Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60045-8

Alteration of chemical behavior of L-ascorbic acid in combination with nickel sulfate at different pH solutions *in vitro*

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ARTICLE INFO

Article history:
Received 15 August 2011
Received in revised form 7 September 2011
Accepted 28 September 2011
Available online 28 March 2012

Keywords:
L-ascorbic acid
Nickel
pH
Spectrum
Chemical behavior
Spectra
UV visible spectrophotometer
Peak wavelength
Free radical
Vitamin C
Nickel sulfate

ABSTRACT

Objective: To evaluate the alteration of chemical behavior of L-ascorbic acid (vitamin C) with metal ion (nickel) at different pH solutions *in vitro*. **Methods:** Spectra of pure aqueous solution of L-ascorbic acid (E mark) compound and $NiSO_4$ (H_2O) (sigma USA) were evaluated by UV visible spectrophotometer. Spectral analysis of L-ascorbic acid and nickel at various pH (2.0, 7.0, 7.4 and 8.6) at room temperature of 29 ℃ was recorded. In this special analysis, combined solution of L-ascorbic acid and nickel sulfate at different pH was also recorded. **Results:** The result revealed that λ_{max} (peak wavelength of spectra) of L-ascorbic acid at pH 2.0 was 289.0 nm whereas at neutral pH 7.0, λ_{max} was 295.4 nm. In alkaline pH 8.6, λ_{max} was 295.4 nm and at pH 7.4 the λ_{max} of L-ascorbic acid remained the same as 295.4 nm. Nickel solution at acidic pH 2.0 was 394.5 nm, whereas at neutral pH 7.0 and pH 7.4 were the same as 394.5 nm. But at alkaline pH 8.6, λ_{max} value of nickel sulfate became 392.0 nm. The combined solution of L-ascorbic acid and nickel sulfate (6 mg/mL each) at pH 2.0 showed 292.5 nm and 392.5 nm, respectively whereas at pH 7.0, L-ascorbic acid showed 296.5 nm and nickel sulfate showed 391.5 nm. At pH 7.4, L-ascorbic acid showed 297.0 nm and nickel sulfate showed 394.0 nm in the combined solution whereas at pH 8.6 (alkaline) L-ascorbic acid and nickel sulfate were showing 297.0 and 393.5 nm, respectively. **Conclusions:** Results clearly indicate an altered chemical behavior of L-ascorbic acid either alone or in combination with nickel sulfate *in vitro* at different pH. Perhaps oxidation of L-ascorbic acid to L-dehydro ascorbic acid *via* the free radical (HSc*) generation from the reaction of H_2ASc + Ni (II) is the cause of such alteration of λ_{max} value of L-ascorbic acid in the presence of metal nickel.

1. Introduction

Nickel is a metallic element belonging to group VIIIb of the periodic table. It is resistant to alkalis, but generally dissolves in dilute oxidizing acids. Nickel carbonate, nickel sulfide, and nickel oxide are insoluble in water, whereas nickel chloride, nickel sulfate, and nickel nitrate are water soluble. The prevalent ionic form is nickel (II). In biological systems, dissolved nickel may form complex components with various ligands and bind to organic material. It is a ubiquitous trace metal and occurs in soil, water, air, and in the biosphere. Levels in natural waters have been found to range from 2 to 10 μg/L (fresh water) and from 0.2 to 0.7 μg/L (marine). Atmospheric nickel concentrations in remote areas range from <0.1 to 3 ng/m^3[1,2]. Nickel and other heavy metals can also generate free radicals directly from molecular oxygen in a two step process to produce superoxide anion. In the continued presence of the heavy metal, the superoxide anions formed can then combine with protons in the dismutation reaction generating hydrogen peroxide in the process. Heavy metals are also able to catalyze the generation of the highly toxic hydroxyl radical from superoxide anion and hydrogen peroxide[3,4]. Vitamin C, also referred to as ascorbic acid or ascorbate, belongs to the water-soluble class of vitamins. Humans are one of the few species who lack the enzyme to convert glucose to vitamin C. Ascorbic acid (AA) is an odorless, white solid having the chemical formula $C_6H_8O_6$. The vitamin is easily oxidized to form dehydroascorbic acid (DHAA), and thus oxidation is readily reversible. Vitamin C has the ability to sequester the singlet oxygen radical, stabilize the hydroxyl radical, and regenerate reduced vitamin E back to the active state[5]. It has been observed that both vitamin C and nickel sulfate influence the functional metabolism in human virtually by oral exposure route[6]. The oral route of absorption for both vitamin C and nickel was usually processed through

