

Tradename: AC Det'Ox Hair

Code: 21030

CAS #: 8013-01-2 & 68333-16-4 (or) 92128-79-5

Test Request Form #: 13295

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

Study Director: *Daniel Shill*

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Test Performed:

Copper Chelation Assay

Introduction

Hard water contains heavy metals which are deposited on hair during washing. Deposition and long-term buildup of these harmful metals results in structural damage of the hair follicle leading to unmanageable, brittle hair. Copper is a key constituent in hard water and causes damage to the cuticle and breakdown of protein within the hair while accelerating the formation of free radicals and ROS. It is also known to discolor hair, often resulting in a green tint on lighter hair types. Chelating products aim to neutralize and remove these metals by reacting with them so they can be washed away. Therefore, cosmetic products designed to chelate metals such as copper are critical in hair care formulations.

Accordingly, a Copper Chelation Assay was performed to demonstrate the ability of **AC Det'Ox Hair** to chelate copper.

Assay Principle

Fourier-Transform Infrared (FT-IR) Spectroscopy and UV-Vis Spectroscopy were used to identify copper chelation by a cosmetic ligand. The spectra of a pure cosmetic ligand, a pure copper solution, and a mixture of the two were obtained to establish chelation occurred. Shifts in FT-IR absorption intensity and wavenumber signify specific changes in molecular bonds while shifts in UV-Vis absorbance signify changes in electronic structure. To further substantiate copper chelation, pH was monitored as copper solution was added to the cosmetic solution. A reduction in pH indicates a reaction has taken place. Together these three parameters confirm copper chelation in a solution.

Materials

- A. Equipment:** Nicolet iS5 FT-IR Spectrophotometer; iD5 ATR Accessory; Shimadzu UV-1800 UV/Vis Spectrophotometer; Accumet AB200 pH /mV/conductivity probe (Fisher)
- B. Reagents:** Copper AA Standard, 1000 ppm (LabChem, LC134007)*; Deionized Water
- C. Other:** Pipettes*

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Three solutions were prepared as follows, 100% **AC Det'Ox Hair**, 100% Copper Standard (1000 ppm), and a 1:1 mixture of **AC Det'Ox Hair** and Copper Standard. The initial pH of **AC Det'Ox Hair** was taken, and changes were monitored after each quarter addition of Copper Solution until the 1:1 ratio was achieved. Following thorough mixing, 1 mL of each solution was dried for one hour at 105°C to remove all water. The dry extracts were analyzed on the FT-IR spectrometer. Finally, the three solutions were further diluted with deionized (DI) water to 0.002% concentrations and analyzed on the UV/Vis Spectrophotometer. Data displayed is representative of all **AC Det'Ox Hair** lots.

To provide an indication of copper chelated by **AC Det'Ox Hair**, area under the curve (AUC) was calculated using the UV/Vis data by adding the areas under the line between each consecutive absorbance values using the following equation:

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (\lambda_{i+1} - \lambda_i) (Abs_i + Abs_{i+1})$$

For absorbance values Abs_1 and Abs_2 at wavelengths λ_1 and λ_2 , the AUC between those two time points is equivalent to the product of difference in time and the average of the two absorbance values. The amount of copper chelated was determined by dividing the AUC values for the Copper Solution by the 1:1 **AC Det'Ox Hair**: Copper Solution.

Results

The data obtained met criteria for a valid assay. **AC Det'Ox Hair** chelated copper ions present in aqueous solution.

Table 1. Solution pH after stepwise additions of Copper Solution.

Dilution (%)	Measured pH
100% AC Det'Ox Hair : 0% Copper Solution	6.49
87.5% AC Det'Ox Hair : 12.5% Copper Solution	4.67
75% AC Det'Ox Hair : 25% Copper Solution	3.58
62.5% AC Det'Ox Hair : 37.5% Copper Solution	2.98
50% AC Det'Ox Hair : 50% Copper Solution	2.58

