Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: AC Melatonin Liposome SF

Code: 61012

CAS #: 7732-18-5 & 123465-35-0 & 73-31-4

Test Request Form #: 5981

Lot #: N200114B

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Marueen Danaher*
Principal Investigator: *Michael Hovis*

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC)

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are dangerous to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Accordingly, the Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **AC Melatonin Liposome SF**.

Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox® equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

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Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instruments, Winooski, VT); Pipettes
B. Buffers:	75 mM Potassium Phosphate (pH 7.4); Deionized H_2O
C. Reagents:	2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) (153 mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4 nM)
D. Preparation:	Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH
E. Microplate:	Corning 96 Well Black Side/Clear Bottom Microplates
F. Software:	Excel Analysis ToolPack (Microsoft)

Methods

Solutions of **AC Melatonin Liposome SF** and Trolox® (positive control) were prepared in 75 mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at concentrations ranging from 12.5 µM to 200 µM in 75 mM potassium phosphate buffer.

For the assay, 25 µL of test material and Trolox® were combined with 150 µL of fluorescein in 75 mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37˚C for 30 minutes. At the end of the incubation period, 25 µL of AAPH (153 mM prepared in 75 mM potassium phosphate buffer) were added to each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 480 nm and an emissions wavelength of 520 nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software (BioTek Instruments) using the below equations:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample's Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro molar Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE. Assays were repeated three separate times with each sample run in duplicate. Duplicates for each replicate were averaged, and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

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Results

The data obtained from this study met criteria for a valid assay. **AC Melatonin Liposome SF** showed very potent antioxidant activity at 1.25% concentration. The ORAC value for 1.25% **AC Melatonin Liposome SF** is 182 µMTE.

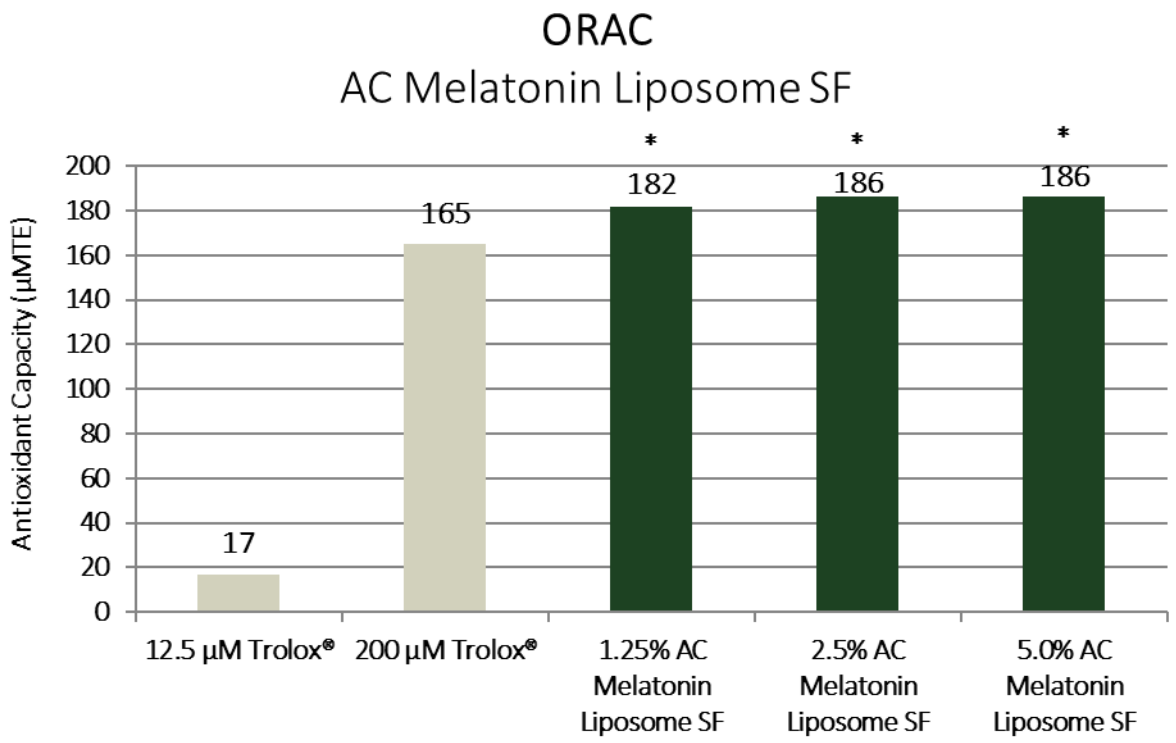

Figure 1. Antioxidant capacity of **AC Melatonin Liposome SF**. * indicates significance ($p \leq 0.05$) compared to 200 µM Trolox®.

Table 1. Results from one-way ANOVA Statistical Analysis Compared to 200 µM Trolox®. * indicates significance ($p \leq 0.05$) compared to 200 µM Trolox®.

	1.25% **AC Melatonin Liposome SF**	2.5% **AC Melatonin Liposome SF**	5.0% **AC Melatonin Liposome SF**
P-value	0.019*	0.018*	0.021*

Discussion

As shown in Figure 1, **AC Melatonin Liposome SF** exhibited greater antioxidant activity at all use levels than 200 µM Trolox®. The antioxidant capacity of **AC Melatonin Liposome SF** increased as the concentration increased, indicating that the ability to minimize oxidative stress is dose dependent. Maximizing the antioxidant capacity on a cellular level allows for ROS to be dealt with at a rate that provides protection from cellular damage. This cellular damage can be seen as physical signs of aging such as wrinkles, loss of elasticity, unwanted pigmentation, and skin unevenness with slow regeneration.

In summary, **AC Melatonin Liposome SF** is capable of providing antioxidant properties and aids in the anti-aging process through protection at the cellular level.