

## Change in Acidity of L-Ascorbic Acid in the Mixed Solvent DMSO - Water Followed by Conductometric Determination of Dissociation Constants

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### Article info

Received: 06/06/2013  
Accepted: 13/06/2013

### Keywords:

L-ascorbic acid  
Dissociation constant  
Conductometric  
Mixed solvent

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**Abstract:** Using conductometric method, the changes in the acidity of L-ascorbic acid in the mixed solvent dimethylsulfoxide (DMSO) - water were monitored. The mixed solvent used was two-component system consisting of nonpolar and polar solvents. Dissociation constants of L-ascorbic acid was determined in pure water and in the mixed solvent DMSO - water with different molar ratios of two solvents:  $x_{\text{DMSO}} = 0.500, 0.333, 0.250, 0.200, 0.166$ , corresponding respectively to DMSO - H<sub>2</sub>O, DMSO - 2H<sub>2</sub>O; DMSO - 3H<sub>2</sub>O, DMSO - 4H<sub>2</sub>O; DMSO - 5H<sub>2</sub>O. Concentrations of freshly prepared solution of ascorbic acid were in the range from 0.00002 to 0.05 mol dm<sup>-3</sup>. Molar conductivity resistance values were measured for a given concentration of freshly prepared solutions of L-ascorbic acid and 24 hours old solutions. Using Ostwald's dilution law values for the molar conductivity at infinite dilution ( $\lambda_0$ ) and dissociation constants (K), i.g. pK (- log K) were calculated. The results obtained for different concentrations of L-ascorbic acid in the mixed solvent DMSO - water indicate that during a time, pK values declined and acidity increased. Following pK values in the dependence on  $x_{\text{DMSO}}$  for a freshly prepared solution of L-ascorbic acid. The curve with two inflection points was obtained corresponding to the system of mixed solvent DMSO and DMSO - 2H<sub>2</sub>O and DMSO - 3H<sub>2</sub>O. In 24 hours old solution of L-ascorbic acid, inflection points were not observed. The results indicate the possibility of different adducts in the mixed solvent or in old solutions as well as accumulation of degradation products over time.

## INTRODUCTION

Since L-ascorbic acid (AA) called Vitamin C was isolated from the adrenal glands by Szent-Györgia in 1928 it generated great interest as physiologically and pharmacologically active substance (Davey, 2000). Most animal species are able to synthesise this vitamin, while the man and other primates, due to enzymatic deficiency, are unable to synthesise it. The lack of this essential microsupstance is manifested through the symptoms of avitaminosis.

After a number of research studies, the structure and synthesis of Vitamin C has been established. L-ascorbic acid has four isomers that show different physiological effect while some of the isomers, in general, show no physiological effect (Animon, 1974; Enler, 1957). L-ascorbic acid can be represented by a structure shown in Figure 1. which shows the formation keto- $\gamma$ -lactone structure.

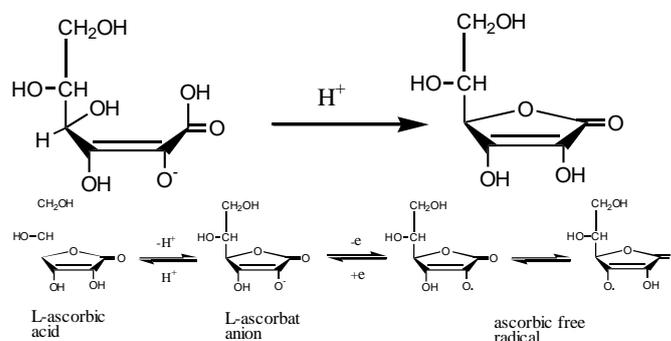


Figure 1. L-Ascorbic acid in all its forms.

According to the Figure 1, it is concluded that L-ascorbic acid has enediol configuration adjacent to the carbonyl group, and can be classified as an acidic reductone. By

increasing pH, increases the speed of oxidation of L-ascorbic acid, in addition, temperature, oxygen in the water, oxygen from the air, light, as well as a presence of polyvalent metal salts contributes to autooxidation process. Oxidation of L-ascorbic acid gives dehydroascorbic acid, and the subsequent hydrolytic cleavage of the ring produced 2,3-diketo-L-gulonic acid that can degrade further giving treonic acid, L-lyxonic acid and L-xylonic acid. L-ascorbate anion with a loss of electrons can give ascorbic free radical (AFR), Figure 1.

