



Vitality

Improving Mineral Performance of Multivitamin/Multimineral Supplements by *Using a New Mineral Delivery System*

The creation of a new complexing environment for minerals that combines the benefits of amino acid chelation and oligofructose complexing (AAOF).



Improving Mineral Performance of Multivitamin/Multimineral Supplements

by *Using a New Mineral Delivery System*

Note: The results of this work have been filed as a patent application. They were presented at the 15th annual meeting of the Society for Free Radical Biology and Medicine (Abstract, in the journal *Free Radical Biology and Medicine*¹³).

The complete findings are currently being prepared for publication in a journal article. This document provides a brief overview of those findings.

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Problem Addressed

Is it possible to increase the solubility of the minerals typically included in dietary supplements while, at the same time, decreasing the rate of free radical generation triggered by these minerals?

Introduction

Minerals are essential for good health. Every living cell depends on minerals for proper structure and function. Minerals are needed for the formation of tissue and bones, healthy nerve function, and proper operation of the cardiovascular system. As a result, a daily multivitamin/multimineral supplement is recommended to help ensure proper nutrient levels.

But unlike minerals in natural foods that are incorporated in bioorganic structures, minerals in dietary supplements are usually in an inorganic form: sulfates, chlorides, oxides, etc. Unfortunately, the majority of minerals in these forms precipitate

(fall out of solution and solidify) at the neutral pH of the small intestine, making absorption questionable.

In addition, some minerals act as a free radical catalyst, triggering the generation of massive amounts of free radicals that can neutralize the effectiveness of the antioxidants (like vitamins C and E) found in the supplement. The minerals primarily responsible for accelerating the generation of free radicals are copper (sometimes listed as cupric on ingredient labels) and iron (often listed as ferrous on ingredient labels).

Results and Discussion

Understanding the problems inherent in mineral supplementation, many have attempted to replicate the mineral form naturally found in fruits, vegetables, and other whole foods. These attempts include complexing them with salts from organic acids (citrates, gluconates, and fumarates) as well as amino acid chelation and Melaleuca's proprietary Fructose Compounding.

While looking for ways to improve upon this, we found research documenting that some forms of fiber—namely oligofructose—may significantly improve mineral absorption in animals and humans.^{1,7,10,12,14,15}

Building upon those findings, a new complexing environment for minerals has been created that combines the benefits of amino acid chelation and oligofructose complexing (AAOF).

Solubility

The stomach is very acidic, with a pH close to 1. In this low pH environment, the majority of minerals are soluble. The problem is that absorption takes place in the small intestine, which has a neutral pH, from 7.0–7.2. In this state, many minerals precipitate or fall out of solution, making them

» Results and Discussion

...oligofructose may significantly improve mineral absorption.

» Solubility

Solubility is at its lowest in inorganic forms of minerals. Organic forms proved better, but the new AAOF form brought the highest levels of solubility.

difficult to absorb.

Using the AAOF complex, the solubilities of key minerals, including calcium, magnesium, copper, iron, manganese, chromium, molybdenum, and selenium were measured. Each substance was first dissolved in acidic stomach conditions. The acidity was then adjusted to mirror pH conditions in the intestine. The amount of mineral still remaining in solution was then measured. All tested minerals were almost 100% soluble.

To measure the solubility of the final supplement form, the AAOF complex was compared to seven popular commercial products, using the same method.

Testing entire supplement formulas that minerals were put into created complications and interference that resulted in drastically different levels of solubility. So the exact same mineral form was found to be more soluble in one supplement formulation, and much less soluble in another formulation (see Figure 1).

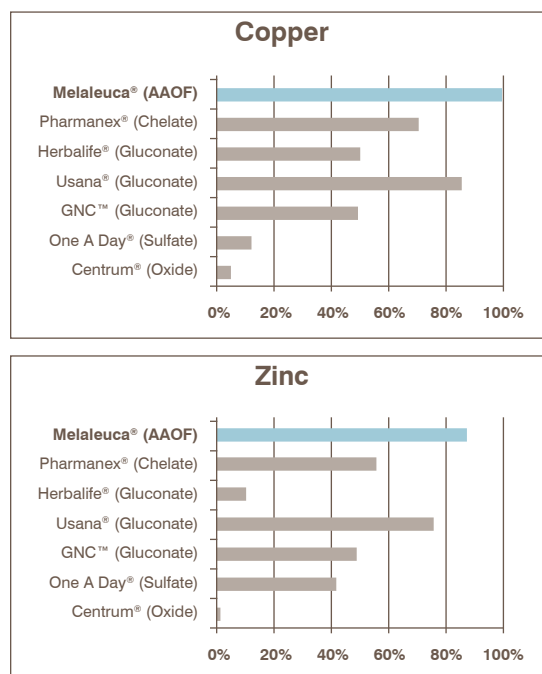


Figure 1. The solubility of copper and zinc at the conditions of the small intestine (pH 7.0–7.2) after exposure of tablets at the conditions of the stomach (pH 1.0).