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Foundation Project: This work was financially supported by Defence Institute of Physiology and Allied Sciences, Government of India, New Delhi [grant No. TC/292/TASK-116(KDS/DIPAS/2006].

different parts of gastrointestinal tract of human which consists of variation of pH microenvironment. The purpose of this study is to evaluate the interaction of L-ascorbic acid (vitamin C) with metal ion (nickel) at different pH solutions *in vitro*. The study may enlighten or extrapolate the ideas of possible interaction of nickel-ascorbic acid in different pH environment of physiological living system *in vivo*.

2. Materials and methods

Analar grade of L-ascorbic acid, $C_6H_8O_6$ (E mark) and nickel sulfate, $NiSO_4$ $6(H_2O)$ compound (Sigma, USA) were used in our experiment. L-ascorbic acid solution was prepared in measuring flask by dissolving 60 mg of L-ascorbic acid in 10 mL of triple distilled water to get 6 mg/mL concentration. Similarly nickel sulfate solution was also prepared in the same concentration (6 mg/mL).

λ_{max} (peak wavelength of spectra) and absorbance values of aqueous solution of L-ascorbic acid were recorded within the range of 280-500 nm wavelength at acidic pH 2.0, neutral pH 7.0, mild alkaline pH 7.4 (it is equivalent to plasma pH of human) and alkaline pH 8.6 at room temperature of 29 ℃ by UV-VIS spectrophotometer (SL-164, ELICO Ltd). Similarly λ_{max} (peak wavelength of spectra) and absorbance values of aqueous solution of nickel sulfate were also recorded within the range of 280-500 nm wavelength at various pH (2.0, 7.0, 7.4 and 8.6) solutions like L-ascorbic acid.

A combined solution of L-ascorbic acid and nickel sulfate was prepared by dissolving 60 mg of L-ascorbic acid and 60 mg of nickel sulfate together in 10 mL of triple distilled water. The concentration of this solution for both L-ascorbic acid and nickel sulfate was 6 mg/mL each. λ_{max} (peak wavelength of spectra) and absorbance values of this combined aqueous solution for both L-ascorbic acid and nickel sulfate were recorded at different pH (2.0, 7.0, 7.4 and 8.6) range. The pH was adjusted with standard solution of 1 N NaOH and N/10 HCl and recorded by digital pH meter (ELICO Ll 120)[7]. All the spectra were saved in a connected PC by using software 'SpectraTreats' version 2.38.1 for further analysis.

3. Results

The result revealed that λ_{max} (peak wavelength of spectra) of L-ascorbic acid at pH 2.0 was 289.0 nm whereas at neutral pH 7.0, λ_{max} was 295.4 nm. In alkaline pH 8.6, λ_{max} was 295.4 nm and at pH 7.4, the λ_{max} of L-ascorbic acid remained the same as 295.4 nm (Table 1). Nickel solution at acidic pH 2.0 the λ_{max} was 394.5 nm, whereas at neutral pH 7.0 and pH 7.4 the λ_{max} values remained unchanged *i.e.* the same as 394.5 nm. But at alkaline pH 8.6, λ_{max} value of nickel sulfate became 392.0 nm (Table 1). The combined solution of L-ascorbic acid and nickel sulfate (6 mg/mL each) at pH 2.0 showed 292.5 nm and 392.5 nm, respectively (Figure 1). Whereas at pH 7.0, λ_{max} of L-ascorbic acid showed 296.5 nm and λ_{max} of nickel sulfate showed 391.5 nm (Figure 2). At pH 7.4, λ_{max} of L-ascorbic acid and nickel sulfate showed 297.0 and 394.0 nm, respectively (Figure 3). Whereas at pH 8.6 (alkaline), L-ascorbic acid was showing 297.0 nm and nickel sulfate was showing 393.5 nm (Table 1 and Figure 4 & 5).

