

# INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213; e-ISSN: 2348-5221)  
www.ijrcrps.com



Research Article

## DEGRADATION STUDY OF ASCORBIC ACID BY UV SPECTROSCOPY

WAJIHA NASHEED<sup>1</sup> AND FATIMA QAMAR<sup>1</sup>

<sup>1</sup>Faculty of Pharmacy Jinnah University for Women, Karachi  
Corresponding Author: wajihanasheed\_05@hotmail.com

### Abstract

Ascorbic acid is a water-soluble vitamin and is a must for the human health. Deficiency of this vitamin in the body can cause scurvy. It is a cofactor in many enzymatic reactions. It also plays many important physiological and metabolic roles and has anti oxidant properties. It has been found helpful in treating common cold and can limit the formation of carcinogenic matters. It undergoes degradation in the presences of heat, temperature, pH, light, metals or humidity. When exposed to heat, UV light and basic pH, vitamin C has undergone degradation. The spectrophotometric assay of the degraded solutions of ascorbic acid has revealed that heat degrades ascorbic acid more as compared to UV light and alkaline medium.

**Keywords:** Ascorbic acid, vitamin, scurvy, degradation, spectrophotometric assay.

### Introduction

Vitamin C or L-ascorbic acid ( $C_6H_8O_6$ , MW = 176.1) is a micronutrient essential for human health. There are two enantiomeric pairs i.e. L- and D-ascorbic acid, and L- and D-isoascorbic acid. L- and D-isoascorbic acid and D-ascorbic acid do not occur in nature and are devoid of vitamin C activity. Both humans and animals are unable to synthesize this water-soluble vitamin and therefore it is an essential dietary component. It is an almost odorless, water soluble white or pale yellow crystalline powder with a pleasant sharp taste. Insufficient intake of vitamin C results in scurvy which is characterized by fatigue, connective tissue weakness, spots on skin, spongy gums and bleeding from mucous membranes [Wang and Still, 2007; Stephen and Utecht, 2001]. The groups which are more prone to insufficient amount of vitamin include smokers [Jacob and Sotoudeh, 2002; Institute of Medicine, 2000], infants fed on evaporated or boiled milk [U.S. Department of Agriculture, 2011; Weinstein et al., 2001], individuals with malabsorption and chronic diseases [Hcoffman, 1985; Deicher and Horl 2003]. The use of citrus fruits and scurvy grass can prevent the occurrence of the deficiency state [Carpenter, 1986].

Ascorbic acid is involved in protein metabolism and is required for the biosynthesis of collagen and certain neurotransmitters [Carr and Frei, 1999; Li and Schellhorn, 2007]. It is also an important physiological antioxidant [Frei et al., 1989] and in the body it has been known to regenerate other antioxidants like - tocopherol. Vitamin C has been found to play an important role in immune function [Jacob and Sotoudeh, 2002]. Higher consumption of ascorbic acid lowers the risk of certain types of cancer [Bram et al., 1980; Peterkofsky and Prather, 1977, Sestilli et al., 1996, Carr and Frei, 1999; Li and Schellhorn, 2007], has beneficial effect on the life and survival time in cancer patients [Cameron and Campbell, 1976; Cameron and Pauling, 1976] and also limits the formation of carcinogens [Carr and Frei, 1999, Hecht, 1997] alone and also in combination with menadione [Gul, 2014]. Certain studies have shown that higher plasma concentration of ascorbate reduces the risk of cataract formation [Carr and Frei, 1999; Jacob and Sotoudeh, 2002; Yoshida et al., 2007]. Ascorbic acid can successfully treat common cold [Pauling, 1971], helps in the absorption of iron and copper [Hallberg, 1985; Harris and Perceval, 1991],

stabilize folates [Stokes et al., 1975] and maintains glutathione in its reduced form [Henning et al., 1991; Johnston et al., 1993].

The recommended daily allowances (RDAs) for vitamin C in adult males and females are 90 mg and 75 mg respectively, 85 mg during pregnancy and 120 mg for lactating mothers, for children aged between 9-13 years its 45 mg [Institute of medicine, 2000]. High intakes of vitamin C do not cause any serious adverse effects as it has low toxicity. However the common adverse effects include diarrhea, nausea and other gastrointestinal complaints. Its high intake may also cause reduced vitamin B<sub>12</sub> and copper levels, erosion of dental enamel and allergic reactions [Institute of medicine, 2000; Jacob and Sotoudeh, 2002].

