Benefits of Trimethylglycine (Betaine)

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Trimethylglycine (betaine) is a very common molecule in nature. It can be found in shellfish (6 to 14 g/kg) and in sugar beets and is a by-product of sugar beet processing, where it reaches a content of 8% in molasses. The pure form, obtained through separation from molasses by industrial chromatography, has been employed in diet supplements in the U.S., in animal feed (0.2 to 15 g/kg) and such industrial applications as anti-corrosive heat exchange liquids.[a] In-depth studies have been carried out in order to understand its unusual behavior in water.

As a zwitterion with a small polar molecule prone to hydrogen bonding, it strongly interacts with water and other similar molecules, thus giving unusual physico-chemical (e.g. solvent power) and sensory properties, such as a silkier feel, to solutions. In biological systems it acts as a methyl donor. Many marine organisms accumulate it when submitted to osmotic stress, as a substitute for absent solutes.[1] Wheat plants increase their resistance to biotrophic fungi by a pre-treatment with betaine.[2] As it is non-toxic (its LD50 is 11.2 ± 0.7 g/Kg), highly water-soluble (up to 55%) and chemically stable, it is a safe, interesting material for skin treatment.

Chemical Structure and Physical Properties

In aqueous solutions betaine forms strong hydrogen bonds (8 to 9 kcal/mole), thus changing the water activity. The COO- group attracts the hydrogen atoms of the surrounding water. Additional water molecules are spatially oriented around betaine under two opposite forces: attraction, by the positive charge that is present on the nitrogen atom, and repulsion, by the methyl groups that are attached to the same atom. In the solid state, betaine crystallizes with one water molecule. In water solution, the intrinsic viscosity and the apparent specific volume are far higher than for its parent compound glycine. Betaine shows a higher amount of static water co-ordination molecules, which move with it under the double influence of hydrophilic and lipophilic interactions.[3] The chemical structure is shown in Figure 1.

The Huggins constant (k'), which is generally interpreted as an interaction factor accounting for the easy exchange of water molecules between the hydration sphere of betaine and the bulk water, is high (k' = 1.156). Also the negative hydration number (h = –0.797) indicates that the statistical average number of water molecules remaining constantly around the betaine molecule is very low.

In other words, both values suggest that one water molecule, when attracted by a betaine molecule, is very rapidly substituted by another water molecule. That is, it has a shorter residence time in the vicinity of betaine than in that of another water molecule.

Betaine is therefore a true water carrier that releases it easily to the surrounding environment when required by changing physical conditions. The water structure in solutions of betaine, at concentrations from 1% to 5%, studied by laser-Raman spectroscopy,[3] proves that a shift occurs from the quasi-crystalline and solid amorphous states of water (highly organized) to the amorphous liquid and the liquid states (less organized). This is shown by the strength and the changing number of hydrogen bonds. This also proves that increasing

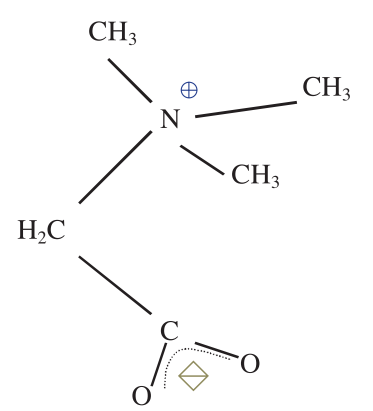

Figure 1. Chemical structure of betaine

[a]Fortum patent (FI960971 1.3.1996 – Pat. FI199260 – Grant date 26/1/1998- US pending US142093 – file date 2/10/1998. Additional Countries: CA,CN,EP,RU,JP,WO)

Key words

Trimethylglycine, water coordination, moisturization, pH buffer, lenitive effect, cosmetic formulation

Abstract

Commonly found in nature, trimethylglycine lends itself to many useful applications in personal-care products from foam boosting to skin moisturization.

Trimethylglycin kommt in der Natur vor und es ist vielfaeltig nutzbar in Produkten der persoenlichen Pflege, von Schaumsteigerung zu Hautbefeuchtung.

Communément trouvé dans la nature, le triméthylglycine se prête à beaucoup d'applications utiles dans les produits pour les soins personnels, de l'augmentation de la mousse à l'humidification de la peau.

