Metrohm

Application Bulletin 98/5 e

Determination of ascorbic acid (vitamin C) and its compounds

Branch

General analytical chemistry; organic chemistry; pharmaceutical industry; food, stimulants, beverages, flavours; biochemistry, biology;

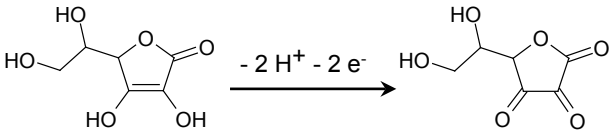
Keywords

Ascorbic acid; titration; bi-voltametric titration; photometric titration; polarography; vitamin C; ISO 6557/2; branch 1; branch 3; branch 4; branch 7; branch 8; 6.0309.100; 6.1115.000

Summary

In addition to the natural occurrence, ascorbic acid (vitamin C) is added to food and beverages as an antioxidant. It can also be found in various pharmaceutical products.

Ascorbic acid, as well as its salts and esters, can be determined by titration or by means of polarography. The determination is based on the oxidation of ascorbic acid to dehydroascorbic acid.



For the titrimetric determination bi-voltametric or photometric equivalence point indication can be used. The bi-voltametric indication is independent of inherent coloration of the sample.

The polarographic method is the most selective of the described methods, since other reducing or oxidizing substances do not interfere.

Bi-voltammetric determination with iodine

Instruments

- Titrator with MET mode
- 10 mL burette
- Stirrer

Electrode

Double Pt-sheet electrode	6.0309.100

Reagents

- Volumetric iodine solution, c(I_2) = 0.01 mol/L
- Glyoxal, w($C_2H_2O_2$) = 40%
- Sodium hydroxide solution, c(NaOH) = 1 mol/L
- Sulfuric acid, w(H_2SO_4) = 25%
- Disodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5\ H_2O$
- Acetic acid, c(CH_3COOH) = 2 mol/L

Solutions

Titrant	c(I_2) = 0.01 mol/L
Glyoxal solution	w($C_2H_2O_2$) = 40% 200 mL w($C_2H_2O_2$) = 40% is adjusted with c(NaOH) = 1 mol/L to pH = 7.0. The solution has to be stored in a dark bottle in a refrigerator.

Standard

Disodium thiosulfate pentahydrate	Disodium thiosulfate pentahydrate is dried at 120 °C for 2 h and cooled down in a desiccator for at least 1 h.

Sample preparation

- Drinks, fruits and vegetable juices can be analyzed directly.

- For tablets and other vitamin preparations, a diluted solution in degased dist. water is prepared. Thereof an aliquot is used for the titrimetric determination.
- Foods, stimulants and animal feeds are extracted using the appropriate procedures.

Analysis

Titer

Approximately 50 mg $Na_2S_2O_3 \cdot 5\ H_2O$ is weighed into a titration beaker with an accuracy of 0.1 mg, 5 mL $c(CH_3COOH)$ = 2 mol/L is added and the solution is diluted to approximately 80 mL with deionized water. The solution is then titrated with $c(I_2)$ = 0.01 mol/L until after the first equivalence point.

Sample

50 mL sample is pipetted into a titration beaker, 2 mL glyoxal solution is added and the mixture is stirred briefly and allowed to stand for 5 min. After the addition of 5 mL sulfuric acid $w(H_2SO_4)$ = 25% it is titrated with $c(I_2)$ = 0.01 mol/L until after the equivalence point.

Parameters

Titer

Mode	MET Ipol
Vol. increment	0.1 mL
Signal drift	50 mV/min
Max. waiting time	26 s
I(pol)	1 µA
EP criterion	30 mV
EP recognition	greatest

Sample

Mode	MET Ipol
Vol. increment	0.1 mL
Signal drift	50 mV/min
Max. waiting time	26 s
I(pol)	1 µA
EP criterion	30 mV
EP recognition	greatest