Fruits and vegetables are the best source of vitamin C. Major contributors are citrus fruits like oranges, lemon, grape fruits and their juices, others include tomatoes, kiwifruits, broccoli, strawberries, green peppers, Brussels sprout, potatoes and cabbage [U.S. Department of Agriculture, 2011]. Ascorbic acid content in food is reduced by prolonged storage and it is also destroyed by heat [Institute of medicine, 2000; Weinstein, 2001]. It is readily absorbed and widely distributed [Kallner et al., 1982]. It is metabolized in liver and to some extent in kidney. The major pathway of its metabolism involves loss of two electrons [Davies et al., 1991]. Oxalic acid and ascorbic acid-2-sulphate are the major metabolites [Oregata et al., 2004]. In plasma, vitamin C exists in the form of ascorbate ion and reaches a concentration of 30–60 µM, with a maximum concentration of 90 µM, which is the renal threshold for complete ascorbic acid reabsorption [Wilson, 2005].

The degradation reactions of vitamin C upon temperature, pH and presences of metals or oxygen [Niemala, 1987; Tauqi and Martell, 1967; Tatum et al., 1969]. When heated in aqueous solution, the products detected includes dehydroascorbic acid, 2,3-diketogulonic acid, therionic acid, oxalic acid, reductic acid, reductones, sugars and sugar acids [Velisek et al., 1974]. Several studies have been conducted to study the degradation pattern of vitamin C caused by UV light [Koutchma and Shmails, 2002; Tandon et al., 2003; Tran and Farid 2003; Ye et al., 2007], heat [Benterud, 1977; Vikram et al., 2005; Torrigosa et al., 2006] and alkali [Jian-Ping and Chen 1998].

Research has been done on the degradation behavior of ascorbic acid in solutions [Finholt et al., 1963], biological fluids [Karlsen et al., 2007], fruit and fruit juices [Johnson et al., 1995; Burdurlu et al., 2006; Choi et al., 2002], foods [De Ritter, 1976] and pharmaceutical products [Mateli et al., 2008]

Spectrophotometry is usually preferred over other methods because it is economical, less time consuming and maintenance is also economical. UV spectrophotometry can also be use for stress degradation testing. According to International Conference of Harmonization (ICH) guideline the active pharmaceutical ingredient is expressed to various forced degradation conditions which are basic acidic and light conditions [ICH, 2003] Forced degradation is able to demonstrate that the selected method is stability indicating it means that this technique is use to identify the increase in the degradation product and the subsequent loss of active components.[Kishore et al., 2013].

### **Parameters involve in Forced Degradation**

The forced degradation studies on drug substance involves acid/base stress testing, photo degradation, temperature and or with humidity, time, pH variation (low and high).

### **Thermal and/or humidity stress testing**

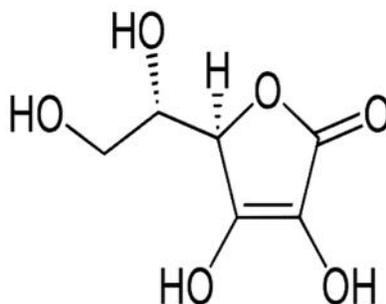
Thermal and/or humidity stress testing is performed by exposing the drug substance to thermal/humidity conditions in due course which causes the substance to degrade forcefully to its main components.

### **Degradation by UV light**

UV degradation is a main problem in numerous UV-unstable products which are made up of natural and synthetic polymers as they break or disintegrate when exposed to continuous sunlight. As the attack is dependent on the degree and degree of exposure, nonstop exposure is a more serious problem than intermittent exposure

### **Acid/base stress testing**

Acid/base stress testing is used to evaluate the forced degradation of a drug substance. This test involves degradation of a drug substance by exposure to basic or acidic medium over time to its primary degradation products. This type of hydrolysis occur in labile carbonyl functional groups which are esters (lactones), amides (lactams) aryl amines, imides, imines alcohols and carbamates.



**Figure 1:** Structure of Ascorbic acid

## Experimental work

### Ascorbic Acid

Active of ascorbic acid has been used for the purpose of degradation studies.

