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Competition of Fe^{3+} UV-Vis Absorption between Ascorbic Acid (AA) and Clofibric Acid (CA)

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Research Article

Abstract

In this short communication, we are showing experimental evidence that Fe^{3+}-Clofibric Acid (CA) complex has the ability to mediate the oxidation of a physiological substance, Ascorbic Acid (AA), using an established protocol. The change in UV-Vis absorption of AA at 265 nm was monitored (after 2 min) as a function of change of the concentration of CA. It also appeared that, over a period of 30 min and 60 min there was further decrease in the overall absorption of the ferric-clofibrate-ascorbate complexes. Further detailed studies are needed in this area.

Keywords: Fe^{3+}; Clofibric Acid (CA); Ascorbic Acid (AA); UV-Vis absorption spectroscopy.

1. Introduction

This short commentary is a part of the special issue of the role of metal ions in biological systems in particular those of the first series of the transition metals in the periodic table. Many researchers have studied the decomposition of AA in the presence of many metal ions which lead to the formation of a polymeric metal oxalate species with peculiar structural features [1]. Others studied the kinetic of AA oxidation using different chemicals and/or metal ions [2-7]. The nature of AA transformation depends on two factors, (1) pH-values, and (2) the type of metal ion involved as it was reviewed extensively by Davies [8].

For example, Martell and Khan have studied the kinetics of the decomposition of AA with many metal complexes such as the cupric (Cu^{2+}), the ferric (Fe^{3+}), the vanadyl (VO^{2+}), and the uranyl (UO_2^{2+}) ions with many numbers of chelating ligands such as 1,10-phnanthroline, ethylene diamine tetra-acetic acid (EDTA), *trans*-1,2-diaminocyclohexametetraacetic acid (CDTA), and di-ethylene-tri-amine-penta-acetic acid (DTPA) among other ligands [4-7]. The most comprehensive metal ion stability constant data base by Martell and Smith published by National Institute of Standard and Technology (NIST) does not contain any data for the reaction of CA with any metal ion [9]. The lack of detailed studies (theoretical or otherwise experimental, *in vitro* or *in vivo*) for the reaction of CA with metal ions was one of the reasons that prompted us to take the initiative to study the reactions of CA with a many of metal ions [10,11].

Here, we are showing a very simple UV-Vis experimental setup (*in vitro*) in which we have scanned the mixture of Fe^{3+}:CA:AA from 250 nm to 350 nm. The reason for the choice of this scanning window is the fact that AA, CA and Fe^{3+} do not have measurable absorption beyond these window (near UV-Vis, Visible, nor the far UV-Vis, nor the near IR region of the spectrum) Also, we have measured the UV-Vis spectra at a constant value of 265 nm which is the maximum value for ascorbic acid absorption.

2. Methods

2.1 Chemicals

Clofibric acid (CA) [$C_{10}H_{11}ClO_3$] Formula weight (F. Wt.)=214.6 $gmol^{-1}$ and iron nitrate nona-hydrate [$Fe(NO_3)_3 \bullet 9H_2O$] F. Wt.=404.0 $gmol^{-1}$ were from Sigma Aldrich (St. Louis, MO 63178 USA). L(+)Ascorbic Acid (AA) [$C_6H_8O_6$] F. Wt.=176.13 $gmol^{-1}$ was purchased from Acros (New Jersey, USA). All solutions were prepared using doubly deionized water (D.I. H_2O). All glassware (beakers, volumetric flasks and cuvettes) were soaked in acid bath for extended period of time. Glassware was washed three times with D.I. H_2O before any use or any solution preparation.

2.2 UV-Vis spectroscopy

All UV-Vis spectroscopic measurements were conducted using a T60 high-performance spectrophotometer in connection with UVWIN software version 5.0, (Advanced ChemTech, Louisville, KY). Samples were prepared in D.I. H_2O at 25°C. The UV-Vis spectra were scanned from 250 nm to 350 nm in quartz cuvettes. The concentration of the Fe^{3+} was fixed at 10 µM which is $=10.0 \times 10^{-6}$ $mol.L^{-1}$. The concentration of AA was fixed at 99 µM which is $=99.0 \times 10^{-6}$ $mol.L^{-1}$. The concentration of CA was variable as shown in Table 1 and Figure 1.

To reach the above mentioned concentrations, the three components (Fe^{3+}, AA and CA) were mixed in the following order: To a series of five equal volumes 250 mL volumetric flasks; 10 mL, 20 mL, 30 mL, 40

mL and 50 mL of 1.835 × 10^{-3} mol.L^{-1} of CA aqueous solutions were added first followed by the addition of 50 µL of 50 mM $Fe(NO_3)_3$ solution to each volumetric flask, followed finally, with the addition of 10 mL of AA to each volumetric flask.