Calculation

Titer

$$f = \frac{m_s}{V_{EP1} \times 2 \times c_{I_2} \times M_A}$$

f: Titer of the selected titrant
m_s: Mass of standard in mg
V_{EP1}: Titrant consumption until the first equivalence point in mL
2: Stoichiometric factor
c_{I_2}: Concentration of the selected titrant in mol/L; here $c(I_2)$ = 0.01 mol/L
M_A: Molar mass of the analyte; here 248.18 g/mol

Sample

$$\beta_{AA} = \frac{V_{EP1} \times c_{I_2} \times f \times M_{AA} \times 1000}{V_S}$$

β_{AA}: Mass concentration of ascorbic acid (AA) in mg/L
V_{EP1}: Titrant consumption until the first equivalence point in mL
c_{I_2}: Concentration of titrant in mol/L
f: Correction factor («titer») without unit
M_{AA}: Molar mass of ascorbic acid in g/mol; here 176.13 g/mol
1000: Conversion factor
V_S: Sample volume in mL

Example determination

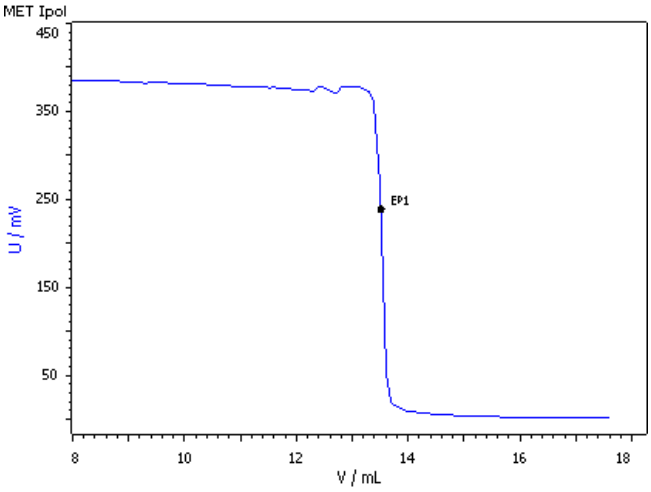


Fig. 1: Titration curve of the bi-voltametric ascorbic acid determination with iodine

Comments

- The addition of the glyoxal solution ensures that no SO_2 is determined together with the ascorbic acid.
- This method does not determine ascorbic acid alone. Under the given conditions other oxidizable compounds will also be determined. For a more selective titration 2,6-dichlorophenol indophenol (DPIP) can be used as titrant.

References

- Schweizerisches Lebensmittelbuch – Method 703.1
  Determination of ascorbic acid in fruit and vegetable juices, iodometric

Bi-voltametric determination with 2,6-Dichlorophenol-indophenol

Instruments

- Titrator with MET mode
- 10 mL burette
- Stirrer

Electrode

Double Pt-sheet electrode	6.0309.100

Reagents

- 2,6-Dichlorophenol-indophenol sodium salt dehydrate, DPIP
- Sodium hydrogen carbonate, $NaHCO_3$
- Oxalic acid dihydrate, $C_2H_2O_4 \cdot 2\ H_2O$

Solutions

Titrant	c(DPIP) ≈ 0.001 mol/L Approx. 330 mg DPIP is dissolved in about 250 mL hot dist. water (50 – 60 °C) containing 100 mg $NaHCO_3$, transferred into a 1000 mL volumetric flask and made up to the mark with dist. H_2O. The solution can be stored up to one week in a brown glass bottle in a refrigerator. The titer of this solution has to be determined daily.
Oxalic acid solution	w($H_2C_2O_4$) = 2% 28 g oxalic acid dihydrate is weighed into a 1 L volumetric flask, dissolved in dist. H_2O. The flask is then filled up to the mark with dist. H_2O.

Standard

Ascorbic acid standard	β(ascorbic acid) = 0.5 g/L 50.0 mg dried ascorbic acid is weighed into a 100 mL volumetric flask and made up to the mark with the oxalic acid solution. This solution has to be freshly prepared.

Sample preparation

- Drinks, fruits and vegetable juices can be analyzed directly.
- For tablets and other vitamin preparations, a diluted solution in degased dist. water is prepared. Thereof an aliquot is used for the titrimetric determination.
- Foods, stimulants and animal feeds are extracted using the appropriate procedures.

