# CM-Glucan a new yeast polysaccharide for cosmetic use

http://www.mib-bio.com

*Dr. Fred Zülli and Christine Saecker, Mibelle AG Cosmetics, Bolimattstr. 1, 5033 Buchs, Switzerland*

Polysaccharides have been used for years as thickening, stabilizing and co-emulsifying ingredients in personal care products since they are easy to formulate, very stable and highly tolerable. In addition many polysaccharides are active substances (Aloe Vera; Hyaluronic Acid). These bioactive agents have become increasingly important in all kind of products. The major activity of these polysaccharides is the improvement of skin moisture.

The objective of this paper is to present a new polysaccharide with additional beneficial properties for skin care preparations. The natural polymer is derived from yeast cell walls and has well documented immuno-stimulatory properties.

## History of yeast products

For centuries, crude preparations from yeast and living yeast cells have been used for both cosmetic and pharmaceutical purposes.

In folk medicine, these products have been found useful in curing many diseases such as tuberculosis, diarrhoea and diabetes.

Yeast preparations have also been used to treat skin conditions of all types such as Impetigo and Acne[1].

In 1941, precise investigations of yeast components led to the discovery of the first defined pharmaceutical yeast product, Zymosan[2]. Further studies have shown that water insoluble Zymosan has immune stimulatory activity.

This product is a raw cell wall extract composed of 50% glucan, other polysaccharides, proteins and lipids.

## Research on glucan

During the last decade, glucan from yeast cell wall preparations has been identified and proved as the immunologically effective part of Zymosan which stimulates macrophage-mediated phagocytotic defence mechanisms[3].

Glucan is a (1-->3)-ß-linked polyglucose of high molecular weight and belongs to the class of drugs known as biological response modifiers (BRMs).

Yeast derived glucan enhance the defence mechanism against bacterial infections[4] and malignant tumours[5].

In other investigations, yeast glucan has also been shown to accelerate wound healing with advanced reepithelization and a reduction in the number of inflammatory cells[6,7]. Modifications of glucan making it water – soluble have resulted in new, very promising products[8]. According to Williams et al.[9], a soluble glucan derivative (phosphorylated glucan) promotes the healing of a variety of wound types such as decubitus, burns or surgical wounds.

Another soluble glucan is undergoing phase I clinical trials in patients suffering from Acquired Immune Deficiency Syndrome (AIDS)[10]. Preparations of conjugates with chemotherapeutic agents[11] are hoped to enhance the effectiveness and reduce the toxicity of these drugs. This is achieved through a positive influence on their pharmacokinetic properties.

## Development of a product for cosmetic use

Medical yeast cells and preparations of them are known to exhibit no toxic or allergic effects when applied to the skin[1].

Thus, the isolation of interesting components from yeast will most probably result in products which are very tolerable for topical application. The preparation of glucan, a well documented component from baker's yeast (*Saccharomyces cerevisiae*) is a straightforward approach to the development of promising products for cosmetic or dermatological purposes.

Despite its polar nature, glucan isolated from cells of baker's yeast is a water-insoluble particulate polymer which is not suitable for personal care products. If this glucan is to become cosmetically applicable, it needs to be converted into a water-soluble and biologically effective form.

In our laboratory, we have developed a process for the isolation of glucan from the cell wall of baker's yeast (Figure 1).

Through derivation of glucan to carboxymethyl glucan, we obtained a water-soluble product. Our new compound is an advanced bioactive yeast product which readily lends itself to a variety of cosmetic applications. The quality of the process ensures the purity of the final product, CM-Glucan.

