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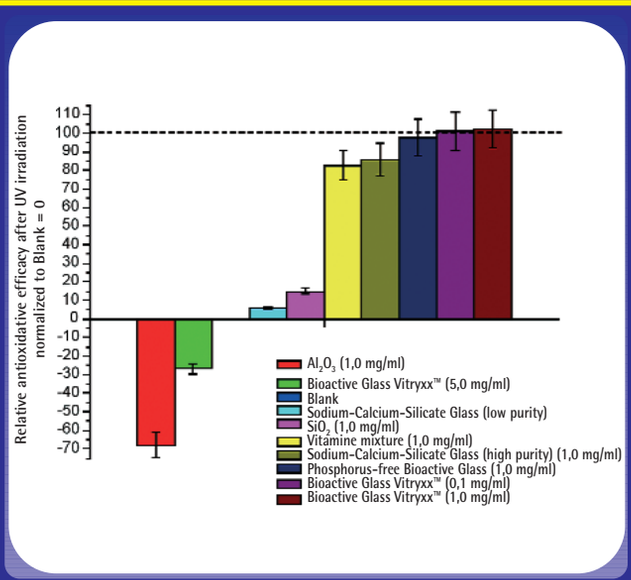

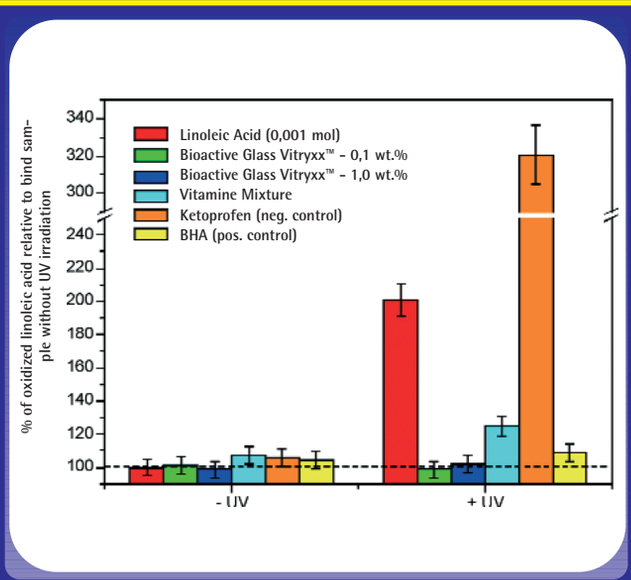

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Bioactive Glasses as a Potential New Class of Anti-Oxidative Ingredients for Personal Care Products

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Keywords: Bioactive Glasses, anti-oxidative, active ingredient

Introduction

Radicals formed on the skin, for example by exposure to UV-sunlight, can seriously damage cells and lead to the formation of wrinkles and premature skin aging. Currently, there are many effective organic active ingredients on the market, such as vitamins C and E, as well as plant extracts such as green tea, grape seed and others. In most cases, these actives obtain their anti-oxidative efficacy by absorbing the existing radicals rather than inhibiting the formation of new radicals.

As a new class of active ingredients, Bioactive Glasses in the form of finely grained powder, have shown clear anti-oxidative effects in *in-vitro* cell systems such as keratinocytes (HaCaT cells). Due to their mineral and inorganic character, this class of actives is insensitive to light and temperature extremes. The combination of those properties has led to the supposition that these may be suited to function as anti-oxidative actives for use in personal care products.

History

Bioactive Glasses in general are known to the biomaterials and medical implant community mainly as a proven bone grafting material that supports the regeneration of bone tissue and accelerates the healing process. Their beneficial biological activity and high level of biocompatibility are well documented in the biomaterial literature (1-3). Newer investigations demonstrated that finely grained powders of bioactive glasses have substantial anti-microbial, anti-inflammatory and mineralizing properties (4).