La trimetilglicina, que se encuentra comúnmente en diversos productos naturales, presenta varias aplicaciones útiles en la formulación de productos de cosmética y tocador, como por ejemplo, aumento de la formación de espuma y humectación de la piel.

concentrations of betaine decrease the ordered organization of the water molecules surrounding betaine molecules. The implication of these behaviors when determining the optimum use concentrations of betaine as w/o emulsions stabilizer will be described later.

Furthermore, betaine does not immobilize water molecules as many humectant polyols (like glycerol) do. This allows the water for the living cells to be completely available. This effect is certainly positive for the water equilibrium of oral mucous cells and provides the theoretical reasons for the employment of betaine in oral-care products.[4]

Differential Scanning Calorimetry shows a water-betaine eutectic at –22°C at 15% concentration. The easy movement of water molecules, which is very unusual for a concentrated salt solution, looks therefore to be maintained by the presence of betaine, and explains the in vivo osmo-protectant capability of betaine even at low temperatures. Indeed the 50% aqueous solution freezes at –32°C.

Surface Tension

Studies on surface tension do not show any significant change in water surface tension (13% decrease at 7% concentration). Therefore, betaine cannot be defined as a surfactant agent. The wetting angle, which signals the affinity of a drop of betaine aqueous solution for a hydrophobic support, is also increased, thus suggesting an increase in water cohesiveness. It could be surmised that the intramolecular arrangement is ruled by the internal attraction between the cationic and anionic group. This attraction creates a preferential “bean-like” structure (Figure 2) where the two ions of the dipole face each other, while the methylenic and methyl groups are located on the external surface.

Continuous water molecule exchange: In dilute solution, one water molecule could temporarily occupy the space between the two ions while the other water molecules are orientated by the attraction of the dipole. The mobility of the three methyl groups around the C-N axis and their preferential but mobile arrangements due to the steric hindrance, explain why water molecules are quickly released from the hydration sphere near betaine to the bulk of the solution. This is just similar to what a fan makes with the the surrounding air molecules. In concentrated solutions, the intermolecular bonds among several betaine molecules became predominant and other co-ordination mechanisms could take place.

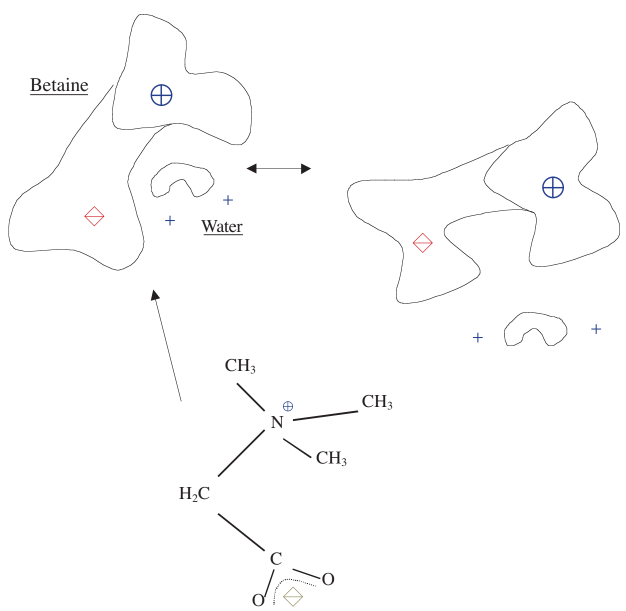

Figure 2. The intramolecular arrangement of betaine, a “bean-like” structure and a “fan” effect. Even if betaine is only slightly acidic in aqueous solution (pH 5.8 at 1%, 6.2 at 10%, 6.3 for 1M solution), betaine is able to buffer acid solutions.

Buffering Behavior

Even if betaine is (only slightly) acidic in aqueous solution (pH 5.8 at 1%, 6.2 at 10%, 6.3 for 1M solution), betaine is able to buffer acid solutions.

The plot of pH against ml of added acid or alkali is reported in Figures 2 and 3, respectively. It is easy to understand why alkaline solutions are not similarly compensated, since the carboxyl anion, an ion of a weak acid, is the only part of the molecule that can modify its structure when pH decreases. The quaternized nitrogen cation stays completely dissociated.

