

Minimization of free radical damage by metal catalysis of multivitamin/multimineral supplements

PROBLEM ADDRESSED

Is it possible to increase the solubility of minerals, typically included in dietary supplements, and at the same time decrease the rate of oxidative catalysis by these minerals of susceptible organics in the mixture, especially antioxidants?

SUMMARY

Multivitamin/multimineral complexes are the most common dietary supplements. Besides quality ingredients and the amount of each ingredient in a product, bioavailability is a major concern.

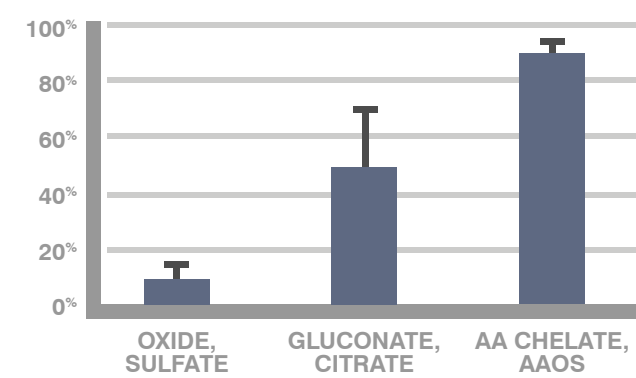
Unlike minerals in natural foods that are incorporated in bioorganic structures, minerals in dietary supplements are usually in an inorganic form: sulfates, chlorides, oxides etc.

Unfortunately, the majority of minerals in these forms precipitate at the neutral pH of the small intestine, making adsorption questionable. In addition, some minerals catalyze free radical generation, depending on their form. Thus, antioxidants in supplements could be oxidized during digestion. We have created a new complexing environment for minerals that consists of an amino acid chelate and non-digestible amino acid oligosaccharide (AAOS). All essential minerals in this form are soluble at intestinal pH. Even though soluble, the commonly used form of copper - gluconate - generates a flux of free radicals similar to the inorganic forms. Monitoring of ascorbate radical generated by different forms of copper shows that ascorbate is oxidized much more slowly with the AAOS matrix.

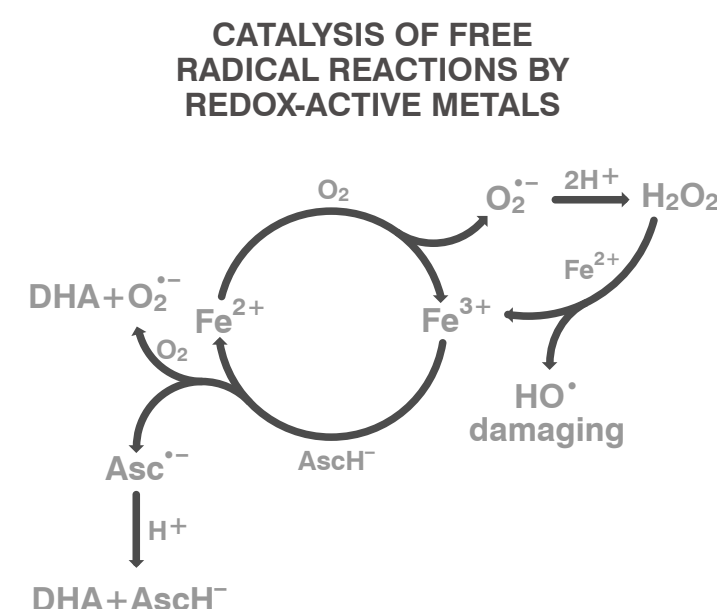
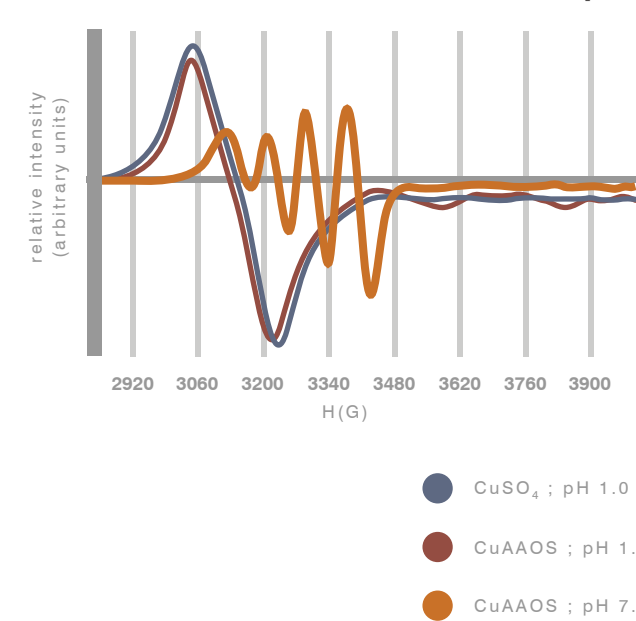
Direct measurement of the oxidation of ascorbic acid (vitamin C) and gallic acid (a typical antioxidant ingredient derived from fruits) by different forms of minerals confirmed the ability of AAOS to slow these oxidations. Similar results were observed with iron-catalyzed formation of hydroxyl radicals (Fenton reaction), as measured by EPR spin trapping. In addition, the relative rates of oxidation of 2',7'-dichlorodihydrofluorescein by H₂O₂ with copper were: sulfate > gluconate > glycinate > AAOS.