3. Results

The UV-Vis spectra scans were collected instantaneously (after 2 min, this is the fastest time we can mix and arrange all cuvettes in the UV-Vis spectrophotometer compartment) by mixing the above mentioned reagents in the order mentioned above. Table 1 shows the detailed amounts of every chemical added to the reaction mixture. Absorption values of the spectra were scanned from 250 nm to 350 nm. To reach the $Fe(NO_3)_3$ concentration of (10 µM) and L-AA concentration of (99 µM); 50 µL of 50 mM $Fe(NO_3)_3$ solution was added to each volumetric flask, followed by the addition of 10 mL of 2.5 mM AA to each volumetric flask. All solutions were freshly prepared prior to the experiment and were incubated at room temperature.

Table 1: Detailed amounts of every chemical added to the reaction mixture. Absorption values of Ascorbic Acid (AA) were taken at 265 nm with the change of the concentration of Clofibric acid.

Vol. of CA (mL)	Concentration of Clofibric acid (µM)	Absorbance at 265 nm after 2 min (taken 3 times)
10.0 mL	89 µM	0.247 ± 0.011
20.0 mL	147 µM	0.231 ± 0.003
30.0 mL	220 µM	0.320 ± 0.003
40.0 mL	294 µM	0.342 ± 0.001
50 mL	366 µM	0.392 ± 0.001

To reach the $Fe(NO_3)_3$ concentration of (10 µM) and L-AA concentration of (99 µM); 50 µL of 50 mM $Fe(NO_3)_3$ solution was added to each volumetric flask, followed by the addition of 10 mL of 2.5 mM AA to each volumetric flask

In another UV-Vis spectrum setup, the UV-Vis spectrum was measured at a constant value of 265 nm (this is the maximum value for ascorbic acid absorption). The ability of the iron complexes of CA to mediate the oxidation of a physiological substance, AA, was further examined by observing the increase in the absorption value as a function of concentration as shown in Figure 2. Figure 2 shows the changes in the concentrations of L-AA, that of the ferric ion solution Fe^{3+} and that of CA were as a function of the Absorbance at the constant value of 265 nm. Both of the above mentioned UV-Vis experiments were done by using quartz cuvettes with optical path length of 1 cm.

Table 2 is showing the correlations of the net absorbance of the reaction mixture over an hour after mixing. After 2, 30 and 60 min, respectively the values of the overall reaction mixtures (AA, Fe^{3+} and CA) appeared to be decreasing over time. This indicates the decomposition of the formed complexes, or perhaps the loss of the AA to its absorption value due to its oxidation.

Table 2: The absorbance of all reagents mixed in this experiment at 265 nm; the absorbance values are decreasing over time.

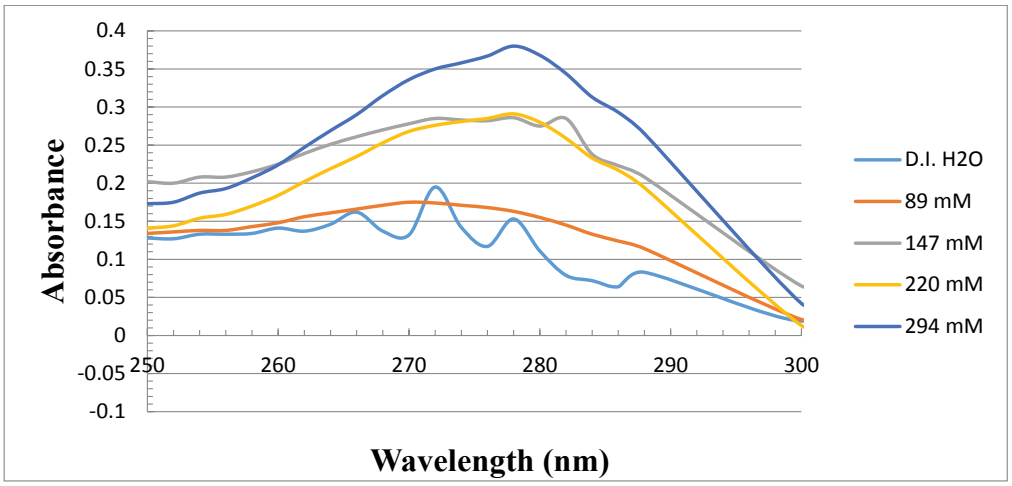
	CA concentration (µM)	Absorbance (after 2 min, taken 3 times)	After 30 min	After 60 min
1	89	0.247 ± 0.011	0.200	0.192
2	147	0.231 ± 0.003	0.229	0.216
3	220	0.320 ± 0.003	0.317	0.303
4	294	0.342 ± 0.001	0.336	0.325
5	366	0.392 ± 0.001	0.377	0.366

