

Active H Characterization & Analysis Report

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This report presents the characterization and analysis data for the magnesium-based Active H product, as distributed through Generations of Health. The purpose of the testing is to accurately characterize the attributes and mechanisms of the newly formulated magnesium hydride silica carbonate compound, labeled as Active H.

The testing involved the following assays:

- Time-domain redox
- Concentration-domain redox
- Total dissolved solids
- Cytochrome reduction
- Oxyhemoglobin
- Nicotinamide adenine dinucleotide reduction capacity

All of the *in vitro* tests individually combine to offer a postulate for the *in vivo* mechanism and reactivity of Active H in the body.

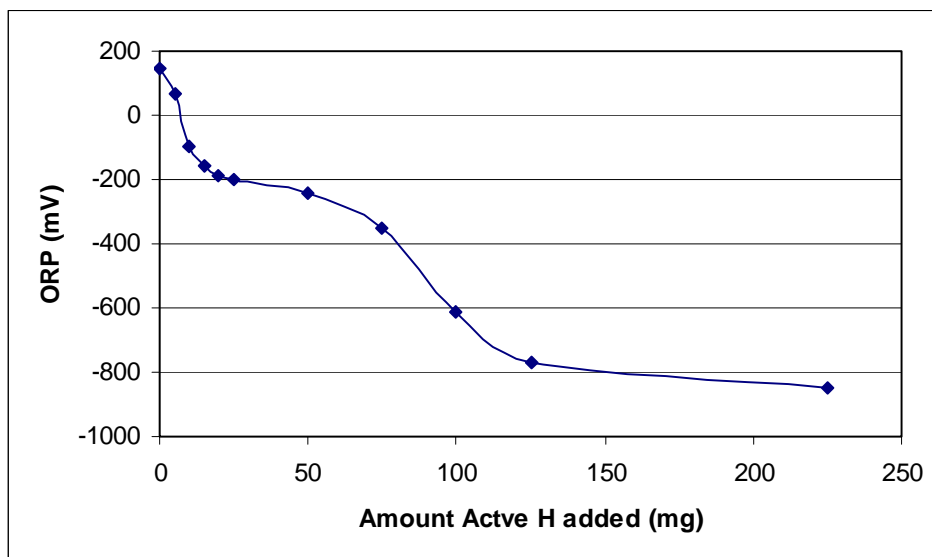
Test 1: Concentration-Domain Redox Analysis

Purpose: This method of analysis characterizes how Active H behaves in solution as a function of concentration. The results will indicate whether the Active H formulation reacts instantly, or will be time-released. This method also indicates the ability of Active H to successfully react with other compounds, such as free radicals. Oxidation/reduction (redox) potentials measure, in millivolts, the ability of a compound to react. Generally, a lower redox reading means that there is more energy stored in the compound to react. Similarly, the pH of a solution measures the amount of free positive hydrogen ions in solution. Together, redox and pH can be used to calculate a value known as rH. rH is the unbiased determination of the state of reduction or oxidation that a compound is in and is indicative of the overall probability that a compound will react.

Materials and Methods: An IQ Scientific IQ400 ORP/pH meter was used to monitor the ORP and pH levels of subsequent additions of Active H into 250 mL of distilled water. All readings

were taken in a 250 mL Erlenmeyer flask being magnetically stirred. An initial reading of pH and ORP were taken. Cumulative additions of Active H, totaling 5, 10, 15, 20, 25, 50, 75, 125 and 225 mg/250 mL were added to the distilled water in 5 minute increments. At each increment, the pH and ORP values, in mV, were recorded. The water had an initial temperature of 26 °C.