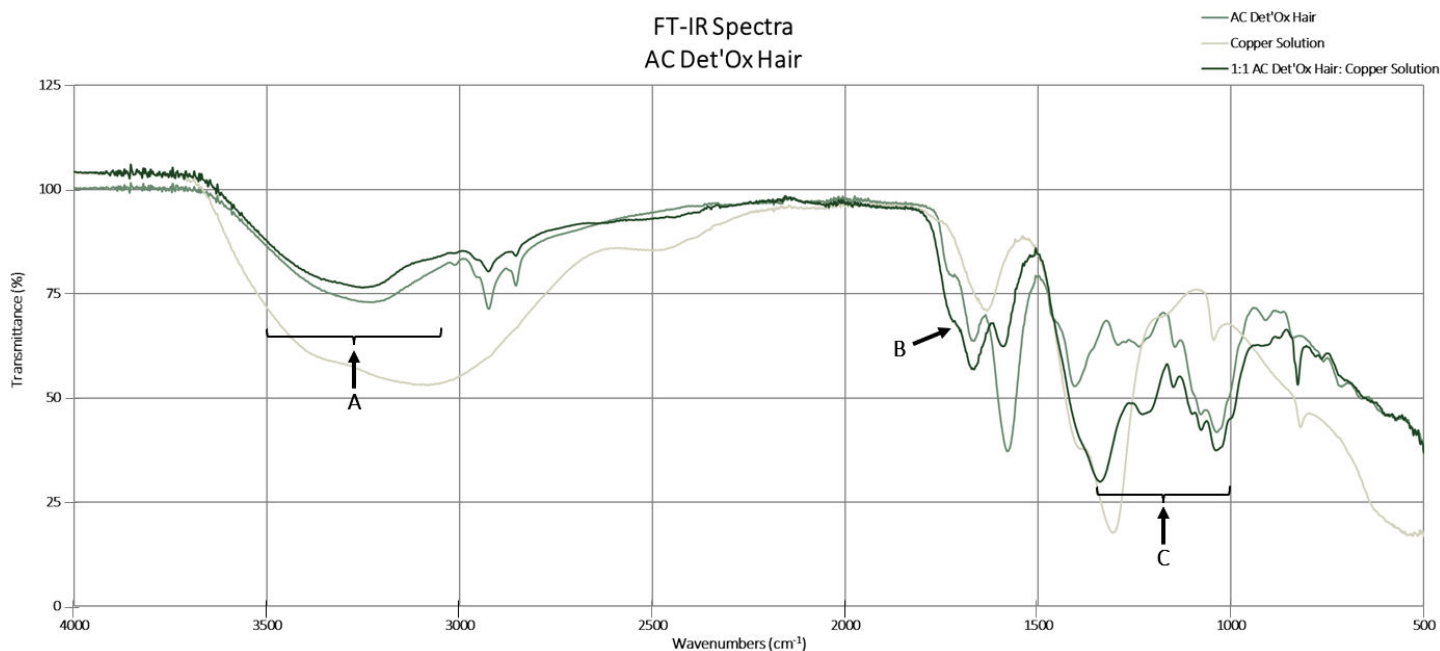


Figure 1. FT-IR Spectra of dried extracts. A) O-H Region (~3200-3500 cm⁻¹). B) C=O Peak (~1700 cm⁻¹). C) Fingerprint Region (~1300-1000 cm⁻¹).