Analysis

Titer

1 to 3 mL standard solution and 50 mL oxalic acid solution are added into a titration beaker. The solution is then titrated with c(DPIP) ≈ 0.001 mol/L until after the first equivalence point.

Sample

The sample containing 0.05 to 0.5 mg ascorbic acid is pipetted into the titration beaker. 50 mL oxalic acid solution is added. The solution is then titrated with c(DPIP) ≈ 0.001 mol/L until after the equivalence point.

Parameters

Titer

Mode	MET Ipol
Stirring rate	4
Start volume	1 mL
Pause	30 s
Vol. increment	0.05 mL
Signal drift	30 mV/min
Max. waiting time	32 s
I(pol)	1 µA
EP criterion	30 mV
EP recognition	greatest

Sample

Mode	MET Ipol
Stirring rate	4
Start volume	1 mL
Pause	30 s
Vol. increment	0.05 mL
Signal drift	30 mV/min
Max. waiting time	32 s
I(pol)	1 µA
EP criterion	30 mV
EP recognition	greatest

Calculation

Titer

$$f = \frac{\beta_{AA} \times V_A}{c_{DPIP} \times V_{EP1} \times M_{AA}}$$

f:	Titer of the DPIP solution
β_{AA}:	Concentration of the standard solution in g/L
V_A:	Added volume of ascorbic acid solution in mL
c_{DPIP}:	Concentration of titrant in mol/L
V_{EP1}:	Titrant consumption until the first equivalence
M_{AA}:	Molar mass of ascorbic acid in g/mol (176.12 g/mol)

Sample

$$\beta_{AA} = \frac{V_{EP1} \times c_{DPIP} \times f \times 1000 \times M_{AA}}{V_A \times d}$$

β_{AA}:	Mass concentration of ascorbic acid (AA) in mg/L
V_{EP1}:	Titrant consumption until the first equivalence point in mL
c_{DPIP}:	Concentration of titrant in mol/L
f:	Correction factor («titer») without unit
1000:	Conversion factor
M_{AA}:	Molar mass of ascorbic acid in g/mol (176.12 g/mol)
V_A:	Aliquot volume in mL
d:	Dilution factor

$$d = \frac{V_S + V_{Ex}}{V_S}$$

V_S:	Sample volume in mL
V_{Ex}:	Volume of extraction solution in mL

Example determination

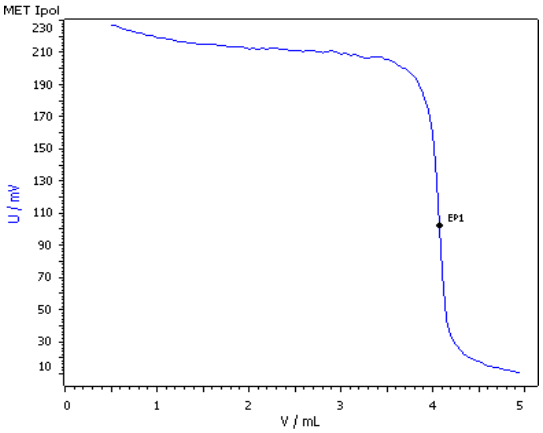


Fig. 2: Titration curve of the bi-voltametric ascorbic acid determination with DPIP as titrant

References

- G.Pongracz
  Neue potentiometrische Bestimmungsmethoden für Ascorbinsäure und dessen Verbindungen
  Fresenius Z. Anal. Chem. 253, 1971, 271 – 274

Photometric determination

Instruments

- Titrator with MET mode
- 10 mL burette
- Stirrer

Electrode

Optrode (520 nm)	6.1115.000

Reagents

- 2,6-Dichlorophenol-indophenol, DPIP
- Sodium hydrogen carbonate
- Oxalic acid dihydrate

Solutions

Titrant	c(DPIP) ≈ 0.001 mol/L Approx. 330 mg DPIP is dissolved in about 250 mL hot dist. water (50 – 60 °C) containing 100 mg $NaHCO_3$, transferred into a 1000 mL volumetric flask and made up to the mark with dist. H_2O. The solution can be stored up to one week in a brown glass bottle in a refrigerator. The titer of this solution has to be determined daily.
Oxalic acid solution	w($H_2C_2O_4$) = 2% 28 g oxalic acid dihydrate is weighed into a 1 L volumetric flask, dissolved in dist. H_2O. The flask is then filled up to the mark with dist. H_2O.