When compared to traditional forms of minerals used in supplements, we conclude that the oxidative loss of antioxidants in solution at physiological pH is much slower when AAOS is used.

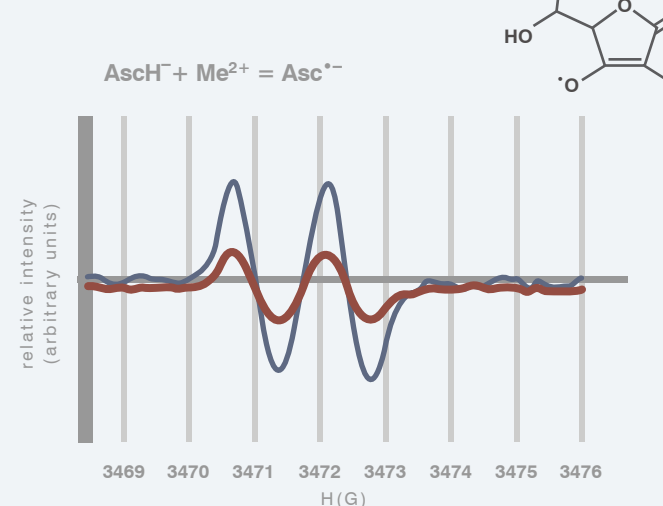
AMINO ACID CHELATES OF METALS ARE MORE SOLUBLE AT INTESTINAL pH THAN INORGANIC FORMS



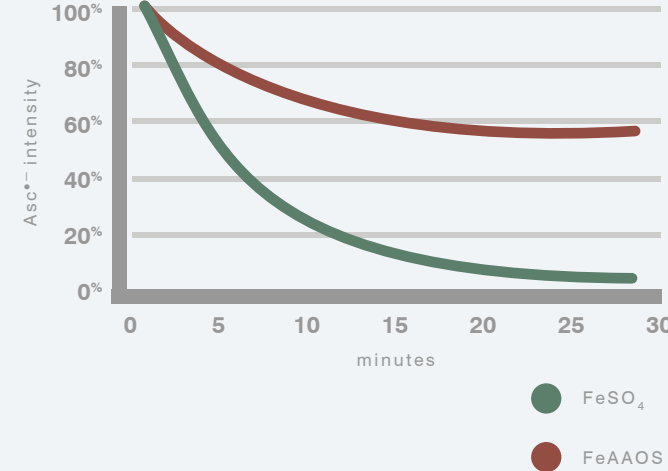
COPPER FORMS SOLUBLE AMINO ACID CHELATES AT NEUTRAL pH