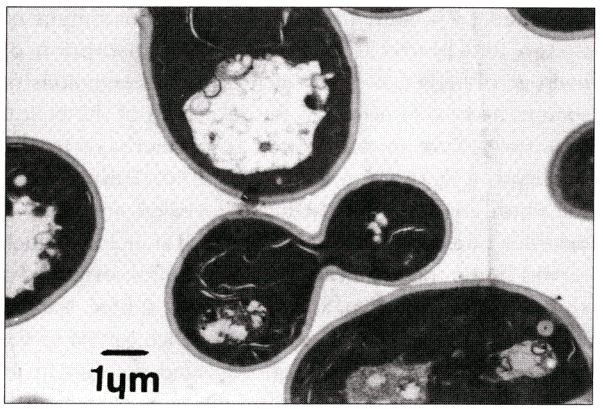


***Figure 1.*** *The cell wall of budding yeast appears as small white border. (Photograph by Dr. Sanglard and Dr. Sengstag, ETH Zürich)*

In its pure form, CM-Glucan is a white powder which is water-soluble in concentrations up to 4%. It is compatible with most cosmetic ingredients as well as the majority of manufacturing conditions: pH values ranging from 2 to 10, high salt concentrations, temperatures as high as 80°C, non-ionic or anionic detergents, and alcohol concentrations up to 40%. In addition, CM-Glucan does not interfere with other active ingredients such as liposomes, vitamins or α-hydroxy acids. Therefore, CM-Glucan can be incorporated directly into most cosmetic products. Solutions of CM-Glucan are slightly viscous and form a thin film on the skin. The smooth sensation on the skin is comparable to that of hyaluronic acid, a frequently used mucopolysaccharide in cosmetic formulations.

Tolerability and efficacy are the two most important parameters in the selection of ingredients for contemporary cosmetic formulations.

The dermatological tolerance of CM-Glucan has been carefully monitored in dermatologically healthy volunteers. The Human Repeated Insult Patch Test[12,13,14] was conducted on 33 panellists using a 2% aqueous solution in order to determine the human irritation, sensitization, photo-irritation and photo-sensitization potential of CM-Glucan. The results have shown that CM-Glucan is neither an irritant/photo-irritant nor a sensitizer/photo-sensitizer and is exceptionally well tolerated.

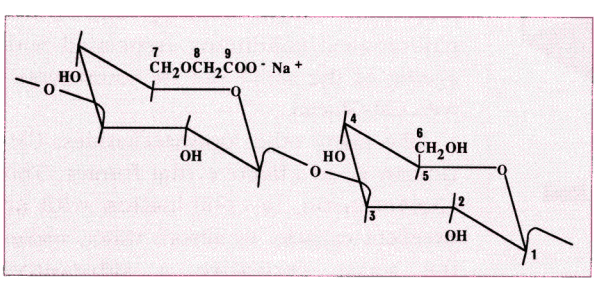


***Figure 2.*** *Structure of CM-Glucan, sodium salt; glucose and carboxymethylglucose units are linked by ß-(1-->3) glycosidic bonds.*

Toxicological tests with glucan and soluble glucan derivatives have shown that systemic administration, over a wide range of doses, does not induce morbidity [8,10,15,16].

Furthermore, we have investigated the cytotoxicity of CM-Glucan on fibroblast cultures in our laboratory. Fibroblasts were grown in serum-free medium with increasing CM-Glucan concentrations up to 0.1%. The examined concentrations did not exert any cytotoxic effects nor did they inhibit growth of fibroblasts.

## Physiochemical Characterization of CM-Glucan

The chemical identity of the structure of CM-Glucan (Figure 2) was analysed using different techniques. The degree of carboxymethyl substitution was determined to be 0.75 by means of a titration/dialysis method. This suggests that on an average, three out of four glucose units are modified to carboxymethyl glucose. A pKa of 4.1 was determined for the acid group.

In order to elucidate the type of subunit linkage of the polymer and to verify the carboxymethylation modification of glucan, samples of CM-Glucan were analysed by $^{13}C$-nuclear magnetic resonance (NMR) spectroscopy (Figure 3). Comparison of signals from CM-Glucan (Tab. 1) with spectroscopic data of other glucans