Standard

Ascorbic acid standard	β(ascorbic acid) = 0.5 g/L 50.0 mg dried ascorbic acid is weighed into a 100 mL volumetric flask and made up to the mark with the oxalic acid solution. This solution has to be freshly prepared.

Sample preparation

- Juices without pulp can be analyzed directly.
- For tablets and other vitamin preparations, a diluted solution in degased dist. water is prepared. Thereof an aliquot is used for the titrimetric determination.
- Foods, juices with pulp, stimulants, and animal feeds are extracted using the appropriate procedures. Colored substances can remain in the sample, because they do not interfere with the determination of ascorbic acid.

Analysis

For both titer and sample a blank determination has to be carried out. If the sample is directly measured (without extraction) the same blank value can be used for titer and sample.

Blank

For the titer the following blank determination is performed: 30 mL oxalic acid solution are titrated with c(DPIP) ≈ 0.001 mol/L until after the equivalence point using the Optrode at 520 nm.

If the sample is extracted, an aliquot of the extraction solution is pipetted into a titration beaker, 30 mL oxalic acid solution is added and followed by the titration with c(DPIP) ≈ 0.001 mol/L until after the equivalence point using the Optrode at 520 nm.

Titer

1 to 3 mL standard solution is pipetted into the titration vessel and 30 mL oxalic acid solution is added. The solution is then titrated with c(DPIP) ≈ 0.001 mol/L until after the equivalence point using the Optrode at 520 nm.

Sample

The sample or an aliquot of the extracted sample solution (containing at least 0.2 mg ascorbic acid) is added into the titration beaker, 30 mL oxalic acid solution is added and the solution is then titrated with c(DPIP) ≈ 0.001 mol/L until after the equivalence point using the Optrode at 520 nm.

Parameters

Mode	MET U
Stirring rate	6
Start volume	0.5 mL
Pause	30 s
Signal drift	30 mV/min
Max. waiting time	32 s
Volume increment	0.05 mL

EP criterion	15 mV
EP recognition	greatest

Calculation

Titer

$$f = \frac{\beta_{AA} \times V_A}{c_{DPIP} \times (V_{EP1} - Blank_{titer}) \times M_{AA}}$$

f:	Titer of the DPIP solution
β_{AA}:	Concentration of the standard solution in g/L
V_A:	Added volume of ascorbic acid solution in mL
c_{DPIP}:	Concentration of titrant in mol/L
V_{EP1}:	Titrant consumption until the first equivalence
$Blank_{titer}$:	Volume of the blank consumption for the titer determination in mL
M_{AA}:	Molar mass of ascorbic acid in g/mol (176.12 g/mol)

Sample

$$\beta_{AA} = \frac{(V_{EP1} - Blank_{sample}) \times c_{DPIP} \times f \times 1000 \times M_{AA}}{V_A \times d}$$

β_{AA}:	Mass concentration of ascorbic acid (AA) in mg/L
V_{EP1}:	Titrant consumption until the first equivalence point in mL
$Blank_{sample}$:	Volume of the blank consumption for the sample determination in mL
c_{DPIP}:	Concentration of titrant in g/L
f:	Correction factor («titer») without unit
1000:	Conversion factor
M_{AA}:	Molar mass of ascorbic acid in g/mol (176.12 g/mol)
V_S:	Aliquot volume in mL
d:	Dilution factor