When considered as a material, glass is a collective term for any number of different compositions in a glassy or amorphous state. As opposed to crystalline materials, glasses do not have a long range order to their molecular structure. In the case of inorganic glasses the network is built up by so called network formers (e.g. SiO_2, P_2O_5). Further additives which can be included in the glass are called network modifiers (e.g. Na_2O, CaO). While constituents and compositional ranges may vary, bioactive glasses are typically composed of oxides of silicon, calcium, sodium and phosphorus. In the form that is approved for medical use, and for which the bulk of safety and efficacy data exists, the composition is 45 wt% SiO_2, 24,5 wt% CaO, 24,5 wt% Na_2O and 6 wt% P_2O_5.

In aqueous environments a rapid ion exchange occurs between Na^+ out of the glass and H^+ from the water, which is the starting point of a multi-step process and leads to an increase of pH in the surrounding medium. The fast release of sodium is accompanied by a somewhat slower release of other species like calcium ions, silica and phosphorous.

Anti-oxidative activity

Although the mechanism of anti-oxidative efficacy is currently not completely clarified, fine-grain bioactive glass powders have clearly shown anti-oxidative effects in *in-vitro* cell systems.

Oxidative modifications of proteins are implicated in a number of detrimental physiologic and pathologic processes. The introduction of carbonyl groups into amino acid residues of proteins is a hallmark for oxidative modifications (5). The oxidation of proteins by UVA irradiation leads among other modifications to the conversion of some amino acid side chains to carbonyl derivatives (6). One widely-used standard test to determine this type of oxidation is the so called carbonyl-protein assay (7). In this test immortal, non-tumorigenic human keratinocytes (HaCaT cells) are irradiated with UVA-light (5 J/cm^2, correspond to 50 min exposure time). The number of oxidized proteins (the amount of carbonyl derivative formation in the cells) is detected and quantified by the reaction with 2,4-Dinitrophenylhydrazine (DNPH) (8). The formed hydrazone derivatives have fairly high molar extinction coefficients and are measured at a wavelength of 365 nm with a UV radiometer. The total amount of proteins assessed with the Bio-Rad Protein assay (based on Bradford dye-binding procedure) is used as reference (9).

To determine the anti-oxidative efficacy of the different materials all samples are

grained to fine powders with an average particle size of approx. 5 µm (d_{50}) and suspended in the cell culture medium.

Table 1 shows the composition of the tested glasses.

The concentrations of the test samples are given in mg/ml. The measured amount of oxidized proteins is determined in nmol DNPH /mg protein. The absolute amount of DNPH is converted into percent (%) relative to the blank sample not irradiated with UVA-light (=100%), since absolute amounts can vary in different test realizations.

Table 2 gives an overview over the tested samples and the amount of formed DNPA in percent relative to the blank sample without UV irradiation. The error in the reproducibility of the test setup in the measured DNPA concentrations is less than 10 % (rel).

Fig. 1 shows the prevention in formation of oxidized carbonyl derivates relative to the blank sample (set to 0). The higher the number, the higher is the anti-oxidative efficacy of the tested material. For a number of 100 the amount of oxidized proteins is kept constant for UV irradiation and no UV irradiation, therefore all oxidation processes induced by UV light can be avoided.

As seen in **Fig. 1**, not only does pure Al_2O_3 powder have no anti-oxidative efficacy, it also shows pro-oxidative effects. Sodium-Calcium-Silicate Glass (low purity) with heavy metal impurities from mass production has nearly no effect on the amount of oxidized proteins in the cell. Pure SiO_2 shows nearly no anti-oxidative efficacy, as well. The well-known effective vitamin mixture 0,1 wt% Tocopherole Acetat [0,5 mg/ml] and Ascorbylpalmitate [0,5 mg/ml] shows a clear anti-oxidative efficacy, similar to the Sodium-Calcium-Silicate Glass (high purity). The reactive phosphorus-free Bioactive Glass shows quite a strong efficacy, however, slightly less then the original Bioactive Glass in concentrations of 0,01 wt% [0,1 mg/ml] and 0,1 wt% [1,0 mg/ml]. When the UV-irradiated and the non-irradiated cells, each treated with Bioactive Glass, are compared with each other, nearly no oxidation can be observed at these concentrations.