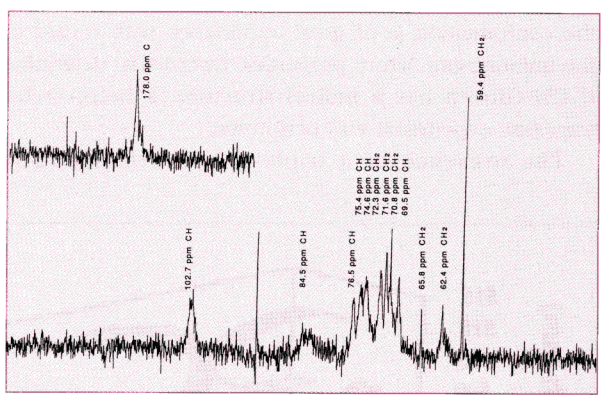


***Figure 3.*** *$^{13}C$-NMR spectrum of CM-Glucan. Samples were dissolved in $D_2O$/DMSO-d6 at 40mg/ml. Spectrum was recorded at 200MHz. Signals arising from $CH_2$ groups were detected by the DEPT technique. (Spectrum by N. Walch, University of Zürich)*

provided by the literature[8,17] confirmed the proposed structure presented in figure 2.

Table 1 compares the chemical shifts in ppm of the carbon atoms in glucan[8] with the signals of CM-Glucan. The numbers of carbon atoms correspond to the numbers in figure 3.

The carboxymethylation of glucan caused the expected additional signals for the atoms C-7, C-8 and C-9.

A small portion of the glucan has single ß-(1-->6) linked glucose units. These branched structures and carboxymethylation at other positions as suggested in figure 3 are responsible for the additional signals in the

**Tab. 1**

| C-atom | Insoluble glucan | CM-Glucan |
|---|---|---|
| C-1 | 103.0 | 102.7 |
| C-2 | 72.8 | 74.6 |
| C-3 | 86.2 | 84.5 |
| C-4 | 68.4 | 69.5 |
| C-5 | 76.3 | 76.5 |
| C-6 | 60.8 | 59.4 |
| C-7 | — | 70.8 |
| C-8 | — | 71.6 |
| C-9 | — | 178.0 |

spectrum which are not identified in table 1.

The presumed high molecular weight of insoluble glucan was not drastically affected through the isolation and modification procedures required to obtain the CM-Glucan product.

A gel permeation experiment revealed an apparent molecular weight of approximately 100'000 Da for CM-Glucan.

## Active structure of CM-Glucan

Previous reports indicate that the immunological and antitumor activity of certain ß-1,3-D-glucan BRM's is related to the conformation of the polymer[18]. Denaturation of the tertiary structure of the triple-helical BRM Lentinan, decreased its activity. This suggests that the conformation is of great importance with regard to the immunostimulatory properties. In order to determine if CM-Glucan has a helical structure, a helix-->coil transition experiment was performed.

The presence of a triple-helical compound is indicated by a shift in the absorption maximum of Congo Red bound to the substance[8]. Disruption of hydrogen bonds caused by increasing concentrations of sodium hydroxide results in the relaxation of the helical structure. This in turn leads to a decrease in the absorption maximum of Congo Red. Helix-->coil transition analysis of CM-Glucan revealed an order--> disorder transition above 1M NaOH. Laminarin, which served as the triple-helical (positive) control, exhibited a shift in absorption maximum around 0.05M NaOH. Examination of a 60'000 Da Dextran which served as the random coil (negative) control revealed no shift in its absorption maximum and showed the same characteristic as Congo Red without polysaccharide (Fig. 4).