L-Ascorbic acid dissociations in two steps depending on pH of water solution and can be changed by addition of hydroxide (Figure 2). On 37 °C  $pK_1$  is 4.18, and  $pK_2$  = 11.6 (Karrer, 1933; Borskok, 1937). Some authors cited different values that are dependable on temperature. This dissociation reaction occurs on  $C_2$  and  $C_3$  hydroxyl groups giving ions of L-ascorbic acid.

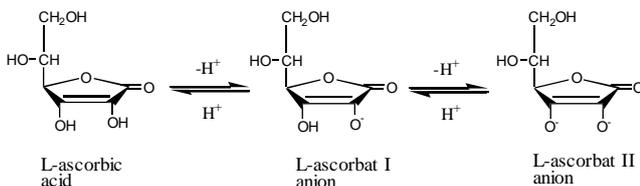


Figure 2. L-Ascorbic acid ions.

The acidity of the solution is determined by the concentration of  $H^+$  ions in solution. The term proposed by King (King, 1965) gives the logic to the name “acidity constant”, avoiding separate descriptions of constants, one for acids, and other for bases. Brönstad-Lowry theory (Albert, 1971) is widely accepted for description of acids and bases. According to this theory, the acid dissociation constant is described by the chemical equation:

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad \text{or} \quad pK_a = pH + \log \frac{[HA]}{[A^-]}$$

and includes equilibrium concentrations of all species present in solution.

This equation can be used in a simplified form if constants are determined in solutions with a concentration of less than  $0.01 \text{ mol dm}^{-3}$  or monovalent ions are used (Albert, 1971).

For the evaluation of conductivity data by using recently developed conductance equations, the most essential parameters are the viscosity and the dielectric constants of the solvent media. To evaluate the conductivity measurements the Kraus and Bray conductance equation can be used:

$$\lambda_c c = \frac{1}{\lambda_c} \lambda_0^2 K - \lambda_0 K$$

where  $\lambda_c$  is molar conductance of the acid at concentration  $c$ ,  $\lambda_0$  is molar conductance of the acid at infinite dilution and  $K$  is dissociation constant. Plots of  $1/\lambda_c$  versus  $c\lambda_c$  for the acid in the various media gave straight lines. The limiting molar conductance and dissociation constants were determined from slopes and intercepts (Jaiswal, 2005).

## EXPERIMENTAL

### Materials

All chemicals used for the preparation of the solution were of the highest purity grade and prepared in redistilled

water. Prepared mixtures of solvent and solute were kept at 20 °C. Mixture DMSO - water was prepared by mixing calculated volume of solvents, and the molar proportion of DMSO: water were the following:  $x_{\text{DMSO}} = 0.500, 0.333, 0.250, 0.200, 0.166$ . The composition of mixed solvent can be presented as DMSO -  $H_2O$ , DMSO -  $2H_2O$ ; DMSO -  $3H_2O$ , DMSO -  $4H_2O$ , DMSO -  $5H_2O$ . Preferred concentrations of L-ascorbic acid were prepared by accurate weighing and dissolving it in these mixed solvent systems. Fresh solutions were used immediately after preparation for experiment. Conductometric measurements were performed using conductometer “Iskra” MA 5962. Conductometric cell was from the same manufacturer type MA 5963. Initially, the conductometric cell was plated, and the value of the cell constants determined. For all measurements, conductivity temperature was maintained at  $25 \pm 0.2$  °C.

To confirm the method that can be used for conductometric determination of dissociation constants for L-ascorbic acid, solution of acetic acid was utilized as a control. By measuring the molar resistance of solutions with different concentrations of acetic acid, the molar conductivity  $\lambda_c$  was calculated. Here, using the method of linear regression, molar conductivity at infinite dilution,  $\lambda_0$  and dissociation constants  $K$  i.e.  $pK$  were calculated. The obtained results for acetic acid showed that conductometric method was suitable for determining the ionization constants of L-ascorbic acid in water and mixed solvent. Table 1 shows the measured and calculated parameters for acetic acid and linear dependence is shown in Figure 1.