#### Material and reagents

Pyrex glass wares were used including measuring cylinder, funnel, volumetric flask, beakers, pipette, and stirrer. For the washing of glass wares we use chromic acid then we wash with water and finally rinsed with double distilled or DI water (freshly prepared). Analytical grade reagents were used which includes 0.1N Sodium hydroxide and de-ionized water or double distilled water.

#### Instruments

Ultraviolet Lamp (Power of 8N, Serial NO: N 045571, LF-204.LS) '4W-254 and 365 nm', Spectrophotometer with a quartz cuvette (T80 UV-VI Spectrometer) 'PG Instrument', Weighing Balance (Item PA214C): 'Pioneer OHAIUS', and Water Bath 'HH-4' having digital and constant temperature tank.

#### Preparation of 0.1 N Sodium hydroxide

0.1 N NaOH was prepared by dissolving 4gm in de-ionized water and the volume was made up to 100 ml.

#### Preparation of Ascorbic acid solution

1.7612 gm of ascorbic acid was dissolved in de-ionized water and the volume was made up to 100 ml.

the absorbance of the solution was measured at the wavelength of 244 nm.

#### Procedure for Degradation Studies:

The ascorbic acid solution was subjected to degradation by treating it with alkaline solution (pH 8.5-9), UV light and heat (in a water bath at 90 °C) and after different intervals of time the absorbance of the solutions were determined at 244 nm.

#### Results and Discussion

The main object of this study was to determine the source by which ascorbic acid undergoes maximum degradation. Ascorbic acid was subjected to different environmental conditions i.e were exposed separately to heat (solution placed in a water bath at 90 °C), UV light and alkaline medium for sixty minutes. After every ten minutes absorbance was determined for each solution. Our study has shown that vitamin C has shown maximum degradation when exposed to heat claiming the % recovery of 93.66%. When ascorbic acid solution was exposed to heat initially it degraded slowly but after 20 minutes of exposure to heat, the solution started degradation rapidly as shown in figure-3. On exposed to UV light it did not show a rapid degradation rather showed a slow degradation till 20 minutes and even afterwards it does not showed rapid photo degradation (Figure-2). In alkaline medium ascorbic acid solution showed a drastic degradation and a linear regression line is obtained. % recovery and log absorbance are shown in Table 1.

**Table 1: Degradation pattern of Ascorbic acid solution**

Time (mins.)	Heat		pH 8.5		U.V light	
	% Recovery	Log	% Recovery	Log	% Recovery	Log
10	99.62	0.430	98.74	0.426	99.88	0.431
20	98.74	0.426	98.07	0.423	99.00	0.427
30	97.18	0.419	97.40	0.420	97.85	0.422
40	96.07	0.414	96.74	0.417	96.74	0.417
50	94.96	0.409	96.07	0.414	95.62	0.412
60	93.66	0.403	95.62	0.412	94.81	0.409

The comparison % recovery and log concentration of the degradation by the methods indicates their accuracy in the following order:

Heat > UV light > alkaline medium

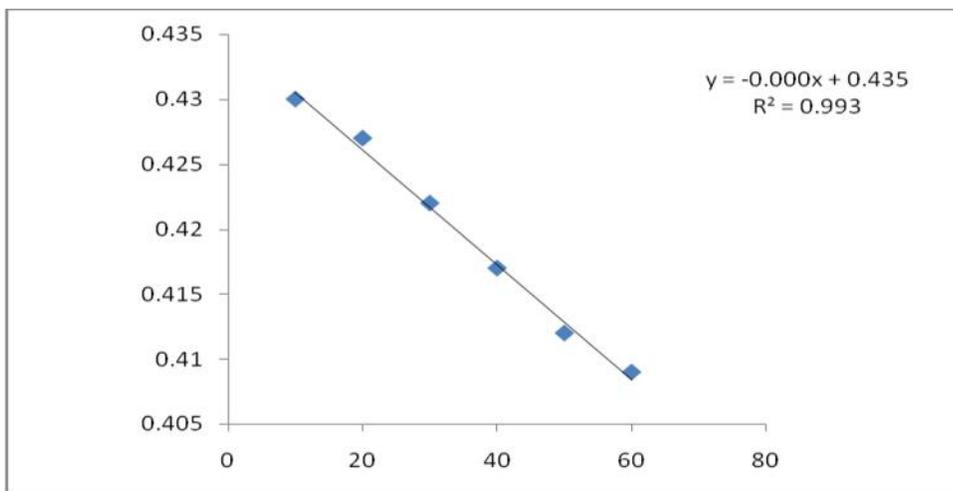


Figure 2: Photodegradation of ascorbic acid

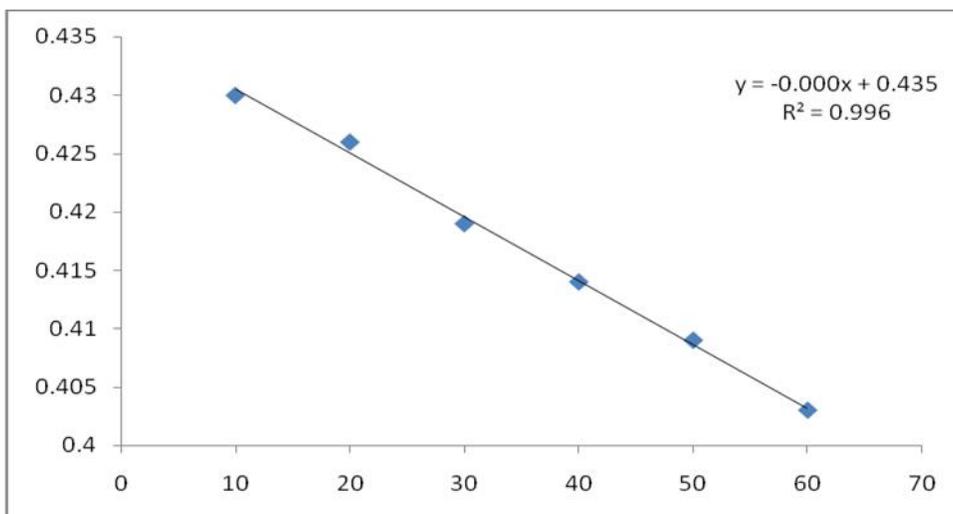


Figure 3: Thermal degradation of ascorbic acid

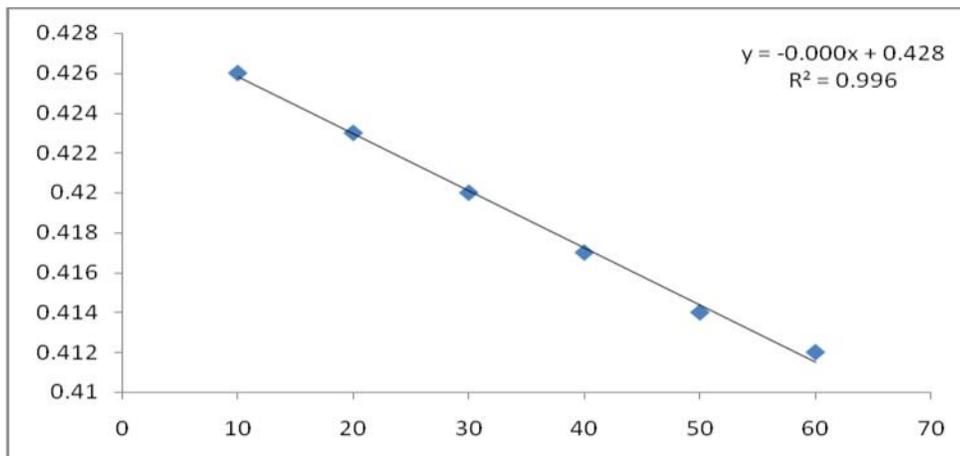


Figure 4: Degradation of ascorbic acid at pH 8.5

## References

- Benterud A., 1977. Vitamin losses during thermal processing, Physical, Chemical and Biological Changes in Food Caused by Thermal Processing. T. Hoymen and O. Kvaales, eds. Applied Sciences, London, p. 199.
- Bram S, Froussard P, Guichard M, Jasmin C, Augery Y, Sinoussi-Barre F, Wray W. 1980. Vitamin C preferential toxicity for malignant melanoma cells. *Nature*. 284(5757): 629–631. doi: 10.1038/284629a0.
- Burdurlu HS; Karadenz f; Koca N. 2006. Degradation of vitamin C in citrus juice concentrates during asean food. *Info storage. J. Food Eng.*; 74: 211-216.
- Cameron E, Campbell A. 1974. The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. *Chem Biol Interact*; 9: 285-315.
- Cameron E, Pauling L. 1976. Supplemental ascorbate in the supportive treatment of cancer: prolongation of survival times in terminal human cancer. *Proc Natl Acad Sci USA*; 73: 3685-3689.
- Carpenter KJ. 1986. The history of scurvy & vitamin C. New York: Cambridge University Press.
- Carr AC and Frei B. 1999. Towards a new recommended daily allowance for vitamin C based on antioxidant and health effects in humans. *Am J Clin Nutr*; 69: 1086-1107.
- Choi, MH; Kim, GH; Lee, HS. 2002. Effects of ascorbic acid retention on juice colour and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. *Food Res. Int.*; 35: 753-759.
- Davies MB; Austin J; Partridge DA. 1991. Vitamin C: Its Chemistry and Biochemistry. Cambridge: The Royal Society of Chemistry.
- De Ritter, E. 1976. Stability characteristics of vitamins in processed foods. *Food Techn.*; 30: 43-54.
- Deicher R, Horl WH. 2003. Vitamin C in chronic kidney disease and hemodialysis patients. *Kidney Blood Press Res*; 26: 100-106.
- Finholt P, Paulson RB and Higuchi T. 1963. Rate of anaerobic degradation of ascorbic acid in aqueous solutions. *J. Pharm. Sci.*; 52: 948-954.
- Frei B, England L, Ames BN. 1989. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA*; 86: 6377-6381.
- Gul, W. 2014. Menadione: role in cancer prevention and method of analysis. *WJPR*; 2(10): 1390-1394.
- Hallberg L. 1985. The role of vitamin C in improving the critical iron balance situation in women. *Int J Vitam Nutr Res*; 27: 177-187.
- Harris ED, Perceval SS. 1991. A role for ascorbic acid in copper transport. *Am J Clin Nutr*; 54:1193S-1197S.