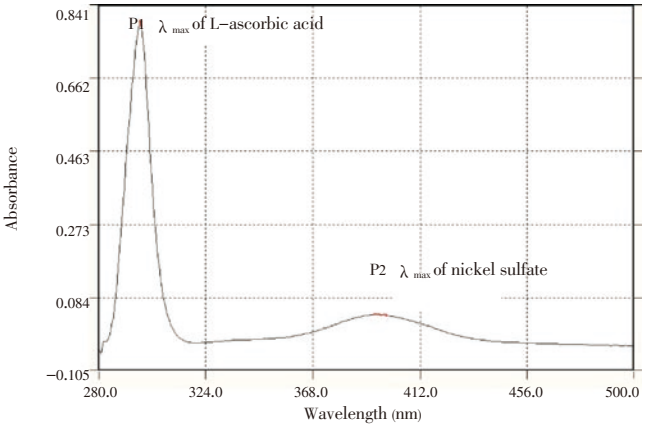

Figure 1. λ_{max} of L-ascorbic acid and nickel sulfate combined solution at pH 2.0.
P1: λ_{max} of L-ascorbic acid; P2: λ_{max} of nickel sulfate.

Table 1
λ_{max} and absorbance values of L-ascorbic acid and nickel sulfate individually and in combination at different pH.

Sl. No.	Chemical	Concentration	pH 2.0		pH 7.0		pH 7.4		pH 8.6	
			λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance
1	L-ascorbic acid	6 mg/mL	289.0	0.307	295.4	1.354	295.4	1.345	295.4	1.784
2	Nickel sulfate	6 mg/mL	394.5	0.136	394.5	0.123	394.5	0.137	392.0	0.143
3	L-ascorbic acid + Nickel sulfate	6 mg/mL each	292.5	0.798	296.5	1.527	297.0	1.558	297.0	1.621
			392.5	0.151	391.5	0.137	394.0	0.152	393.5	0.163

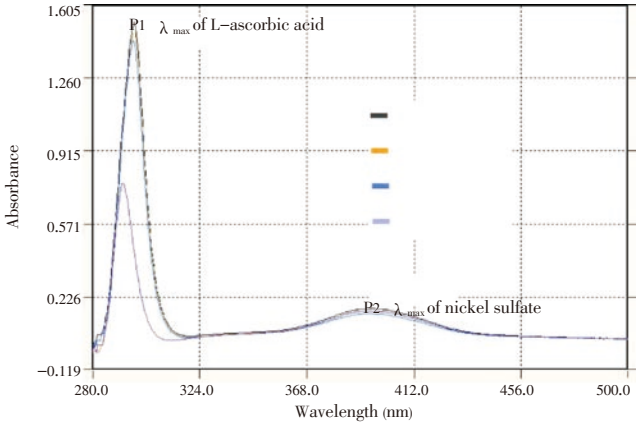

Figure 2. λ_{max} of L-ascorbic acid and nickel sulfate combined solution at pH 7.0.
P1: λ_{max} of L-ascorbic acid; P2: λ_{max} of nickel sulfate.

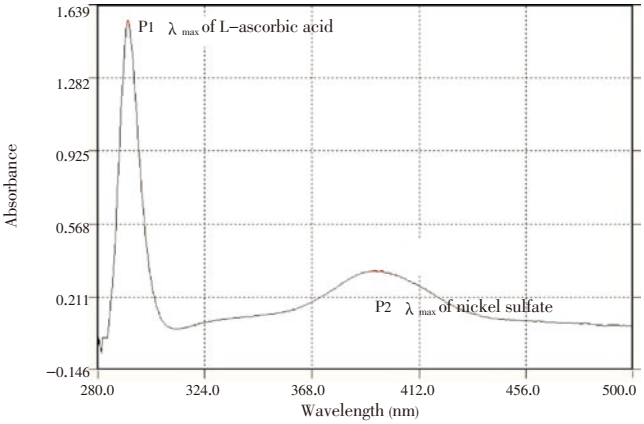

Figure 3. λ_{max} of L-ascorbic acid and nickel sulfate combined solution at pH 7.4.
P1: λ_{max} of L-ascorbic acid; P2: λ_{max} of nickel sulfate.

