TRIMETHYLGLYCINE: A VEGETAL STRESS-MOLECULE PERFORMING A WIDE RANGE OF COSMETIC ACTIVITY

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Introduction

Trimethylglycine, commonly named betaine, is the most simple amphoteric molecule. It is completely vegetal (1,2), as it is produced in the sugar industry by industrial chromatography of molasses. While abundantly used in foods and diet supplements, many interesting applications in cosmetics have recently been investigated, like its capability to increase the volume and stability of foams in surfactant solutions. For its special chemical structure (it is the internal salt of a weak acid and a strong alkali) trimethylglycine is a solvent and buffering agent for strong acids and Lewis' acids. It allows to improve the efficiency of α- and β-hydroxy acids in increasing the physiological rate of epidermal cell renewal, while keeping a low skin-irritation level. In oral care cosmetics, it acts as a mucous membrane protectant (3). For its special water co-ordination capability, its solubilising power, polymer swelling capability, after-feel improvement in hair products, skin moisturization and elasticity enhancing properties, trimethylglycine provides unusual characteristics to many products intended for skin maintenance (4).

Aim of the study

In this work, some applications of trimethylglycine are described, together with the experimental results concerning in-vivo efficacy studies in cosmetic recipes. Trimethylglycine is effective in different cosmetic aspects: firstly, it is a sensorial modifier for aqueous solutions and o/w emulsions. Massage time and slipperiness are increased, even in blends with other hydrotropes. Stickiness is contemporarily decreased. Firstly, we wanted to demonstrate its moisturising and protecting effects for both skin and hair. Moreover, it was studied its capability to protect the oral mucosa from the irritant effect of surfactants used in oral care. Finally, we could demonstrate its help in dissolving and buffering Lewis' acids used in cosmetics.

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Structure-activity relationship

Trimethylglycine structure represents the most simple amphoteric molecule.
Its chemical structure is reported in Fig.1.

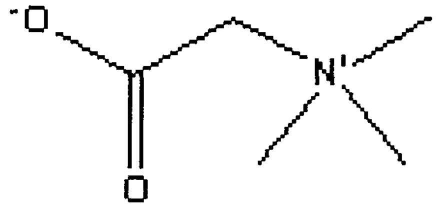

***Fig. 1** Chemical structure of trimethylglycine*

In dilute solutions, the COO^- group is strongly attracted by the quaternary ammonium ion but cannot move too closely to it because of the strain that such orientation would induce on the bond angles of the central carbon atom. One water molecule, for its high dipole moment, is attracted and can find easily place into the space between the two ions. Indeed, betaine crystallises with one water molecule The dipole also attracts many surrounding water molecules, as demonstrated by the intrinsic viscosity and the apparent specific volume of the molecule, that are far higher than for its parent compound glycine. In fact betaine shows a higher amount of static water co-ordination molecules, which move with it in the solution under the double influence of hydrophilic and lipophilic interactions (5).
Nevertheless, when measuring the time spent by a specific water molecule near the trimethylglycine molecule (via measurements of the Huggins constant, the apparent specific volume and the hydration number), this happens to be very short. In other words, water molecules are attracted by the dipole, but they move away from it very quickly. This is probably due to the oscillatory variable space between the anion and the bulky cation. Indeed, the three methyl groups can rotate around the N-C axis and their hydrogen atoms, which support a fraction of positive charge, can approach very near to the COO^- group or occupy intermediate positions. Each water molecule that occupies the space between such groups is so forced to leave it under the effect of steric hindrance. Immediately after, a new water molecule can enter the space that is newly enlarged by the successive movement of the methyl groups.

The definition of 'water carrier' and of 'water fan' for trimethylglycine seems therefore appropriate. Of course, this is true at pH values where the COO^- is completely ionised. In concentrated solutions, more complex phenomena may happen because of extended interactions among different molecules.

Chemical properties

As the buffering capability toward acids and the skin protection ability toward AHA was demonstrated, we tried the use of trimethylglycine in association with Lewis' acids, like aluminium and aluminium/zirconium chlorohydrates, in aqueous solution. The searched property was the capability to buffer such solutions, whose pH that could be detrimental to the skin. pH values of the obtained solutions were monitored in comparison to standard aqueous solutions Moreover, trials were conducted to find out if betaine enabled to increase the solubility level of compounds which are well-known to be hardly dissolved (6). Usually, 50% trimethylglycine solutions were prepared for the tests, but also lower concentration were monitored in case of interesting results. The speed of dissolution of same compounds was also controlled. Test solutions were obtained by the following compounds: aluminium and aluminium zirconium chlorohydrate and poly-chlorohydrates, thioglycolic acid, dodecyl benzene sulphonic acid.
Results were the following:

Aluminum Chlorhydrate

Ingredients	**% w/w**	**pH-value**
Trimethylglycine	44	
Aqua	44	6,4
Aluminium Chlorohydrate	12	

Ingredients	**% w/w**	**pH-value**
Aqua	88	
Aluminium Chlorohydrate	12	4,4

A faster solubility rate of Aluminium Chlorohydrate was noticed when trimethylglycine was previously dissolved in water, when compared to the simple solution of the Chlorohydrate in pure water.