However, if too high a loading factor for Bioactive Glass is chosen, in this test

Gew.-%	Bioactive Glass Vitryxx™	Phosphous free Bioactive Glass	Sodium-Calcium-Silicate Glass (high purity)	Sodium Calcium-Silicate Glass (low purity)
SiO_2	45,00	47	71,80	71,22
Na_2O	24,50	26,5	14,40	14,10
CaO	24,50	26,5	9,70	9,60
P_2O_5	6,00	-	-	-
MgO	-	-	4,10	4,00
Al_2O_3	-	-	-	0,90
TiO_2	-	-	-	0,04
Fe_2O_3	-	-	-	0,10
ZnO	-	-	-	0,01
BaO	-	-	-	0,03
Summe	**100,00**	**100,00**	**100,00**	**100,00**

Table 1 Glass compositions

	sample	% DNPA rel. to Blank	% DNPA rel. to Blank
sample	Conc.	-UV	+UV
Blank	-	100	190
Bioactive Glass Vitryxx™	0,01 wt% (0,1 mg/ml)	77	89
Bioactive Glass Vitryxx™	0,1 wt% (1,0 mg/ml)	82	87
Bioactive Glass Vitryxx™	0,5 wt% (5,0 mg/ml)	151	217
Phosphorus Free Bioactive Glass	0,1 wt% (1,0 mg/ml)	82	92
Sodium-Calcium-Silicate Glass (high purity)	0,1, wt% (1,0 mg/ml)	95	104
Sodium-Calcium-Silicate Glass (low purity)	0,1 wt% (1,0 mg/ml)	140	184
SiO_2 (cristobalite)	0,1 wt% (1,0 mg/ml)	88	175
Al_2O_3	0,1 wt% (1,0 mg/ml)	123	258
0,5 mg/ml Tocopherol Acetat [Vitamin E] 0,5 mg/ml Ascorbylpalmitate [Vitamin C]	0,1 wt% (1,0 mg/ml)	103	107

Table 2 Relative amount of formed DNPA in test realizations

0,5 wt% [5 mg/ml], the opposite effect, i.e. a pro-oxidative, can be detected.
SiO_2 and Al_2O_3 were chosen as relatively inert materials to show that the anti-oxidative effect of Bioactive Glass is not caused by light-scattering or light-absorption effects, preventing the cells from irradiation by UV-light.
It is well-known from literature, that polyvalent ions play an important role in the formation process of radicals (10,11). Thus, it can be suggested that the difference between the Sodium-Calcium-Silicate Glass (low purity) from mass production and the Sodium-Calcium-Silicate Glass (high purity) is caused by polyvalent metal impurities.
The Sodium-Calcium-Silicate Glass (high purity) and the phosphorus-free Bioactive Glass are relatively similar with respect to their components; however, due to the high content of silica in the Sodium-Calcium-Silicate Glass (approx. 72 wt% - silica forms a strong network in the glass), it is rather inert in comparison with the phosphorus-free Bioactive Glass (SiO_2 of about 45 wt%).
Another anti-oxidative efficacy test was carried out to corroborate the results of the above carbonyl-protein assay. In this test, the oxidation of the lipid linoleic acid is determined.
Photodynamic lipid peroxidation (i.e. light-induced peroxidation of cellular membrane lipids) is one of the early steps of cell injury due to radicals and active oxygen species (12). The reaction of these species with lipids, called lipid peroxidation, is a well-studied phenomenon. The linoleic test is based on the photo-oxidation of this unsaturated acid that by a Type II (radical) reaction generates a conjugated double bond hydroperoxide. This formation can be readily monitored by the optical density (OD) with a spectrophotometer at 233 nm (13).
Ketoprofen, which is known to induce a strong pro-oxidative effect, was used as the negative control, and Butylhydroxyanisol (BHA), which shows a strong anti-oxidative efficacy, was chosen as the positive control.
Table 3 shows the tested samples with the used concentrations. The results are expressed as percentage of the level of the unstressed situation (14). The error in the reproducibility of the test setup in the optical density of hydroperoxide is less than 5 % (rel).
The well-known anti-oxidative vitamin mixture Tocopherole Acetat / Ascorbyl Palmitate shows a clear and strong effect in this test, as well as the Bioactive Glass tested in the two concentrations 0,1 wt% and 1,0 wt% [1 mg/ml and 10 mg/ml]. In **Fig. 2** these test results are plotted.