$$d = \frac{V_S + V_{Ex}}{V_S}$$

V_S:	Sample volume in mL
V_{Ex}:	Volume of extraction solution in mL

Example determination

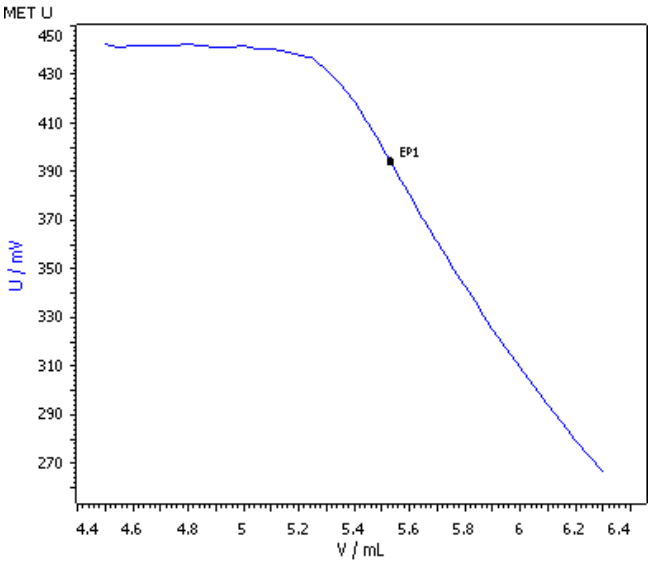


Fig. 3: Titration curve of the photometric determination of ascorbic acid in blood orange juice

Comments

- Samples containing iron, tin or copper salts as well as sulfite, thiosulfate or sulfur dioxide cannot be analyzed using this method.
- Instead of 2,6-dichlorophenol indophenol, other oxidizing titrants can be used as well, e.g. iodine, iodide/iodate, chloramine-T, Fe(III). Here apart from ascorbic acid other oxidizable compounds present in the sample will also be determined.
- Juices with pulp should be extracted because the pulp disturbs the measurement signal leading to large signal drifts and long titration times.

References

- ISO 6557/2
  Fruits, vegetables and derived products – Determination of ascorbic acid content – Part 2: routine methods
- Schweizerisches Lebensmittelbuch – Method1560.1
  Determination of ascorbic acid in food stuff and cosmetics, titrimetric
- L. Erdey, G. Svehla
  Ascorbinometric Titrations, Akademiai Kiado, Budapest, 1973

Polarographic determination

Instruments

- VA instrument
  capable of operating a mercury electrode and supporting DP mode

Electrode

WE	Multi-Mode Electrode pro Mercury drop capillary	6.1246.120 6.1226.030
AE	Separate Pt rod electrode	6.0343.000
RE	Ag/AgCl reference electrode c(KCl) = 3 mol/L Electrolyte vessel filled with c(KCl) = 3 mol/L	6.0728.020 6.1245.010

Reagents

- Sodium acetate anhydrous, CH_3COONa, for analysis, CAS 127-09-3
  or
  Sodium acetate trihydrate, $CH_3COONa \cdot 3H_2O$, for analysis, CAS 6131-90-4
- Acetic acid, w(CH_3COOH) = 100%, for analysis, CAS 64-19-7
- L-ascorbic acid (vitamin C), $C_6H_8O_6$, for analysis, CAS 50-81-7
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

Solutions

Acetate buffer pH = 4.6	8.2 g sodium acetate anhydrous or 13.61 g sodium acetate trihydrate is dissolved in ultrapure water. 6 mL acetic acid is added and the solution is made up to 100 mL with ultrapure water.

Standard

Ascorbic acid standard solution	β(Ascorbic acid) = 1 g/L 50 mg L-ascorbic acid is dissolved in degased ultrapure water and made up to 50 mL. This solution has to be freshly prepared every day.

Sample preparation

- Drinks, fruits and vegetable juices can be analyzed directly
- For tablets and other vitamin preparations, a diluted solution in degased ultrapure water is first prepared, an aliquot of which is then used for the determination.
- Foods, stimulants and animal feeds are extracted using appropriate procedures.

Analysis

Measuring solution

10 mL ultrapure water

1 mL acetate buffer pH 4.6

0.5 mL sample

10 mL ultrapure water, 1 mL acetate buffer pH = 4.6 and 0.5 mL sample are pipetted into a polarographic vessel. After degasing the measuring solution with nitrogen for 300 s a DP polarogram is recorded using the parameters given below.