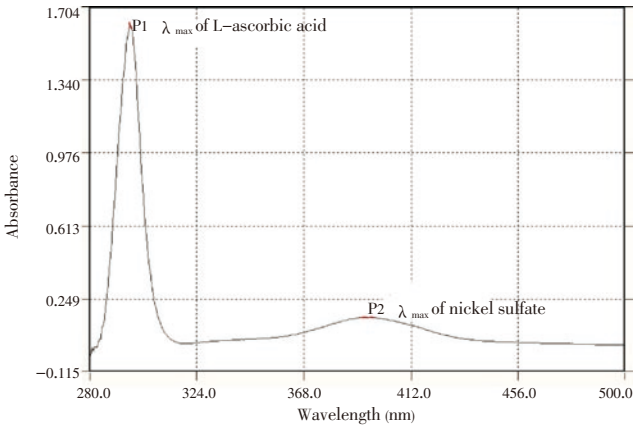

Figure 4. λ max of L-ascorbic acid and nickel sulfate combined solution at pH 8.6.
P1: λ max of L-ascorbic acid; P2: λ max of nickel sulfate.

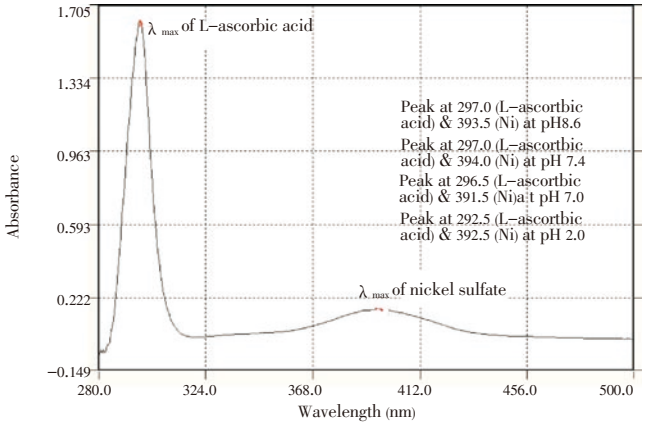

Figure 5. λ max of L-ascorbic acid + nickel sulfate at different pH (superimposed).

4. Discussion

Results showed that the lowest λ max value of L-ascorbic acid was at acidic pH 2.0 in comparison with normal (pH 7.0) or other alkaline pH (7.4 and 8.6). It indicated a bathochromic shift of λ max value of L-ascorbic acid as the pH varies from acidic to alkaline range passing through neutral. Similarly we have also noticed that the λ max values for nickel sulfate remained unchanged in the pH range of 2.0 to 7.4 but it showed a little hypsochromic shift at pH 8.6. These results reflect that pH of the medium at which L-ascorbic acid and nickel sulfate are dissolved definitely influences their chemical characteristics.

In case of combined solution, L-ascorbic acid showed a definite bathochromic shift whereas interestingly λ max values of nickel sulfate did not show any definite shift in altered pH solutions.

The results clearly indicate an altered chemical behavior of L-ascorbic acid and nickel sulfate either alone or in combination *in vitro* as changes of pH. Probably oxidation of L-ascorbic acid to L-dehydro ascorbic acid *via* the free radical (HSc*) from the reaction of H_2ASc + Ni (II) is the cause of alteration of such chemical behavior[7].

Reaction is as follows:

$H_2ASc + Ni(II) \leftrightarrow HASc^* + Ni(I) + H^+$ ('*' – free radical)

$2HASc^* \leftrightarrow H_2ASc + ASc$

In recent years, particular attention has been paid to studies on the complexation of important bioelements with biologically active ligands[8,9]. The presence of the dienol group in the molecule of the L-ascorbic acid may be the reason for possible complexation of the compounds with metal ions[10]. Although few authors have dealt with the study on metal ascorbic acid interaction *ex vivo* but the characteristics of these complexes are not yet completely understood. Relatively great attention has been paid to ascorbinate complexes of transition metals[7,8] which is reflected by an experiment of Berger *et al* who had observed that vitamin C (ascorbic acid) can act as an antioxidant or pro-oxidant *in vitro*, depending on the absence or the presence, respectively, of redox-active metal ion[11].

Our observation clearly shows that the mechanism of interaction of L-ascorbic acid-metal (Ni II) is relatively dependent on the acidic pH of the reacting solution. It is known that the acidic pH of solution strongly influences the spectrophotometeric characteristic of L-ascorbic acid but less influences on metal ions. Hence we can postulate from this study that pH status of the physiological microenvironment in living system possibly influences the chemical behavior of L-ascorbic acid alone or in combination with heavy metal like nickel sulfate *in vivo*.

Conflict of interest statement

We declare that we have no conflict of interest.

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