Results: The initial water reading had an ORP of 146.3 mV and a pH of 7.46. The initial calculated rH (see Appendix 1 for rH formula) was 26.46, indicating a very oxidized environment. The initial addition of 5 mg Active H caused the ORP and pH readings to change to 68.6 mV and 7.89, respectively. The final rH, after the addition of 225 mg of Active H was -2.41, indicating an exceptionally reduced environment. The final ORP reading was -846.1 mV and the pH was 9.51. The plot of the ORP versus the amount of Active H added resulted with a dual, isobestic polynomial relationship:



Discussion and Significance: The plot of ORP versus Active H concentration indicates a time-released mechanism of action for the magnesium hydride carbonate compound. The nature of the polynomial decrease in ORP eludes to a second-order kinetic action taking place. Notice the initial drop in ORP, followed by a lower rate, then another increased rate. This is very significant for the idea of a consistency in bio-reactivity and the ability of Active H to react in the body over an extended period of time. Compared to the old formula of Active H, this

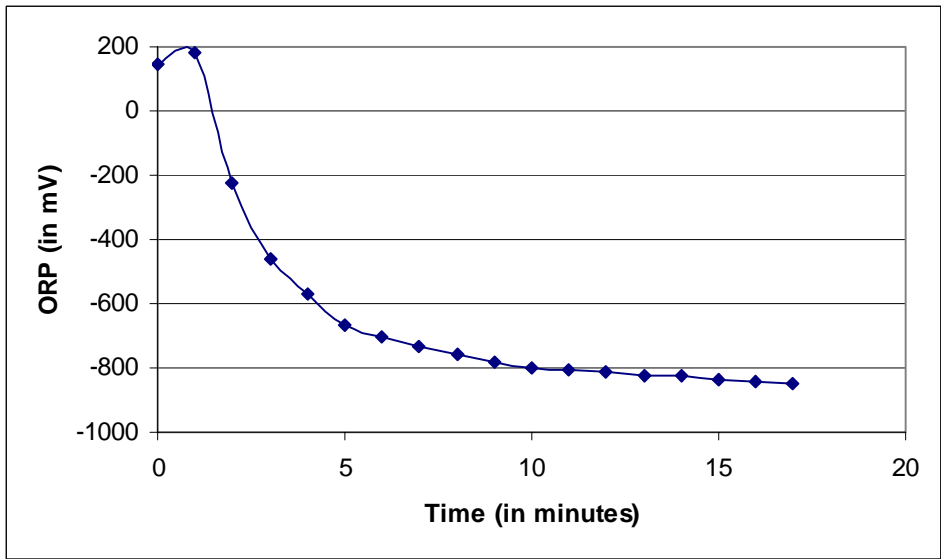
mechanism would allow a greater distribution of the active hydrogen in the compound within the body. The net change in ORP from an initial reading to a final reading of -992 mV is a profound change in redox.

Test 2: Time-domain Redox Analysis

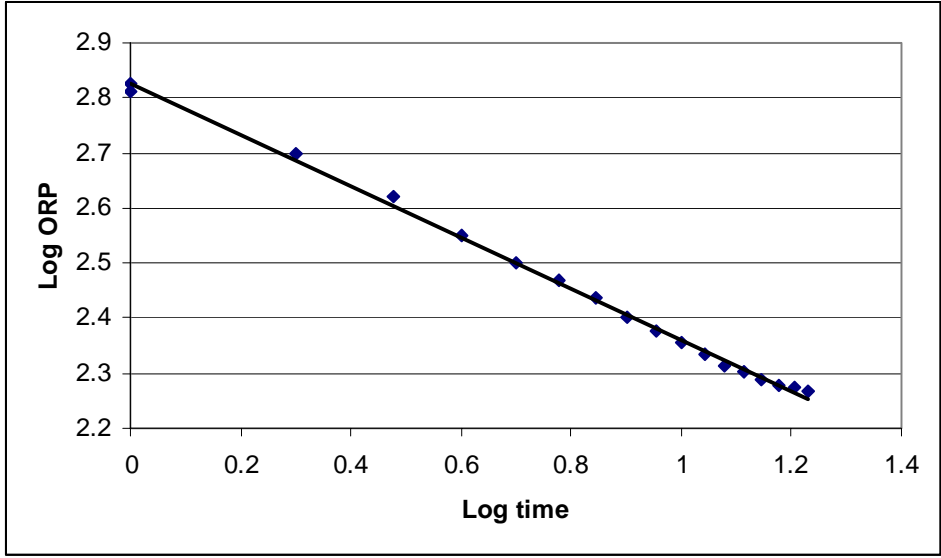
Purpose: The purpose of the time-domain analysis is to understand how Active H responds in solution over time. The data collected in this assay as well as the other assays offer “pieces of a puzzle” that can be looked at as a whole to evaluate the effectiveness of a compound. The ORP versus time of 250 mg of Active H added to 250 mL of distilled water will aid with insight into the reactivity of the compound.