Table 1. The measured resistance values and calculated physical values used for calculations of  $\lambda_0$  and  $K_c$  for different acetic acid concentrations

$c \times 10^{-5}$ ( $\text{mol dm}^{-3}$ )	R ( $\Omega$ )	$\kappa \times 10^{-6}$ ( $\Omega^{-1} \text{cm}^{-1}$ )	$\lambda_c$ ( $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ )	$X = 1/\lambda_c$	$Y = \lambda_c c$
5000	805	382	7.635602	0.130965	0.38178
1000	1820	169	16.88643	0.059219	0.168864
100	6050	50.8	50.79884	0.019685	0.050799
50	8700	35.3	70.65126	0.014154	0.035326
10	21600	14.2	142.2838	0.007028	0.014228
5	34100	9.01	180.254	0.005548	0.009013

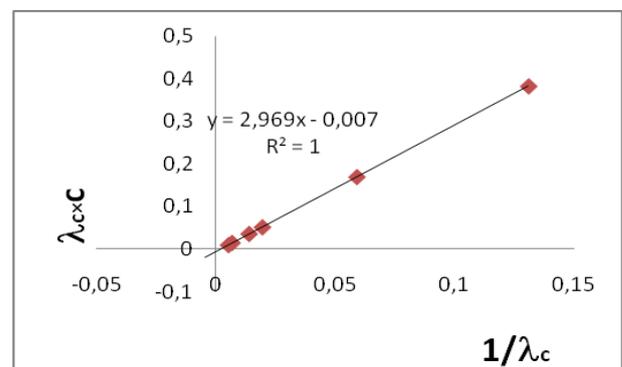


Figure 1. Plot of  $\lambda_c c = f(1/\lambda_c)$  for a water solution of acetic acid.

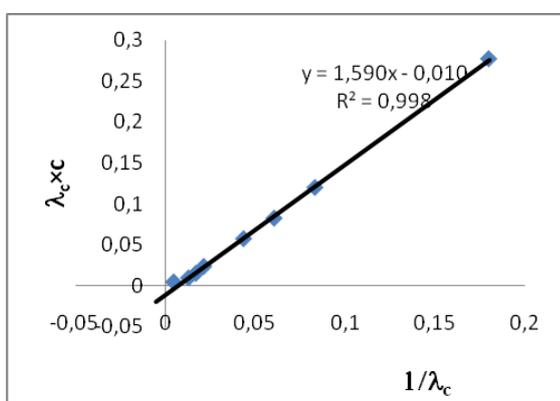
After extrapolation, the value for  $\lambda_0 = 418.74 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$  was obtained while dissociation constant was  $K_a = 1.693 \times 10^{-5} \text{ mol dm}^{-3}$  or  $pK_a = 4.77$  which is in agreement with literature values (Albert, 1971).

This process of solving algebraic computation that limits molar conductivity at infinite dilution and  $pK$  was

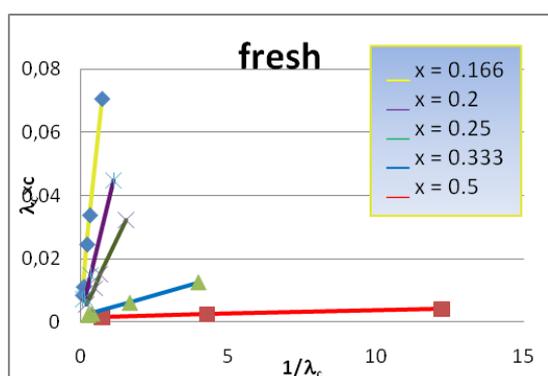
conducted in order to determine the dissociation constants that are related to L-ascorbic acid in pure water and mixed solvent immediately after preparation and in 24 hours old solution. The values obtained are represented in Table 2 and Figure 2.

**Table 2.** The measured resistance values and calculated physical values used for algebraic and graphic computed values for  $\lambda_0$  and  $K_a$  for a different concentrations L-ascorbic acid (freshly prepared solutions)

$c \times 10^{-5}$ (mol dm <sup>-3</sup> )	R ( $\Omega$ )	$\kappa \times 10^{-6}$ ( $\Omega^{-1} \text{cm}^{-1}$ )	$\lambda_c$ ( $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ )	$X = 1/\lambda_c$	$Y = \lambda_c \cdot c$
2	17900	4.19441	209.7207	0.004768	0.004194
12	8150	9.21227	76.76892	0.013026	0.009212
25	5120	14.6641	58.65625	0.017048	0.014664
50	3230	23.2446	46.48916	0.02151	0.023245
250	1310	57.313	22.92519	0.04362	0.057313
500	910	82.5055	16.5011	0.060602	0.082505
1000	625	120.128	12.0128	0.083245	0.120128
5000	270	278.074	5.561481	0.179808	0.278074

