The treatment with eye cream at 0.5% decreases the lattice surface compared to the control (no treatment). According to the results obtained under the conditions of the test, the eye cream used at 0.5% tends to increase the collagen contraction. The best results are observed at 0.5% with a significative decrease of the lattice surface by 6.68% compared to the control at 16 hours. The cream visually decreased the lattice surface over time, which means an increase in the collagen contraction (**Figure 1-3**). Our results are compatible with the literature. Park et al. suggested that these alginate oligosaccharides might have the potential to prevent skin aging by promoting collagen synthesis through the inhibition of collagen degrading enzyme [26].

4. CONCLUSION

Our study showed that the eye cream formulation obtained from hydrolyzed algin called alginic acid is a potent anti-pollution & anti-aging agent and can be used in patients with lentigines and pigment spots and wrinkles. Future controlled clinical trials are needed to evaluate the efficacy of the eye cream.

In particular, the search for anti-pollution & anti-aging agents of natural origin is progressing rapidly, which points to the need for further studies exploring the utilization of the therapeutic agents from Brown algae.

AUTHOR CONTRIBUTIONS

Concept: GG, SA; Design: GG, SA; Supervision: GG; Materials: SA; Data Collection and/or Processing: GG, SA; Analysis and/or Interpretation: GG; Literature Search: GG; Writing: GG; Critical Reviews: GG, SA.

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CONFLICT OF INTEREST DECLARATION

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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