Materials and Methods: 250 mg of Active H compound was added to 250 mL of distilled water in a 250 mL Erlenmeyer flask. The water, at 26°C, was stirred magnetically. Before adding the Active H, base readings of ORP and pH were taken with an IQ Scientific ORP/pH meter. After the addition of the Active H, ORP and pH reading were taken every 1 minute for a total of 17 minutes.

Results: The data collected were tabulated and statistically analyzed. The addition of 250 mg Active H into 250 mL of distilled water resulted in a logarithmic relationship between the decrease in ORP reading and the time, as shown below:



In all replicates thus far, there has been a small Gibb’s energy difference to overcome before the decrease in ORP. Taking the log/log relationship between both axes, a linear regression is formed with a $y = -1.0757x + 3.0997$, $R^2 = 0.99$ equation:



Such that each time unit evolved a -1.07 times relative drop in ORP value.

Discussion and Significance: Consistent with the concentration-domain experimentation, the time-domain assay indicates a clear relationship of time release and ORP drop for the length of the experimentation. The redox rate appears to have slowed after 15 minutes, although still was declining. The ability of a compound to release consistently over time has been a troubling

problem in the nutraceutical and pharmaceutical industries. The impact of a consistent reaction mechanism over time is that there is a more significant even distribution of the active ingredient. The new formulation of Active H successfully performs three main objectives: 1) Creates a reduced environment, 2) Has increased potential for reactivity and 3) reacts evenly over time.

Test 3: Total Dissolved Solids

Purpose: The purpose of the total dissolved solids analysis is to define the amount of the added material is completely dissolved and suspended in solution. For most bio-chemical reactions to happen with the highest efficiency, it is important to have the compound dissolve in water. The more dissolved the active ingredient, the more efficient the reaction kinetics.

Materials and Methods: In two separate aliquots, 250 mg each of the new and old formulations of Active H were added to 250 mL of distilled water. Dissolved solids readings were taken initially, at 5 minutes and 10 minutes for both Active H formulations. Readings were taken with a Hanna Instruments TDS1 meter with automatic temperature compensation and reported in parts per million (ppm).

Results: In both tests, the initial water reading was 4 ppm dissolve solids. The Active H formulation results were:

	Initial (ppm)	5 minutes (ppm)	10 minutes (ppm)
New formulation	4	270	290
Old formulation	4	273	288