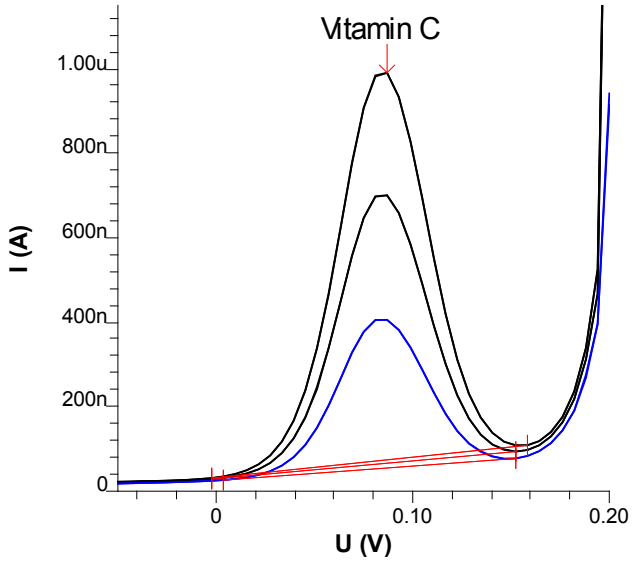
The concentration of ascorbic acid is quantified by two additions of ascorbic acid standard solution β(ascorbic acid) = 1 g/L.

Parameters

Determination	
No. of additions	2
No. of replications	2
Voltammetric	
Electrode	DME
Measuring mode	DP – Differential Pulse
Stirring speed	2000 min^{-1}
Hydrodynamic measurement	No
Sweep	
Equilibration time	10 s
Start potential	-0.05 V
End potential	0.2 V
Pulse amplitude	0.05 V
Pulse time	0.04 s
Potential step	0.06 V
Potential step time	0.6 s
Sweep rate	0.01 V/s
Substance + calibration	
Calibration	Standard addition
Name	Vitamin C

Peak potential	0.1 V
Tolerance	0.05 V
Baseline	Linear Automatic

Example determination

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Vitamin C
c =  243.506 mg/L
+/-    0.999 mg/L (0.41%)
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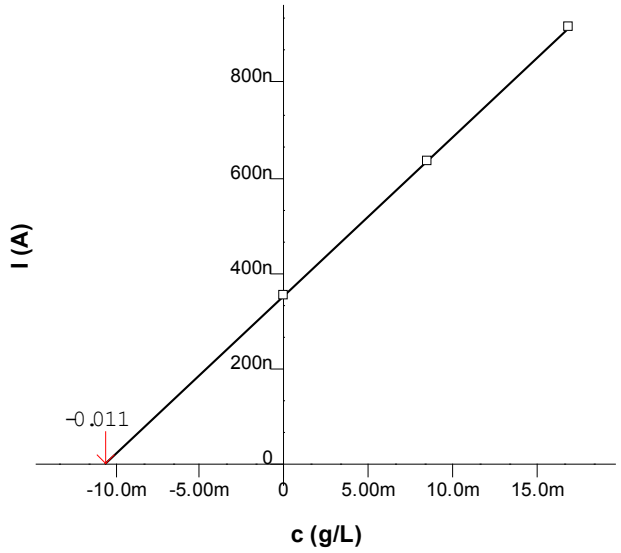

Fig. 4: Polarogram and calibration curve of a determination of ascorbic acid in orange juice.

Comments

- Ascorbic acid is sensitive against oxygen and light. Therefore it is recommended to degas the deionized water for the preparation of the standard solution and keep the standard in the dark. The standard solution should only be used on the day of preparation.
- Larger quantities of chloride ions (e.g. in sauerkraut) interfere with the polarographic determination of ascorbic acid. They are removed from the sample solution by precipitation with silver nitrate and subsequent filtration.

References

- D. Amin
  Application of differential pulse polarography to the assay of ascorbic acid
  Microchem. J., 28, 1983, 174 – 179.
- S. Kozar, A. Bujak, J. Eder-Trifunovic, G. Kniewald
  Determination of L-ascorbic acid in fresh and processed fruit and vegetables by differential pulse polarography
  Fresenius Z. Anal. Chem., 329, 1988, 760 – 763.

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