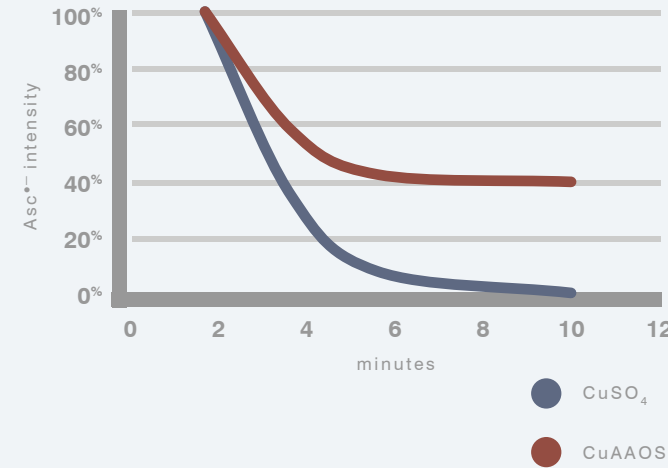
MONITORING OF ASCORBATE RADICAL SHOWS THAT AAOS SLOWS THE CATALYTIC ACTION OF METALS



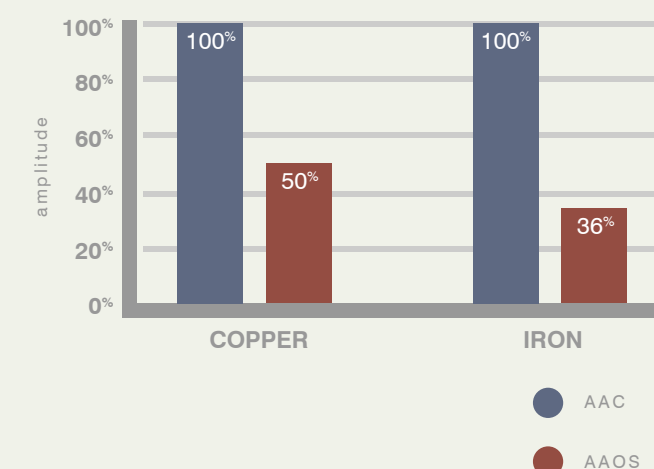
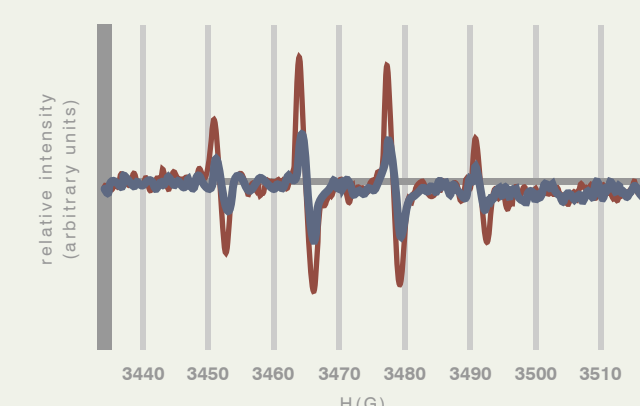
IRON