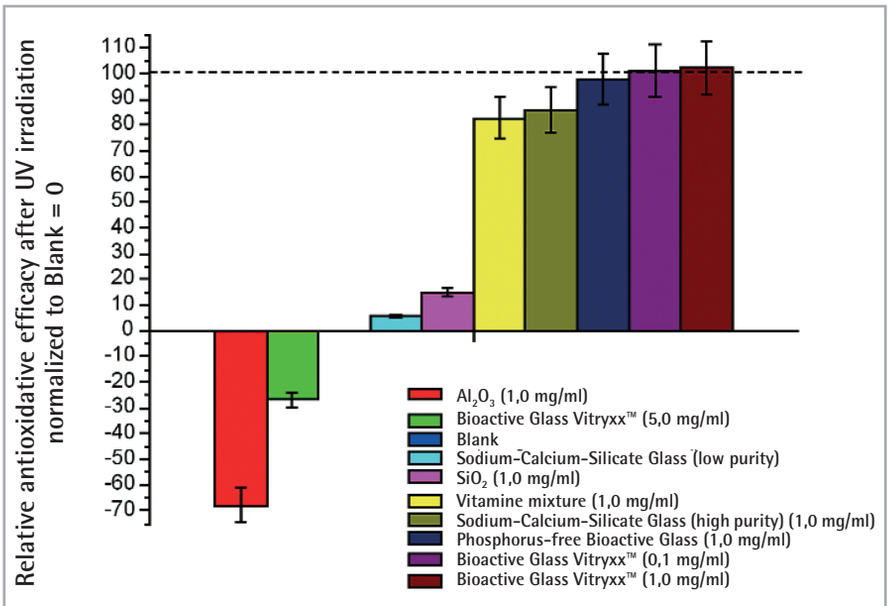

Fig. 1 Relative antioxidative efficacy after UV irradiation

	Conc.	-UV OD (233 nm)	-UV (%)	+UV OD (233 nm)	+UV (%)
Blank (Linoleic acid)	10^-3 mol	0,624	100%	1,250	200%
Bioactive Glass Vitryxx™	0,1 wt% (1,0mg/ml)	0,627	101%	0,615	99%
Bioactive Glass Vitryxx™	1,0 wt% (10mg/ml)	0,621	99%	0,634	102%
Tocopherol Acetat/ Ascorbyl Palmitate	0,5 wt% (5mg/ml)	0,670	107%	0,779	125%
Ketoprofen (neg. control)	10^-5 mol	0,661	106%	2,000	321%
BHA (pos. control)	0,01 wt%	0,650	104%	0,676	108%

Table 3 Amount of oxidized linoleic acid (OD) and percentage relative to the unstressed situation

Formulating Bioactive Glass

While incorporating bioactive glass powder into a product formulation, agglomeration can easily occur due to the active surface chemistry of the material; therefore, a performance active, Schott Vitryxx™M has been developed specifically for cosmetic applications. Schott Vitryxx™M utilizes mica as a spacer be-

tween the glass particles to improve its dispersibility.(4)

In the data shown, best anti-oxidative results were obtained with bioactive glass *in vitro* at an effective concentration of 0,1 wt%. Depending on the carrier system (formulation), a higher loading factor of Vitryxx™ M may be necessary to obtain the same degree of efficacy. If a loading factor of e.g. 1,0 wt% is chosen for an o/w cosmetic formulation bioactive glasses can demonstrate a high alkaline characteristic, as a result of the ion exchange between Na^+ and H^+, which poses a major challenge to effective formulation.