It is interesting to note that the 1.8 pH value of a 10% solution of glycolic acid is increased to 3.0 by the addition of 17% betaine, as described in the following:

	Betaine Concentration	pH
Glycolic Acid 10%	0	1.8
+ Betaine	2%	2.2
	5%	2.5
	10%	2.8
	17%	3.0

This property has been exploited in the formulation of milder AHA solutions. However, betaine does not increase the conductivity of water significantly, thus showing no influence on water dissociation constants (Table 1) and an intramolecular electric compensation, at least in dilute solutions.

As noted above, an electrical compensation of the dipole inside the molecule probably takes place instead of intermolecular bonds. In hydrated betaine, the C-C-O angle is modified and a lower energy is required to bend it, as shown by related IR spectra of anhydrous and monohydrate betaine.[3] This could mean that the attraction force applied to the positive charge by the carboxyl anion is decreased by the water binding.

Concentrated Aqueous Solution

High concentrations of betaine in water lead to a new super-solvent with a high dielectric constant. A fifty percent solution dissolves up to

2% allantoin easily at room temperature, four times its maximum water solubility. This property is useful to prepare master solutions of allantoin without heating high amounts of water, and provides the additional humectant effect of betaine to final products (at 0.15% final allantoin content, betaine concentration will be around 3.7%, which gives superior skin feel). Salicylic acid is also cold-soluble in such concentrated solutions up to 5%.

This value is remarkable if compared with its standard water solubility, which is about 0.2%, thus reaching a 25-fold increase. We used this property and the resultant stability of such solutions to prepare mixed AHA and BHA transparent formulations (Formula 1). Surprisingly, 50% betaine water solution was found to be less irritating to the skin than pure water.[5]

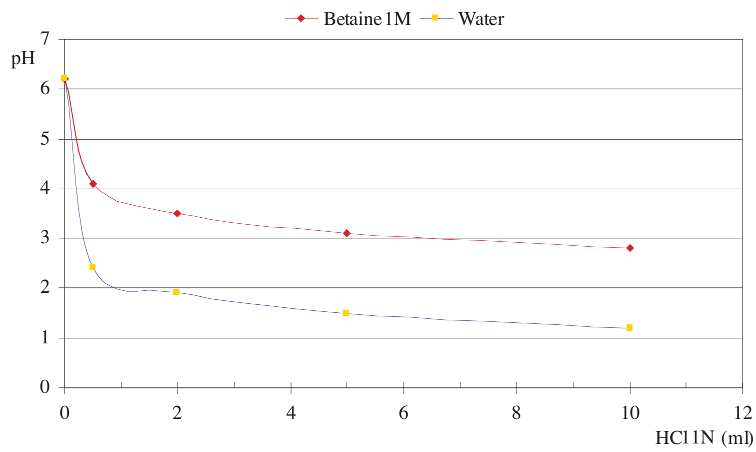

Figure 3. pH values after addition of HCl 1N to 100 mls of 1M betaine aqueous solution

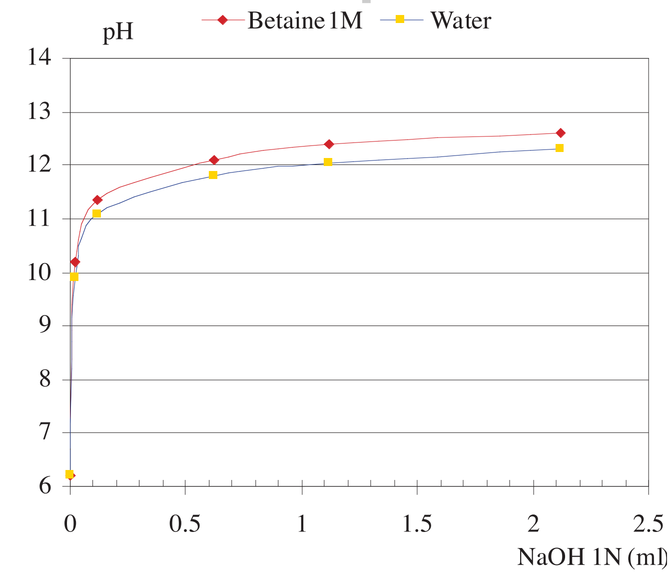

Figure 4. pH changes after the addition of NaOH (1N) to 100 mls of 1M betaine aqueous solution

Swelling of Hydrophilic Polymers

Betaine increases the swelling speed of some hydrophilic thickeners and enhances their thickening power by 20% on average. Figures for carboxymethylcellulose and neutralized carbomer are reported in Figures 5 and 6.