Solubility was found to be at its lowest in inorganic forms of minerals. Organic forms proved better, but the new AAOF form brought the highest levels of solubility.

» Free Radical Generation

The ability of minerals to trigger free radicals differs greatly depending on the mineral form.

Free Radical Generation

Free radicals are highly reactive molecules with lifespans as short as one-billionth of one second. An overabundance of free radicals is considered dangerous because they have the ability to damage the molecules and tissues of the body. In fact, the majority of degenerative diseases can be linked to free radical damage.

Minerals (especially copper and iron) are known triggers of free radical generation, but their ability to trigger free radicals differs greatly depending on the mineral form.

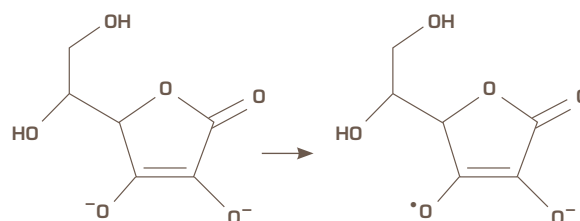
The main technology for studying free radicals is electron paramagnetic resonance (EPR), also known as electron spin resonance (ESR). Many experimental models use this technology. This study utilized a few key models:

- Ascorbate radical model (oxidation of vitamin C)⁵
- Hydroxyl radical model (Fenton reaction, spin trapping technique)^{6,11}
- Tocopheryl radical model (oxidation of vitamin E)^{8,16}

For demonstration purposes, the results from the ascorbate radical model will be outlined.

Ascorbate Radical Model

The oxidation of ascorbic acid catalyzed by iron or copper first leads to the formation of ascorbate radical.



Below is the EPR signal of the ascorbate radical. The amplitude of the waveform goes down as the ascorbic acid (vitamin C) is consumed.

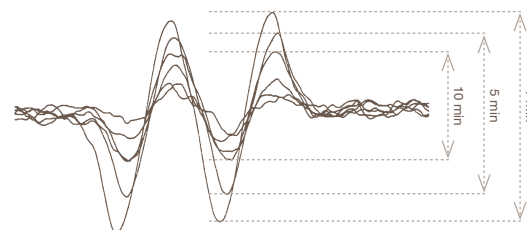


Figure 2. EPR signal of ascorbate radical.

» Ascorbate Radical Model

Ascorbic acid (vitamin C) survives much longer when complexed with AAOF.

Figure 3 shows the loss of ascorbate radicals over time in the presence of different mineral forms.

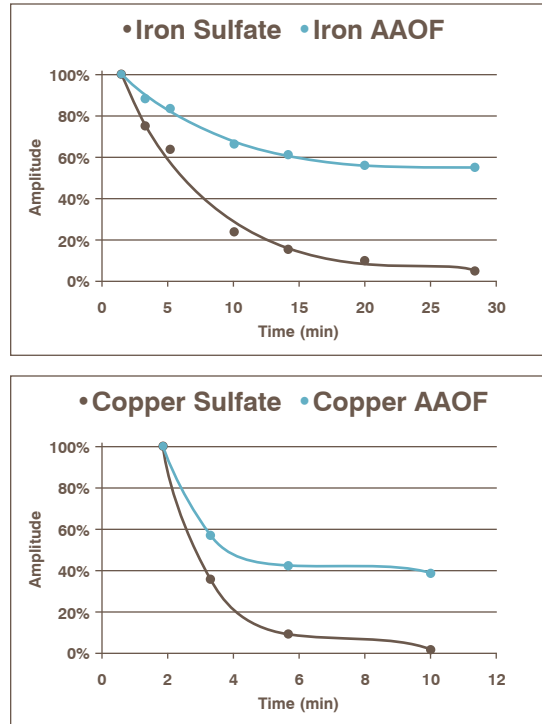


Figure 3. The rate of loss of the ascorbate radical is much faster when in the presence of the inorganic forms of iron or copper.

This illustrates that ascorbic acid (vitamin C) survives much longer when complexed with AAOF. In about 30 minutes, the traditional forms of copper and iron destroy almost all of the ascorbic acid, but the AAOF form remains stable at about 60%.