As a special buffering behaviour of trimethylglycine was observed in solution with aluminium chlorohydrate, several solutions at different concentrations of trimethylglycine were prepared (buf) in order to study the concentration/pH relationship. Aluminium Chlorohydrate ($Al_2(OH)_5Cl \cdot 2\text{-}3H_2O$) was dissolved in trimethylglycine solutions and the pH values were measured.

The test formula was the following

Ingredients	**% w/w**
Aqua	To 100
Betaine	Variable
Aluminium Chlorohydrate	12

Trimethylglycine amount	**PH**
0%	4.4
5 %	4.6
10%	4.8
20%	5.3
30%	5.7
40%	6.2

The higher the percentage of Trimethyglycine, the higher the solubility rate of aluminium chlorohydrate as well as the pH-value of solutions. This finding is of remarkable importance and can be exploited in the formulation of skin friendly deodorants and antiperspirants. As a matter of fact, the addition of trimethyglycine to aqueous solutions, while allowing the quick solubility of aluminium chlorohydrate, gave rise to an interesting buffering effect, as the pH-value was brought back to physiological standard. Aluminium/zirconium chlorohydrates showed similar behaviours.

Thioglycolic acid

Ingredients	**% w/w**	**pH value**
Betaine	40	
Aqua	42	3,7
Thioglycolic Acid 80%	18	
Ingredients	**% w/w**	**pH value**
Aqua	82	1,5
Thioglycolic Acid 80%	18	

It is very interesting to note that the solvent power of trimethylglycine helps reducing the off-odour of thiglycolic acid. The moisturising and protective effect of trimethylglycine for the skin could be of help for some practical cosmetic application of this innovative thioglycolate salt.

Dodecylbenzensulphonic acid

Ingredients	**% w/w**	**pH value**
Betaine	45	
Aqua	45	4,4
Dodecylbenzene sulfonic acid	10	
Ingredients	**% p/p**	**pH value**
Aqua	90	0,6
Dodecylbenzene sulfonic acid	10	

Solution is obtained by heating at 60°C, as dodecylbenzene sulfonic acid is not soluble in the cold. Indeed, this special neutralisation process could lead to unique detergent applications, that are being explored. The buffer effect of betaine toward acidic compounds is once again demonstrated.

Materials and methods

In the following studies concerning hair and skin evaluations, volunteers were conditioned in a bio-climatic room (24°C, 50% rh) for 30', and all measurements were taken at the same conditions.
Measures on the forearm: each volunteer was required not to either cleanse or moisturise the forearms for five hours prior to the beginning of the test. The assessment was performed on the volar area of each forearm, where areas measuring 9 cm^2 were delimited. The areas to be treated were chosen in such a way that right and left arm were alternated among the subjects.
Data were compared by Variance Analysis and Tukey test (Anova-Manova) and t-test for paired data.

The listed instruments were used

a) Corneometer, CM 825 Combi 3, Courage and Khazaka, Köln
b) Cutometer SEM 575, Courage and Khazaka, Köln
c) Tewameter TM 210, Courage and Khazaka, Köln
d) Image analysis (SILFLO Flexico Ltd, United Kingdom, program Quantilines Monaderm, videocamera High Performance CDD, COHU)
e) Chroma meter CR-300 Minolta

In the dental study, twenty healthy non-smoking subjects (mean age 30,5 years) were recruited. The study was a crossover, double-blind. A paper disc impregnated with 300 µl of the test solution was put in a 18-mm Finn Chamber which was applied to the buccal mucosa for 15 min with a special made plastic clip. Electrical impedence was measured before and up to 45 min after the 15 min exposures with the test solutions.
The instrument used in this case was an experimental multi-frequency electrical impedance spectrometer developed at the institute of Dentistry, University of Turku, Finland.