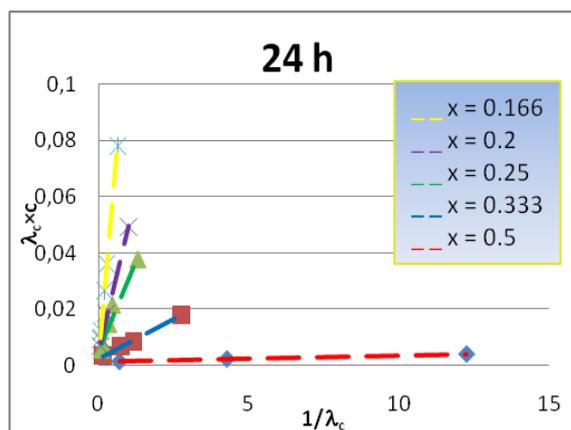


**Figure 2.** Representation of equation:  $\lambda_c C = f(1/\lambda_c)$  for water solution of L-ascorbic acid.



**Figure 3a.** Linear dependance where  $\lambda_c C = f(1/\lambda_c)$  for 24 hours old L-ascorbic acid solutions in mixed solvent with different mole ratios of DMSO.

After extrapolation, the obtained values were as follows:  $\lambda_0 = 149.48 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$  and  $K_a = 7.15 \times 10^{-5} \text{ mol dm}^{-3}$  or  $\text{pK}_a = 4.147$  that are in an agreement with literature values. Using the same procedure, conductometric measurements were performed for different concentrations (0.0005, 0.001, 0.005, 0.01, and 0.05 mol dm<sup>-3</sup>) of L-ascorbic acid in the mixed solvent DMSO - water ( $x_{\text{DMSO}} = 0.166, 0.200, 0.250, 0.333$  and  $0.500$ ), and the obtained experimental data were used to calculate the acidity constants of freshly prepared and 24 hours old solution of L-ascorbic acid. Results are shown in graph 3a and 3b graph.



**Figure 3b.** Linear dependance where  $\lambda_c C = f(1/\lambda_c)$  for 24 hours old L-ascorbic acid solutions in mixed solvent with different mole ratios of DMSO.

By using linear extrapolation method, the values for concentration dissociation constants i.e. acidity constant K or pK for L-ascorbic acid in the mixed solvent of varying moles of constituent components are obtained.

## RESULTS AND DISCUSSION

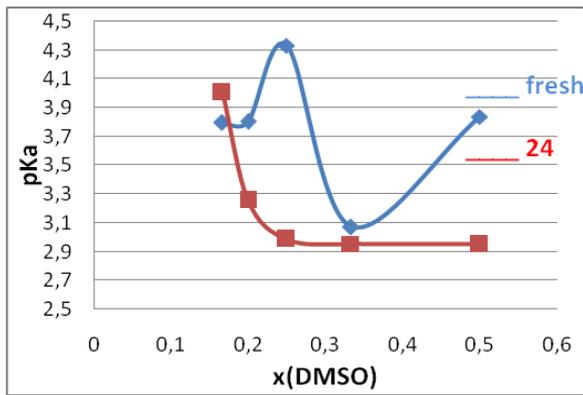
Based on the conductometric measurements, the dissociation constant of acetic acid in water were determined by solving Ostwald's law of dilution equations using the method of the least squares. The calculated pK value of acetic acid in water at 25 °C was 4.771 which is in good agreement with literature data (4.75) (Albert, 1971). The results indicate the possibility of monitoring pK with deviation of 0.02 pK units.

Since our goal was monitoring changes in acidity mixed solvent, rather than determining absolute values of pK, selected method was suitable for the study. The same method and the same procedure were used to calculate pK of L-ascorbic acid at 25 °C and the resulting value of  $\text{pK}_a = 4.147$  coincided with literature data with value of 4.12 (Kumler, 1935). Measurements were performed with a freshly prepared solution to avoid "autoxidation" of L-ascorbic acid in aqueous solution leading to reduced pK values. pK values were determined for L-ascorbic acid in the system of mixed solvents: DMSO - water with different molar components of the mixed solvent and calculated corresponding pK values are given in Table 3.

**Table 3.** pK of freshly prepared and 24 hours old solutions of L-ascorbic acid in two-component mixed solvent DMSO - water with different molar ratios of solvents

$x_{\text{DMSO}}$	$\text{pK}_{\text{fresh}}$	$\text{pK}_{24}$	$\Delta \text{pK}$
0.166	3.795	4.008	-0.213
0.200	3.805	3.258	0.547
0.250	4.333	2.987	1.346
0.333	3.072	2.953	0.119
0.500	3.836	2.951	0.885

Concentrations of dissolved L-ascorbic acid in each two-component solvent were 0.0005, 0.001, 0.005, 0.01 and 0.05 mol dm<sup>-3</sup>. Calculated values of pK listed in Table 3 show decline in pK values over a time and an increase in the acidity of the solution, which is caused by decomposition products of L-ascorbic acid.