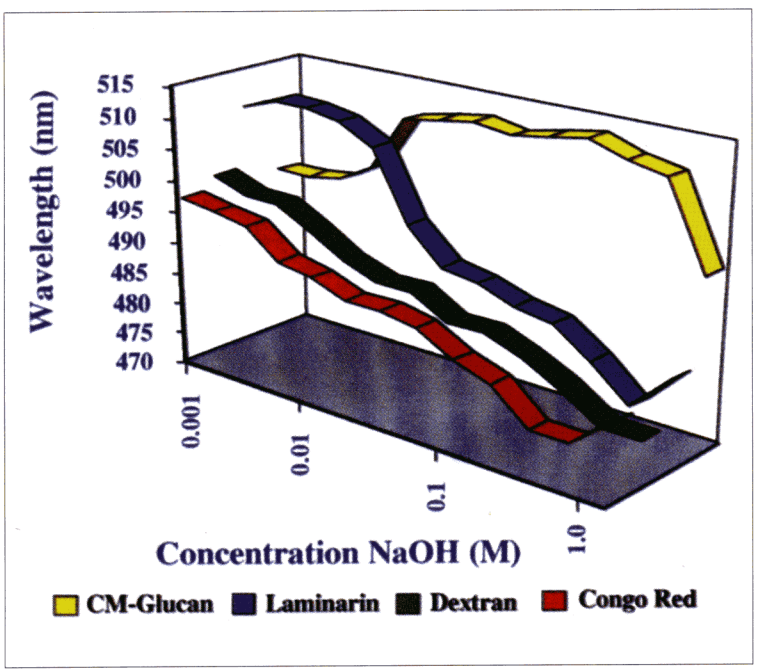


***Figure 4.*** *Changes in the absorption maximum of the Congo Red-CM-Glucan and Congo Red-Laminarin complexes at various concentrations of sodium hydroxide. Dextran and Congo Red serve as negative controls.*

## CM-Glucan's profile in cosmetic formulations

In the last few years, suncare products have gained considerable attention. This can be attributed to a perceived depletion of ozone in the stratosphere which has resulted in an increased concern about premature skin ageing and skin cancer. Today's consumers demand not only products which protect the skin from the sun but also formulations which offer additional skin care.

Evidence has been presented which suggests that CM-Glucan may impart such multiple benefits to sun-stressed skin. For example, UV-light related injuries can be alleviated by CM-Glucan. In addition, the skin's balance can be restored after UVB radiation. Due to its immune stimulatory activity and wound-healing properties, CM-Glucan is an effective ingredient of after-sun products for the treatment of irritated and/or sunburned skin.

Yeast products are traditionally used in the treatment of skin with signs of acne. Now this treatment is continued with the pure bioactive agent in a concentrated form suitable for cosmetic applications. The less severe eruptions of acne can be acceptably treated with relatively simple cosmetic preparations. CM-Glucan can be used in everyday products to treat juvenile acne. Inflammation and infection of the affected skin can be alleviated with CM-Glucan containing products.

Decreased phagocytotic activity of macrophages, increased susceptibility to infections, slow healing and other pathological conditions associated with ageing of the skin[19] can be counteracted with CM-Glucan.

Like many other polysaccharides, CM-Glucan is an effective film former. This characteristic, in combination with an excellent capacity to absorb water, makes the yeast derivative a substantive humectant.

## Conclusion

CM-Glucan is a new potent biological agent with a promising future in cosmetic and dermatological applications. At the same time it offers the continuation of a well known and accepted tradition to use preparations of yeast origin.

CM-Glucan meets the requirements of today's consumers for more "natural" or "holistic" but active compounds. By supporting the body's own defence mechanism, CM-Glucan acts in a natural, non-invasive way. The polysaccharide is prepared from renewable resources and is free of dangerous contaminants.

## References


1. Hänsel, R. Polysaccharide, die immun-stimulierend wirken: Eine Übersicht über entsprechende Fertigarzneimittel. Farmaceutisch Tijdschrift voor Belgie 64, 313-326 (1987)

2. Pillemer, L. and Ecker, E.E. Anticomplementary factor in fresh yeast. J. Biol. Chem. 137, 139-142 (1941)

3. Sherwood, E. R., Williams, D.L. and Di Luzio, N.R. Glucan stimulates production of antitumor cytolytic cytostatic factors by macrophages. J. Biol. Resp. Mod. 6, 358-381 (1986)

4. Kokashis, P.L., Williams, D.L., Cook, J.A. and Di Luzio, N.R. Increased resistance to staphylococcus aureus infection and enhancement in serum lysozyme acticity by glucan. Science 199, 1340-1342 (1978)