Because of the combination of high pH and high ion content, deactivation and thus destabilization of the thickening mechanism of lotion/cream formation can occur. Due to the fact that the acrylates copolymer is effective over a wide range of pH's from neutral to high alkaline, alkali-swellable acrylates copolymers such as Carbopol® produced by Noveon can be used to provide a compatible thickening system to suspend bioactive glasses and stabilize high pH lotions/creams. Carbopol® Aqua SF-1's relatively high ion tolerance makes it a highly suitable thickening agent for Vitryxx™ M. By using such a thickener, the loss in thickening efficiency can be eliminated and the desirable viscosity of the end product can also be maintained (4).

Other thickening systems, such as Xanthan gum, a high molecular weight polysaccharide starch derivative, can be used as rheology control agents in aqueous systems and as stabilizers for emulsions and suspensions. Due to its rheological pH independence and salt tolerance, Xanthan gum is also compatible with bioactive glasses.

The presence of significant amounts of ions and the higher pH ranges of aqueous solutions containing bioactive glasses will preclude the use of cationic emulsifiers.(4)

Another critical factor for the successful and effective formulation of bioactive glasses is the incorporation technique, i.e., the addition sequence and the adequate mixing of the ingredients. Typically, bioactive glasses are first mixed with water and the resulting mixture is then added to the product formulation at later stages – this order of addition is vital to achieving optimal benefit from the bioactive glasses (4).

Overall, bioactive glasses have shown good gelling abilities for emulsion/polymer sytems. High shear homogenisation can also be used to create good emulsions and to insure thorough dispersion of particles (4).

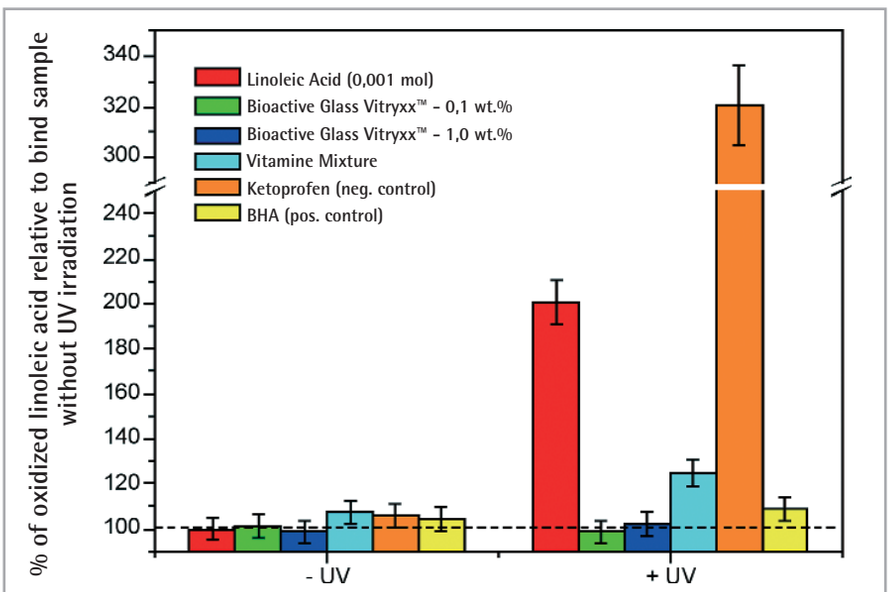

Fig. 2 Percentage of oxidized linoleic acid relative to blind sample (without UV irradiation)

Conclusion

It can be concluded that this new inorganic active ingredient Vitryxx™ shows a high anti-oxidative efficacy in standard *in-vitro* test systems. In both test methods, the »carbonyl protein assay« and the »linoleic acid photoperoxidation test«, a clear anti-oxidative efficacy could be demonstrated. The quantitative efficacy of Vitryxx™ is comparable to the well-known vitamin mixture Tocopherole Acetat / Ascorbyl Palmitate. Furthermore, this active material, being an inorganic, is stable in the presence of light, oxygen and temperature extremes.

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