Influence on Foam and Irritation Potential of Surfactants

Betaine as an anti-irritant: Blends containing betaine at 2% to10% and various surfactants at 15% of active substance were prepared and the foam formation and drainage after 5 min were measured (Table 2). The foam volume was increased only in blends with sodium laureth sulfate, cocamido-propylbetaine and sodium cocoamphoacetate, while other surfactants (sulphosuccinates, carboxylates, olefin sulfonates and non-ionic surfactants) were either negatively affected to a low extent or not at all affected. The viscosities of solutions with such surfactants were not significantly modified, thus proving that a stabilizing effect or an orientation of betaine molecules occurs only at the water/air interphase.

The addition of betaine (3.5%) to mixed surfactant solutions is known to reduce damage to red blood cells (Figure 7) and to decrease the zein number, which is indicative of a reduced aggressive power toward proteins (Figure 8).

The surfactants have an effect on skin cells (membranes and proteins) and may even damage them. In the RBC test bovine blood is added to solutions of surfactants and their effect on the blood is quantified. Pape[10] and coworkers were able to observe via UV spectroscopy hemolysis and denaturation of hemoglobin. The ratio of Hemolysis (L) to Denaturation (D) is the L/D value. This value reflects the dermatological compatibility of the tested material. A high number indicates good dermatological compatibility: >100 non irritant, >10 slightly irritant, >1 moderately irritant, >0.3 irritant, <0.3 very irritant.

Zein is a protein derived from corn and is fairly insoluble in water. Gotte[11] and coworkers found that a surfactant solution causes a part of zein to be dissolved. They even noticed a correlation between irritancy potential of a surfactant and its ability to dissolve zein. From

Table 1. Conductivity of betaine solutions at 20°C, compared to distilled water, a standard hydrochloric acid solution and a sodium chloride solution

Material	Conductivity in distilled water (microSiemens)
Betaine, 0.08 M	90
Betaine, 0.4 M	44
Betaine, 0.8 M	54
Sodium chloride, 0.01 N	7.800
Hydrochloric acid, 0.01 N	3.300
Distilled water used for the experiment	14

Table 2. Influence of betaine on foam volume and stability when blended at various concentrations with selected surfactants at 15%

Surfactant (at 15%)	Betaine (%)	Viscosity (mPas)	Foam, initial (cm^3)	Foam, after 5 min (cm^3)
Sodium laureth sulfate	0	20	64	52
	2	21	67	43
	10	25	80	69
Polysorbate-20	0	12	40	16
	2	16	44	21
	10	20	48	16
PEG-8	0	14	8	0
	2	14	6	0
	10	14	0	0
Sodium C_{14-16} olefin sulfonate	0	22	48	40
	2	21	48	40
	10	22	48	40
Disodium laureth sulfosuccinate	0	20	48	40
	2	21	48	32
	10	30	40	32
Sodium cocoamphoacetate	0	16	44	36
	2	19	56	44
	10	21	60	48
Laureth-5 carboxylic acid	0	12	35	28
	2	12	35	30
	10	12	32	28
Cocamidopropyl betaine	0	15	56	48
	2	13	68	56
	10	21	60	44

Formula 1. Transparent AHA Solution

Betaine	50.00%
Water (aqua)	25.00
Urea	15.00
Salicylic acid	5.00
Glycolic acid	5.00

Comment: Viscosity (RVT Brookfield) = 50 mPas; pH = 5.4
Stable, transparent solution, no precipitate at room temperature and at 4°C

Formula 2. Toothpaste for Sensitive Teeth

Water (aqua)	q.s 100.00%
Glycerin/sorbitol	15.0-25.0
Hydrated silica	14.0-18.0
Sodium bicarbonate	10.0-16.0
Polyglycol (various M.W)	3.0-6.0
Betaine	4.0-6.0
Sodium lauryl sulfate	1-2
Fragrance (*parfum*)	qs
Silica	0.5-1.5
Sodium monofluorophosphate	qs
Polymeric resin	qs
Sodium saccharin	qs
Preservative and bactericide	qs

UV measurement, a value is gained (unit mg N/100ml) and a following classification is attained: 0-200 units, non-irritant; 200-400 units, slightly irritant; >400 units, irritant.

