

H2 Analytics 2505 Anthem Village Dr. Ste E385 Henderson, NV 89052

Laboratory Report

Introduction This report summarizes the analysis of the H2 Dent Care tablet manufactured & distributed by H2 World Health & Beauty Co., Ostrava, Czech Republic, as conducted by H2 Analytics, Henderson, NV. These tests were requested by Natural Wellness Now Health Products Inc., Coquitlam, BC. The purpose of the analysis was:

- 1) Determine the amount of hydrogen gas (H₂) produced by a single tablet when crushed into a powder and immersed in human saliva for 1 minute & 3 minutes (to simulate chewing the tablet) measured using gas chromatography and static headspace analysis (HS-GC).
- 2) Determine the *maximum* amount of hydrogen gas (H₂) that can be produced by a single tablet when crushed into a powder and placed into an acid solution measured using a water displacement apparatus and HS-GC.

Product Description

The product name is H2 Dent Care, lot # 2043601, date code 30112022. The product was received for testing 5/17/2021. According to the manufacturer, when placed into the mouth and chewed, the tablet releases hydrogen gas (H₂), a therapeutic agent. The client sent one box containing 60 tablets (box contains two blister packs, each containing 30 tablets). One tablet measures 9.25 mm (dia.) x 3.8 mm (ht.), weighs 290 mg, and is light-gray in color. The tablet contains elemental magnesium (Mg) that produces hydrogen gas when it comes in contact with water (Mg + $2H_2O \Rightarrow Mg(OH)_2 + H_2$).

Test Equipment and Conditions

Instrument: SRI 8610C gas chromatograph, Hayesep-D 6M packed column, Tungsten-Rhenium TCD; nitrogen carrier gas (99.999%) used for all measurements; column pressure, 20 mL/min @ 20 psi; column/detector oven temperature, 80°C; GC temperature permitted to stabilize for 90 minutes before calibration and testing.

GC test method: static headspace (HS-GC)

Headspace equilibrator: H2 Analytics HA-1001, 60 rpm; equilibration time, 5 min;

GC calibration; 3-point calibration curve @ 250, 500 & 1000 ppm using 1000 ppm precision H₂ gas (Gasco, Oldsmar, FL); calibration performed on the day of testing. In-house software used to convert headspace concentrations to H₂ milligrams.

Syringe: Hamilton #1001, 1000 uL gastight, 1" x 27 ga Luer-Lock

Temperature/pH meter: Oakton pH 6+ meter w/temperature probe; 3-point calibration @ 4.0, 7.0, 10.0

Headspace vials: Sigma-Aldrich, 40 mL VOA borosilicate w/septum cap; generic 518 mL polycarbonate w/septum port

Gas-evolution apparatus - Water displacement using 150 mL flask w/stopper, 250 mL graduated beaker, and associated vinyl tubing; displacement water: distilled (generic); reaction water: 20 mL acetic acid/distilled water (1:1)

Acetic acid (distilled white vinegar), generic source, 5%; distilled water, generic source

Acetic acid/distilled solution (1:1): pH = 2.25

Human saliva: Collected on the day of testing; pH = 6.78

Laboratory temperature: 25°C ± 1°C, RH - 16%; elevation = 864M, measurements adjusted to SATP.

Note: 1) To avoid causing surface oxidation, nitrile gloves were worn when handling tablets. 2) For each requested test, three tests were performed, and the mean & standard deviation was calculated.

Test Section

Test 1: Measure H₂ production using HS-GC when immersing one crushed tablet in human saliva contained within a headspace vial and allowed to react for 1 minute and 3 minutes.

Description: On the day of testing, human saliva was collected in a borosilicate beaker. For each test, 5 mL of saliva was placed into the headspace vial and one tablet was removed from the blister pack and crushed into a fine powder using a mortar & pestle apparatus. The powder was then poured into the vial and the septum cap immediately closed. The mixture was shaken and allowed to react for the specified time (either 1 or 3 minutes). After the specified reaction time, the headspace was sampled, injected into the GC for analysis, and the results recorded.

Results: H₂ Production: 1 min: Mean 0.06 mg (0.03 mmol, 0.73 mL); SD 0.03

3 min: Mean 0.12 mg (0.06 mmol, 1.44 mL); SD 0.03

Test 2: Measure H₂ production when immersing one crushed tablet into an acidic solution until the hydrogen evolution reaction goes to completion using both water displacement and HS-GC methods.

Notes: 1) Due to a phenomenon called passivation, hydroxide produced during the hydrogen evolution reaction can collect on the surface of the magnesium, slowing or even stopping the reaction. Because hydroxide is more soluble in an acidic environment, an acid-water solution ensures the maximum production of hydrogen by preventing passivation.

2) Prior to testing, it was determined that, when placing a crushed tablet into an acidic solution of one part distilled water and one part acetic acid, the reaction producing the H₂ gas would go to completion after approximately two minutes. Therefore, in order to ensure that the hydrogen evolution reaction had gone to completion, all tests measuring the maximum production of H₂ gas using acid were allowed to proceed for five minutes before measuring the production of H₂ gas.

Description:

(Water-displacement method): The water displacement apparatus was prepared by filling a 1000 mL graduated cylinder with distilled water and placing it upside down into a second 500 mL beaker containing 300 mL of distilled water and held by a laboratory stand. A stopper was connected to a ¼" vinyl hose whose open end was placed into the bottom of the inverted cylinder. The acidic gas-evolution solution was prepared by mixing 10 mL of distilled water with 10 mL of acetic acid in a 150 mL flask. One tablet was crushed into powder form using a mortar/pestle, placed into the acid solution and the stopper immediately placed securely into the neck of the flask. The reaction was allowed to proceed for 5 minutes after which the amount of water displaced by the produced gas was measured and recorded. Because some H₂ gas produced by the powder dissolves into the acid solution, the dissolved H₂ concentration of the solution was also measured using HS-GC and added to the amount measured by the displacement apparatus and the results were recorded.

(HS-GC method): The acidic gas-evolution solution was prepared by mixing 2.5 mL of distilled water with 2.5 mL of acetic acid in a 518 mL polycarbonate headspace vial. One tablet was crushed into powder form using a mortar/pestle, placed into the acid solution, and the cap was immediately secured onto the vial. The reaction was allowed to proceed for 5 minutes after which the headspace was sampled and measured using HS-GC and the results were recorded.

Results:

H₂ Production (displacement): Mean 0.38 mg (0.19 mmol, 4.60 mL); SD 0.01

H₂ Production (GC): Mean 0.32 mg (0.16 mmol, 3.90 mL); SD 0.01

APPROVED *

Approved By: _____ Title: Director of Testing Report Date: 5/23/2021