We used the following methods to measure the oxidative damage caused by free radicals:

- Loss of vitamin C in solution^{3,4}
- Loss of trolox (a water-soluble analog of vitamin E) in solution¹⁶
- Loss of flavonoids in solution
- Free radical oxidation of indicator dyes^{2,9}

Typical experiments showing the loss of vitamin C are shown in figure 3. In figure 4 we show typical experimental results of the free radical-mediated oxidation of indicator dyes.

Free Radical Oxidation of Indicator Dyes

In this process, different forms of copper were mixed with hydrogen peroxide and indicator dye (dichlorodihydrofluorescein or DCF). DCF, after being oxidized by free radicals, turns to an orange pigment, which can be measured spectrophotometrically. The intensity of this orange color was measured by its absorbance at 500 nm (OD-500 nm). The results can be seen in Figure 4.

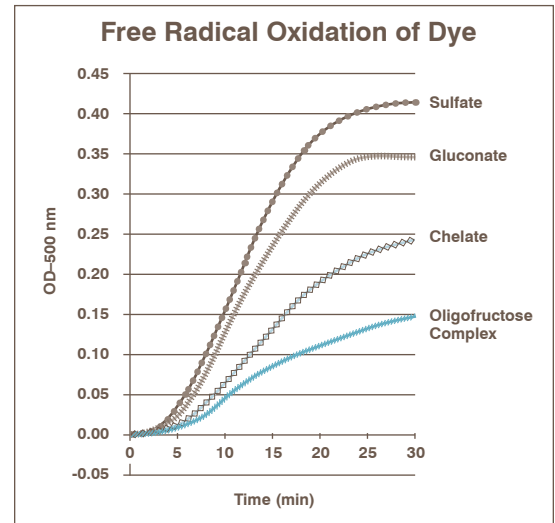


Figure 4. The sulfate form of copper brought about the most rapid oxidation of DCF; the oligofructose complex form had the least oxidation.

The inorganic form of copper (sulfate) has the highest oxidative ability. Surprisingly, copper gluconate (which is an organic form of copper) generated almost the same amount of free radicals as sulfate. Copper chelate behaved much better, and AAOF complex demonstrated the lowest oxidative ability.

Oxygen-Monitoring Model

When a substance oxidizes it will consume the oxygen dissolved in the water of a solution. To analyze the rate of oxidation of the entire multivitamin/multimineral supplement, an oxygen-monitoring model was used. Because this model measures the loss of dissolved oxygen, it measures *all* oxidative loss regardless of the individual ingredient source.

A commercially available men's multivitamin/multimineral supplement using copper oxide was compared with Melaleuca's multivitamin/multimineral supplement containing copper AAOF complex.

» DCF Loss Model

Copper chelate behaved much better than copper (sulfate) and copper gluconate. But AAOF complex demonstrated the lowest oxidative ability.

» Oxygen-Monitoring Model

This model measures all oxidative loss, regardless of the individual ingredient source.

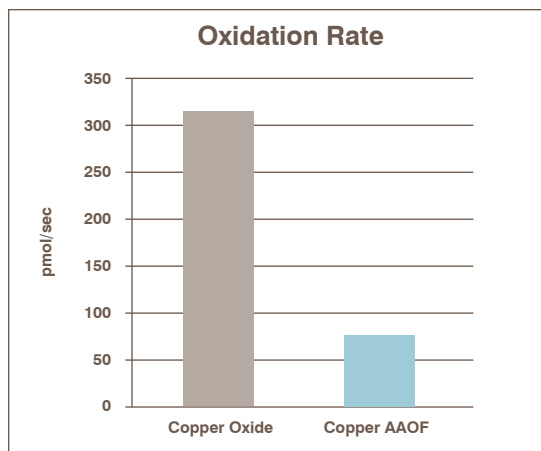


Figure 5. Rate of oxygen consumption for the two supplements.

A similar concentration of copper was taken from each formula and combined with identical amounts of ascorbic acid (vitamin C). These formulas were then dissolved in acidic stomach conditions (pH 1). The acidity was then adjusted to mirror conditions in the intestines (pH 7.0–7.2). The oxygen concentration in the solution was then measured electrochemically with a Clark electrode.

The oxygen consumption rate was more than four times greater for the copper oxide supplement (315 pmol/sec) than it was for the AAOF complex supplement (75 pmol/sec).

Conclusions

- Compared to traditional forms (sulfates, chlorides, oxides, etc.) of minerals (copper, iron, and zinc), Melaleuca's AAOF mineral form delivers significantly higher levels of solubility.
- Melaleuca's AAOF mineral form significantly reduces the rate of free radical generation compared to traditional mineral forms.
- Melaleuca's AAOF mineral form drastically lowers oxidative loss of vitamins and other key nutrients compared to traditional mineral forms.