Furthermore, a progressive decrease of SLES-induced skin irritation is detected by human patch test[5] when the amount of betaine is increased (Table 3). The aforementioned capabilities to protect the mucous membranes are achieved by adding betaine to SLS in toothpaste and mouthwash (Formula 2). A patent[6] was granted on the basis of the anti-irritant properties of betaine blended with many surfactants and in other cosmetic applications.

Shampoo and Conditioner Formulations

Wet detangling properties can be achieved in conditioning shampoo formulations (Formula 3). The foam-increasing properties of betaine, which are found in blends with amphoterics and SLES, are exploited to formulate a delicate, easy-rinse baby shampoo, containing a low amount of surfactants and no traditional lather boosters. A controlled study was carried out with the half-head technique of shampoo formulations both with 2% betaine and without it. The silky feel and the final conditioning effect of the betaine-based product were significantly improved.[7]

Transparent hair conditioners are the result of a balanced blend between traditional cationics and betaine. Also in this case, the hair feel and wet/dry combing ease were improved (Formula 4). A patent[8] was granted to Wella for the hair-improving properties of betaine in cosmetic formulations.

Skin Tonics

The high humectancy and the skin relief properties of betaine are also very useful in cosmetics. The best moisturizing effect (in terms of TEWL recovery) has been measured in tape-stripped skin. This has introduced practical applications for an after-AHA-treatment moisturizing formula (Formula 5).

In vivo measurements[9] of the efficacy of 4% betaine aqueous solutions in increasing corneometric values and in decreasing macrorugosity (wrinkles depth) of face skin showed significant results, not statistically different from 4% glycerine aqueous solutions. No significant changes of skin barrier (TEWL) and elasticity have been detected on volunteers' forearms, when tested on non-stripped skin.

Toothpaste

Betaine has been employed at 4% in toothpaste and tested in a clinical study against a placebo on volunteers suffering from chronic dry mouth syndrome. While all the clinical parameters and oral bacterial charge remained unchanged, subjective perception and objective symptoms of dry mouth showed significant improvements. Indeed, because betaine can have protective qualities for living cells, it has no bactericidal effect, as the minimal inhibitory concentration against several microorganisms is above 10%. However, its skin relief properties on skin deprived of stratum corneum, its mobile water coordination activity and its reduction of SLS-induced irritation make such results understandable.

On the other hand, sugars such as sorbitol, which are frequently used in toothpaste,[4] bind water strongly and do not release it easily. An example of such application is reported (Formula 2).

O/W Emulsions

Betaine, at low concentrations (1-4%) in o/w creams, is able to combine its humectant properties and water coordination capability without the residual skin stickiness often associated with using other hydrotropes like glycerin. Moreover, the presence of betaine commonly results in residual skin silkiness and softness perception, even at low concentrations (Formula 6, 7). High concentrations of betaine do not show destabilizing of the emulsion at any temperature, while the skin feel perception is characterized by long-lasting humectancy without tackiness. A balanced blend, at a total concentration around 4%-5%, of betaine and either sorbitol or propylene glycol or glycerine may result in a whole range of skin perceptions (from velvety to soft feel), drying time and emolliency. The spreading ease of emulsions over the skin is slightly reduced in comparison with glycerine-based creams, but softness and emolliency are perceptibly improved.

Emulsions used at very low temperatures could benefit from such a useful property.

W/O Emulsions

Betaine can replace magnesium sulphate and sodium chloride in the cold stabilization of w/o emulsions (Formula 8). This formulation is not stable at a high temperature (45°C), probably for the high mobility of water in the interphase. The addition of the usual small amounts of magnesium stearate or pyrogenic silica provides the hot stabilization of such formulations. In general, the amount of emulsifier can also be reduced when using betaine as a stabilizer. The amount of betaine is critical for each tested emulsifier. The best stabilization is achieved in the range from 1 to 1.5% for polyglycerol derivatives, and about 2% for silicone emulsions.

An additional advantage is the disappearance of a desiccating effect and a drying sensation created by magnesium salts in contact with the skin. On the contrary, the aqueous phase gives the typical silky perception of betaine when spread over the skin.