**Figure 4.** Plot of pK values of freshly prepared and 24 hours old solutions of L-ascorbic acid in two-component mixed solvent DMSO - water in dependence on DMSO molar ratio in a solvent mixture.

It is evident from the diagram  $pK = f(x_{DMSO})$  that, for a freshly prepared solution of L-ascorbic acid, the data curve is obtained with the inflection points that correspond to the composition of a mixed solvent system of DMSO and DMSO - 2H<sub>2</sub>O and DMSO - 3H<sub>2</sub>O. The data curve for  $pK = f(x_{DMSO})$  for solutions of 24 hours old L-ascorbic acid in Figure 4, shows no inflection points. It is most likely that the solution contains degradation products of L-ascorbic acid and various products of solvolysis. Measurement results show that one can, on the basis of conductivity measurements and determination of pK values, define the specific interaction between the components and mixed solvent.

This study can contribute to the understanding of relationship that exists between the physical-chemical properties (Milićević, 1983) of the solution and interactions between the components and mixed solvent.

## CONCLUSION

Selected physico-chemical method for the conductometric monitoring of acidity of L-ascorbic acid proved to be reliable. Because of the tendency toward autooxidation, measurement of L-ascorbic acid needs to be conducted in freshly prepared solution in order to avoid adverse effects that could disturb the measured values.

Monitoring changes in acidity of L-ascorbic acid in freshly prepared solution showed that at a given molar ratio of components in a mixed solvent solution, there is an interaction between the components of solution that

## Summary/Sažetak

Praćena je promjena kiselosti L-askorbinske kiseline u mješovitom vodenom rastvaraču dimetilsulfoksid (DMSO) - voda konduktometrijskom metodom. Odabir mješovitog rastvarača uzet je kao dvokomponentni sistem nepolarnog i polarnog rastvarača. Određivana je konstanta disocijacije L-askorbinske kiseline u čistoj vodi i u mješovitom rastvaraču DMSO - voda u različitim molalnim udjelima mješovitog rastvarača:  $x_{DMSO} = 0.500; 0.333; 0.250; 0.200; 0.166$  što odgovara respektivno kombinaciji DMSO - H<sub>2</sub>O; DMSO - 2H<sub>2</sub>O; DMSO - 3H<sub>2</sub>O; DMSO - 4H<sub>2</sub>O; DMSO - 5H<sub>2</sub>O. Koncentracije svježe pripremljenih rastvora askorbinske kiseline kretale su se u intervalu od 0.05 do 0.00002 mol dm<sup>-3</sup>. Konduktometrijski je određivana molarna provodljivost mjereći vrijednosti otpora za date koncentracije svježe pripremljenih rastvora različite koncentracije L-askorbinske kiseline i odstajalih 24 sata. Koristeći Ostwaldov zakon razblaženja izračunate su vrijednosti za graničnu molarnu provodljivost ( $\lambda_0$ ) i konstantu disocijacije (K) tj.  $-\log K$  (pK). Rezultati dobiveni za različite koncentracije L-askorbinske kiseline u mješovitom rastvaraču DMSO - voda pokazuju da sa vremenom dolazi do opadanja pK vrijednosti, odnosno do povećanja kiselosti. Prateći pK u funkciji  $x_{DMSO}$  za svježe pripremljen rastvor L-askorbinske kiseline dobiva se kriva sa dvije prevojne tačke (infleksije) koje odgovaraju sistemu mješovitog rastvarača DMSO - 2H<sub>2</sub>O i DMSO - 3H<sub>2</sub>O. Kod rastvora L-askorbinske kiseline koji su odstajali 24 sata nije primijećena prevojna tačka. Dobiveni rezultati ukazuju na mogućnost asocijata u mješovitom rastvaraču ili pak kod odstajalih rastvora na degradacione produkte koji nastaju tokom vremena.

contribute to the effect of unexpected or anomalous values of acidity.

Our experiment showed that changes in acidity is recorded in the mixed solvent systems that can be best described by quantitative composition: DMSO - 2H<sub>2</sub>O and DMSO - 3H<sub>2</sub>O. Twenty four hours old solutions of L-ascorbic acid in the mixed solvent showed a progressive increase in acidity with increasing moles of DMSO in a two-component system with the assumption of a possible increase in the acidic degradation products of L-ascorbic acid, but, without additional measurements and parameters, this hypothesis could not be confirmed.

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