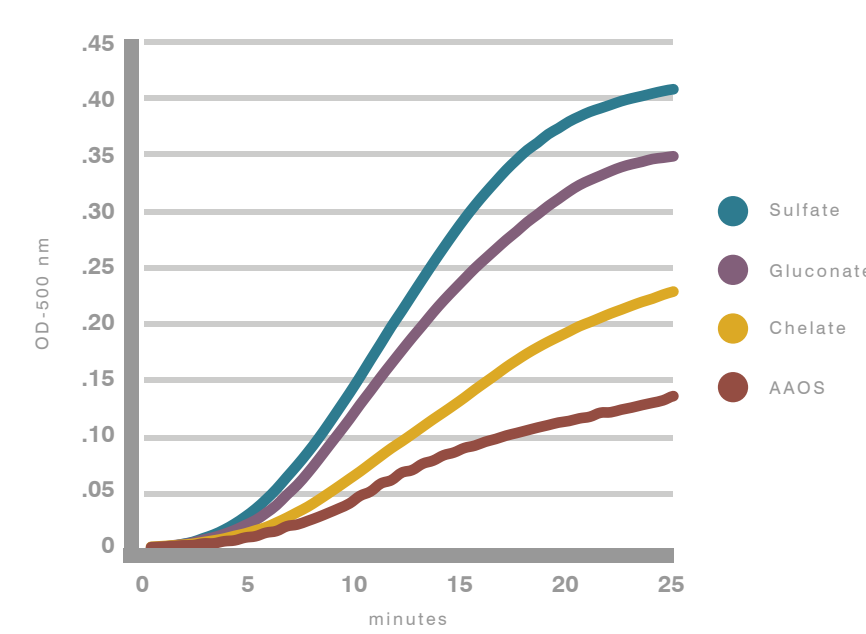
COPPER



AAOS INHIBITS THE FORMATION OF DMPO/HO• GENERATED BY THE FENTON REACTION



THE OXIDATION OF DCF BY COPPER AND H₂O₂ HAS RELATIVE REACTION RATES: SULFATE > GLUCONATE > GLYCINATE > AAOS



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MATERIALS AND METHODS

Solubility

Each substance or tablet powder was: dissolved in 0.01 M HCl; pH adjusted to 1.0 – 1.2; shaken for one hour; then the pH adjusted to 7.0 – 7.2 with NaOH; shaken for one hour; then centrifuged for 15 min at 13,000 g. Metal concentrations were measured by ICP. The solubility at intestinal pH was determined as percent of each mineral remaining in the supernatant.

EPR

All EPR measurements were done in 50 μ L capillary tubes at room temperature using a Bruker ER-200 X-band EPR spectrometer. Instrumental conditions were:

- EPR settings for ascorbate experiments: microwave frequency 9.71 GHz; center field 3472 G; scan range 10 G; scan time 20 s; mod amp 1.25 G; time constant 0.5 s; microwave power 10 mW; and instrument gain 2×10^6 .
- EPR settings for DMPO spin trapping (hydrogen peroxide plus iron/copper): microwave frequency 9.71 GHz; center field 3472 G; scan range 100 G; scan time 100 s; mod AMP 1.25 G; time constant 0.5 s; microwave power 10 mW; instrument gain was 2×10^6 .
- EPR settings for transition metals (Fe³⁺, Cu²⁺, Mn²⁺): microwave frequency 9.71 GHz; center field 3415 G; scan range 1000 G; scan time 100 s; modulation 2.5 G; time constant 0.5 s; microwave power 102 mW; instrumental gain varied for different samples from 10^3 to 3.2×10^5 ; temperature was ambient.

Dichlorodihydrofluorescein Oxidation

2',7'-Dichlorodihydrofluorescein diacetate was hydrolyzed in 20 mM NaOH at room temperature for 20 min. Mineral stock solutions were prepared in water. DCF (200 μ L of 90 μ M in 20 mM carbonate buffer, pH 7.0) and 80 μ L of mineral solution (100 μ M in 20 mM carbonate, pH 7.0) were mixed in a standard UV-Vis cuvette. The reaction was initiated by addition of 20 μ L of 0.3% H₂O₂. Absorbance at 500 nm was monitored for 30 min.

CONCLUSIONS

- Amino Acid Oligosaccharides (AAOS) increases the solubility of metals compared to typical formulations for dietary supplements.
- AAOS slows the iron catalyzed oxidation of ascorbate
- AAOS slows the copper catalyzed oxidation of ascorbate.
- AAOS inhibits the formation of DMPO/HO[•] generated by the Fenton reaction.
- AAOS appears to be a superior matrix for the formulation of dietary supplements.