A special case of w/o stabilization properties is described in Formula 9, where a w/o emulsion lipstick was stabilized by the addition of betaine, that also keeps the water content constant during the high temperature phases of lipstick manufacture.

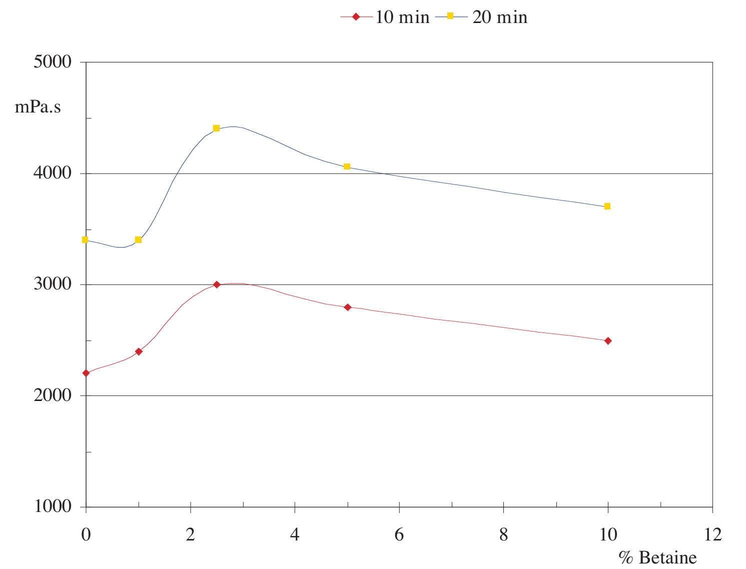

Figure 5. Viscosity values during the swelling phase of 1% water and water-betaine dispersions of sodium carboxymethylcellulose. (Brookfield RVT Spindle #3, 5 rpm, 40°C). Measurements taken 10 min and 20 min after dispersion of powder in the solutions. Most effective betaine concentrations are between 2 and 4%.

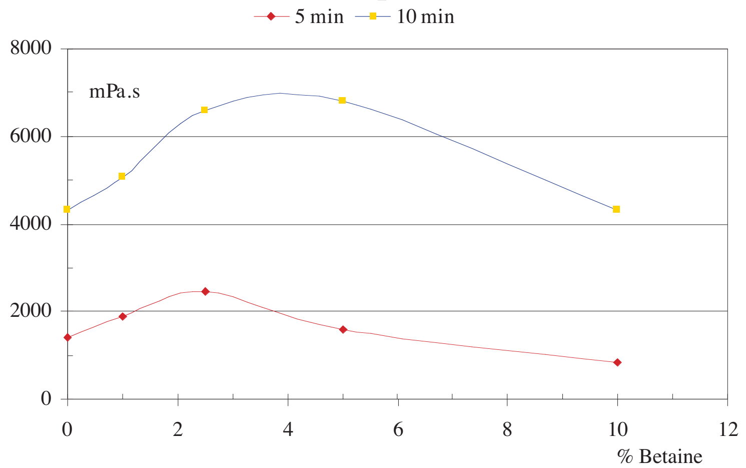

Figure 6. Viscosity values during the swelling phase of water and water-betaine dispersions of 0.14% sodium carbomer. (Brookfield RVT Spindle #3, 5 rpm, 20°C). Measurements taken 5 min and 10 min after dispersion of the powder in the liquids. Most efficient betaine concentrations are between 2.5 and 6%.

Conclusion

Through structural studies conducted on known molecules from nature, new trends in cosmetic applications are now becoming available. Betaine is an example of such a trend and exhibits a wide number of interesting properties: buffering capability, bio-compatibility, water coordination and orientation, anti-irritation properties with surfactants, antiwrinkle effect, special skin feel and an uncommon solubilizing effect. Its use in cosmetics offers formulators exceptional properties for creating innovative formulations.

Acknowledgements: This paper has been written with the support of Finfeeds Finland Ltd. and the scientific help of Professor Mohamed

Table 3. SLES-induced skin irritation scores from mixed betaine-SLES solutions patch tested in humans

% Betaine in SLES	Score at 24 hours	Score at 48 hours
0	4.6	1.9
2.0	2.9	1.4
3.5	2.7	1.3
5.0	2.2	1.1
7.0	0.8	0.5

Mathlouthi from Reims University. I thank Mrs. Kirsti Jutila of Finfeeds Finland Ltd. for her valuable help.