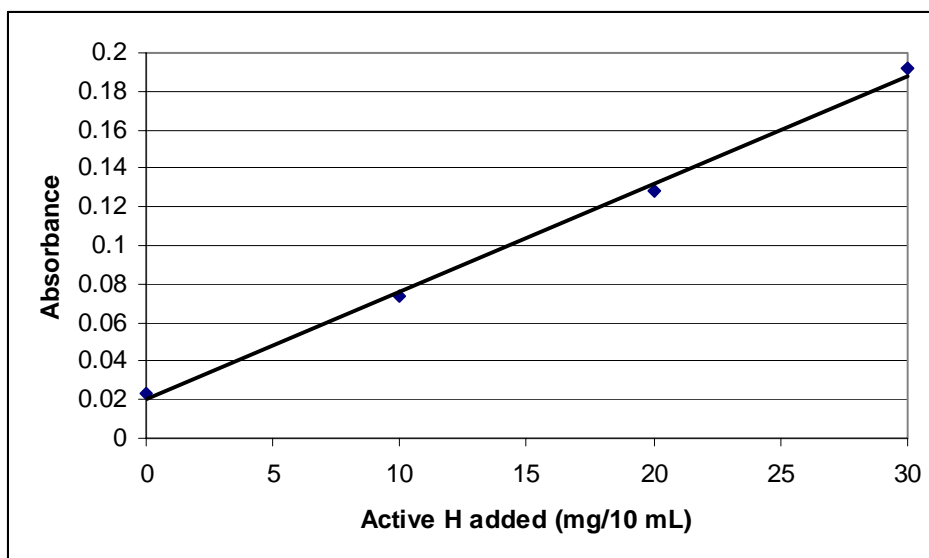
Discussion and Significance: The similarity between the results indicates a consistency in the dissolution of the compound over time, in both formulations. This method of analysis can indicate any potential sources of biochemical inefficiency if the results were to show a discrepancy between total dissolved solids or if there were an indication of low parts per million dissolved solids.

Test 4: Cytochrome *c* Reduction Analysis

Purpose: Cytochrome *c* is a porphyrin derivative protein that has an integral role in metabolic activity. Particularly, cytochrome *c* is responsible for the transfer of an electron in the mitochondrial electron transport chain. The cyto-proteins are primed for optimum function by being kept in a reduced state that is rich in electrons. When the cytochromes are in a reduced state, they effectively react with their surroundings and have increased reaction kinetics.

Materials and Methods: Pure, harvested cytochrome *c* (>99.8%) was used to create a solution in an isotonic 0.1M phosphate buffer with 5% citrate and EDTA, pH 7.6. 6.5 mg of the pure cytochrome *c* was added to 10.0 mL of the fore mentioned buffer in a volumetric flask and was allowed to dissolve. 1.2 mL of the cytochrome/buffer solution was added to a 1 cm path length quartz cuvette and placed into a Jasco V-530 spectrophotometer and referenced to 1.3 mL of the isotonic buffer solution. A baseline reading was taken from 500 nm to 600 nm, at which time a small crystal of potassium ferricyanide was added to the cuvette to fully oxidize the cytochrome *c*. 5.0 mg aliquots of Active H were added to the cuvette, covered with parafilm and inverted several times to insure a homogeneous mixture. Samples were allowed to sit for 5 minutes and lightly shaken to dislodge microscopic, colloidal bubbles in the solution. After the five minute wait, the cuvette was again zeroed to the isotonic buffer and readings taken. This was repeated for 10 mg, 20 mg and 30 mg/10 mL concentrations.

Results: The absorbance of cytochrome *c* at 550 nm is indicative of the reduced version of the protein. A plot of the absorbance at 550 nm versus the concentration of Active H added created the following graph:



Whereas the equation for the linear trend line is $y = 0.0056x + 0.0206$ with a $R^2 = 0.9969$. These results are in virtual perfect correlation with Beer's law of absorbance spectroscopy and indicate a consistent trend in cytochrome *c* reduction.

Discussion and Significance: These results clearly demonstrate the *in vitro* ability of the newly formulated Active H to reduce the cytochrome *c* protein. This reduced state is invaluable in the body for everything from the enzymatic control of reactions, to overall kinetics to the prime function of the protein: as an electron regulator in the electron transport chain. Subsequent iterations of this experiment indicate a full, stoichiometric relationship between the amount of Active H added and the amount of cytochrome *c* reduced. Adding sodium hydrosulfite to the cytochrome *c*/ Active H solution would fully reduce any remaining oxidized protein in the mixture. For each iteration of the experiment, it was noted that any sodium hydrosulfite added did not increase the absorbance at 550 nm, therefore indicating that the Active H fully reduced the cytochrome *c*.