Reference List

- 1 Abrams, S. A.; Hawthorne, K. M.; Aliu, O.; Hicks, P. D.; Chen, Z.; Griffin, I. J. An inulin-type fructan enhances calcium absorption primarily via an effect on colonic absorption in humans. *J. Nutr.* **137**:2208-2212; 2007.
- 2 Brubacher, J. L.; Bols, N. C. Chemically de-acetylated 2',7'-dichlorodihydrofluorescein diacetate as a probe of respiratory burst activity in mononuclear phagocytes. *J. Immunol. Methods* **251**:81-91; 2001.
- 3 Buettner, G. R. In the absence of catalytic metals ascorbate does not autoxidize at pH 7: ascorbate as a test for catalytic metals. *J. Biochem. Biophys. Methods* **16**:27-40; 1988.
- 4 Buettner, G. R. Ascorbate oxidation: UV absorbance of ascorbate and ESR spectroscopy of the ascorbyl radical as assays for iron. *Free Radic. Res. Commun.* **10**:5-9; 1990.
- 5 Buettner, G. R.; Jurkiewicz, B. A. Ascorbate free radical as a marker of oxidative stress: an EPR study. *Free Radic. Biol. Med.* **14**:49-55; 1993.
- 6 Buettner, G. R.; Mason, R. P. Spin-trapping methods for detecting superoxide and hydroxyl free radicals in vitro and in vivo. *Methods Enzymol.* **186**:127-133; 1990.
- 7 Devareddy, L.; Khalil, D. A.; Korlagunta, K.; Hooshmand, S.; Bellmer, D. D.; Arjmandi, B. H. The effects of fructooligosaccharides in combination with soy protein on bone in osteopenic ovariectomized rats. *Menopause.* **13**:692-699; 2006.
- 8 Kagan, V. E.; Kuzmenko, A. I.; Shvedova, A. A.; Kisin, E. R.; Tyurina, Y. Y.; Yalowich, J. C. Myeloperoxidase-catalyzed phenoxyl radicals of vitamin E homologue, 2,2,5,7,8-pentamethyl-6-hydroxychromane, do not induce oxidative stress in live HL-60 cells. *Biochem. Biophys. Res. Commun.* **270**:1086-1092; 2000.
- 9 Komarov, A. M.; Hall, J. M.; Weglicki, W. B. Azidothymidine promotes free radical generation by activated macrophages and hydrogen peroxide-iron-mediated oxidation in a cell-free system. *Biochim. Biophys. Acta* **1688**:257-264; 2004.
- 10 Kruger, M. C.; Brown, K. E.; Collett, G.; Layton, L.; Schollum, L. M. The effect of fructooligosaccharides with various degrees of polymerization on calcium bioavailability in the growing rat. *Exp. Biol. Med. (Maywood.)* **228**:683-688; 2003.
- 11 Mak, I. T.; Komarov, A. M.; Kramer, J. H.; Weglicki, W. B. Protective mechanisms of Mg-gluconate against oxidative endothelial cytotoxicity. *Cell Mol. Biol. (Noisy. -le-grand)* **46**:1337-1344; 2000.
- 12 Nzeusseu, A.; Dienst, D.; Haufroid, V.; Depresseux, G.; Devogelaer, J. P.; Manicourt, D. H. Inulin and fructooligosaccharides differ in their ability to enhance the density of cancellous and cortical bone in the axial and peripheral skeleton of growing rats. *Bone* **38**:394-399; 2006.
- 13 Rabovsky, A. B.; Komarov, A. M.; Ivie, J.; Buettner, G. R. Minimization of free radical damage by metal catalysis of multivitamin/multimineral supplements (abstract). *Free Radical Biology and Medicine* **45**:S128. (8 A.D.)
- 14 Roberfroid, M. B.; Cumps, J.; Devogelaer, J. P. Dietary chicory inulin increases whole-body bone mineral density in growing male rats. *J. Nutr.* **132**:3599-3602; 2002.
- 15 van den Heuvel, E. G.; Muys, T.; van, D. W.; Schaafsma, G. Oligofructose stimulates calcium absorption in adolescents. *Am. J. Clin. Nutr.* **69**:544-548; 1999.
- 16 Witting, P. K.; Upston, J. M.; Stocker, R. Role of alpha-tocopheroxyl radical in the initiation of lipid peroxidation in human low-density lipoprotein exposed to horse radish peroxidase. *Biochemistry* **36**:1251-1258; 1997.