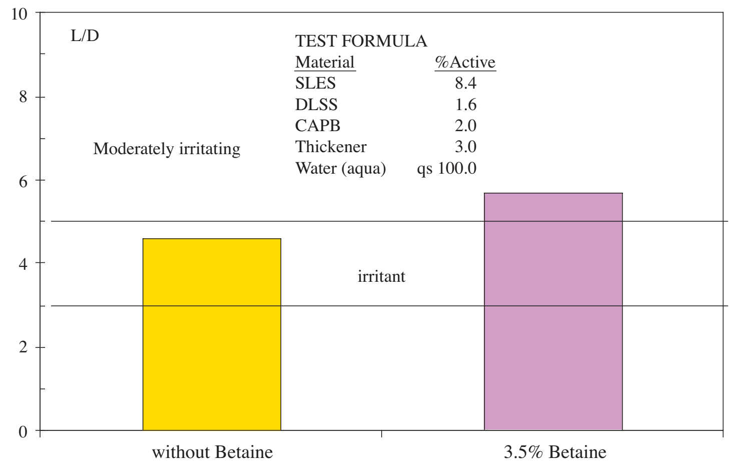

Figure 7. Red Blood Cell test result of a mixed surfactants solution, without and with 3.5% betaine
The ratio of Hemolysis (L) to Denaturation (D) is the L/D value

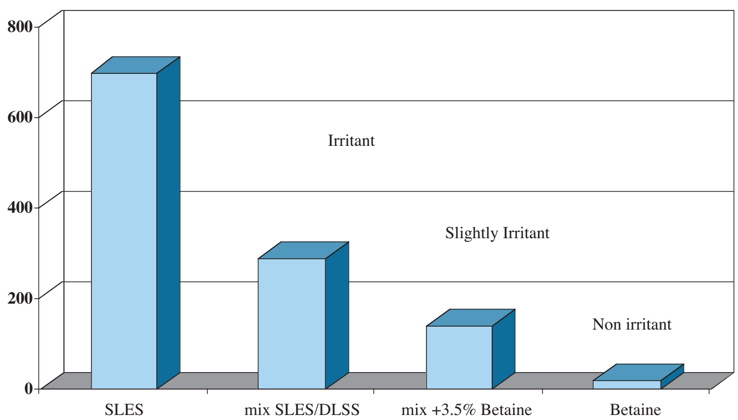

Figure 8. ZEIN test of SLES alone, blended with disodium laurylsulphosuccinate (DLSS), blend of SLES, DLSS and betaine, and of betaine alone
Label Unit = mg N/100ml

Formula 3. Conditioning Shampoo

Water (aqua)	78.14%
Betaine	5.00
Sodium laureth sulfate	9.45
Cocamide DEA	3.00
PEG-7 glyceryl cocoate	1.00
Cocamidopropyl betaine	2.10
Quaternium-15	0.20
Chamomilla recutita extract	1.00
Ceramide II	0.02
Lactic acid	0.09
Preservative	qs

Comment: Viscosity (RVT Brookfield) = 1,100 mPas; pH = 6.7

Formula 4. Hair Conditioner

Water (aqua)	93.30%
Polyquaternium-10	0.70
Betaine	5.00
Hydroxyethylcellulose	0.80
Diazolidinyl urea	0.20
Lactic acid	qs

Comment: Viscosity (RVT Brookfield) 5.0 rpm = 11,000 mPas ; pH = 4.5

[redacted]

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Formula 5. Tonic Lotion

Water (aqua)	qs 100.00%
Glycerin	2.50
Betaine	2.50
Panthenol	0.20
Allantoin	0.05
Disodium EDTA	0.10
PVP	0.20
Hyaluronic acid	0.05
Citrus aurantium dulcis flower water	5.50
Citrus grandis juice/bisabolol	1.00
Helichrysum italicum extract	0.50
Ethoxydiglycol	1.00
Olea europaea leaf extract	0.05
Preservatives	qs
L-arginine	0.16