UV-Vis Spectra AC Det'Ox Hair

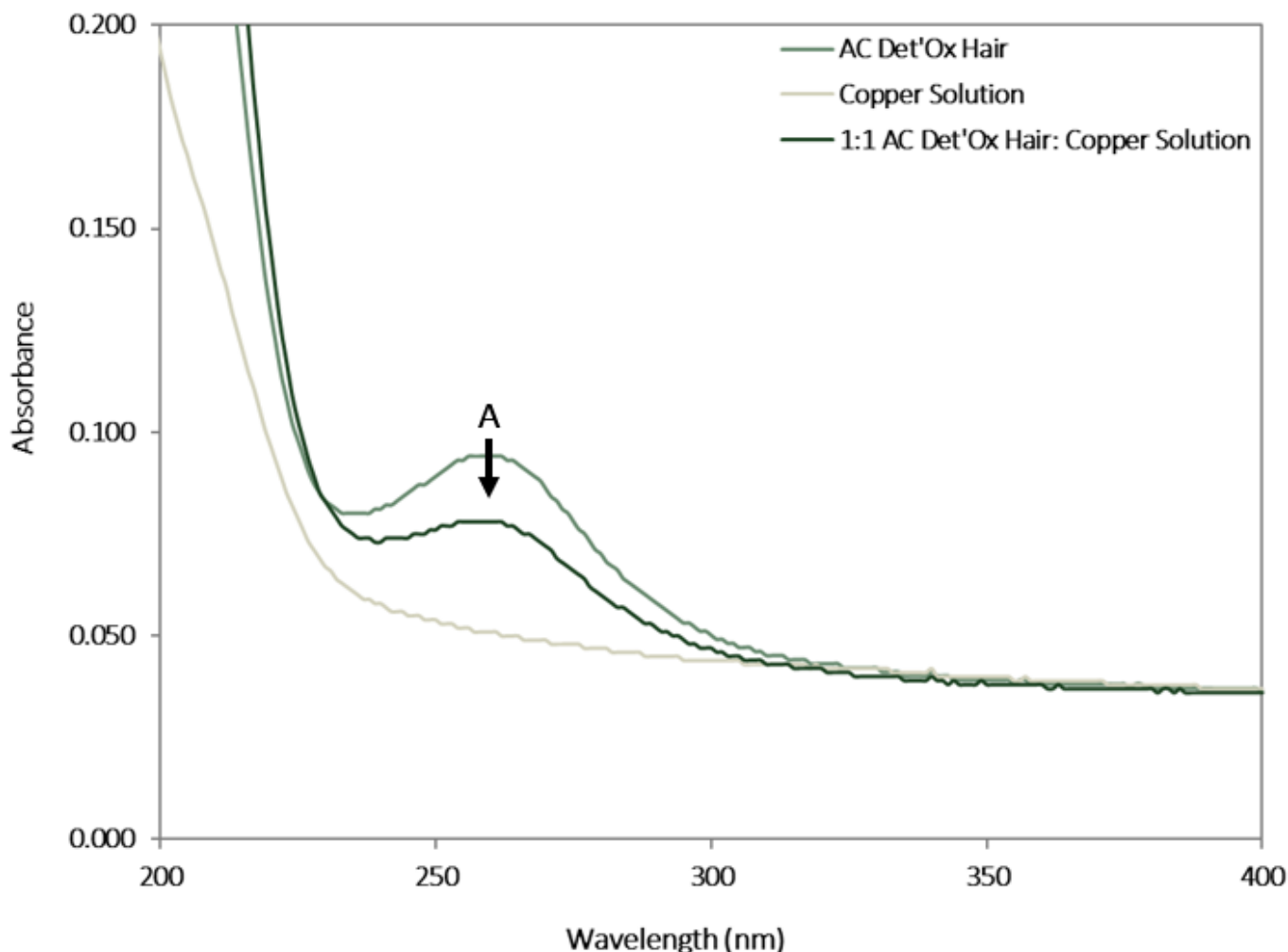


Figure 2. UV-Vis Spectra of 500x diluted solutions. **AC Det'Ox Hair**, **Copper Solution**, and **1:1 AC Det'Ox Hair: Copper Solution**. A) Reduced peak intensity indicative of copper chelation.

Discussion

The copper chelating ability of **AC Det'Ox Hair** was evaluated and confirmed. As demonstrated in Table 1, pH decreased as the copper solution was added to **AC Det'Ox Hair** indicating a reaction was occurring. FT-IR spectroscopy confirmed several key molecular changes after the reaction. The O-H Region ($\sim 3200\text{-}3500\text{ cm}^{-1}$) exhibited a broadening and shifting indicative of coordination between hydroxyl groups and copper ions (Figure 1A). Moreover, the prominent C=O peak around 1700 cm^{-1} shifts to a lower wavenumber, a classic sign of metal chelation with a carboxyl group (Figure 1B). Finally, altered intensities and slight shifts in the Fingerprint Region ($\sim 1300\text{-}1000\text{ cm}^{-1}$) are indicative of C-O stretching and ring vibrations consistent with the formation of a metal complex (Figure 1C). With respect to UV-Vis Spectra, Figure 2 further confirms chelation via a reduction in intensity of the peak at 250-300 nm from pure **AC Det'Ox Hair** to the 1:1 mixed solution. Utilizing AUC calculations, **AC Det'Ox Hair** chelated 46% of the copper present in solution.

Taken together, these data indicate **AC Det'Ox Hair** chelates copper ions in solution.