The absorbance were measured at 2 min, after 30 min passed and after 60 min passed on solution preparations

We have shown in a separate study, in the special issue of the electronic journal of biology (eJBio), that the maximum absorption of CA appeared at the range of 275 nm to 278 nm as shown in Figure 1. Also, the maximum absorption of Fe^{3+} appeared at the range of 300 nm to 305 nm. It is known from literature that the maximum absorption of AA appeared at 265. For that above mentioned reasons, there is a clear overlap between the absorptions of both AA and that of CA. Further kinetic and detailed experimental setups are needed in this area to further monitor the decomposition of ascorbic acid.

4. Discussion

Herein, we are showing in Figure 3 the predicted position of in binding of Fe^{3+} to both AA and that of CA. the curved arrows are showing the possible chelating sites. There are three chelating sites on AA vs. one chelating site on CA. The change of the net and overall UV-Vis absorption of (Fe^{3+}:CA:AA) reaction mixture was scanned and monitored from 250 nm to 350 nm. Also in a separate experiment, we monitored the changes of the absorbance(s) with the changes in reagent's concentrations at the maximum value of 265 nm (265 nm is maximum absorption of that of AA). We are showing a new experimental evidence (*in vitro*) that there is tangible changes in



Fe^{3+}=10 µM, Ascorbic acid=99 µM.
The concentration of Clofibric acid was varied with the following values: 0 µM (control), 89 µM, 147 µM, 220 µM and 294 µM

Figure 1. UV-Visible absorption spectra of Fe^{3+}:Clofibric acid:Ascorbic acid.

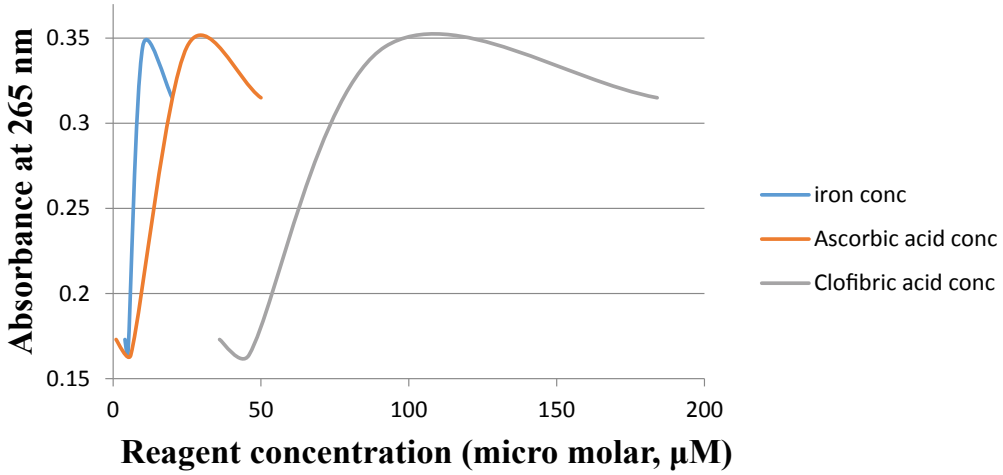


Figure 2. Correlation of the concentrations in micro-molar units (µM) of all species involved in this experiment with the maximum absorption of Ascorbic acid at 265 nm.

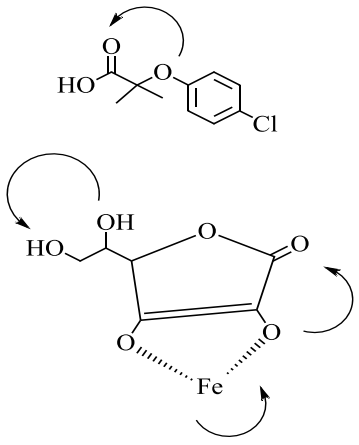


Figure 3. Proposed chelating sites of the ferric ion (Fe^{3+}) to both ligands studied in this article, AA and CA.

the Ultraviolet-Visible absorption spectroscopy for the reaction mixture of CA-Fe^{3+} and the biologically known AA ligand. One might predict form the decrease in the net absorption of the reaction mixture as over time as shown in Figure 3 the decomposition of AA to de-hydroascorbate as it is known as one of its decomposition by products. Figure 3 is showing the possibilities of the binding of Fe^{3+} with both AA and CA ligands. Further experimental (both *in vitro* and *in vivo* works) and theoretical calculations are needed in this area which are underway in our lab to enhance the understanding of this reaction system.

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