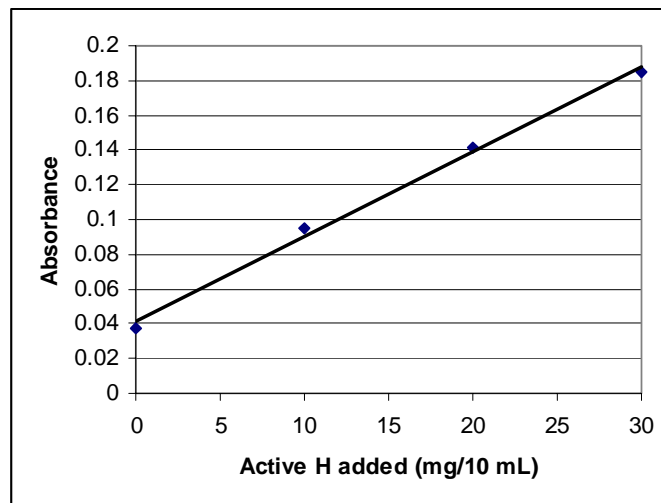
Test 5: Oxyhemoglobin Characterization

Purpose: In human erythrocytes (Red Blood Cells), hemoglobin is the predominant Fe (iron) protein that acts to transport oxygen through the body. While hemoglobin is carrying oxygen, it is referred to oxyhemoglobin and conversely, deoxyhemoglobin when it has delivered its

oxygen. Whether or not a hemoglobin molecule has attached oxygen or not is redox controlled though intracellular signaling using the ORP potentials as its means of communication. These tests measured the differences in increased oxyhemoglobin when in the presence of Active H.

Materials and Methods: In a 1 cm path length cuvette, 1.6 mL of the 0.1M isotonic phosphate buffer with citrate and EDTA was mixed with 0.10 mL of type O, human erythrocytes. Standard additions of Active H were prepared in 0.10, 0.20 and 0.30 mg/mL concentrations in isotonic buffer. A baseline reading was taken from 500 nm to 600 nm and zeroed to a phosphate buffer blank. All of the readings were taken 15 minutes after the addition of the Active H compound.

Results: The data from the oxyhemoglobin maxima of 576 nm plotted as a function of the concentration of Active H added provide the following graph:



This correlation of data is consistent with all other analyses and has a linear trend with a regression equation of $y = 0.0049x + 0.0413$ with a correlation coefficient of $R^2 = 0.9963$.

Discussion and Significance: The ability to control the redox initiated uptake of molecular oxygen in blood is an interesting characteristic demonstrated by the Active H. It is postulated that as higher concentrations of Active H are added to the mixture and as the pO₂ pressure increases that the rate of oxygenation of hemoglobin will slow down, as the relationship is sigmoidal overall and linear only in regions of low concentration. The benefit and resultant

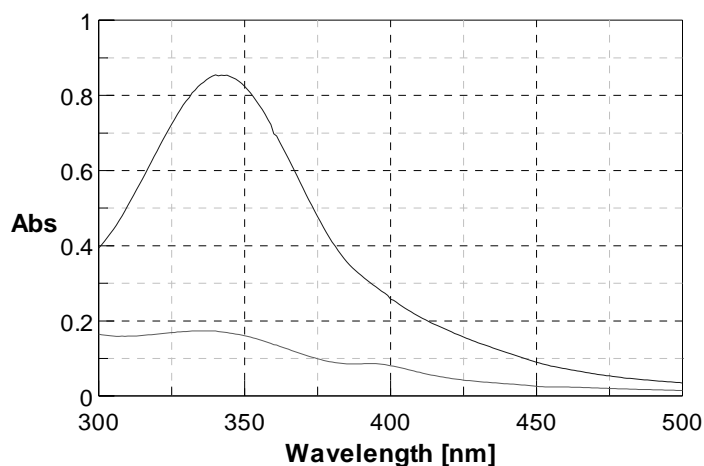
postulative effect of this characteristic is that more blood flow will be carried with less energy to the far regions of the body.