5. Di Luzio, N.R. Comparative tumor inhibitory and anti-bacterial activity of soluble and particulate glucan. Int. J. Cancer 24, 773-779 (1979)

6. Leibovich, S.J. and Danon, D. Promotion of wound repair in mice by application of glucan. J. Reticuloendothel Soc. 27, 1-11 (1980)

7. Wolk, M. and Danon, D. Promotion of wound healing by yeast glucan evaluated on single animals. Med. Biol. 63, 73-80 (1985)

8. Williams, D. L., McNamee, R.B., Jones, E.L., Pretus, H.A., Ensley, H.E., Browder, I.W. and Di Luzio, N.R. A method for the solubilization of a (1-->3)-ß-D-glucan isolated from Saccharomyces cerevisiae. Carbohydrate Res. 219, 203-213 (1991)

9. Williams, D.L. and Browder, D. Soluble phosphorylated glucan: methods and compositions for wound healing US-Patent 4,833,131 (1989)

10. Williams, D.L., Sherwood, E.R., Browder, W.I., McNamee, R.B., Jones, E.L. and Di Luzio, N.R. Pre-clinical safety evaluation of soluble glucan. Int. J. Immunopharm. 10, 405-414 (1988)

11. Nagai, K., Tanaka, J., Kiho, T. and Ukai, S. Synthesis and antiumor activities of Mitomycin C (1-3)-ß-D-glucan conjugate. Chem. Pharm. Bull. 40, 986-989 (1992)

12. Kligman, A. M. and Epstein, W. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1, 231-239 (1975)

13. Kaidbey, K.H. and Kligman, A.M. Photo-maximimization test for identiying photoallergic contact sensitizizers. Contact Dermatitis 6, 161-169 (1980)

14. Billhimer, W.L. Phototoxicity and photoallergy. in: Clinical safety and efficacy testing of cosmetics. Waggoner, W.C. (ed.), Marcel Dekker, New York and Basel, 43-74 (1990)

15. Feletti, F., De Bernardi die Valserra, M., Contos, S., Mattabon, P. and Germogli, R. Chronic toxicity study on a new glucan extracted from Candida albicans in rats. Drug Res. 42(II), 1363-1367 (1992)

16. Mansell, P.W.A., Ichinose, H., Reed, R.J., Krementz, E.T., McNamee, R. and Di Luzio, N.R. Macrophage-mediated destruction of human malignant cells in vivo. J. Natl. Canc. Inst. 54, 571-580 (1975)

17. Aouadi, S., Heyraud, A., Seigle-Murandi, F., Steiman, R, and Fournet, B. Structural analysis and rheological behaviour of an extracellular polysaccharide from Drechslera spicifera. Carbohydrate Polymers 17, 177-183 (1992)

18. Tabata, K., Ito, W., Kojima, T., Kawabata, S. and Misaki, A. Ultrasonic degradation of schizophyllan, an antitumor polysaccharide produced by Schizophyllum commune Fries. Carbohydrate Res. 89, 121-135 (1981)

19. Danhof, I.E. Potential reversal of chronological and photo-aging of the skin by topical application of natural substances. Phytotherapy Res. 7, 53-56 (1993)


## Authors biography


***Dr. Fred Zülli, Mibelle Cosmetics***
***Head of Biochemistry Department***

Academic background: Study of Chemistry at the Engineering School in Winterthur (TWI). Study of Molecular Biology at the ETH in Zürich. Ph thesis and research assistant at the ETH on: "Genetic engineering of thermostable enzymes. Postdoctoral experience at the Centre de Recherche Nestec SA on: "Molecular biology and genetics of commercial baker's yeast strains"

***Christine Saecker, Mibelle AG Cosmetics***
***Member of the Biochemistry Department***

Academic background: Studies of Biology and Chemistry at the University of Hamburg, Germany. Graduated (1. Staatsexamen) from the University of Hamburg. Graduated (2. Staatsexamen) as teacher for higher education of subjects. Preparing of toxicological and ecotoxicological expertises about existing chemical substances at the Laboratory of Pharmacology and Toxicology in Hamburg