- Hcoffman FA. 1985. Micronutrient requirements of cancer patients. *Cancer*; 55 (1 Suppl): 295-300.
- Hecht SS. 1997. Approaches to cancer prevention based on an understanding of N-nitrosamine carcinogenesis. *Proc Soc Exp Biol Med*; 216: 181-191.
- Henning SM, Zhang JZ, McKee RW, Swendseid ME, Jacob RA. 1991. Glutathione blood levels and other oxidant defence indices in men fed diets low in vitamin C. *J Nutr*; 121: 1969-1975.
- ICH. Stability testing of new drug substances and products Q1A (R2). 2003. International Conference on Harmonization, IFPMA, Geneva.
- Institute of medicine. 2000. Food and nutrition board: Dietary reference intake for vitamin C, vitamin E, Selenium and Carotenoids. Washington, DC: National academy press.
- Jacob RA, Sotoudeh G. 2002. Vitamin C function and status in chronic disease. *Nutr Clin Care*; 5: 66-74.
- Jian-Ping Yuan and Feng Chen. 1998. Degradation of ascorbic acid in aqueous solution. *J. Agric. Food Chem.* 46(12): 5078-5082.
- Johnson JR, Braddock RJ and Chen CS. 1995. Kinetics of ascorbic acid loss and non enzymatic browning in orange juice serum: Experimental rate constant. *J. Food Sci.*, 60: 502-204.
- Johnston CS, Meye CG, Srilakshmi JC. 1993. Vitamin C elevates red blood cell glutathione in healthy adults. *Am J Clin Nutr*; 58: 103-105.
- Kallner A; Hartmann D; Hornig D. 1982. Kinetics of ascorbic acid in humans. In: Seib PA, Tolbert BM, eds. *Ascorbic Acid: Chemistry, Metabolism and Uses*. Advances in Chemistry Series, Washington DC: American Chemical Society. 335-348.
- Karlsen A, Blomhoff R, Gundersen TE. 2007. Stability of whole blood and plasma ascorbic acid. *Eur J Clin Nutr.* ;61(10):1233-6.
- Kishore kumarhotha et al. 2013. Forced degradation studies: practical approach- overview of regulatory guidance and literature for the drug products and drug substances. *Int. Res. J. Pharm.* 4 (5).
- Koutchma T and M Shmails. 2002. Degradation of vitamin C after alternate treatments of juices. Paper presented at IFT conference, New Orleans.
- Li Y, Schellhorn HE. 2007. New developments and novel therapeutic perspectives for vitamin C. *J Nutr*; 137: 2171-2184.
- Mateli N; Birghila S; Popescu V; Dobrina S; Soceanu A; Oprea C; Magearu V. 2008. Kinetic study of vitamin C degradation from pharmaceutical products. *Rom. J. Phys.*; 53(1-2): 343-351.
- Niemela K. 1987. Oxidative and non-oxidative alkali-catalyzing degradation of L-ascorbic acid. *J. Chromatogr.*; 399: 235-243.
- Ortega GR, Deimling MJ, Delgado JN. 2004. In: Block JH, Beale JM (Eds), *Wilson and Gisvold's textbook of organic, medicinal and pharmaceutical chemistry*