Test 6: Nicotinamide Adenine Dinucleotide Reduction Analysis

Purpose: This analysis served to study the relationship between Active H and the reduction of the coenzyme nicotinamide adenine dinucleotide (NAD^+). When NAD^+ is reduced, it is considered to be in the NADH form which is the internal energy production enzyme that is responsible for metabolic activity, including ATP production, the allosteric control of other enzymes and the redox regulation of the Krebs's Cycle and the electron transport chain in mitochondria. The ability of a compound to reduce NAD^+ to the NADH form is very important physiologically as the higher the NADH concentration that is created, the more energy produced by the organism. Thus, the reduction ability is directly correlated to increased biochemical activity, namely energy.

Materials and Methods: Absorbance spectroscopy was once again used to monitor the amount of reduced versus oxidized NAD in an *in vitro* sample. Spectra acquired were taken from 300 nm to 500 nm. 21.2 mg of beta- NAD^+ sodium salt was dissolved in 25 mL of isotonic phosphate buffer. A reference spectrum was taken blanked to phosphate buffer. 10.0 mg of Active H was added to the NAD^+ solution, inverted to assure homogeneous mixing, then scanned with the same parameters as the reference spectrum.

Results: The coenzyme NAD^+ does not absorb light in the visible region. The reduced form, NADH, absorbs significantly in several regions, particularly at 340 nm. The spectrum obtained for this experiment is detailed below:



Where the reduced NADH is significantly more concentrated by at least a 4 fold increase. Each iteration and replicate of this experiment provided similar results.

Discussion and Significance: The ability of a compound to reduce the coenzymatic NAD^+/NADH redox pair provides the essentials internally for the body to operate optimized and efficiently. Contiguous to the increase of NADH comes increased purine metabolite concentrations, including ATP, ADP, AMP, and more. On a molar basis, the new and old formulations are consistent with the reduction of NAD^+ to NADH. The new formulation maintains the reduction more stabilized over time.

Experimentation Summary

All of the experimentation on the new formulation of Active H provides consistent and significant evidence that Active H, more than ever, plays in integral role in our health. The initial tests characterize the redox properties of Active H and the levels of redox obtained. The next experiments use the foundation of the redox control as mechanistic explanations for the increases in oxyhemoglobin, the reduction of cytochrome c and NAD^+ . All of these evidentiary pieces provide the objective support of the profound biochemical abilities of the Active H compound.

More studies, including in vivo clinical studies are currently in development and underway to further characterize and truly understand the mechanism and reactions of the magnesium hydride carbonate in Active H.

Appendix 1: Nernst and rH

Since ORP alone is not indicative of the true reducing power of a compound due to proton interactions from changes in pH, a variation of the Nernst equation (1) proves to be an effective means to measure the reducing potential for a compound that is reported in units of rH, a logarithmic scaled report denoting absolute reducing potential.

$$E_h = 1.23 - \frac{RT}{F} \text{pH} - \frac{RT}{4F} \ln \frac{1}{P_o} \quad (1)$$

Where E_h is the measured oxidation-reduction potential, F is the Faraday constant, R is the universal gas constant and T is absolute temperature. The value of 1.23 accounts for potential of oxygen under one atmosphere being 1.23V greater than in a solution of the same pH. rH is defined explicitly as the negative logarithm of the oxygen pressure, P_o (2).

$$\text{rH} = -\log P_o \quad (2)$$

Active H has been shown to have an rH value as low as -2.75, a profound result indicating its ability to be a more effective reducing agent than other compounds. Comparatively, magnesium silica hydride maintains a greater reducing potential thousands of times greater than vitamin C (rH 23), ubiquinone (rH 19) and beta-carotene (rH 26).