Formula 6. O/W Hand Cream

Water (aqua)	qs 100.00%
Steareth-2	3.00
Steareth-21	2.00
Isopropyl myristate	4.00
Prunus dulcis oil	2.00
Caprylic/capric triglyceride	3.00
Cyclomethicone	4.00
Preservatives	qs
Sodium carbomer	0.15
Glycerin	2.50
Betaine	2.50
Fragrance (*parfum*)	qs

Comment: Viscosity RVT Brookfield (Helipath C) = 22,000 mPas; pH = 7.2

Formula 7. O/W Cleansing Milk

Water (aqua)	qs 100.00%
Steareth-2	2.50
Steareth-21	1.50
Dimethicone	15.00
Olea europaea fruit oil	2.50
Hydrogenated soybean oil	2.50
Triclosan	0.20
Phenoxyethanol	0.70
Tocopheryl acetate	0.10
Glycerin	2.00
Betaine	2.00
Allantoin	0.10
Disodium EDTA	0.10
Xanthan gum	0.40
Citrus grandis juice/bisabolol	3.00
Olea europaea leaf extract	0.10
Citrus aurantium dulcis flower water	2.00
Preservatives	qs
Sodium hydroxide	qs

Comment: Viscosity RVT Brookfield (Helipath C) = 14,000 mPas; pH = 5.9

References

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Formula 8. W/O Day Emulsion

Water (aqua)	qs 100.00%
Sorbitan sesquioleate	4.00
Polysorbate 80	1.00
Cera microcristallina	1.00
Cera alba	2.00
Limnanthes alba seed oil	2.14
Butyrospermum parkii	0.16
Polydecene	30.00
Magnesium stearate	0.50
Silica dimethyl silylate	0.50
Glycerin	2.00
Betaine	2.00
Preservatives	qs

Comment: Viscosity RVT Brookfield (Helipath C) =112,000 mPas; pH (10%) = 6.8

Formula 9. Emulsion Lipstick

A. Ricinus communis seed oil	31.70%
Octadecanol	10.50
Glyceryl hydrogenated rosinate	7.50
Cera alba	5.50
Candelilla cera	5.40
Octadecyl ricinoleate	5.00
Ozokerite	4.60
Caprylic/capric trigylceride	4.20
Carnauba	3.50
Lanolin alcohol	2.40
Mineral oil	2.40
Cetyl alcohol	1.50
Quaternium-18 hectorite	1.20
Titanium dioxide, talc, color	13.00
Isopropyl lanolate	1.00
Propylene carbonate	0.30
Preservative, antioxidant	0.30
B. Water (aqua)	50.00
Betaine	5.00
Sericin	5.00
Sorbitan isostearate	15.00
PEG-2 hydrogenated castor oil	15.00
Ozokerite	5.00
Hydrogenated castor oil	5.00

Procedure: Combine in the proportion of 80% w/w A (base color) and 20% w/w B (water-based emulsion).

1. DE Robertson, D Noll, MF Roberts, JA Menaia and DR Boone, Detection of the osmoregulator betaine in methanogens, *Appl Environ Microbiol* 56(2) 563-565 (1990)
2. K Manninger, M Csosz and E Tyihak, Induction of resistance of wheat plants to pathogens by pretreatment with N-methylated substances, *Acta Biol Hung* 49(2-4) 275-280 (1998)
3. M Mathlouthi, Physicochemical Properties of Betaine-Water Solutions and Their Possible Applications to Cosmetic Formulations, final report of contract with Finnsugar Bioproducts, University of Reims (1997)
4. E Söderling, A Le Bell, V Kirstilä and J Tenovuo, Betaine-containing toothpaste relieves subjective symptoms of dry mouth, *Acta Odontol Scand* 56 (1998)
5. Toxicol Laboratories Ltd, Test Ref V3-V10-8811 (1998)
6. EP 0531387 B1, KM Jutila (1996)
7. Hazel Pool Associates, Study report DE248 AA (1998)
8. EP 0056595 B1, 1984 E Konrad, H Mager and D Hoch (1984)
9. ISPE srl, Study report 15/00/00 (2000)
10. Pape, Hoppe, *Drug Res* 40(l), nr.4 (1990), 498
11. Gotte, Ernst, *Chem Phys Appl*. Surface Act Subst Proc Int Cong 4 (1964) 83-90

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