- 11<sup>th</sup> ed., Lippincott William and Wilkins, Baltimore, MD, USA, Chap 26.
- Pauling L. 1971. The significance of the evidence about ascorbic acid and common cold. *Proc Natl Acad Sci USA*; 68: 2678-2681.
- Peterkofsky B, Prather W. 1977. Cytotoxicity of ascorbate and other reducing agents towards cultured fibroblasts as a result of hydrogen peroxide formation. *J Cell Physiol*. 90(1):61–70.
- Sestili P, Brandi G, Brambilla L, Cattabeni F, Cantoni O. 1996. Hydrogen peroxide mediates the killing of U937 tumor cells elicited by pharmacologically attainable concentrations of ascorbic acid: cell death prevention by extracellular catalase or catalase from cocultured erythrocytes or fibroblasts. *J Pharmacol Exp Ther*. 277(3):1719–1725.
- Stephen R, Utecht T. 2001. Scurvy identified in the emergency department: a case report. *J Emerg Med*; 21: 235-237.
- Stokes PL, Melikian V, Leeming RL, Portman-Graham H, Blair JA, Cooke WT. 1975. Folate metabolism in scurvy. *Am J Clin Nutr*; 28: 126-129.
- Tandon K.; R. Worobo ;J. Churlay; O. Padilla-Zakour. 2003. Storage quality of pasteurized and UV-treated apple cider. *J. Food Processing Preservation*; 27: 21-35.
- Taqi Khan MM; Martell AE. 1967. Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalyzed oxidation. *J. Am. Chem. Soc.*; 89: 4176-4185.
- Tatum JH; Shaw PE; Berry RE. 1969. Degradation products from ascorbic acid. *J. Agric. Food Chem*; 17: 38-40.
- Torrigosa, F.; Esteve, M. J.; Frigola, A.; Cortes, C. 2006. Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field as compared with pasteurized juice. *J. Food Engineering*; 73: 339-345.
- Tran M. T. and M. Farid. 2004. Ultraviolet treatment of orange juice. *Innovative Food Sci. Emerging Technol.*; 5(4): 495-502.
- U.S. Department OF Agriculture, Agricultural research service. 2011. USDA National nutrient database for standard reference, Release 24, Nutrient data laboratory home page, <http://www.ars.usda.gov/ba/bhnrc/ndl>
- Velisek J; Davidek J; El-Zeany BA; et al. 1974. Antioxidant activity of some brown pigments in L-ascorbic acid solutions. *Z. Lebensm Unters Forsch*; 154: 151-156.
- Vikram, V. B.; Ramesh, M. N.; Prapulla, S. G. 2005. Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. *J. Food Engineering*; 69: 31-40.
- Wang AH and Still C. 2007. Old world meets modern: a case report of scurvy. *Nutr Clin Pract*; 22: 445-448.
- Weinstein M, Babyn P, Zlotkin S. 2001. An orange a day keeps the doctor away: scurvy in the year 2000. *Pediatrics*; 108: E55.
- Wilson JX. 2005. Regulation of vitamin C transport. *Annu Rev Nutr*. 25:105–125.
- Ye Z; T. Koutchma, B. Parisi; J. Larkin; L. J. Forney. 2007. Ultraviolet inactivation kinetics of *E. coli* and *Y. pseudotuberculosis* in annular reactor. *J. Food Sci.*; 72: E271-E278.
- Yoshida M, Takashima Y, Inoue M, Iwasaki M, Otani T, Sasaki S. 2007. JPHC study group. Prospective study showing that dietary vitamin C reduced the risk of age-related cataracts in a middle-aged Japanese population. *Eur J Nutr*; 46: 118-124.