Bioengineering measurements
Hair studies

1) Water retention capability on the hair
The aim of this study was to evaluate the water retention capability on the hair of trimethylglycine, 4% aq, in comparison with glycerine solution at the same concentration. The test was carried out on 10 tresses of Italian virgin hair, grouped in two groups of five each. Each group was treated with the test solution or with the reference.
Preliminary treatment
The dry tresses are immersed for 10' in deionized water, then padded with blotting paper. Then, they are left to equilibrate for 10' in the air.
Tresses are then weighed ten times, at time intervals of 5' and the weights recorded.
Treatment
Tresses are treated 6 times as follows: immersion in the test solutions for 10 minutes, then 3 minute rinsing with deionized water, then padding with blotting paper and equilibration for 10' at the air.
Each tress is then weighed 10 times at constant time intervals of 5'.
Results
The basal value and values measured at the different control times were statistically compared by means of the Analysis of Variance and the Tukey test. The groups of data were considered significantly different for a probability value of $p<0.05$. Even if results were not statistically different for all groups of data, when measuring the area under the curve (total effect) of the plots of water content at increasing times, the results (7) show that the average increase of water retention into the hair treated with trimethylglycine is 40%, while the same value for glycerine is 6% .

2) Hair strengthening and conditioning
A series of studies have been carried out in order to detect the hair strengthening capability and conditioning power of solutions containing trimethylglycine (8). Both caucasian and oriental hair were used in the test. The most interesting results were obtained with a group of three tresses made of oriental hair. They were bleached together, twice in succession, in order to provide damaged samples for the study, then washed with 15% active solution of sodium laurethsulphate and left dry naturally. A 5% aqueous solution of trimethylglycine was tested, against water as a placebo control.
Tresses were wetted and 1g of SLES solution wash massaged for 60", then rinsed and massaged for 30" with the betaine solution, which was left on the hair for 15', then rinsed for 60". Tresses were combed, then left to dry naturally.
The above cycle was repeated for a total of five cycles. The placebo tresses were submitted to the same treatment, using pure water instead of betaine solution. 50 hairs were taken randomly from the tresses and measured. The results were the following.

Oriental hair

Tress	Elastic Modulus (Mpa)	Elastic Gradient (gmf/mm)	Plateau Stress (gmf/sq micron)	Stress @ 15% (gmf/sq micron)	Stress @ 25% (gmf/sq micron	Break stress (gmf/sq micron
Treated with 5% betaine solution	4,660	58.2	1.09E-002	1.46E-002	1.46E-002	2.86E-002
Treated with water placebo	2,420	31.7	1.01E-002	1.05E-002	1.33E-002	2.70E-002
Base tress (untreated)	3,020	40.0	1.05E-002	1.08E-002	1.36E-002	2.72E-002

Comparing the tress treated with trimethylglycine solution to that treated with the water placebo, four parameters show a significant difference, at $p<0.05$. These are elastic modulus, elastic gradient, stress at 15% and stress at 25%. For all these parameters, values for the trimethylglycine treated hair are higher, indicating that trimethylglycine has helped to strengthen the hair in both the elastic and yield region.

Skin studies

1) Repeated arm wash test
Preliminary trials on a reduced number of subjects (four) show that trimethyl-glycine can reduce by 40% the TEWL increase in the subjects treated respectively with shower bath formula and the same containing 4% trimethylglycine, after 18 repeated washing of the forearm skin. Skin redness increase was also reduced by 30% (9).

2) Corneometry and image analysis
In another indicative trial with reduced number (five) of subjects, a glycolic acid 14.2% solution containing trimethylglycine was compared to a glycolic acid solution at the same concentration, buffered at the same pH (3.9) with sodium hydroxide (10). Volunteers used the products on their face twice a day for 45 days. Trimethylglycine containg solution increased significantly the skin moisturization level by 6.9%.In a previous test (11), glycerine at 4% aq was not significantly different from trimethylglycine 4% aq in increasing the water content of the skin, measured by Corneometry, when six volunteers used the two solutions for 30 days on their forearms. The same happened when the same test was carried out on the face by additional six volunteers, applying the solutions on the crow-feet area for 45 days, twice a day. In this case, a significant reduction (6.1%) of the mean basal value of macrorugosity (mean of greys) was measured by image analysis (12).

Dental measurements

In this study (13), the aim was to evaluate whether trimethylglycine has any protective effect in short term exposure of the human oral mucosa in vivo with two different irritating detergents, sodium lauryl sulphate (SLS) and cooamidopropylbetaine.

Distilled water showed no effect on oral mucosa in 15 min exposure as measured by electrical impedence. Both 0.5 and 1% SLS solutions showed a significant reduction of three of the four parameters after 15 min exposure ($p<0.05$), 2% SLS showed a reduction of all four indices ($p<0.001$). Trimethylglycine, at 4% had no effect on detergent induced irritation with the 2% or 0.5% SLS solution, while it reduced significantly ($p<0.05$) three of the four parameters when blended with 1% SLS solution.

Conclusions

New application and evaluation studies conducted on trimethylglycine allow to open new fields for its cosmetic used. Trimethylglycine exhibits a number of useful characteristics for cosmetic applications: buffering capability, anti-irritant properties with surfactants, uncommon solubilising effect, special skin feel and protection of water content in the skin. Its use in cosmetics will be offering additional instruments to innovative formulations.

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